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An Autopsy Study of Cranio-Cerebral Injuries Due to Road Traffic Accidents

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Abstract

Cranio-cerebral injuries are a morbid state, resulting from gross structural changes to the scalp, skull and the contents of the cranium. In present scenario, accidents are now considered as one of major contributing factors of Non-Communicable Diseases which have become major health problems of developing countries like India. According to the national crime bureau statistics In India, over 1,46,133 people were killed in various road traffic accidents during 2015 and the count raised to 1,50,785 in 2016. Information on the contributing factors is mandatory to reduce this burden. A prospective study of all Road Traffic accidents coming for autopsy at Government Medical College, Anantapuram. Majority of victims were male in the age group between 31-40 years. Most of them had Linear fractures of the skull along with Sub dural haemorrhage. Two Wheeler riders were the most common victims. Most of the victims died due to Cranio-Cerebral Injury. Most of the times, it is the negligence, which results in an accident. The negligence may be on the part of the victim or may be there on the part of other person who occupied the second vehicle. Sometimes innocent pedestrians are also victimized to these deaths. An accident for all practical purposes is preventable, provided if it is anticipated and proper precaution and preventive measures are adopted.

Key words : *Road traffic accidents, Intracranial Hemorrhage, Skull fractures, Pedestrian*

Introduction

Road traffic accidents (RTA) can be defined as ‘’ An accident that occurred on a way or street open to public traffic; resulted in one or more persons being killed or injured and at least one moving vehicle was involved. Thus RTA is collisions between vehicles, between vehicles and pedestrians; between vehicles and animals; or between vehicles and geographical or architectural obstacles [1] .” Road Traffic Accidents (RTA) are increasing in alarming ways. Globally nearly 1.2 million people killed in RTA during the year 2002. Developing and underdeveloped countries accounted for 80% of these deaths and 21% of total injury projected estimations reveals that fatalities due to RTA will be the 3rd leading cause of death by 2020 moving from its present 9th position [2] .Developing countries are very different from the industrialized countries with regard to the environment and mix of vehicles in the traffic stream [3] . Number of ‘Traffic Accidents’ in the country have been increased by 3.1% (from 4,81,805 in 2014 to 4,96,762 in 2015) during 2015 compared to 2014.[4] Road Traffic Accidents (RTAs) are an important public health problem requiring urgent attention in developing countries such as India, which has the highest proportion of deaths due to RTAs in South East Asia. One of the main causes of death and disability is road traffic injuries, with an unequal number of incidences in developing countries.[5]. In 2000, RTA were the ninth leading cause of disability-adjusted life years lost and are projected to become third by 2020.[6] Every year, more than 20 million people are injured or disabled and 1.17 million are killed because of RTAs.[6] In developing countries, more than 85% of the deaths and nearly 90% of the

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disabilities are caused by RTAs globally.[7] In India, of the mortality of 2,123.60 from all causes, 1,463 are caused by RTA. The World Health Organization (WHO) report on Global Status Report on Road Safety, which is the first of its type, states that, with more than 130,000 deaths annually, India has overtaken China and now has the worst RTA rate worldwide.[8]

Materials and Method

All the post-mortem examinations conducted in Ananthapuram medical college mortuary on deaths due to road traffic accidents during 2016 year were analysed. The data regarding pattern of injuries leading to death, blood alcohol levels in the dead victims, age group affected, statistics of deaths among pedestrians and two wheeler drivers were collected for analysis. The study was conducted with the permission and help of medical records section and autopsy surgeons who have done post-mortem examination in various cases of deaths due to road traffic accidents. All cases of head injuries and injuries over other parts of the body due to RTAs are taken for the study. Decomposed bodies are not included in the present study. A total number of 234 cases were studied during the year 2016. This study included detailed history regarding the incident and complete clinical history including operative procedures, if any and detailed external and internal examination of the body.

Observation and Results

The current study conducted on 234 deaths due to road traffic accidents for which autopsy done in mortuary of Anantapuram government medical college, Andhra Pradesh during the year of 2016.

Out of 234 deaths **majority victims were** males. Males accounted to 150 (64%) and females were 84 (36%). As per age wise observation 73 deaths(males 45, females 28) occurred in the age group of 31-40 years. In the age group of 41 to 50 years the number of deaths were 58 (males 37, females 22). Next age group is 21 to 30 years in which 40 deaths (males 25, females 15) occurred; in the age group of 51 to 60 years 25 deaths(males 15, females 10) occurred; in the age group of 10 to 20 years 19 deaths (males 16, females 3) occurred; 14 people (males 8, females 6)more than 61 years are also died. Whereas 4 children (males 4 and no females) less than 10 years also died. If we take up the graph the peak is rising towards 31 to 40 years age group both in males and females.

During study of distribution of injuries to other body parts, Maximum number of victims had injuries associated with other body parts (159 cases) followed by head injuries(75 cases).

In our study different types of extra cranial injuries either alone are in combination were found. Among those abrasion was found in (134) followed by contusion (94), laceration(83), fractures(31), crushing injury (8) incised(6), penetrated(4) injuries respectively.

Out of 234 cases studied, 18 were intact without any fracture of the skull. 216 cases had skull fractures. Among them linear/fissure fractures topped the list of skull fractures (112), followed by , Communited fractures (58), Depressed (20), combined (15), sutural separation (9), crush injuries (expressed fractures) (9) and the least being the ring fractures (2) and no gutter fractures seen and these fractures found either individually or in combination with each other

After opening the skull cavity the different kinds of intra cranial haemorrhages were observed in the study group. The type of intracranial haemorrhages found either individually or in combination with each other. Among these, Subdural haemorrhage either singly or in combination with others was found in 103 head injury cases. Next comes subarachnoid haemorrhage and it was found in 93cases, followed by intra cerebral haemorrhage(14), Extra dural haemorrhage(12) intra ventricular haemorrhage(5), Pontine haemorrhage (5) Cerebellar haemorrhage(2).

It was observed in current study the cranio cerebral injury was the leading cause of death in most of the victims that is in173deaths. Followed by haemorrhagic shock in 47 victims then neurogenic shock.

Table:1. Age and Sex wise distribution cases of fatal RTAs

S.No	Age	Male		Female		Total	
		No of cases	%	No of cases	%	No of cases	%
1	<10	4	1.71	0	0	4	1.71
2	11-20	16	6.84	3	1.28	19	8.12
3	21-30	25	10.68	15	6.41	40	17.09
4	31-40	45	19.23	28	11.97	73	31.20
5	41-50	37	15.81	22	9.40	59	25.21
6	51-60	15	6.41	10	4.27	25	10.68
7	>60	8	3.41	6	2.56	14	5.97
	Total	150		84		234	100

Table:2. Head injury association with other body part injury

S.no	Item	No Of Cases	Percentage
1	Head injury with other body parts injuries	159	67.95
2	Only head injury	75	32.05
3	Total	234	100

Table:3. Types of extra cranial injuries

s.no	Type of injuries Associated with headinjuries.	Total	Percentage(%)
1	Abrasion	134	57.26
2	Contusion	94	40.17
3	Laceration	83	35.47
4	Incised injury	6	25.64
5	Penetrated injury	4	17.09
6	Crushing injury	8	34.19
7	Fractures	31	13.25

Table:4.Frequency of different types of fracture

s.no	Types of fracture	Total	Percentage(%)
1	Linear/ fissure	112	47.87
2	Communited	58	24.78
3	Depressed	20	08.54
4	Gutter	0	0
5	Ring	2	0.86
6	Sutural separation	9	3.84
7	Combined	15	6.42
8	No fracture	18	7.69
9	Total	234	100

Table:5. Distribution of the Intracranial haemorrhages with respect to site in cases of fatal RTA

s.no	Type of Hemorrhage	Number of cases	Percentage (%)
1	Extra Dural Hemorrhage (EDH)	12	5.13
2	Sub Arachnoid hemorrhage(SAH)	93	39.74
3	Sub Dural hemorrhage(SDH)	103	44.02
4	Pontine hemorrhage	5	2.13
5	Intra cerebral hemorrhage(ICH)	14	5.98
6	Cerebellar hemorrhage	2	0.85
7	Intraventicular Hemorhage(IVH)	5	2.14

Table:6. Different causes of Death in cases of fatal RTA

s.no	Cause of death	Number of cases	Percentage(%)
1	Cranio cerbral Injury(CCI)	173	73.93
2	Neurogenic Shock(NS)	9	38.46
3	Hemorrhagic Shock(HS)	47	20.09
4	Septic Shock(SS)	5	2.14
5	Total	234	100

Discussion

Head Injuries are more prone in road traffic accidents. These are due to the exchange of force between the body parts which are moving and the stationary hard objects present on and around the road. Or the human body may be stationary and the moving object come and hit the person wherein there is an exchange of force which results in the deformity of the body parts and the result is the injuries spread over the body surface or internal organs of the body.

The results of our study on the cranio cerebral injury due to RTA were analyzed and compared with other studies conducted at various places in India and abroad.

Table:1 in our present study maximum number of head injury victims were males in the age group of 31-40 years. The reason could be that the males of in this age group are prime earners of the family and remain outdoors during most of the day, risk taking behavior, Male dominance is explained by the fact that, males are more exposed to hazards of road. Similar results were observed in the various studies [9,10,11] while study by shamim monga et al[12] contrasts our finding where the common age group was between 21-30 years.

Table:2 In our study injuries to other body parts also has happened along with head injury, similar finding found in study by Shobhna et al (13). As most of the victims are from two wheeler accidents and pedistrians, victims had involvement of other body parts after hitting was more common.

Table:3 In present study grazed abrasion was most common associated injury either single or in combination with other injuries. Similar findings were observed in study conducted by Oberoi et al [14] . However study conducted by Shobhna et al [13] laceration was most common associated injury. Whenever there is a road traffic accident there is blunt force acting on the victim so it causes injuries like abrasion, contusion, lacerations.

Table:4 In regards to fracture of skull bone, we found that linear fracture was most common, similar findings were observed in studies conducted by Wankhede et al [15], Menon et al [16], Shobhna et al [13]. Type of fracture occurring after vehicular accident depends on various factors e.g. speed, safety measures used, ground over which fall occurs. In severe accidents combination of fractures is observed. In another study which was carried out by kuchewar et al[17], almost 79.87% of victims were with skull fracture . Fracture of the vault of skull was more common than the base of the skull and most common type of fracture was linear. These results are similar to the results of our study.

Table:5 In current study, most common type of haemorrhage was subdural (either alone or with combination) which is similar to study conducted by Ravikumar et al (18) and Arvind kumar et al (19) while study by Wankhede et al[15] contrasts our finding where Sub Arachnoid Haemorrhage was most common.

Table:6 in our study the commonest cause of death was Cranio Cerebral Injury, it is consistant with the study conducted by B. Srinu Naik et al.[20]

The findings in the present study most of the vulnerable group road users are, two wheeler riders, and pedestrians . Similar findings observed in the study by Gururaj et al.[10] . These groups of road users form the major bulk on Indian roads, and hence, their exposure is higher. Unlike occupants in cars and other heavy vehicles, these road users are directly exposed to the traffic environment,

Summary and Conclusion:

The present study can be summarized and concluded as, head injury deaths due to road traffic accidents are occurring in significant number in day to day medicolegal autopsies. Among Highest number of deaths belonged to age group of 31 to 40 years with male preponderance. Majority Of Head injuries are associated with other body parts injuries and abrasion being the commonest. Linear fracture was most common type of skull fracture, and the Sub Dural Haemorrhage was the leading type of intracranial haemorrhage. Cranio cerebral injury being the commonest cause of death and two wheeler riders being the most vulnerable group of affected road users.

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Original Research Article

Estimation of age from Shoulder Joint by Radiographic Assessment of Epiphyseal Fusion of Related Bones in Population of Chhattisgarh: A Cross Sectional Study

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Abstract

Age estimation of an individual is of medico legal importance in both civil & criminal cases. Ossification centres appear & fuse in a fairly definite sequence at a particular age group from which age of an individual can be determined. This study aims to investigate the relationship between the stage of epiphyseal union at shoulder joint & biological age in Chhattisgarh population. The study was carried out in 100 healthy subjects (40 girls and 60 boys) aging from 13 to 20 years. The obtained results from the radiographs revealed that the complete fusion of epiphysis of shoulder joint is seen at 18-20 years. Females were consistently developing epiphyseal union at a younger age than their male counterparts, with two years of difference. Results also suggest that the age of epiphyseal union is found to vary greatly all over the world indicating the need for separate standards of age of epiphyseal union for separate regions.

Key Words: *Epiphyseal Union, Shoulder Joint, Proximal End of Humerus.*

Introduction

In law, crime and punishment is entirely based on criminal responsibility and this in turn depends on the age of a person. Determination of age of an individual from epiphyseal union is a well accepted fact in the field of medical and legal professions. Epiphysis of the bone unites during age periods which are remarkably constant for a particular epiphysis. This is possible due to complex but dependable system by which the osseous framework of his body develops, grows & matures. Extensive work for the determination of age by epiphyseal union has been carried out in abroad and different states of India which revealed differences in the ages of epiphyseal union. The differences may be an account of varying dietetic, geographic, hereditary and other factors. Present study has been undertaken in indigenous population of Chhattisgarh from ossification around shoulder joints roentgenographically.

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Aims & Objectives:

1. To evaluate age specific difference in epiphyseal union at shoulder joint.

2. To estimate age from epiphyseal union at shoulder joint.

3. To compare bisexual difference in epiphyseal union at shoulder joint.

4. To compare the findings in the epiphyseal union at shoulder joint in Central Indian population with other parts of India on the basis of previous studies.

Material & Method

The present study was carried out in the Department of Forensic Medicine & Department of Radiology, SSIMS, Bhilai, Chhattisgarh (Central India). A total of 120 individuals participated in this study. The subjects included were students of 13-20 years of age from schools & colleges from Bhilai city. They are born to parents living in Central India and have lived here since birth. The subjects do not have any disease/deformity pertaining to bones or chronic disease affecting the general health. An informed consent was taken from all

subjects prior to each investigation.

Procedure of Radiography: After taking written consent, thorough physical examination & radiological evaluation was done. X-Rays of right shoulder joint were taken with the help of X-Ray machine in the Department of Radiology. Minimum shots were taken to expose the joints involved in study. Minimum & appropriate voltage settings of X-Ray machine were applied so as to avoid unnecessary radiation exposure of the subjects. All the radiological procedure was undertaken according to the prescribed standards. Skeletal maturity was evaluated according to the Jits & Kulkarni's[7] classification of four stages: Appearance, Non fusion, Partial fusion & complete fusion ("A", "NF", "PF", "CF" respectively). The master chart was prepared and tabulated as per code given above. The data was examined and tallied by experts in Forensic Medicine and Radio-diagnosis. It was classified, analysed and compared with known standards. At the end conclusions were drawn, which were compared with available results of various previous studies.

Radiographs showing stages of fusion of proximal end of humerus:

Fig 1. (Non fusion)

Fig 2. (Partial fusion)

Fig 3. (Complete fusion)

Results

TABLE 1: Age & gender wise distribution of subjects.

Age in years	Males		Females	
	No	%	No	%
13-14	6	10	4	10
14-15	7	11.67	4	10
15-16	9	15	8	20
16-17	10	16.67	7	17.5
17-18	12	20	7	17.5
18-19	8	13.33	5	12.5
19-20	8	13.33	5	12.5
Total	60	100	40	100

Table 2: Epiphyseal fusion at proximal end of humerus:

Males				**Females**		
Age (years)	**NF**	**PF**	**CF**	**NF**	**PF**	**CF**
13-14	5 (8.33%)	1 (1.67%)	0 (0%)	2 (5%)	2 (5%)	0 (0%)
14-15	4 (6.67%)	3 (5%)	0 (0%)	0 (0%)	4 (10%)	0 (0%)
15-16	2 (3.33%)	6 (10%)	1 (1.67%)	0 (0%)	3 (7.5%)	5 (12.5%)
16-17	0 (0%)	6 (10%)	4 (6.67%)	0 (0%)	2 (5%)	5 (12.5%)
17-18	0 (0%)	2 (3.33%)	10 (16.67%)	0 (0%)	0 (0%)	7 (17.5%)
18-19	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
19-20	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
Total	60 (100%)			40 (100%)		

In males, proximal end of humerus shows: non fusion in 11 (18.33%) cases in age group of 13-16 years, partial fusion in 18 (30%) cases in age group of 13-18 years & complete fusion in 31 (51.67%) cases in age group of 15-20 years. Complete fusion is seen in all the subjects in age group of 18-20 years.

In females, distal end of humerus shows: non fusion in 2 (5%) cases in age group of 13-14 years, partial fusion in 11 (27.5%) cases in age group of 13-17 years & complete fusion in 27 (67.5%) cases in age group of 15-20 years. Complete fusion is seen in all the subjects in age group of 17-20 years.

Table 3: Epiphyseal fusion at acromion process:

Males				**Females**		
Age (years)	**NF**	**PF**	**CF**	**NF**	**PF**	**CF**
13-14	6 (10%)	0 (0%)	0 (0%)	4 (10%)	0 (0%)	0 (0%)
14-15	7 (11.67%)	0 (0%)	0 (0%)	2 (5%)	2 (5%)	0 (0%)
15-16	7 (11.67%)	2 (3.33%)	0 (0%)	1 (2.5%)	7 (17.5%)	0 (0%)
16-17	0 (0%)	8 (13.33%)	2 (3.33%)	0 (0%)	5 (12.5%)	2 (5%)
17-18	0 (0%)	9 (15%)	3 (5%)	0 (0%)	1 (2.5%)	6 (15%)
18-19	0 (0%)	2 (3.33%)	6 (10%)	0 (0%)	0 (0%)	5 (12.5%)
19-20	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
Total	**60 (100%)**			**40 (100%)**		

In males, acromion process shows: non fusion in 20 (33.34%) cases in age group of 13-16 years, partial fusion in 21 (35%) cases in age group of 15-19 years & complete fusion in 19 (31.66%) cases in age group of 16-20 years. Complete fusion is seen in all the subjects in age group of 19-20 years.

In females, acromion process shows: non fusion in 7 (17.5%) cases in age group of 13-16 years, partial fusion in 15 (40.5%) cases in age group of 14-18 years & complete fusion in 18 (45%) cases in age group of 16-20 years. Complete fusion is seen in all the subjects in age group of 18-20 years.

Table 4: Fusion of Coracoid Process:

Males				**Females**		
Age (years)	**NF**	**PF**	**CF**	**NF**	**PF**	**CF**
13-14	4 (6.66%)	2 (3.33%)	0 (0%)	2 (5%)	2 (5%)	0 (0%)
14-15	4 (6.66%)	3 (5%)	0 (0%)	0 (0%)	4 (10%)	0 (0%)
15-16	2 (3.33%)	7 (11.67%)	0 (0%)	0 (0%)	2 (5%)	6 (15%)
16-17	0 (0%)	2 (3.33%)	8 (13.33%)	0 (0%)	0 (0%)	7 (17.5%)
17-18	0 (0%)	0 (0%)	12 (20%)	0 (0%)	0 (0%)	7 (17.5%)
18-19	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
19-20	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
Total	60 (100%)			40 (100%)		

In males, coracoid process shows: non fusion in 10 (16.65%) cases in age group of 13-16 years, partial fusion in 14 (23.33%) cases in age group of 13-17 years & complete fusion in 36 (60%) cases in age group of 16-20 years. Complete fusion is seen in all the subjects in age group of 17-20 years.

In females, coracoid process shows: non fusion in 2 (5%) cases in age group of 13-14 years, partial fusion in 8 (20%) cases in age group of 13-16 years & complete fusion in 30 (75%) cases in age group of 16-20 years. Complete fusion is seen in all the subjects in age group of 16-20 years.

Discussion

Table 5: Comparison of average ages of fusion of epiphyses around shoulder joint in males and females:

Sr. No	**Researcher**	**Humerus (years)**	**Acromion (years)**	**Coracoid (years)**
1.	Davies & Parson (1927)1	19-21	-	-
2.	Flecker (1932)2	17-19	17	18-20
3.	Pillai (1936)3	17	18	14
4.	Galstaun (1937)4	17-19	17-19	-
5.	Cardoso Hugo (2008)5	20-23	19-20	17-18
6.	Buri S et al (2017)6	18-19	19-20	16-17
7.	Present study	17-19	18-20	16-18

Proximal end of humerus: The observations of present study matches with works of Flecker (1932)[2] in Australian population, Pillai (1936)[3] in South Indian population & Galstaun (1937)[4] in Indian population. Studies conducted by Davies & Parson (1927)[1] in English population, Cardoso Hugo (2008)[5] in Portugese population & Buri S et al (2017)[6] in Rajasthani population show comparatively late fusion of epiphysis by 1-3 years.

Acromion: The observations of present study matches with work of Pillai (1936)[3] in South Indian population. Studies conducted by Galstaun (1937)[4] in Indian population, Cardoso Hugo (2008)[5] in Portugese population & Buri S et al (2017)[6] in Rajasthani

population show comparatively late fusion of epiphysis by 1-2 years.

Coracoid: The observations of present study matches with works of Buri S et al (2017)[6] in Rajasthani population. Studies conducted by Flecker (1932)[2] in Australian population & Cardoso Hugo (2008)[5] in Portugese population show late fusion by 1-2 years whereas there is early fusion at 14 years in study conducted by Pillai (1936)[3] in South Indian population.

Summary and Conclusions

1. This study was conducted exclusively on the young indigenous population of Chhattisgarh region.

2. The epiphyseal union of bones at right shoulder joint in males is completed in all instances (100%) at the age of 19-20 years.

3. The epiphyseal union of bones at right shoulder joint in females is completed in all instances (100%) at the age of 18-20 years.

4. As the sample size is limited further studies are necessary. Region wise studies should be conducted for better correlation and comparison.

5. Due to changing lifestyle pattern, dietary, climatic, behavioural factors age of ossification is changing as mentioned in the available literature. So as to evaluate these changes, studies are recommended in every region of India at regular time period for academic and judicial interest.

6. The opinion about age should be given always in the range. From this study it can be concluded that the opinion about age can be given in a range having margin of error of 1-2 years.

7. Radiological interpretations are observer dependent so the set standards should be considered under expert guidance to arrive at conclusion in such radiological studies.

8. For estimation of age relevant joints should be radiologically examined for different centres and opinion should be arrived considering the status of multiple centres.

Ethical Clearance- Taken from institutional ethical committee.

Source of Funding- Self.

Conflict of Interest- Nil.

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Original Research Article

Age Estimation from Radiological Evaluation of Epiphyseal Union of Related Bones Around Elbow Joint: A Cross Sectional Study from Central India (Chhattisgarh)

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Abstract

Radiological evaluation of the skeletal age represents an important tool for the estimation of the chronological age in the living. The aim of our study was to evaluate the relationship between the chronological age and degree of epiphyseal fusion around elbow joint in population of Chhattisgarh (Central India) and to compare our results with the results of similar studies. The study was carried out in 100 healthy subjects (40 girls and 60 boys) aging from 13 to 20 years. The obtained results from the radiographs revealed that the complete fusion of epiphysis of elbow joint is seen at 16-20 years. Females were consistently developing epiphyseal union at a younger age than their male counterparts. Results also suggest that the age of epiphyseal union is found to vary greatly all over the world indicating the need for separate standards of age of epiphyseal union for separate regions.

Key Words: *epiphyseal union, elbow joint, distal end of humerus.*

Introduction

Accurate age estimation of living persons provides an authentic official document to prove their chronological age, important for humanitarian, administrative & legal purposes. Radiographic evaluation of the skeletal age is one of the most reliable methods of the four pillars for age estimation. Nevertheless this method is only an estimate, having an inherent degree of error due to variation in human skeletal development. The aim of our study was to evaluate the relationship between the chronological age and the degree of epiphyseal fusion around elbow joint in population of Chhattisgarh (Central India) and to compare our results with the results of similar studies. Present study has been undertaken in an indigenous population of Chhattisgarh from ossification around elbow joint radiographically.

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Aims & Objectives

5. To evaluate age specific difference in epiphyseal union at elbow joint.

6. To estimate age from epiphyseal union at elbow joint.

7. To compare bisexual difference in epiphyseal union at elbow joint.

8. To compare the findings in the epiphyseal union at elbow joint in Central Indian population with other parts of India on the basis of previous studies.

Material & Method

The present study was carried out in the Department of Forensic Medicine & Department of Radiology, SSIMS, Bhilai, Chhattisgarh (Central India). A total of 100 individuals participated in this study. The subjects included were students of 13-20 years of age from schools & colleges from Bhilai city. They are born to parents living in Central India and have lived here since birth. The subjects do not have any disease/deformity pertaining to bones or chronic disease affecting the general health. An informed consent was taken from all subjects prior to each investigation.

Procedure of Radiography: After taking written consent, thorough physical examination & radiological evaluation was done. X-Rays of right elbow joint were taken with the help of X-Ray machine in the Department of Radiology. Minimum shots were taken to expose the joints involved in study. Minimum & appropriate voltage settings of X-Ray machine were applied so as to avoid unnecessary radiation exposure of the subjects. All the radiological procedure was undertaken according to the prescribed standards. Skeletal maturity was evaluated according to the Jit & Kulkarni's[17] classification of four stages: Appearance, Non fusion, Partial fusion & Complete fusion ("A", "NF", "PF", "CF" respectively). The master chart was prepared and tabulated as per code given above. It was classified, analysed and compared with known standards. At the end conclusions were drawn, which were compared with available results of various previous studies.

Results

TABLE 1: Age and gender wise distribution of subjects.

Age in years	Males		Females	
	No	%	No	%
13-14	6	10	4	10
14-15	7	11.67	4	10
15-16	9	15	8	20
16-17	10	16.67	7	17.5
17-18	12	20	7	17.5
18-19	8	13.33	5	12.5
19-20	8	13.33	5	12.5
Total	60	100	40	100

Table 2: Epiphyseal Fusion of the Composite epiphysis (Capitulum and Lateral region of Trochlea, Medial region of Trochlea, Lateral Epicondyle) with shaft of humerus:

Males				Females		
Age (years)	NF	PF	CF	NF	PF	CF
13-14	2 (3.33%)	4 (6.67%)	0 (0%)	0 (0%)	2 (5%)	2 (5%)
14-15	0 (0%)	2 (3.33%)	5 (8.33%)	0 (0%)	1 (2.5%)	3 (7.5%)
15-16	0 (0%)	2 (3.33%)	7 (11.67%)	0 (0%)	0 (0%)	8 (20%)
16-17	0 (0%)	0 (0%)	10 (16.67%)	0 (0%)	0 (0%)	7 (17.5%)
17-18	0 (0%)	0 (0%)	12 (20%)	0 (0%)	0 (0%)	7 (17.5%)
18-19	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
19-20	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
Total	60 (100%)			40 (100%)		

In males, composite epiphysis shows: non fusion in 2 (3.33%) cases in age group of 13-14 years, partial fusion in 8 (13.33%) cases in age group of 13-16 years & complete fusion in 50 (83.33%) cases in age group of 14-20 years. Complete fusion is seen in all the subjects

in age group of 16-20 years.

In females, composite epiphysis shows: partial fusion in 3 (7.5%) cases in age group of 13-15 years & complete fusion in 37 (92.5%) cases in age group of 13-20 years. Complete fusion is seen in all the subjects in age group of 15-20 years.

Table 3: Epiphyseal fusion of Medial Epicondyle with shaft of humerus:

Males				**Females**		
Age (years)	**NF**	**PF**	**CF**	**NF**	**PF**	**CF**
13-14	1 (1.67%)	5 (8.33%)	0 (0%)	1 (2.5%)	3 (7.5%)	0 (0%)
14-15	0 (0%)	5 (8.33%)	2 (3.33%)	0 (0%)	2 (5%)	2 (5%)
15-16	0 (0%)	2 (3.33%)	7 (11.67%)	0 (0%)	1 (2.5%)	7 (17.5%)
16-17	0 (0%)	0 (0%)	10 (16.67%)	0 (0%)	0 (0%)	7 (17.5%)
17-18	0 (0%)	0 (0%)	12 (20%)	0 (0%)	0 (0%)	7 (17.5%)
18-19	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
19-20	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
Total	60 (100%)			40 (100%)		

In males, Medial Epicondyle shows: non fusion in 1 (1.67%) cases in age group of 13-14 years, partial fusion in 12 (20%) cases in age group of 13-16 years & complete fusion in 47 (78.33%) cases in age group of 14-20 years. Complete fusion is seen in all the subjects in age group of 16-20 years.

In females, Medial Epicondyle shows: non fusion in 1 (2.5%) cases in age group of 13-14 years, partial fusion in 6 (15%) cases in age group of 13-16 years & complete fusion in 33 (82.5%) cases in age group of 14-20 years. Complete fusion is seen in all the subjects in age group of 16-20 years.

Table 4: Epiphyseal fusion of Head of Radius:

Males				**Females**		
Age (years)	**NF**	**PF**	**CF**	**NF**	**PF**	**CF**
13-14	3 (5%)	3 (5%)	0 (0%)	1 (2.5%)	3 (7.5%)	0 (0%)
14-15	1 (1.67%)	6 (10%)	0 (0%)	0 (0%)	2 (5%)	2 (5%)
15-16	0 (0%)	3 (5%)	6 (10%)	0 (0%)	0 (0%)	8 (20%)
16-17	0 (0%)	0 (0%)	10 (16.67%)	0 (0%)	0 (0%)	7 (17.5%)
17-18	0 (0%)	0 (0%)	12 (20%)	0 (0%)	0 (0%)	7 (17.5%)
18-19	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
19-20	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
Total	60 (100%)			40 (100%)		

In males, Head of Radius shows: non fusion in 4 (6.67%) cases in age group of 13-15 years, partial fusion in 12 (20%) cases in age group of 13-16 years & complete fusion in 44 (73.33%) cases in age group of 15-20 years. Complete fusion is seen in all the subjects in age group of 16-20 years.

In females, Head of Radius shows: non fusion in 1 (2.5%) cases in age group of 13-14 years, partial fusion in 5 (12.5%) cases in age group of 13-15 years & complete fusion in 34 (85%) cases in age group of 14-20 years. Complete fusion is seen in all the subjects in age group of 15-20 years.

Table 5: Epiphyseal fusion of olecranon process of ulna:

Males				**Females**		
Age (years)	**NF**	**PF**	**CF**	**NF**	**PF**	**CF**
13-14	2 (3.33%)	4 (6.67%)	0 (0%)	2 (5%)	2 (5%)	0 (0%)
14-15	1 (1.67%)	6 (10%)	0 (0%)	0 (0%)	2 (5%)	2 (5%)
15-16	0 (0%)	3 (5%)	6 (10%)	0 (0%)	0 (0%)	8 (20%)
16-17	0 (0%)	0 (0%)	10 (16.67%)	0 (0%)	0 (0%)	7 (17.5%)
17-18	0 (0%)	0 (0%)	12 (20%)	0 (0%)	0 (0%)	7 (17.5%)
18-19	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
19-20	0 (0%)	0 (0%)	8 (13.33%)	0 (0%)	0 (0%)	5 (12.5%)
Total	60 (100%)			40 (100%)		

In males, olecranon process shows: non fusion in 3 (5%) cases in age group of 13-15 years, partial fusion in 13 (21.67%) cases in age group of 13-16 years & complete fusion in 38 (63.33%) cases in age group of 15-20 years. Complete fusion is seen in all the subjects in age group of 16-20 years.

In females, olecranon process shows: non fusion in 2 (5%) cases in age group of 13-14 years, partial fusion in 4 (10%) cases in age group of 13-15 years & complete fusion in 34 (85%) cases in age group of 14-20 years. Complete fusion is seen in all the subjects in age group of 15-20 years.

Discussion

Table 6: Showing comparison of ages (years) of union of epiphyses around Elbow joint given by various workers with findings of present study:

Researcher	**Composite epiphysis**		**Medial epicondyle**		**Radial head**		**Ulnar Olecranon**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Lal & Nat (1934) (U.P.)1	17	-	-	-	17	-	-	-
MJS Pillai (1936) (South India)2	14	14	17	17	17	17	-	-
Galstaun (1937) (Bengal)3	16	14	16	14	16-17	14-15	16	15
Basu & Basu (1938) (Bengal)4	-	13-14	-	13 ½ -14	-	13 ½ -14	-	13 -14
Lal & Townsend (1939) (U.P.)5	-	15	-	-	-	16	-	-
Gupta et al (1974) (U.P.)6	-	-	18-19	17-18	16-17	17-18	-	-
Sahni & Jit (1995) (Punjab)7	-	-	-	16	-	16	-	-
Nemade K et al (2007) (Vidarbha)8	16-17	14-15	17-18	15-16	17-18	15-16	-	-
Sidhom & Derry (1931) (Egyptian)9	17-18	20-21	-	-	19-20	-	-	-
Ledger & Wasson (1941) (Pakistan)10	14-15	14-15	17-18	14-15	17-18	14-15	-	-
Frazer (1958) (European)11	17	14	18	15	16-17	14-15	-	-
Gray (1995) (European)12	16	14	20	20	17	14	-	-
Present study	16-17	15-16	16-17	16-17	16-17	15-16	16-17	15-16

Composite epiphysis: The observations of present study for males, matches with works of Lal & Nat (1934)[1] in Uttar Pradesh, Nemade K et al (2007)[8] in Vidarbha, Sidhom & Derry (1931)[9] in Egyptians & Frazer (1958)[11] in Europeans. For females, the observations match with the findings of MJS Pillai (1936)[2] in South Indians, Galstaun (1937)[3] in Bengalis, Basu & Basu (1938)[4] in Bengalis, Lal & Townsend (1939)[5] in Uttar Pradesh, Nemade K et al (2007)[8] in Vidarbha & Ledger & Wasson (1941)[10] in Pakistani population.

Medial epicondyle: The observations of present study for males, matches with work of MJS Pillai (1936)[2] in South Indians, Galstaun (1937)[3] in Bengalis, Nemade K et al (2007)[8] in Vidarbha & Ledger & Wasson (1941)[10] in Pakistani population. For females, the observations match with the findings of MJS Pillai (1936)[2] in South Indians, Gupta et al (1974)[6] in Uttar Pradesh, Sahni & Jit (1995)[7] in Punjabis, Nemade K et al (2007)[8] in Vidarbha & Ledger & Wasson (1941)[10] in Pakistani population.

Radial head: The observations of present study for males, matches with work of Lal & Nat (1934)[1] in Uttar Pradesh, MJS Pillai (1936)[2] in South Indians, Galstaun (1937)[3] in Bengalis, Gupta et al (1974)[6] in Uttar Pradesh, Nemade K et al (2007)[8] in Vidarbha, Ledger & Wasson (1941)[10] in Pakistani population, Frazer (1958)[11] in Europeans & Gray (1995)[12] in Europeans. For females, the observations match with the findings of Galstaun (1937)[3] in Bengalis, Lal & Townsend (1939)[5] in Uttar Pradesh, Sahni & Jit (1995)[7] in Punjabis, Nemade K et al (2007)[8] in Vidarbha, Ledger & Wasson (1941)[10] in Pakistani population & Frazer (1958)[11] in Europeans.

Ulnar Olecranon: The observations of present study for both males & female, matches with work of Galstaun (1937)[3] in Bengalis.

Summary and Conclusions

9. This study was conducted exclusively on the young indigenous population of Chhattisgarh region.

10. The epiphyseal union of bones at right elbow joint in males is completed in all instances (100%) at the age of 16-20 years.

11. The epiphyseal union of bones at right elbow joint in females is completed in all instances (100%) at the age of 15-20 years.

12. As the sample size is limited, further studies are necessary. Region wise studies should be conducted for better correlation and comparison.

13. Due to changing lifestyle pattern, dietary, climatic, behavioral factors, age of ossification is changing as mentioned in the available literature. So as to evaluate these changes, studies are recommended in every region of India at regular time period for academic and judicial interest.

14. The opinion about age should be given always in the range. From this study it can be concluded that the opinion about age can be given in a range having margin of error of 1-2 years.

15. Radiological interpretations are observer dependent so the set standards should be considered under expert guidance to arrive at conclusion in such radiological studies.

16. For estimation of age, relevant joints should be radiologically examined for different centres and opinion should be arrived considering the status of multiple centres.

Ethical Clearance- Taken from institutional ethical committee.

Source of Funding- Self.

Conflict of Interest- Nil.

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Manufacturers and Endorsers Liability: Shifting the Onus Approach

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Abstract

Endorsement being seen as a platform of presentation of traits and performances of a product it draws an analogy with 'invitation to offer' with the product or service voicing their presence via celebrity personalities attracting attention. Though the case of Donoghue v. Stevenson remains the original precedent on manufacturer's negligence absence of any contractual obligations to the consumer making him responsible towards the ultimate beneficiary, the ratio and obiter could should be analysed in sharing a look-alike obligation on endorsers for injury ensuing from use of such product or service. Since the debate of Nestles' Maggi brand, there has been a look out at the existing liability regime for the celebrity endorsers of such products. In the wake of flooding consumables in the free competitive market, falsification of critical data, misleading and non-obvious statements and superficial promising results often prejudice consumers' choice as well as preference, thereby affecting their rights to claim for healthy, consumer friendly products and income justified and habit of consumerism. Moreover, being a '*ideal' 'God like'* and '*larger than life'* figure to common man, personalities from entertainment, sports and other popular industry invite a stricter interpretation on their 'responsibility quotient' because of the edge over other professional advertisements in terms of greater credibility and trust.

Keywords: *Endorsement, Misleading advertisements, Due diligence, Consumerism, Customers, Celebrities.*

Introduction

Endorsers bridge the gap between manufactures and the consumers and often create a strong and relatable emotional bond with consumers with their earned likeability, and ways of work and life and become arbiter of quality, taste and performance of products or services. With the growing effect of the social media in the purchase journey of every individual the companies too are evolving new manners of effective saleability and one of the effective methods adopted is through endorsement through different channels. And many big celebrities are associated with the giant companies to vouch for their products and brands. But where brands are taking advantage of celebrities, by rampantly using the social median to reach the stakeholders and customers buying behaviours dependent on celebrity endorsement, the endorser possess some responsibility and have liability against their misleading products. Because the customer places a higher value on products endorsed by celebrities and it is as if they are receiving a short of advice from a brand regarding that product when the customer is a fan of the celebrity.

So the endorsers in a way increase awareness trust and familiarity of the products they are vouching for. People also believe that the products promoted by the celebrities will make them or allow them to get the traits of the particular celebrity. So the customer in a way shows greater recall of product those are endorsed by celebrities. And thus, through the endorsement of brands by celebrities and endorser, companies are taking undue advantage.

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Justification in favour of liability:

1. There is an undue influence on the "freedom to choose" of consumers.

2. A joint or vicarious liability on the celebrity is a justified step for any for any ensuing immediate damage or harm from the use of product or services by the consumers.

3. There is a shared malicious interest of the endorsers with manufacturers in money making.

Justification against Liability:

1. Endorsers are not the approval authority or testing authority and certainly lack the technical expertise on the same.

2. It is understood that the primary safety test has been done by the authority as because it has been stamped to be market ready.

3. Endorser being a mere medium of transmission of information should not be made a scapegoat.

4. There is an express and implied 'Indemnity clause' in the Contract between the manufacturers and endorsers justifying a compensatory

5. The age old revolutionary concept of 'Caveat Emptor' puts a responsibility on consumers' shoulder to be informative.

Liability of the celebrities for the claims made by them in advertisements: The question of liability of the celeb-endorser for the lapses of a manufacturer is long been debated ever since Nestles Maggie brand of instant noodle had quality issues and the product falling the foul of food testing authorities was banned by the Food Safety and Standards Authority of India.[1]

An extremely important legal debate has been raised concerning the liability of endorsers. Various legal experts opine that even if the celeb-endorsers are made liable under certain provisions of existing legal provisions, it becomes difficult to prove the presence of clear intent to harm anyone via the endorsements they are making in respect of the deficient product. The rights and obligations of the endorsers are very well looked after by the contract between the endorser and the company. But in cases where a particular endorser is engaged not directly by the company but by any advertising agency, the terms and conditions of the contract in such cases would be different. Moreover, in some cases, there might be non- existence of any contractual relationship between endorser and company. In absence of any contract, how far a brand ambassador is liable for deficient products is a big question.

Civil Liability Regime in India: Endorsers cover up allegations of misleading promotion under their plea as mere expression of opinion and a freedom of speech guaranteed under Article 19 of the Constitution of India But that too comes certain exceptions with a reasonable restriction being imposed in the interests of general public. Health of citizens is thus, matter of paramount consideration and no person may be allowed to trade at the cost of public health.

False and misguided claims range from overrated results of fairness products ('fair skin in four weeks')[2], food supplements guarantying 'taller stronger and sharper children, Easy Slim Tea, use of Yantras and Stone/Topaz for male child to magical cure of ailments (Dettol TVC's super 'protects from 100 Germs', Patanjali's Chawanprash's[3] claim on '51 herbs') and many more making advertisements void of any rational logic or ethics.

a) Tortuous Liability

Under the Law of Torts, duty of care and negligence could be clubbed against the endorser with a burden of proof on consumer to prove 'malice' on the part of endorser which is difficult to prove. Moreover, the endorsers in a manner are acting as agents of the company and thus invite no liability under the tort law.

b) The Sales of Goods Act, 1930

Implied condition of merchantability befitting the description and 'conditions and warranties' clause under the law is a major safeguard for consumers' interests. Also, occasionally making buyer rely on the skills and judgment of seller and informing seller of his 'particular purpose' makes the seller contractually responsible.

c) Consumer Protection Act, 1986

Of all the legislations dealing with misleading advertisements and liability parameters of endorsers, the Consumer Protection Act, 1986 evolves to be most efficacious whose sole purpose is to safeguard consumers' interests and settlement of disputes. Under section 2(1)(r) the 'Unfair Trade Practices' include the misleading advertisements which falsely represent

that goods are of particular standard, quality or grade along with the representation that certain goods have performance, characteristics uses or benefits which such goods actually don't have.

The Consumer Protection Councils under the Act, thus, play a vital role in deciding the extent of liability of the celebrities in case of misleading advertisements but only if the role of celebs in endorsing the products of certain brands and attracting liability in case of misleading advertisements specifically provided in he Act as the existing Act does not provide clear guidelines around rights and liabilities of celeb-endorsers. Considering the impact of celebrity endorsement on the consumer choices and behaviour, situation is more damaging in case of misleading advertisements of various products and unrealistic claims. Also the intent behind the advertisements and promotional activities is not only in a view to put forth an artistic expression but to have a better impact of their product in a very short span of time so that a whole new bunch of consumers just extend their wish to buy it because a particular celebrity uses the same.

Keeping in view the current scenario of celeb endorsement and celeb behaviour, the Consumer Protection Bill, 2018 which was introduced in the Lok Sabha for the first time on January 5, 2018, seeks to replace the existing Consumer Protection Act of 1986 in order to address the emerging consumer vulnerabilities. Till now in India, no celebrity endorser has ever been prosecuted for a misleading and exaggerated claim in an advertisement. But once the proposed bill is passed, the responsibility of celeb endorsers to our society could be enforced by a legal backing and also the trend of celebrity endorsement will become more guarded.

Moreover, the Bill is obviously a welcome move towards taking the issue of misleading advertisement but there are certain loopholes in the said bill which has been attracting criticism over and again since the bill was introduced in the Parliament for the first time in the year 2015.

a) The proposed Bill though seems to have attempted some modification in ensuring professional and ethical liability on the endorsers; it certainly tried to limit the consumer's freedom to enforce its sanctioning rights via some compromising clauses. [4]

b) Defence of Due Diligence:[5] On the question of liability for "false or misleading endorsement prejudicial to the interest of any consumers", endorsers have been given an opportunity to negate malice and prove their fair intention in not knowing about the veracity of the product by pleading "due diligence' and precaution.

c) No vicarious liability too could be ensued on the endorsers.

d) Empanelled Mediator: Filing of complaint through an executive agency who is an officer belonging to the Central Consumer Protection Authority and who is being duly authorised by the Chief Commissioner is violative of a person's 'right to sue' by limiting it through an unaffected intermediary.

e) Compounding of Offence: Compounding of offence by the brand ambassador for first offence is an easy escape with the brand ambassador or endorser shelling out monetary damages at the cost of consumer's physical and mental health.

Criminal Liability Regime in India:

a) Indian Penal Code:

Any person selling or offering for sale noxious or unfit food can be prosecuted according to the provisions of the "Indian Penal Code" if that person does the same knowingly. In the case involving the instant noodle Maggie, a case under Section 270 which incriminates the malignant act that is likely to spread infection of diseases that are dangerous to the life of the persons was filed against the endorsers. Also the criminal liability cases involving Section 273 dealing with the "sale of noxious drinks", Section 276 involving the "selling of drugs as a different drug or preparation" and Section 420 involving "cheating and dishonesty" was reportedly being filed against the celebrity endorsers.

But the liability cases made under Section 420 and Section 376 dealing with cheating with regard to property and the sale of different drugs or preparations respectively are falling apart as it was not the clear case here. On the other hand, in Sections 270 and Section 273 there is a specific requirement of the involvement of the accused directly with a clear intent which is missing in some cases and difficult to prove in some other.

b) Other Special Statutes:

The Food Safety and Standards Act, 2006 under Section 24, puts a restriction on advertisements that are misleading. The Act says "no person shall be allowed

to engage in misleading representation concerning the standard 'quality, quantity, grade and composition', and 'need for, or the usefulness of a food product". Moreover, it also restricts the endorser from making such statements that gives any guarantee to the consumers as to the efficacy of the product.

The penalty for false advertisement is provided under Section 53 of the said Act which can extend up to 10 lakhs (it is believed by some legal experts that the amount of penalties in such cases are typically picked up in the contractual indemnities provided by the manufacturers or the brand owners).

Regulatory Framework in India: "Advertising Standards Council of India", (ASCI) is a non-statutory Tribunal established in the year 1985 also deals in regulating the mechanism for ensuring just and ethical advertisement practices and takes up complaints against any perceived misrepresentation in an advertisement.[6]

It has power to give direction for modification and withdrawal of any advertisements hereby assuring truthful representations of the product. ASCI works on its code of Advertising Practice and is applicable to advertisements read heard or viewed in India irrespective of their origin. According to Gowree Gokhale, a senior partner of an International Law Firm Nishith Desai Associates, basing on the principles of "decency, fairness, honesty and responsibility"[7] the advertisements are evaluated by the ASCI. Although the orders of the ASCI's are generally void of enforceability, the Ministry of Indo and Broadcasting has made the violation of "Advertising Code" a penalty restricting the Cable service providers and TV channels to promote.

International Norms: In the United States, the Federal Trade Commission has certain guidelines for endorsement by celebrities. The FTC made amendments in the year 2009 giving the consumers the liberty to even hold celebrities liable in cases of misleading advertisements. It has prescribed standards for the liability of endorsers. Advertisement are evaluated on the principle of "*good reason to believe that*" in order to examine whereas the endorser has sufficient reasons to believe that the said feature of a particular commodity he is endorsing and thus guidelines make the celebrities responsible enough to investigate products first and also requiring celebrity to be a *bona fide* user.[8]

In Europe, there is voluntary self-imposed code to be followed by the celebrities whereby they are refrained from endorsing "medicines, medical treatments, tobacco and alcohol" which have harmful effect over the health of the people.

In China, there is equal and joint responsibility with food producers if food causes harm with an option to file claim either against enterprise manufacturer or celebrity recommending the same. Misleading testimonials too have same fate. The Food Safety law of China talks about endorsers' liability in case of misleading testimonials and recommendations. In an endorsement of a shampoo, Jackie Chan faced a legal battle for the shampoo brand he was endorsing which was alleged to be containing cancer causing ingredients whereas the advertisement showed it chemical free. But the suit against the endorser failed on the ground of non-existence of any legal relationship between the endorser and the company. China does not have any procedure for determining liability of the celeb endorsers such as "false and misleading test" and is yet to evolve laws regarding celebrities by fixing joint responsibilities in all spheres of trade.[9]

Korea, on the other hand, has a "self- regulatory institution" responsible for issuing guidelines with respect to endorsements which also has the power to decide which products are to be expressly advertised and which are not, thereby making false advertisements a rarity. It can thus, be said that Korea has comparatively more stringent mechanism in controlling misleading advertisements.

Conclusion

In lights of the expanding economic horizons of the country, it is the need of the hour to establish an economy where none is taking advantage of and none is left bereft of resources. Endorsers certainly are creating target base and building markets for big multinational companies through their perceived influence on consumers' mind, interpreting 'innocent misrepresentation" and 'negligent misrepresentation" would be a guidance for lawmaker to see through a better and sound enforceability and plea of privity of contract or defences against strict liability falling short of their rationality when endorsements involve recklessness and whimsical professional ethics. Before acting as a spokesperson to the brand's voice or being a mere carrier of information there is certainly a 'line of commitment' to morality and ethics. Thus, while it is time to ensure strict regulations against people who sacrifice their integrity for money, celeb-endorsers and advertisers take joint responsibility to marketing and

promoting 'truth' and 'veracity' in products and services.

Ethical Clearance: Not required as the researcher has just referred to some published works. The research is doctrinally undertaken, completely by the researcher herself**.**

Source of funding: Self

Conflict of Interest: Nil

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Crossword Puzzle: An Innovative Assessment Tool to Improve Learning of Students in Forensic Medicine

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Abstract

Background: As per Medical Council of India (MCI) for setting teaching learning standards according to need of society, now has focus shift from traditional teaching methods to adopting teaching learning method that is been termed as competency-based learning. Faculty training programme by MCI are also providing the right platform for learning the new methodology in medical education.

Objectives: To prepare an interesting and effective tool in assessing the knowledge of Forensic medicine among the medical undergraduate students to produce competent and confident Indian Medical Undergraduates.

Methodology: Crossword puzzle exercise was given to 2nd year students, who were in 5th semester. 38 students participated in this study. A questionnaire was given to the participants to evaluate the response consisting of 15 questions; responses were obtained on Likert scale 5 to 0.

Results: Out of 38 students 92% of students found the puzzle interesting. 76.4% students found this puzzle as a useful tool to learn forensic medicine. 73% students agreed it to be a useful tool for the assessment of their knowledge. 81% students were in favour to use such puzzle in competitive exams. In terms of retaining the knowledge of topics, 84 % students found this puzzle useful.

Conclusion: Crossword puzzle improves memory power, performance and critical thinking of students with fun. This can be tried as a question in periodic internal examination for internal assessment as well as well as for the purpose of structured viva examination in university examination.

Key words: *MCI, traditional teaching, medical undergraduate, cross word, assessment*

Introduction

Teaching and learning are mutual procedures that influence each other, and assessment determines both students and teacher's effort [1]. The purpose of assessment is to impart quality education to students , to give feedback to the educational managers about the curriculum, and to categories the students according to their talent and to monitor their own learning. Assessment also provides a high degree of fairness and objectivity in testing and produces data to enable continuous quality improvement [2]. The welfare and indeed the future health of people depend on the quality of medical graduates which in turn depends on quality of medical education. Educationists believe that simply by changing the assessment style for the learners can affect the way students engage with the subject contents [3]. Assessment drives learning and learning drives practices. By changing the format of assessment or examination, can lead the students to engage their study more thoughtfully; their focus can be shifted to clinical rather than theoretical issues [4].

The crossword puzzle is a century old game and was first used in ancient Egypt. In 1913, Kathrynne

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created a modern crosswordpuzzle[5]. In recent years, it has become an interesting game and we come across it in many newspapers, magazines, books, and journals. Some magazines even offer prizes for solving it as an incentive for the participants. Recently educationists have started using these puzzles an educational tool especially in medical education and nursing courses.[6-12] Crossword puzzle can also be used as an innovative tool for assessment of undergraduate medical students.

This exercise helps them to remember the forensic medicine terms easily, understand the concept and helps the teachers to evaluate the students' knowledge and understanding. It can detect the misconceptions and help to clarify the concepts of the medical student .[13,14] The benefits of such active learning are that it promotes the communication ability, memory, cooperation among learners and their critical thinking power.[15]

Such games are important in acquiring skill and psychomotor knowledge, brain functions like understanding, thinking, remembering and analyzing the facts.[16,17] The crossword puzzles are in various forms including cryptic form, symmetrical form.

Since medical education has changed dramatically in last few decades, the assessment of knowledge of medical students also needs to be changed and it needs to be effective as well as interesting. Crossword puzzle will prove as an effective tool for assessment in terms of recall and analysis of knowledge. Forensic medicine is a very important subject for the medical student for their career, and for the medico-legal system to have more experts. The crossword short answer question (SAQ) stimulates the cognitive function. Due to previous experience in crossword puzzles[11], the author got an idea to use his puzzle in the examination as short question answer.

The literature search did not show any crossword puzzle tool in form of short question-answer assessing the knowledge of the forensic medicine in medical students. This is an innovative tool for the above purpose and hence this study was planned. We tried to evaluate the crossword puzzle tool for assessment of their knowledge and also analyzed their feedback for the crossword exercise given to them.

Materials & Method

The present study was a prospective cross-sectional analysis of feedback questionnaire. This study was carried out in the Department of Forensic Medicine, MAMC Agroha after approval from institutional ethics committee. The crossword puzzle exercise was formulated with the terms and words from taught syllabus of Forensic Medicine was formed. This puzzle had varying degrees of difficulty which were evenly distributed; key related to each question was prepared, including facts, hints or statement related to the question. This puzzle was distributed to faculty of forensic medicine for validation. Suitable changes after validation were incorporated. A feedback questionnaire was prepared by the authors consisting of 15 questions evaluating student's perception on the given cross word puzzle, its impact on retaining and recalling the knowledge and further utilization of such puzzle in different disciplines of Under graduate curriculum. The questionnaire was also given to senior faculty of the various streams of medical under graduate subjects for validation. The validated feedback questionnaire was used for responses. All the feedback responses were graded on Likert scale from 5 to 0 in which grade 5 was awarded to those who strongly agreed, 4 to those who agreed, 3 to those who moderately agreed,2 to those who least agreed,1 to those who strongly disagreed and those who did not respond were given grade 0. A total of 38 students of 5th semester participated voluntarily after briefing them about the method and purpose of this crossword puzzle and feedback questionnaire. Each question was awarded score 1 (for correct) or 0 (for incorrect), with no negative marking. All the participants were given a crossword puzzle sheet, a paper for keys, and a questionnaire. Participants were instructed not to use internet, mobile or any books. They were allotted 45 minutes to solve the puzzle. The puzzle had 9 horizontal and 8 vertical words to fill with the help of 17 key statements (Figure1 and Figure 2). After completion of 45 minutes, a questionnaire for feedback was given to the participants and 15 minutes to read the questions carefully and to answer. The crossword entries were checked and each participant was marked out of 17. Marks scored by students and response to feedback were analyzed with suitable analytic tests.

Results

Out of total 38 students; 11 students secured 16-17 marks, 17 students obtained marks between 13-15 and 5 students obtained 10-13 marks. Of the total, 47.23% did not agree that it was a wastage of time. 81.6% students felt this method lees time consuming as compare to traditional examination methods. 65.8% students also

agreed to take part in preparing cross word puzzles by their own. Other responses of the feedback are depicted in figures 1 to 3.

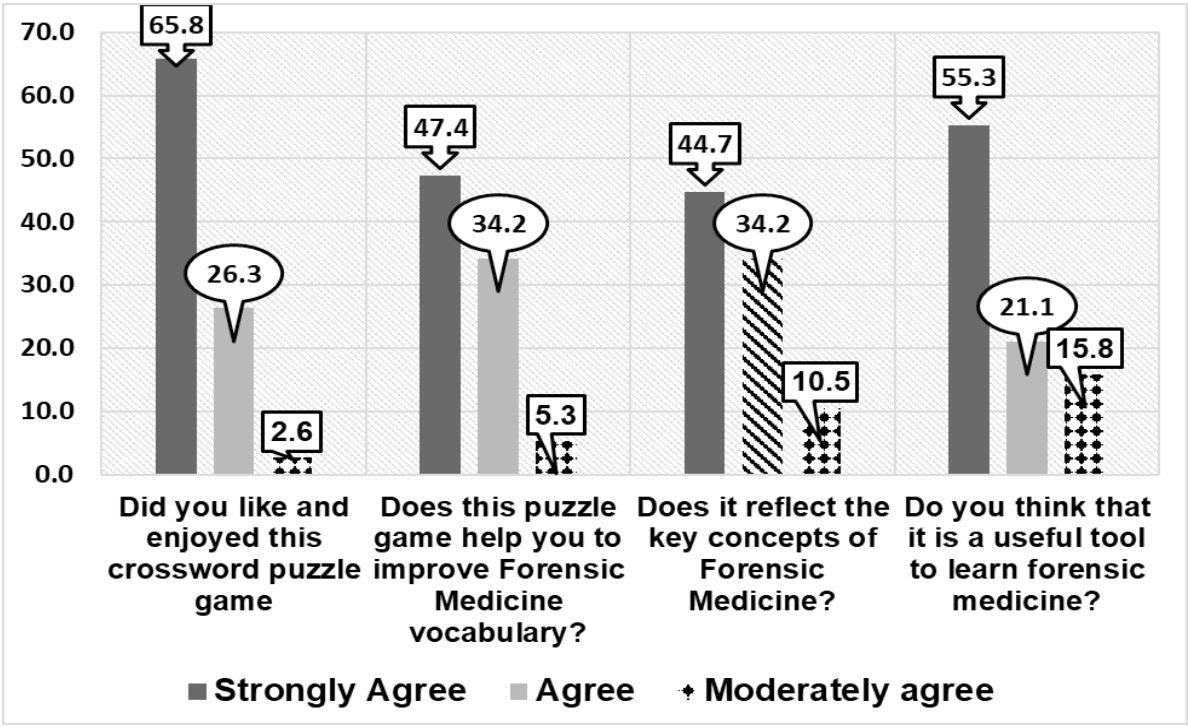

Figure 1: Students perception on cross word puzzle in Forensic Medicine

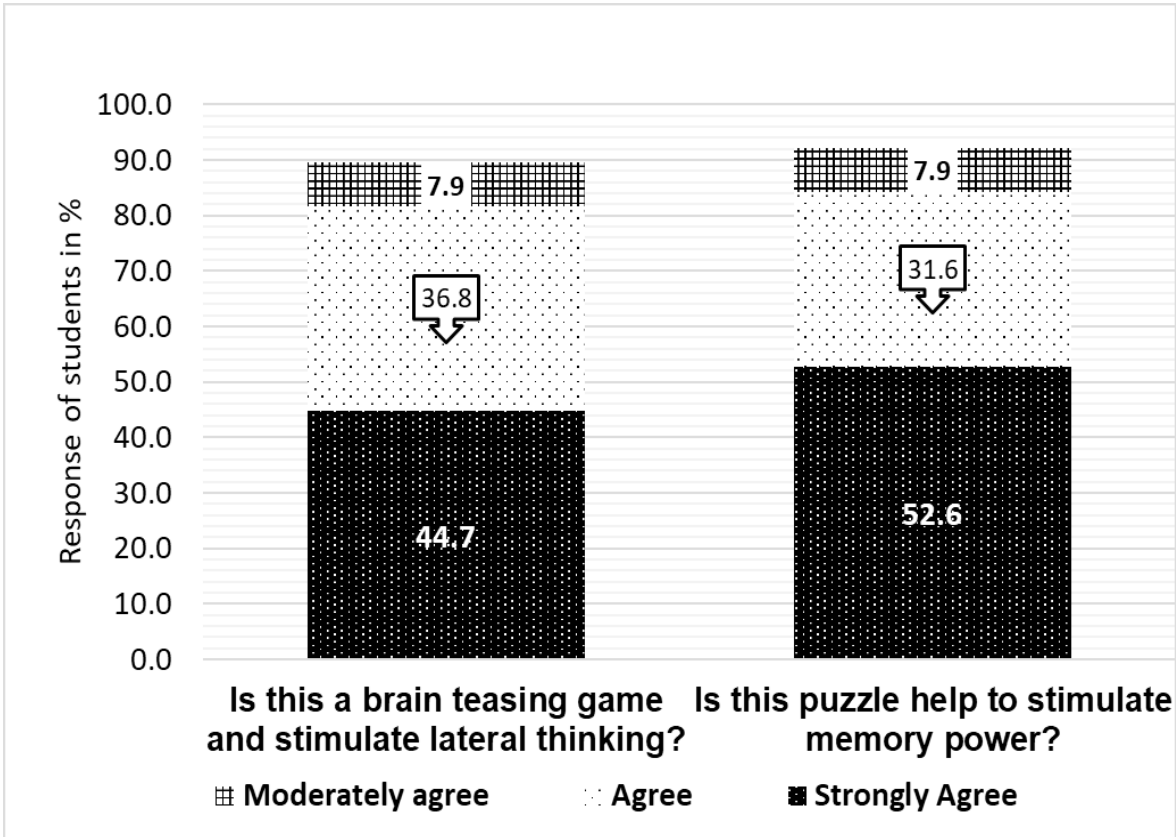

Figure 2: Students' perception on recalling the information by cross word puzzle

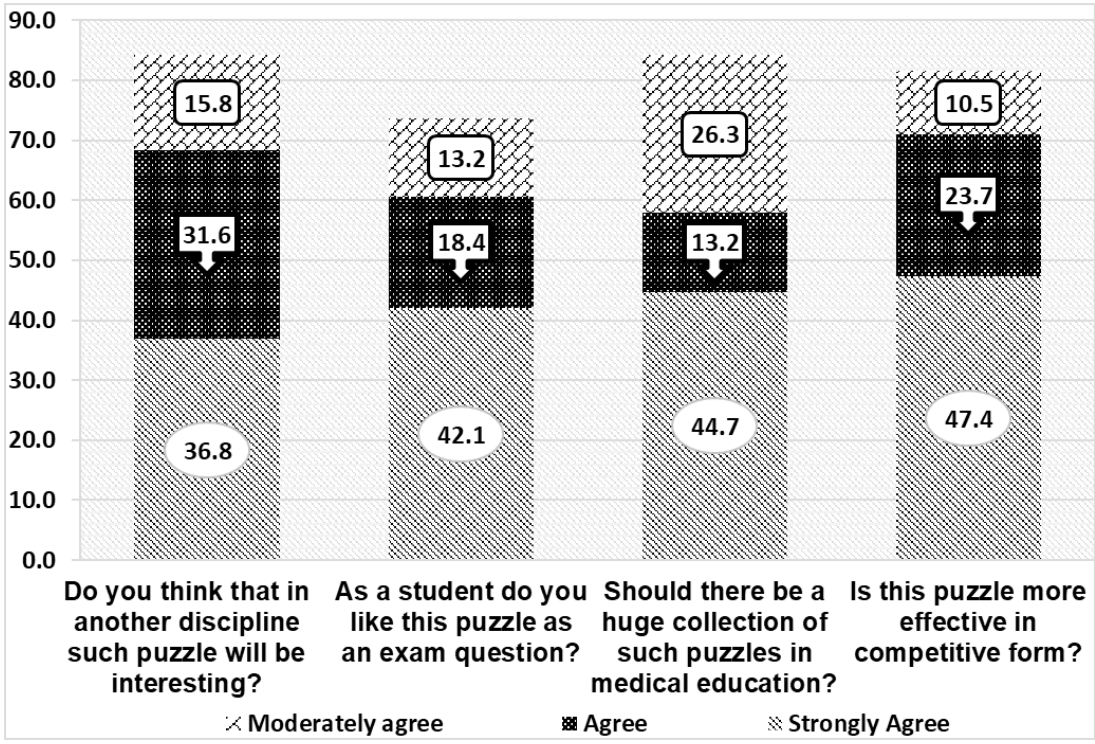

Figure 3: Students' feedback on implementing such crossword puzzle in other disciplines

Discussion

The crossword puzzle is a very interesting word game and many people enjoy this game. Crossword puzzle as a teaching tool is very beneficial in remembering the words and terms with the help of keys. The key consists of facts, hints or statements in the form of a word, facts, terms or incidences and when a student reads the key, he recalls the answers easily. As we know that memorizing any fact needs its correlation and memorization of other related facts[12]. Once he remembers the related words, he has to fit the answer in the crossword by counting the number of alphabets mentioned in the key. This is how it stimulates thinking and improves the memory. This same mechanism of recalling is useful in an examination which will automatically improve the performance in an examination too[13].

We did this survey to study the individual performance of an undergraduate medical student without any help or without the cooperation of groups. So, it becomes easy to know the performance of a single student, because in a group usually, one or two students are active, and others are passive and so the assessment is not for all[13].

Bailey et al used four types of games like crossword puzzles, words scramble, word search and hidden message puzzles, in gastrointestinal physiology teaching. According to them, in the crossword puzzle, inability to identify the word indicates the weakness and that can be corrected by proper teaching.[10]

Htwe et al did the study on crossword as a teaching tool on the group of students and revealed that is an excellent tool for teaching pathology with fun.[11] Saxena et al. also did the same study on groups of students and they concluded that students liked the crossword puzzle as a teaching tool.[12]

We believe that for teaching and learning purpose this survey can be used on a group of students, but for the assessment of the students, the student must solve the crossword puzzle individually. In our study, the main aim was to study the crossword puzzle as a tool to assess the students in an examination, so we did not make the groups of students. Playing with such puzzles, psychomotor skill and knowledge both are increased.[12] We did this study for the subject of forensic medicine which is a brain teasing discipline where the student must churn the thought process for the complicated case scenario with various medico-legal aspects, like a detective. Nowadays many of the medical schools and colleges emphasize on student oriented medical education where the active involvement and participation of every

student is necessary, especially in small group teaching, for better and detailed understanding of concepts in medical science. The students are encouraged to solve the problems by themselves.[18,19]

Crossword puzzle improves cognitive function as per clinical trial done by Wolinsky et al.[20] They used computerized crossword puzzle in a clinical trial on middle aged and old people to study the cognitive processing speed and concluded that crossword puzzle delays the decline in the cognitive process and improves the speed of the cognition.

Conclusions

Majority of participants liked the crossword puzzle as an examination question. It improves memory power, performance and critical thinking of students with fun. This can be tried as an examination question in periodic internal examination for internal assessment and can be used for the purpose of structured viva examination in university examination in other disciplines of medical undergraduate curriculum paving the way for creating more competent medical graduates, the need of the society at present time.

Ethical Clearance: Taken by by Institutional ethical committee

Source of Funding: self

Conflict of Interest: Nil

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A Study to Assess the Patients Compliance to Medications among Hypertension Patients at Selected Areas in Kanchipuram District Tamilnadu

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Abstract

Hypertension is the most prevalent cause for cardio vascular disease, affecting at least 600 million people worldwide and is an important contribute to cardiovascular mortality and mortality. Compliance to antihypertensive medication has been found to be a major concern.[3]

The study was conducted to assess the patient's compliance to medications among hypertension patients at selected areas in Kanchipuram district Tamilnadu. The objectives of the study were to assess the patient's compliance to medications among hypertensive patients and to associate patient's compliance to medications with selected demographic variables.

The research design selected for the study were Descriptive research design. The sampling technique used for the study were purposive sampling with the sample size of 60. Data collection was done by identifying the demographic variables and by using Morisky Medication Adherence scale, the patient's compliance to hypertension medications was assessed.

The results revealed that assessing patients compliance to medications, the study shows that 48(80%) of hypertensive patients are following medium compliance of hypertension medication and 12(20%), of the patients are having low compliance and none of the patients 0% are following high compliance to medication. Regarding association with demographic variables, factors like sex and blood pressure are highly associated with compliance to medications and there is no significant association with the demographic variables like age, educational status, types of family, religion, socio economic status

Hence community education is needed to improve the patient's ability to cope up of medications and make informed decision regarding hypertensive medications and to motive the community people to introduce changes in diet and life style modification.

Keywords: *Patients, Hypertension, Medications, Compliance*

Introduction

Hypertension is the most common cardiovascular disease, emerging as a major public health problem in developing as well as developed countries. The WHO report that the prevalence of any disease, hypertension ranks four in the world. Pooled epidemiological studies show the average prevalence of hypertension in India is 25 % in urban and 10 % in rural population. Hypertension is a significant public health problem in urban and rural areas of India. It is directly responsible for 57 % of all stroke deaths and 42% of coronary heart disease deaths in India. It is also leading cause of blindness, renal failure and congestive heart failure.[3]

Successful control of blood pressure is of para amount importance in the reduction of morbidity and

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mortality rates and many studies have demonstrated the impact of antihypertensive agents on improving clinical outcomes. Howere the achieved by optimal compliance to prescribed medications according to healthcare provider's instructions.[4]

In developing countries, the degree of non-compliance is assumed to be higher particularly due to shortage of health resource and difficulties in access to health care professional is necessary. patient's education is a process that improves patient's ability to cope up and make informed decision regarding their disease medication and motive the patients to introduce changes in diet and lifestyle modifications.[6]

Materials & Method

The methodology of research indicates the general patterns of organizing the procedure for getting valid and reliable data for investigation. Research approach for the present study was a descriptive research approach. The descriptive research design seems to be the most appropriate design for this study. The study was conducted in the selected area in pooncheri villages, Kanchipuram district, Tamilnadu. All the hypertensive patient residing in the selected villages and who fulfilled the sampling criteria was included in the study.

Sampling Criteria

Inclusion Criteria

- Patients who are diagnosed as Hypertension
- Hypertensive patients who are under single hypertensive drug

Exclusion Criteria

- Hypertensive patients who are having other chronic diseases.
- Hypertensive patients who cannot speak and understand Tamil/English

By using purposive sampling technique 60 samples were selected. The tool used for the study consists of the demographic variables like age, sex, educational status, types of family, religion and socio economic status and blood pressure. To assess the Compliance to medications, Morisky Medication Adherence scale was used.

Study Findings:- Regarding demographic variables

- Maximum no of hypertensive patients were in the age group of 51years and above 41 (68%)
- Maximum female 32 (53%) are hypertensive patients
- Majority of educational status was Non formal education 24 (40%)
- Majority of family of hypertensive patients are single family 40 (66.66%)
- Majority of religion of hypertensive patients are Hindu 41 (68.33%)
- Majority of taking hypertensive medications regularly 42 (70%)
- Majority of person from which a they are getting medication for hypertension are 38 (63.33%)
- Majority of blood pressure are 46 (76.66%)

- Regarding assessing patients compliance to medications, the study shows that 48(80%) of hypertensive patients are following medium compliance of hypertension medication and 12(20%), of the patients are having low compliance and none of the patients 0% are following high compliance to medication. Regarding association with demographic variables, factors like sex and blood pressure are highly associated with compliance to medications and there is no significant association with the demographic variables like age, educational status, types of family, religion, socio economic status.

Discussion

Hypertension is the most prevalent cause for cardio vascular disease, affecting at least 600 million people worldwide and is an important contribute to cardiovascular mortality and mortality. Compliance to antihypertensive medication has been found to be a major concern.

The mean proportion of control hypertension among all hypertension patients being only 13% because of poor medication compliance and is one of the leading causes of failure to achieve blood pressure control.

Conclusion

The study reveals that health care facilities should concentrate on continuous treatment for non communicable diseases, health education to the public

on regular medications can able to prevent complications from cardiovascular diseases and other conmplications.``

Efforts to be made enhance the capabilities of doctors, village health nurses and other paramedical through pre-services and in-service training programme to enhance not only prescribing medications and follow up of medications is essential.

Conflict of Interest: Nil

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Ethical Clearence: Chettinad Academy of Research and Education, Institutional Human Ethics Committee

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Restrictions Imposed by Human Biology in Achievement of Gender Justice as Human Rights

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Abstract

In contemporary times with frequency as high as that of everyday , we are witnessing debates on implementation of gender justice. Be it college forums, news channels or for that matter parliament everywhere and everyone at both conscious and sub-conscious levels are aware of the problems resulting because of imbalance in power equations between the genders. The focus area of this research paper will be India and legislation of India which has made many laws, bye-laws and rules to bring feminine and masculine gender on the same footing but yet there are gaps through which gender justice is getting hit hard despite the Criminal Amendment Act of 2013 and 2018. Complication by the NALSA judgment have occurred as a result of which the definition of gender justice needs over-hauling but the Indian society is unwilling for it. Through this research paper , researchers trace out the problem is actually lying in the very meaning of sex, sexuality and gender as understood by the society of India and the solution is lying in being neutral towards sex and gender.

Keywords:- *Sex, Gender , Sexuality, Gender Justice*

Introduction

From the history of human to present, there are ample evidences which are leaving no doubt on the fact that there is some sort of interconnection between working of the human society irrespective of the fact that in which ethnicity and time zone that society is working in. There are certain rules of a society of course unwritten in majority of the human civilizations which are based on the sex of a person. For example, as to how the property is to be distributed, which jobs particular sex and gender can do or cannot do, even the "public places" like parks, circus, theaters, malls etc.. are being restricted by sex and gender of a person.

Nomenclature of Sex And Gender

This research paper is focusing on the how the concept of gender justice has been developed over a period of time. Before that there are certain concepts which shall be understood before focusing on the research problem of this research paper. And they are:-

a) **Sex**

As known commonly, it is a natural phenomenon which distinguishes between body of man and woman. It is also known as sexual activity which results in progeny. Medilexicon[10] defines Sex as a character biological in nature based on their gonads, chromosomes, hormones, primary and secondary characteristics and identifies human bodies as male and female. Sex is also a psychological progression of a person which triggers the behaviour automatically for the purpose of sexual contentment. Wharton[11] in her work defines sex as a characteristic which is biological relying on hormones, chromosomes, gonads etc..and if clearly distinguished then are termed as normal sex being male and female and if parameters are in divergence, the human body is labeled as Intersex.

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b) **Gender**

Gender is a role which males and females are expected to play in society for ensuring its smooth functioning. Tierney[12] defines Gender as social and civilizing expression which dictates the manner in which defined biological body is suppose to function.

Role of Sex and Gender

As the Indian society began to take shape, individualism vanished and a pattern developed wherein food production became the responsibility of the males, considering their physical potency while distribution went to females because of their biological capacity of giving birth, making womb, a resource to cash as the principle was to survive. These clashes for survival, highlighted the need of human biology where male sex attained preferences in working of the societies and accordingly roles named as gender were formulated for the sake of convenience. It was this convenience, roles in the form of gender began to define and since it meant survival, male and female began to enforce them on the younger generations and inculcated divine , morality and law to aid the compliance of these roles and penalized all acts of deviance.

As a matter of fact, in India the presence of third sex is known by its gender role which is to provide amusement and is labeled as Kinnars, Enunchs, Hijras, Khwaja-sarah nd recently as Intersex, transgenders etc. They were in haraams of the kings and queens, in circus etc..but were in no way daughter-in-laws or sons-in-law of a "normal" family.

Effect of Gender on Sexuality

Sexual behaviour which aided in continuance was accepted as normal known as Heterosexual activity with a rider of reproduction classifying it to be moral sexuality. This termed penile-vaginal intercourse as normal and moral while rest any other sexual activity as immoral even between a heterosexual couple because that was not resulting in progeny.

Because of this moral weightage, sexuality began to give in and same sex relation modernly known as homosexuality were frowned upon and became sin. To ensure that homosexuality and all other non-productive heterosexual activities are discouraged, legal structure was roped in and sanctions as hard as burning alive, amputation , hanging , jail sentences and not to forget fine and social detestation, were introduced to make sure that gender is abided within the four squares as laid down.

However, this control of gender on sexuality backfired and visible victims of this control were females who were then laiden with the expectation to remain chaste till their official union with a 'man' in form of marriage and procreate as many times possible. It denied them the access to medical sector without getting their gender role scrutinized first at the help-desk and then at doctor's chamber. The other casualty were biological males who were sexually attracted towards males. Known as homosexuality, this behaviour was morally and legally penalized.

Last casualty of gender roles are Third sex/ Intersex, whose sexuality itself has no where been defined. The denial is so strong that no proper scientific research have been conducted to understand the sexuality of Intersex. Even the medical fraternity which is of course the members of normal society have concluded their many research attempts by stating that they are abnormal and in need of medical aid in terms of psychological corrections, hormonal and surgery procedures. The ambiguous genitilia is a medical emergency and sex reassignment is prescribed after consulting with parents of the child with the personal opinion of the doctor. Here most of the times the child is too young to understand the complexities of the procedure yet, a "normal" biological sex is assigned to the child with enforcement of parallel gender role. But what is forgotten that what is allotted will be in arms with what is felt internally. The end result is of a contradiction and conflict of soul and when the child defies the allotment the labels, who is then a transgender[13].

Presence of Third Sex and Gender

It will not be incorrect to say that presence of third sex and gender openly have been felt in the society from last 40-50 years only before that they were open secret of human society which were denied any sort of identity / labeling by the normal society to maintain its self-proclaimed integrity. They have been awarded names from kinnars to hijras , Khawaja-sarahs to kathoeys , sissys to muxes and have faced discrimination along with both physical and sexual violence.

It is only recently that the third sex and gender have been identified as Human and their rights have been advocated through various international conventions.

For instance, united nation resolution on human rights, sexual orientation and gender identity 2012[14], wherein it was sketched that it is the State which is to protect the individuals irrespective of their LGBTQI sexual orientation and any violation will be treated as violation of Universal Declaration of Human Rights. Then there is international policy of United nations on Men having sex with Men 2006[15], where in states are directed to take all the measures on the fact that homosexual men are not discriminated on basis of their sexuality and proper medical treatment is provided to them as needed. It has been further said that there shall be awareness programmes in local neighbourhood to make society aware that homosexuals shall not be discriminated just because of their sexuality. There is declaration of commitment on HIV/AIDS[16] wherein it was for first time in the recorded history of international legal documents, stated that the transgender person are in need of State protection and medical aid as they are the ones who are being discriminated solely on the basis of their sexuality.

India also know acknowledges, the presence of the third sex and gender via NALSA[17] judgment wherein an umbrella term "Transgender" was chosen to identify distinct sexuality of LGBTQUIA. It was in NAZ judgment[18] wherein Section 377 of IPC,1860 was declared as unconstitutional by Delhi High Court, but only till the consensual part. This was overturned in Suresh Kumar[19] wherein the court opined, that it is the legislative decision and courts shall not interfere as they only interpret law. In 2018 came Navtej Singh Johar[20] wherein again Section 377 of IPC,1860 was declared unconstitutional till the consensual part giving a choice to make to members of LGBTQIA community.

The reason of such fluctuations in dictates of judiciary pertaining to the issues of LGBTQUIA community is the fact that there are moral dilemmas of the society and judges are part of the society, so it is very normal that such dilemmas step in law making process. This conflict between legality and morality was first time recorded in Hart-Devlin debate wherein Wolfeden Committee in England clearly stated that legal dicta has nothing to do with same sex behaviour and hence the Buggery Act got evaporated from the legal text of Britain. This small statement caused uproar in British Society because it was disturbing the way a society works. This lead to debate[21] between two wise heads namely HLA Hart and Lord Devlin where later opined that segregation of morality from legality will lead to confusion in for a person as there will be no internal, moral force which will make him/ her to oblige with law. HLA Hart, who rejected the arguments made by the former and stated that Devlin is going by the principle of majority rules but the minority no matter how small cannot be overlooked and cannot be imposed on everyone.

In India, it was in 2018 only when the consensual homosexuality was acknowledged. Ideally, it is a great step taken by the judiciary in bestowing Article 21 and 19 on LGBTQIA community and gave them liberty of life and choice but the confusion on sex and sexuality can be seen in the dictum of apex court as well. The celebrated NALSA judgment which recognizes all non-heterosexual activities under the term of transgender and identifies kinnars, hijras etc.. as third gender, while in the interviews conducted by the researcher proves the fact that the judgment has failed them. They opine that their attempt was to identify themselves as women but labeling them as transgender have defeated their struggles. They are still outside the purview of society and hence justice is still a distant dream for them.

Gender Justice

Oxfam[22] defines gender justice as a human right for women in order to grant life full of liberty, dignity and freedom to make choice. While generally, gender justice is taken as something which aims to bring equality between man and woman. Point to note here is that the concept of gender justice in itself is talking about two known biological sexes and genders only. It is correct that gender justice is talking about equality, justice , liberty etc.. all fantasized concepts to restore dignity of human life but they are all incomplete attempts as not all lives are taken into consideration. This results in biasness where the base of discrimination is lying in human genitals as a result of which implementation of gender justice around the world has failed miserably and no concrete results are yet been achieved even in India. For instance, legislation in an attempt to do gender justice formulated Article 14, 15 and 16 of constitution to open up work, education opportunities and public place for Indian women but the glaring question is are they still open?. In a recent survey[23], it was conveyed that more number of educated women are dragging themselves out of work force, to be home-makers as there is no one to look after young ones. So, what sort of gender justice are we as a society advocating?. Answer to this situation is lying in gender neutrality, which is relatively new concept but rather simple to understand and it means applying all the principles of justice and

equality without giving any preference to sex and gender of person.

Cambridge[24] dictionary defines gender neutrality as something which has nothing to do with men and women in particular. The definition in itself is confusing and hilarious, as it does not explains what it actually means. European Institute of Gender Equality[25] defines it as, policies which are not in particular effecting men or women. However it doesnot stop here and says further that these policies effect male and female sex uniformly. This definition on international forum clearly demonstrates the importance of normal sexes in the society and the confusion or rather unwillingness to give acceptance to third sex and gender as a human body.

Even the concept of justice, in form of gender justice is under challenge. However before that we need to have a basic understanding of what is gender justice? United nations defines it as a concept wherein an end is brought to the inequality among biological males and females be it in home, office or societal norms itself.

Here the problem is that years of usage feminine gender is allotted the role of victim and it is the male biology who is a offender. In India, the legal acceptance of homosexuality and all other non-heterosexuality has highlighted the fact that even the biological male can be victim of gender discrimination and the biological female a hardened offender. Now this very notion has jolted the concept of gender, which is finding difficult to accept that masculinity can be a victim and feminity an offender. "Normal/ mainstream" Society is still finding it difficult to accept that in heterosexuality which is still the normal and moral conduct for the society, a man can be victim and a women an offender, as it questions the very role the society had created for them.

Elevation to Gender Neutrality

The question which arises before us is now that what can be done to solve the confusion which the pre-conceived notions of sex, gender , gender justice have on the society. And the answer to this problem is lying in extension of gender justice and moving towards the understanding and accepting of "Gender Neutrality" which means being neutral to one's gender without giving any preference to biological and sociological sex.

This concept is focusing on bringing formal equality wherein the concept will be applied as whole and focus will be on human body and its rights rather than on his or her rights where substantive equality executes itself. This execution of substantive equality is so unaware of the concept of intersex and LGBTQUIA that there are no roles defined for them in the society. Let alone be the roles, no name are also allotted for intersex and transgenders unlike the designations of Mr., Mrs., Miss, His or Her , He or She for 'normal' male and female sexes. The names if any available to intersex and transgenders are like abnormal, kinaar, hijras, she-male, he-female and all sorts of slangs and derogatory words which are being used to taunt rather then name them.

Conclusion

To brief up this piece, it seems that this newly found species / variation of human body in India has shook up the strings of society on the basis of which its execution has been drafted. Until now the very concept of human rights to accomplish itself was literally looking below the belt of a human body and if it was able to trace the distinguish genitilia of a male and a female only then that body classified itself to be a human and having any human rights. Even the black and white text of Indian laws including the constitution of India which sculpts the principle of equality from Article 14 to 16 but give it a shape of male and female only. The very concept of human also known as person in law in itself needs a change. Both law and society has to understood the genitilia, testosterone, estrogen and heterosexuality are not the only criteria to identify someone as humanoids. There are feelings of love and respect in the heart, value of life honour and dignity and that certainly cannot be based on sex and sexuality of a person.

Conflict of interest:-NIL

Source of funding:-Self

Ethical Clearance :- In this self funded research paper , researchers have taken care of ethics required in research and have taken duly credited the respective authors at appropriate places and since it is a self funded work, no permission from any committee was required.

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Original Research Article

Knowledge & Perception Of Medical Students Towards The LGBT (Lesbian, Gay, Bisexual & Transgender) Patients & Their Rights: A Cross Sectional Study

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Abstract\

Diversity is a part of our everyday lives. Having good attitudes towards LGBT (lesbian, gay, bisexual & transgender) individuals is one of the ways of accepting and respecting human diversity. In this way, the quality of offered services will be same for all the people. Attitudes of medical professionals towards homosexuals can influence their willingness to provide these individuals with medical help. The study evaluates the medical students' knowledge about homosexuality and their attitudes towards it. The sample consisted of 200 participants (104 from second year & 96 from first year) who anonymously completed the preformed questionnaire. The study showed that the second year students have more knowledge than the first year students. Degree of knowledge had significant predictive effect on attitudes in the analyzed predictive model & is important tool in shaping the attitudes towards LGBT patients and reducing stigmatization.

Keywords: *Lesbians, Gays, Bisexuals, Transgenders, attitude.*

Introduction

A person's sexual orientation affects the attitudes of others toward him or her. In today's world, heterosexuality is the norm and most minority groups like LGBT people are subjected to stereotypes. Their population ranges from 4 to 17%[1]. Homosexuality is thought to be "against the nature" by most of the religions in the world & has been a debatable issue since the ancient time. Perceptions toward LGBT people are various according to types of societies, cultural and moral development or political situation. According to Harry (1989)[2], homosexuality is not rare or unusual for people who have that kind of sexual orientation, but they often face the prejudice from multiple sources such as at home, school and work. Weinberg (1972)[3] gives the first definition of homophobia that indicated it as irrational negative attitude towards people with homosexual orientation. Studies use different terms to indicate hostility, antipathy or discriminatory behaviour directed towards LGBT people. Homonegativity includes negative attitudes, values and beliefs of the heterosexual majority towards same-sex couples, along with the negative reactions towards them[4]. These negative feelings have been investigated more deeply & distinguished into variety of terms such as biphobia, transphobia etc[5] each one depicting differences and the special problems that follow. Perception and knowledge towards LGBT patients in health care is an important but infrequently assessed issue in India. So an effort is made with our study to know the same in medical students at a medical college.

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Aim: To know the knowledge & perception of medical students towards the LGBT patients.

Objectives: To assess:

1. Awareness of medical students regarding LGBT people & discrimination faced by them.

2. Acceptance Level of medical students regarding LGBT people & ways to address the problem of discrimination.

3. Awareness of medical students regarding recent Supreme Court judgment regarding S. 377 IPC & its social impact.

4. Attitudes of medical students towards LGBT people.

Materials & Method

Participants: The study includes 200 randomly selected medical undergraduate students with gender distribution of 61.5% (N=123) females and 38.5% (N=77) males. 96 (48%) of the participants were from first year and 104 (52%) were from second year. The participants' ages ranged from 19 to 23 years. Majority of the participants (89.5%) were from urban setup.

Design and settings: Between April & June 2018, 200 medical undergraduates attending their first & second year were asked to participate in the survey to assess their knowledge, attitudes & perception pertaining to LGBT people & their healthcare.

Ethical issues: Permission from Institutional ethical committee was taken. All participants received verbal and written information on the study. Their participation was anonymous and voluntary, and their responses were confidential.

Data collection: We collected data from the students by using preformed anonymous questionnaire having six sections. It was designed by the authors to obtain information that was supposed to have effects on the scores of Knowledge, Attitudes and Perception towards LGBT patients in clinical practice. The survey took place in a large classroom with enough space per student to allow for privacy and it took approximately 30 minutes to complete in presence of authors.

Results

Table 1: Demographics of the Respondent:

Demographic parameter	First year	Second year	Total
Gender			
Male	42	35	77 (38.5%)
Female	54	69	123 (61.5%)
Age			
19	21	4	25 (12.5%)
20	51	16	67 (33.5%)
21	12	40	52 (26%)
22	12	29	41 (20.5%)
23	0	15	15 (7.5%)
Domicile			
Rural	6	15	21 (10.5%)
Urban	90	89	179 (89.5%)

The study includes 200 randomly selected medical undergraduate students with the gender distribution of 61.5% (N=123) females and 38.5% (N=77) males. 96 (48%) of the participants were from first year and 104 (52%) were from second year. The participants' ages ranged from 19 to 23 years with majority (33.5%) are of 20 years of age. Majority of the participants (89.5%) were from urban setup.

Table 2: Awareness:

Awareness parameter	**First year**		**Second year**		**Total (200)**
	Yes	No	Yes	No	
Are you aware of the acronym LGBT & LGBT movement?	96	0	104	0	100%
Knowledge & understanding regarding following terms:					
Lesbian	96	0	104	0	100%
Gay	96	0	104	0	100%
Bisexual	96	0	104	0	100%
Transgender	70 (35%)	26 (13%)	104 (52%)	0 (0%)	100%
Queer	40 (20%)	56 (28%)	90 (45%)	14 (7%)	100%
Homosexual	96	0	104	0	100%
Heterosexual	96	0	104	0	100%
Homophobia	75 (37.5%)	21 (10.5%)	95 (47.5%)	9 (4.5%)	100%
Are LGBT people more likely to be infected with or carry sexually transmitted diseases (STDs)?	52 (26%)	44 (22%)	90 (45%)	14 (7%)	100%
Are LGBT people more likely to be involved in substance abuse?	50 (25%)	46 (23%)	76 (38%)	28 (14%)	100%
Are you aware of the Section 377 IPC & recent judgment regarding it by the Supreme Court of India?	36 (18%)	60 (30%)	102 (51%)	02 (1%)	100%
What is the principle source of information on LGBT?	Peers, Internet, Electronic media		Lectures, Peers, Internet, Electronic media, Print media.		

All the participants were aware of the acronym LGBT & regarding LGBT movement. All students were having knowledge & understanding regarding terms: lesbian, gay, bisexual, homosexual & heterosexual. 13% students (all from first year) were unaware of the term transgender. 42% students mostly from first year were unaware of the term queer. 30% students mostly from first year were unaware of the term homophobia. 71% students say that LGBT people are more likely to be infected with or carry STDs like AIDS, Gonorrhea, Syphilis etc. 63% students say that LGBT people are more likely to be involved in substance abuse like alcohol, tobacco, cocaine, solvent abuse etc. 69% students mostly of second year were aware of the Section 377 IPC & recent judgment regarding it by the Supreme Court of India. Among the different sources of information regarding LGBT, lectures were principle source for second year students whereas peers were principle source for first year students. Internet, electronic media & print media are the other important sources of information.

Table 3: Acceptance Level: Will you accept or not accept the following situations?

Situations	Acceptable	Neutral	Unacceptable	Don't know/ no comment
Your family member is from LGBT group	108 (54%)	41 (20.5%)	38 (19%)	13 (6.5%)
Your friend is from LGBT group	138 (69%)	37 (18.5%)	22 (11%)	3 (1.5%)
Your neighbour at hostel is from LGBT group	157 (78.5%)	33 (16.5%)	5 (2.5%)	5 (2.5%)
Your teacher is from LGBT group	161 (80.5%)	25 (12.5%)	9 (4.5%)	5 (2.5%)
A LGBT person holds a senior position/ position of authority in the organization you work for.	151 (75.5%)	30 (15%)	10 (5%)	9 (4.5%)
You encounter LGBT patient during clinics	185 (92.5%)	9 (4.5%)	4 (2%)	2 (1%)
Your routine patient turns out to be from LGBT	168 (84%)	24 (12%)	2 (1%)	6 (3%)
Same sex marriages	117 (58.5%)	42 (21%)	34 (17%)	7 (3.5%)

While evaluating acceptance level, most of the students were acceptable to the LGBT person as a friend (69%), neighbor (78.5%), teacher (80.5%), senior/ authoritarian (75.5%) & patient (92.5%). But the acceptance level drops (54%) when this LGBT person is a family member & also same sex marriages are unacceptable to 41.5% students.

Table 4: Discrimination Faced: Following are some possible situations faced by LGBT people:

Situations	**Serious**	**Average**	**No problem at all**	**Don't know/ no comment**
A doctor discriminates LGBT patients & they receive lower quality care	183 (91.5%)	7 (3.5%)	2 (1%)	8 (4%)
An institute refuses to employ a doctor qualified for the job due to his/her sexual orientation	176 (88%)	19 (9.5%)	1 (0.5%)	4 (2%)
A medical club/ association/ organization refuses to accept him/ her as its member	158 (79%)	32 (16%)	6 (3%)	4 (2%)
A warden/ landlord refuses accommodation	124 (62%)	66 (33%)	0 (0%)	10 (5%)
A student is discriminated against at school/ college	179 (89.5%)	21 (10.5%)	0 (0%)	0 (0%)
Verbal/ physical/ cyber bullying of LGBT person at school/ college	177 (88.5%)	19 (9.5%)	0 (0%)	4 (2%)
Comparatively they have higher chances of developing anxiety & depression	149 (74.5%)	33 (16.5%)	9 (4.5%)	9 (4.5%)

Most of the participants find discrimination faced by LGBT people as serious issue in various situations like at medical practice (91.5%), employment (88%), clubs/ associations (79%), accommodation (62%), school/ college (89.5%), bullying (88.5%) & resulting anxiety & depression (74.5%).

Table 5: Ways to address the problem of discrimination faced by LGBT people:

Ways	Agree	Neutral	Disagree	Don't know/ no comment
Society should ensure equal opportunities	192 (96%)	6 (3%)	0 (0%)	2 (1%)
Public education	187 (93.5%)	10 (5%)	1 (0.5%)	2 (1%)
Teaching scientific facts about LGBT as a part of sexuality education in schools	182 (91%)	16 (8%)	2 (1%)	0 (0%)
The government should introduce separate legislation to outlaw discrimination on the ground of sexual orientation (admission/ employment/ promotion/ public facilities etc)	132 (66%)	35 (17.5%)	33 (16.5%)	0 (0%)
There should be separate reservation for LGBT people in education & jobs	14 (7%)	37 (18.5%)	144 (72%)	5 (2.5%)

Most of the students are in agreement that equal opportunities (96%), public education (93.5%) & teaching scientific facts about LGBT as a part of sexuality education in schools (91%) are effective ways to address the problem of discrimination. 66% think that there should be separate legislation to outlaw discrimination on the ground of sexual orientation. But maximum students (72%) are against separate reservation for LGBT people in education & jobs.

Table 6: Attitudes towards LGBT people:

Attitudes	Agree	Neutral	Disagree	Don't know/ no comment
Work ability is not directly related to sexual orientation	183 (91.5%)	7 (3.5%)	6 (3%)	4 (2%)
Promiscuity is not directly related to sexual orientation	115 (57.5%)	30 (15%)	19 (9.5%)	36 (18%)
LGBT are psychologically normal people	148 (74%)	28 (14%)	14 (7%)	10 (5%)
Sexual intercourse between them is against order of nature	53 (26.5%)	46 (23%)	82 (41%)	19 (9.5%)
Violence/ discrimination based on sexual orientation	17 (8.5%)	24 (12%)	147 (73.5%)	12 (6%)
Homophobia contributes to discrimination/ violence	98 (49%)	26 (13%)	35 (17.5%)	41 (20.5%)

91.5% participants think that LGBT people are equally competent at work. 74% think that LGBT people are having normal psychology. 57.5% students think that there is no relationship between sexual orientation & sexual promiscuity. 41% students think that sexual intercourse between two LGBT people is with the order of nature. Most of the students (73.5%) are against the violence or discrimination based on sexual orientation. Nearly half of the participants (49%) think that homophobia contributes to discrimination & violence.

Discussion

Supreme Court of India verdict (2018)[6]: Consensual adult gay sex is not a crime and article 14 (right to equality) & 21 (right to life & personal liberty) of Indian Constitution contradict the present view of Section 377. There is lack of Indian literature[7] that has systematically investigated issues related to LGBT people & patients in medical practice and this is an effort to study the knowledge and attitude of medical students

towards the same in an Indian set up. Knowledge about homosexuality emerged as the strongest predictor of a positive attitude towards them. The Association of American Medical Colleges has recommended that "medical school curricula ensure that students master the knowledge, skills and attitudes necessary to provide excellent, comprehensive care for LGBT patients" by including "comprehensive content addressing the specific healthcare needs of LGBT patients" and "training in communication skills with patients and colleagues regarding issues of sexual orientation and gender identity."[8]

First year students (10% - 28%) were unaware of the terms like transgender, queer & homophobia. 30% students (first year) were unaware of the Section 377 IPC & recent judgment regarding it by the Supreme Court of India. That's the reason why 24% to 46% of the participants remained neutral when asked regarding social impact of recent Supreme Court judgment regarding Section 377 IPC. From the pattern of responses, our study proves beyond doubt that degree of knowledge had significant predictive effect on attitudes in the analyzed predictive model & is important tool in shaping the attitudes towards LGBT patients and reducing stigmatization.

Conclusion

Through this study the researcher found the following findings:

- The study showed that the second year students have more knowledge than the first year students. Degree of knowledge had significant predictive effect on attitudes.

- Academic lectures play vital role in understanding the different related terminologies, problems (medical, psychological & social) faced by LGBT people & different ways to address such problems.

- Most of the students were acceptable to LGBT person as a friend, neighbor, and teacher, senior / authoritarian & patient. But this acceptance level drops (54%) when this LGBT person is a family member.

- Most of the participants find discrimination faced by LGBT people as serious issue & 66% think that there should be separate legislation to outlaw discrimination on the ground of sexual orientation.

- Most of the participants think that LGBT people are psychologically normal & their work ability is not directly related to their sexual orientation.

- Most of the participants also support the LGBT rights movement.

Recommendations:

- Public education

- Teaching scientific facts about LGBT as a part of sexuality education in schools.

- The government should introduce separate legislation to outlaw discrimination & Violence.

- Counsellors & counselling centres in medical colleges & schools.

- Accessibility to appropriate and accurate information regarding sexual orientation at early stages through library and Internet resources as well as workshops and forums.

- To change societal attitude media has to play a responsible role by reporting on LGBT issues and promoting a culture of tolerance and freedom for minorities.

- Legal funds need to be created that can take on Public Interest Litigation on LGBT issues.

- Training needs to be conducted for health professionals to increase their understanding of LGBT identity as potential risk factor for self-harm, suicidal behavior and depression.

- Respective authorities should ensure that health, mental health and social care services are provided in a way that is accessible and appropriate to LGBT people.

- National & state government should develop initiatives to support employers in making workplace and workplace culture more supportive and inclusive of LGBT people.

- Police force needs to be sensitized on LGBT issues and general principles of fundamental human rights.

- Stigmatizing or pathologizing language regarding LGBT persons should be avoided.

- Local authorities, policy makers, schools and families need more education on gender identity equally,

applying policies and programs in friendly manner

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Causes and Factors in Road Traffic Accidents at a Tertiary Care Center of Western Uttar Pradesh

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Abstract

Road Traffic Accidents are one of the leading causes of morbidity and mortality in the world. The etiological factors may be classified into human and environmental out of which human factors are much more responsible for number of accidents. This study was carried out on Road Traffic Accidents cases reported to casualty department of Muzaffarnagar Medical College, Muzaffarnagar (U.P) from 1st July 2016 to 30th June 2017. The main objectives of the study were to know the causes and factors responsible for the accidents. Data were collected and analyzed. A Total of 416 cases were included in the study. Human error is responsible for accidents in 78% of cases in which drivers were at fault in 57.0% of cases followed by the pedestrians and passengers of vehicles who were responsible for 18% and 3.0% cases respectively. Faults in vehicles and poor weather accounted for accidents 8.0% & 7.8% of total cases respectively. Bad roads were responsible for accidents in 4.0% of total cases. None of the driver or passengers of the vehicles was wearing helmets or seat belts which are a very alarming trend. The important human factors responsible for accidents were over speeding, rash driving, violation of traffic rules, carelessness, wrong crossing, playing on roads, alcohol intake, fatigue or sleepiness.

Keywords: - *Road traffic accidents, Causes, Risk factors, Human error, Drivers.*

Introduction

Road Traffic Accidents are one of the leading causes of morbidity and mortality in the World. WHO data show that in 2016 nearly 1.35 million people died worldwide as a result of Road Traffic Injuries. However, the rate of death relative to the size of the world's population has remained constant.1 In 2002, Road Traffic Injuries were the ninth leading cause of disability-adjusted life years (DALYs) lost, accounting for over 38 million (DALYs) lost, or 2.6% of the global burden of disease. Low and middle income countries account for 91.8% of the DALYs lost to road traffic injuries worldwide. According to WHO data for 2002, Road Traffic Injuries accounted for 2.1% of all global deaths and ranked as the 11th leading cause of death. These road traffic deaths accounted for 23% of all injury deaths worldwide. Road traffic injuries constitute a major public health and development crisis and are predicted to increases if road safety is not addressed adequately.2 A total of 4.64,674 road accident cases were reported during 2015. Road accident cases in the country have increased by 3.1% (4,64,674 in 2015 from 4,50,898 in 2014) during 2015 compared to 2014.The fatalities in road accidents have increased by 5.1% (from 1,41,526 in 2014 to 1,48,707 in 2015) during 2015 over 2014.It is observed that rate of deaths per thousand vehicles has decreased marginally from 1.0in 2011 to 0.8 in 2015, although the number of vehicles in the country have increased by 28.6% (from 1,41,866 in 2011 to 1,82,445 in 2013) and the quantum of road accidents has increased by 5.6% during same period.4, 64, 674 road accidents caused 1,48,707 deaths and injuries to 4,82,389 person during 2015.Generally road accidents have caused more injuries then deaths but in Punjab and Uttar Pradesh road accidents caused more death compared to persons injured.3 Accidents constitute a complex phenomenon of multiple causation.

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The etiological factors may be classified into two broad categories human and environmental.4 The present study was conducted to explore the various causes and factors responsible for RTAs for better prevention and management of Road traffic accidents.

Material & Method

The present study was conducted in the department of Forensic Medicine at Muzaffarnagar Medical College, Muzaffarnagar a tertiary care teaching hospital from 1/7/2016 to 30/6/2017. During this period a total of 416 cases of Road traffic accidents were reported to casualty department. This study was a prospective study. The relevant data was collected from casualty medical officer, treating doctor, police, relative accompanying the patients and from case files. In few cases adequate information was not obtained and such cases were put under unknown group. A detailed Performa was prepared for filling the observations on the present study. The information thus collected was statistically analyzed.

Observation and Result

During our study period 416 cases were admitted to the hospital with diagnosis of Road Traffic Accidents. The causes of accidents were analyzed in detail. In our study human error was responsible for accidents in 78% cases out of these drivers were at fault in 57% cases followed by pedestrians and passengers responsible for accidents 18% and 3% cases respectively. Present study showed that vehicle and weather were responsible in 8% and 7.8% cases respectively. Poor and defective roads lead to 4% of fatal accidents and in 2.2% cases the cause was unknown. (Table-1)In this study the common fault of drivers responsible for fatal accidents were due to over speeding (49.8%), rash driving (30.8%), loss of control (6.8%), violation of traffic rules (5.5%), alcohol intoxication (4.2%), fatigue or sleepiness (2.9%). (Table-2)In present study out of 75 pedestrians responsible for accidents, 58.8% were careless in crossing roads, 20% were crossing from wrong side, and 16% of children were playing on roads and 5.2% were vulnerable due to old age and diseases. (Table-3) Out of 12 passengers responsible for fatal accidents, 50% were catching or getting down of running buses, 33.4% were travelling on foot board of buses, 8.3% were catching the buses from wrong side of the road and 8.3% of passengers were projecting outside the body of the vehicle when they fell out. (Table-4)In this study out of total 34 cases of accidents attributed to faults of vehicles, 29.8% of vehicles were overloaded or overcrowded, 26.7% of passengers ejected out of open jeeps during collision. These open jeeps are used to ferry passengers illegally. Wrongly parked vehicle on roads leading to accidents at night accounts for 17.8% cases. In this study 14.2% of vehicle has failure of brakes or steering, 8.5% had a tire burst at high speed leading to accidents due to overturning or collision with vehicles or trees. In 3% of cases, projecting loads from rear of trucks accounted for the accidents. (Table-5) Regarding the effect of the poor weather, 46.7% cases occurred at night due to poor vision and fog or mist account for 44.2% cases and 9.1% of cases due to heavy rain or storms in this study. Our study showed that out of 17 cases of accidents due to poor road condition, 34.4% occurred due to stray animals on road and 29.5% due to potholes and damaged roads. In 23.5% cases merging of rural roads directly to a highway with high speed traffic were responsible for accidents. 6.3% cases each occurred due to diversion or construction material on the road and illegal speed breakers, respectively. (Table-6)

Causes and Factors In Road Traffic Accidents at a Tertiary Care Center of Western Uttar Pradesh

Table - 1 Causes of fatal Road Accidents

Causes	Cases	Percentage (%)
Human Error	324	78.0
1. Fault of Drivers	237	57.0
2. Fault of Pedestrians	75	18.0
3. Fault of Passengers	12	3.0
Role of vehicles	34	8.0
Role of Weather	32	7.8
Role of Roads	17	4.0
Unknown	09	2.2
Total (n = 416)	416	100

Table – 2 Faults of Drivers

Faults	Cases	Percentage (%)
Over Speeding	118	49.8
Rash Driving	73	30.8
Loss of Control	16	6.8
Violation of Traffic Rules	13	5.5
Alcohol Intoxication	10	4.2
Fatigue/Sleepiness	07	2.9
Total (n = 237)	237	100

Table – 3 Faults of Pedestrians

Faults	Cases	Percentage (%)
Carelessness	44	58.8
Wrong crossing	15	20.0
Playing of Roads	12	16.0
Disease	04	5.2
Total (n = 75)	75	100

Table – 4 Faults of Passengers

Faults	Cases	Percentage (%)
Catching / alighting Running Bus	6	50.0
Travelling on foot Boards	4	33.4
Catching / alighting from wrong side	1	8.3
Projecting outside the Vehicle	1	8.3
Total (n = 12)	12	100

Table – 5 Faults of Vehicles

Faults	Cases	Percentage (%)
Overcrowding / over loading	10	29.8
Ejected out of open jeeps	09	26.7
Wrongly Parked on Road	06	17.8
Failure of Brakes / Steering	05	14.2
Tire Burst	03	8.5
Projecting loads	01	3.0
Total (n = 34)	34	100

Table 6 – Factors related to Roads

Faults	Cases	Percentage (%)
Stray Animals on Road	6	34.4
Potholes / Damaged Road	5	29.5
Merging of Rural Road with Highway	4	23.5
Diversions / Material on Road	1	06.3
Illegal Speed Breakers	1	06.3
Total (n = 17)	17	100

- **Conflict of interest:** Nil.
- **Source of Funding:** Self-funded.
- **Ethical Permission:** Institutional ethical committee.

Discussion

Road Traffic Accidents are mainly caused by the rapid increase in vehicles on roads, personalized modes of transport, lack of road discipline and improper roadway features. India accounts for about 10% of road accidents fatalities world wide and 85% of all road accidents occurred in the developing countries.[2] In the present study road traffic accidents accounted for 416 cases. The factor of human error is found to be most significant (78%) as compared to defects in vehicles, roads or weather conditions similar to other study.[6-9,13] The drivers were at fault in 57% of cases & were responsible

for over speeding in 49.8% cases, rash driving in 30.8% cases. Violation of traffic rules, alcohol intake, fatigue or sleepiness were other causes.[7,8] Pedestrian comprising of elderly & children are most commonly affected group of road accident victims. Majority of them are themselves responsible for accidents due to carelessness (58.8%), Wrong crossing (20%), children usually playing on roads (16%) and diseases in elders (5.4%). [8,9,13] The common faults of passengers were catching running bus (50%), travelling on foot boards (33.4%), catching from wrong side (8.3%)and projecting outside the vehicle (8.3%).[7,8,13] In the cases of accidents attributed to faults of vehicles, 29.8% of vehicles were overcrowded or over loaded, in 26.7% of cases victim ejected out of open jeeps at the time of collision. A small number of vehicles had mechanical failure in form of steering or brake failure (14.2%) or tire burst (8.5%) at high speed.[6,7,13] Poor weather condition like night or poor vision (46.7%), fog or mist (44.2%), heavy rain or storm (9.1%) responsible for accidents which is consistent with other studies.[7,13,14] Factors related to roads like stray animals on road (34.4%), potholes or damaged road (29.5%), merging of rural road with highway (23.5%) and diversions or material on road (6.3%), illegal speed breakers (6.3%) were responsible for accidents. Findings are consistent with other studies.[7,14] The most disturbing finding in the present study was that none of the drivers and passengers of motor bikes or four wheelers were wearing helmets or seat belts leading to fatal injuries.[10-13]

Conclusion

The tragedy of Road Traffic Accidents is that they particularly involve young, perhaps the young and adventurous ones more. Human error is the most common cause of road accidents out of which drivers are at fault in many cases, followed by faults of pedestrians and passengers. The defects in vehicles, poor road infrastructure and poor weather accounts for a smaller number of cases. Fatal accidents represent not only tragic family loss but also a serious economic loss to the community as their education and training have been wasted. Such incidences can be prevented by giving proper education, awareness, training of safety standards by administrators and law enforcement agencies. There should be road safety programmes regularly in which all the cities of our country must be ranked and sensitized on the basis of various criteria like standards of roads, functioning of traffic signals, obeying of traffic rules by the citizen etc.

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Toxicological Trends of Poisoning in a Tertiary Care Center of Western Uttar Pradesh

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Abstract

Poisoning is a significant global public health problem which leads to nearly a million deaths per year. Incidence of poisoning is high in India. This study was carried out on poisoning cases reported to casualty department of Muzaffarnagar Medical College, Muzaffaranagar from 1st January 2016 to 31st December 2016.The main objectives of the study were to analyze pattern and demographic variables of poisoning cases. Data were collected and analyzed. A total of 72 cases (male: 46, female: 26) were included in the study. Maximum cases were of age group 21-30 years (30.5%). More cases from rural area (75%) were reported. Summer months saw maximum number of cases (37.5%). Poisoning by Organophosphorus was seen in maximum cases (31.9%) followed by Aluminium phosphide (29.1%)and in maximum cases reason for poisoning was Intentional (52.7%) and route of exposure was by oral ingestion (84.7%).The study concludes that the burden of poisoning demands strategies for identification, prevention,and its rational management.

Key Words: *Poisoning, Organophosphorus, Aluminium phosphide, Casualty*

Introduction

Poisoning is a major problem all overthe world, though the type of poison and the associated morbidity and mortality varies from place to place and changes over a period of time. Worldwide, an estimated three million cases of pesticide poisoning occurs every year, resulting in an excess of 250000 deaths. Of these 90% of fatal poisonings occurs in developing countries particularly amongst agriculture workers.[1] An estimated total of 877000 people committed suicide worldwide in 2002 and around 28% of these cases are from South East Asia Region.[2] The exact incidence of this problem in India remains uncertain but, it is reported that 1 to 1.5 million cases of poisoning occurs every year, of which nearly 50,000 dies.[3] The last quarter of century has seen tremendous advances in the fields of agriculture, industrial technology and medical pharmacology. Theseadvances have been paralleledwith remarkable changes in trends of acute poisoning in developing countries, including India.[4] The commonest cause of poisoning in India is pesticide due to easy availability, agriculture based economy and poverty. In developing countries like India occupational poisoning is common due to illiteracy, unsafe practices like storing the pesticides at home and handling them without safety gear.[5] Intentional and unintentional pesticide poisoning has been acknowledged as a serious health problem in many agriculture communitiesof low and middle income countries. WHO and other humanitarian organizations are coercingtheauthorities of different countries to ban the highly toxic pesticide and imposing restrictions to their access.Controlling accessto pesticide is not only critical in reducing self-directedviolence, it is a key to preventing unintentional poisoning. Studies indicate, the ban must be accompanied by evaluation of agriculture needs and replacement with low risk alternative for pest control.[6] The paucity of information on risk population, circumstances and toxics is a hurdle to effective poisoning prevention and targeted intervention programs. So the study is sought to characterizepoisoning with regard to

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demographic factors and common toxic agents in our region.

Material & Method

The present study was conducted in Muzaffarnagar Medical College, Muzaffarnagar, a tertiary care teaching hospital from 1st January 2016 to 31st December 2016. During this period a total of 72 cases of poisoning were reported to casualty department. This study was a prospective study. The relevant data was collected from casualty medical officer, treating doctors and from case file. Data related to name, age, sex, religion, marital status, economic status, type of poison ingested, mode of ingestion, rural & urban distribution of cases, occupation was collected in standardized proforma& analyzed. Brought dead cases were not included in this study.

Observations & Results

During our study period seventytwo cases were admitted to the hospital with diagnosis of acute poisoning.Males were more prone to poisoning (63.9%) as compared to females (36.1%). (Table 1) The incidence of poisoning according to age revealed that there was an increasing trend of poisoning with increase in the age up to 30 years with a peak incidence in the age group 21-30 years which represented 22 (30.5%) cases in this study and then declined. (Table 2) Among the 72 cases admitted to the hospital with diagnosis of acute poisoning 46 (63.9%) cases were married followed by unmarried 25 (34.7%). Incidences are more with rural areas 54 (75%) then urban areas 17 (23.6%). Out of 72 cases incidences was more in middle class 47 (65.3%) followed by lower cases 24 (33.3%). In present study incidence of poisoning was more in farmers 26 (36.1%) and laborers 15 (20.8%) (Table 3) Intentional poisoning was more common 38 (52.7%) followed by accidental 30 (41.7%). (Table 4) Out of all cases reported, oral ingestion was the most common route of exposure 61 (84.7%) followed by inhalation 10 (13.9%). (Table 5) Poisoning was more during summer season 27 (37.5%) followed by winter 24 (33.3%) and monsoon 21 (29.2%). The poisons used were organophosphorus (31.9%), Aluminium phosphide (29.1%), Alcohol (13.9%), rat poison (6.9%), Kerosin (2.8%). Among these Organophosphoruspoisons are the most commonly abused poison followed by Aluminiumphosphide. (Table 6)

Toxicological Trends of Poisoning in a Tertiary Care Center of Western Uttar Pradesh

Table 1: Sex wise Distribution of Cases (N=72)

Sex	No. of Cases	Percentage (%)
Male	46	63.9
Female	26	36.1
Total	72	100

Table 2: Age wise Distribution of Cases (N=72)

Age (Years)	No. of cases	Percentage (%)
0-10	2	2.7
11-20	19	26.4
21-30	22	30.5
31-40	12	16.6
41-50	7	9.8
51-60	3	4.2
>60	6	8.4
Unknown	1	1.4
Total	72	100

Table 3: Distribution of Cases According to Occupation (N=72)

Occupation	No. of Cases	Percentage (%)
Farmers	26	36.1
Laborers	15	20.8
House Wife	10	13.9
Students	9	12.5
Unemployed	6	8.3
Service	5	7.0
Unknown	1	1.4
Total	72	100

Table 4: Distribution of Cases According to Reason for Poisoning (N=72)

Reason		No. of Cases		Percentage (%)	
Intentional		38		52.7	
Accidental		30		41.7	
Unknown		4		5.6	
Total		72		100	

Table 5: Distribution of Cases According to Route for Poisoning (N=72)

Route	No. of Cases	Percentage (%)
Oral Ingestion	61	84.7
Inhalational	10	13.9
Bite/Sting	1	1.4
Total	72	100

Table 6: Distribution of Cases According to Type of Poisoning (N=72)

Poison	No. of Cases	Percentage (%)
Organo Phosphorus	23	31.9
Aluminum Phosphide	21	29.1
Alcohol	10	13.9
Rat Poison	5	6.9
Mosquito Repellant	4	5.6
Benzodiazepine	3	4.2
Kerosene	2	2.8
Cannabis	1	1.4
Corrosives	1	1.4
Insect bite	1	1.4
Unknown	1	1.4
Total	72	100

problem is also common in males who are suffering from infertility (2). Presently, male infertility can be treated with the help of medication by diagnosing the core reason for infertility such as erectile dysfunction or hormonal imbalance (3).

Glutathione is present in the cells of mammals and it is a tripeptide thiol, which is a non-protein sulfhydryl compound. It consists of three amino acids named glutamine, cysteine, and glycine. During the oxidative stress, the glutathione uses the power by the cells to act as a defensive appliance against oxidative destruction. There are two types of Glutathione, i.e. GSH and GSSG. Glutathione is present not only in somatic cells but also in germ cells. The defensive mechanism is consist of enzymatic and non-enzymatic functions, characterized by the Glutathione. In males, the deficiency of Glutathione results in the dysfunction of the central part of the sperm that ultimately results in the defective sperm movement. Glutathione protects the spermatozoa plasma membrane by forming a protective layer [(4)]. As per previous researches, it is proven that Glutathione has the ability to maintain the biological importance of the germ cells [(5)]. Men, who are suffering from genital tract inflammation, unilateral varicocele, and sperm dysfunction can be cured with the help of Glutathione.

The coenzyme Q 10 is a nutrient produced in the body in a natural way. It is also present in different food items. The main function of the Coenzyme Q 10 is that it performs like an antioxidant. It defends the cells of the body from any destruction and plays a key role in metabolism [(6)].

In the initial studies, many researchers have found that coenzyme Q 10 supplementation is quite beneficial to treat the infertility problem in males. Some males are suffering from low sperm count. It not only helps to increase the sperm count but also enhances the mobility so the sperms become efficient enough to enter the egg. About 200-300 mg Coenzyme Q10 intake is beneficial to eliminate infertility problem [(7)]. With the help of Coenzyme Q 10, the sperm quality becomes better.

Aim/Objective

The aim of the present randomized placebo-controlled study is to identify the efficiency of Glutathione and Coenzyme Q10 in increasing the sperm motility rate. The quantitative research enhances the possibility of the Glutathione and Coenzyme Q10 efficacy related to motility, morphology, and concentration of the sperms.

Background Literature

According to various studies, male fertility is improved by the effect of ubiquinone. The motility, morphology, and concentration of the sperms get better with the help of Coenzyme Q10 action in an oxidized form. The reduced form of Coenzyme Q10 characterizes around 90% of the Coenzyme Q10. As per different experimental results, it has been noted that the low dose of Coenzyme Q10 that is 90mg per day for a duration of 12 weeks is quite effective to enhance the quality of sperms [(8)].

Another research revealed that the high dose of Coenzyme Q10 that is 300 mg per day for about 12 weeks is effective enough to treat the infertility issues in males because it has a powerful effect of antioxidant. According to the research conducted by Gyozdjaova, it has been revealed that the combination of carnitine and ubiquinol is very effectual to treat the male infertility problem by increasing the function of mitochondria in the sperms [(8)].

A randomized placebo-controlled study was conducted to observe the effect of Coenzyme Q10 on sperm concentration. It has been observed that the Coenzyme Q10 has a protective effect and it acts as a shield against oxidative stress to enhance the morphology as well as the concentration of the sperms. It took duration of 3 months to get significant outcomes. Another research was conducted by Nadjarzadeh, it is based on a double-blind placebo-controlled experiment. It has been observed that Coenzyme Q10 has no significant effect on sperm quality or concentration [(9)].

In human beings, the low quantity of the glutathione in the seminal fluid leads to the abnormality of the sperms because of the low level of glutathione ultimately unstable the middle portion of the sperm that effects the motility rate of sperms [(10)]. The high dosage of Glutathione 600mg per day is proved to be effectual enough to treat the infertility disorder in males (9).

In the study conducted by Yarosh and colleagues, the role of glutathione S-transferase has been observed in idiopathic male infertility. There were three gene polymorphisms have been selected that include GSTM1, GSTT1, and GSTP1 to analyze their effect on the Russian men who are suffering from idiopathic infertility. 203 samples of DNA were collected from 203 infertile males and 227 DNA samples were collected from 227 fertile males. The PCR-Restriction Fragment Length

Polymorphism technique was applied in the research. It has been noted that GSTMI, GSTT1, and GSTP1 genes are responsible to develop idiopathic infertility in men [(11)]. It has been noted that smoking is the major cause of its phenotypic effect that can be associated with the risk of infertility disorder.

Material and Method

Participants

The present randomized placebo-controlled clinical trial was conducted in Saladin province of Samarra city (Iraq) between Jan 2016 to Dec 2018. The study deployed 51 infertile male subjects for the administration of oral glutathione (250mg sachets) for 6-months. Another group of patients 50 received oral Co-enzyme q 10 (200 mg sachets) for 6 months, a third group was taken a placebo (sugar sachets) for another 6 months. All patients included in this study were of a normal female factor with idiopathic oligoasthenoteratozoospermia, patients with a chronic disease like mumps, hydrocele, neoplasm, trauma from prolonged riding, hypospadias, vas deference obstruction, varicocele, and genital tract infection were excluded from this study, also those who received treatment recently. Patients were from 30-40 years old. Before the start of the study, semen samples were taken for all three groups for baseline assessment. Another assessment was done at the end of the 6 months period. All samples were given to a lap. Within 20 minutes, the interpretation was done in accordance with the WHO 2010 criteria [(12)], semen morphology was done according to Kruger criteria [(13)]. Data analysis was undertaken through SPSS 23.0 in the windows interface. Mann Whitney U-test, Kruskal–Wallis test, and chi-square test were used when needed for assessing the data significance for P value <0.05.

Informed Consent

Informed consent was obtained from all study subjects while categorically explaining them the interventions, objectives, and concerns. After taking the informed consent of the research participants, the data were collected.

Semen samples were taken for all three groups for baseline assessment. Another assessment is done after the end of six months duration. Semen morphology was performed as per Kruger criteria. Moreover, the precipitation and centrifugation were carried out to assess the seminal plasma of Glutathione and Coenzyme Q10 with the help of Ellman technique.

Several interactive interviews were conducted to get the informed consent of the participants of the current research. Some of the essential data were collected from the participants while taking the interview that includes age, marriage time, job type, and social habits.

Results

Table (1) showing the characteristic of patients before start of therapy:

Domains	Co-enzyme q 10 group(n=50)	Glutathione group (n=51)	Placebo group (n=50)	P value
Age (years)	32.2±10.2	31.4±11.6	30.1±7.6	>0.05
% of normal Motility (grade a+b)	28	26	28	>0.05
Sperm Concentration (million per ml)	50.4±12.3	50.2±10.1	51.5±8.2	>0.05
% of normal Morphology (kruger criteria)	10	12	10	>0.05
Semen Volume (ml)	2.24±1.23	2.25±0.85	2.21±0.92	>0.05

This table shows that there is no significant statistical difference among study parameters before the start of the study.

Table (2) showing percent of improvement of semen parameters at the end of the study after 6 months of different treatment interventions.

Domains	Co-enzyme Q10 group (n=50)	Glutathione group (n=51)	Placebo group (n=50)	P value
% of motility improvement	36	38	4	P=0.01
% of morphology improvement	28	24	6	P=0.03
% of concentration improvement	24	26	2	P=0.01
% of volume improvement	6	4	4	P>0.05

This study outcome revealed oral glutathione and co-enzyme q 10 as an effective treatment for improving the sperm parameters (motility, morphology, sperm concentration) which were statistically significant except for semen volume where P value> 0.05. Also, the study showed no significant statistical difference between the use of glutathione and coenzyme q10 as a single agent therapy (P >0.05)

Discussion

The infertility problem in men is because of defective mobility, concentration, and morphology of sperms. Such types of factors are very susceptible to the free radicals that are oxygen molecules and consisted of one or more additional electrons. As per statistics, around 25% of the males are affected with the infertility issues worldwide. There are various reasons that affect the spermatogenesis process such as nutrition, environment, genetics, and physiological issues [(1)].

A study conducted to treat the Iraqi infertile men. In the study, around 60 males were selected. The semen was obtained from the participants and it was divided into three categories. The first category is in vitro sperm characterization, the second category utilized density gradient centrifugation technique and in the last category, the combination of density gradient centrifugation and TAD 600mg Glutathione is used [(14)]. It has been concluded that TAD 600mg Glutathione had a very positive effect to enhance the quality and function of sperms.

Another study was conducted by Trang and his colleagues; the idiopathic male infertility was analyzed in the Vietnam males. 300 blood samples were collected in which 150 were control samples and 150 were infertile samples. The aim of e research is to identify two common SNPs that are associated with infertility. It has been revealed that NAT2 and GSTP1 are susceptible to cause infertility in males [(15)]. Such genetic markers will be useful to investigate the cause of infertility in future researches. Research accompanied by the Kolesnikova and his colleagues. They have observed the Glutathione effectiveness on the men who are suffering from infertility issue. It has been found that a balanced amount of Glutathione is essential for proper functioning [(16)]. Without a balanced quantity of Glutathione, the detoxification of toxic substances will not be possible. To cure infertility in men, it is better to balance the glutathione level in the body. The study conducted by Sinha in 2018 to analyze the role of antioxidants in male infertility, it has been revealed that glutathione has a promising effect as a defensive mechanism [(17)]. It has the ability to cure the reproductive damage that is caused by ROS.

According to the research carried out by Ring and his colleagues, the contribution of the male infertility issue is about 50-60% in whole infertility. In most of the cases, the abnormal semen results in the infertility problem in males. The efficiency of the Coenzyme Q10 has been proved to play a major role to treat the males' infertility [(18)]. The supplementation of Coenzyme Q10 is very effective to treat sperm concentration and quality.

A small study conducted in which azoospermic males were selected for the experiment. 183 patients were selected for the study. It has been observed that coenzyme Q10 has a quite positive effect on the experimental group who were treated with coenzyme Q10 [19]. The motility and morphology of the sperms were enhanced significantly after getting the treatment. The current study indicates the significant improvement in the quality, quantity, morphology, and concentration of sperms with the treatment of glutathione and coenzyme q10.

Conclusion

From this study, we can conclude that both glutathione and co-enzyme q 10 are effective treatment options for improving sperm motility, morphology and concentration. There is no statistical difference between them as a single agent therapy; the researchers need to investigate the scope of co-administering other antioxidants like carnitine, selenium, and vitamin B complex with oral glutathione or co-enzyme q 10 to minimize the prevalence of male infertility in the selected population.

Conflict of Interest - (nil – There are "No Conflict Of Interest")**.**

Source of Funding - By all

Ethical Clearance: Committee members are approved to perform a study about**:**

"The Effect of Glutathione versus Co-Enzyme Q10 on Male Infertility Original Study"

After discussion of study plan with researchers:

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Incidence and Variations of Aberrant Renal Arteries in Kidney Donors Cases

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Abstract

The origin of renal arteries, vessel diameter, and point of entry to the kidney has all been analyzed as potential parameters which are a clinically relevant issue. This anatomical feature may be essential for fully understanding the process of renal perfusion, as it is not only the number of renal arteries that influences the extent of renal blood supply, also their diameter. The aberrant renal arteries that supply the inferior pole are more important because it provides arterial supply to the proximal part of the ureter. The objective of this study is to identify influences and prevalence of aberrant renal arteries of the kidney donors' cases to perform role of the aberrant renal artery.

Prevalence of aberrant renal artery was identified in 26.3% of the cases, incidence was more frequent in left than right side; about 78.7 % of cases presented unilaterally. The remaining cases 21.2 % were RAs presented bilaterally. Diameter of main RA in kidney presenting the RA was 5.73 mm and that of kidney with only single renal artery was 6.19 mm.

Keywords: *Aberrant renal artery, renal arterial variations, renal angiography, kidney, incidence.*

Introduction

As a wide range of renal vascular variations has been reported by anatomists and radiologists; prevalence of these variations is extremely divergent in various populations. Historically, first recorded aberrant renal artery (aRA) was reported by Eustachius [1] in one of his famous plates which remained unprinted in Papal Library until 1714. Since then various cases of aRA have been reported; including variations in origin, number, and branching pattern of renal arteries [2&3]. Therefore, as much as larger samples studied will give more clarifications and changes [3].

aRA defined by presence of more than one renal artery (RA) supplying kidney which may arise from aorta; above or below of main RA [2, 4&5], or may arise from coeliac, superior mesenteric [2], inferior mesenteric, and common iliac arteries [2&3]. name "aberrant" described in literature whereby additional artery branches from abdominal aorta either superior or inferior to main RA and enters kidney directly through the capsule at the upper or lower poles of the kidney, or as "accessory" whereby it enters the hilum directly. Anatomical variants in the renal vasculature considered being extremely common variants [1, 6&7]; the prevalence of aRAs are found in 30% (ranging 20-30%) of the individuals [2-5].

Embryologically, as the kidneys ascend from the pelvis during the embryological development, they receive their blood supply initially from common iliac arteries. Later, they receive new branches from the aorta, and the inferior branches disappear. This continuous generation and degeneration of renal arteries as the kidney ascend explains the high incidence of the variations in the blood supply to the kidneys. Therefore, the complicated embryology variations of this organ are relatively higher when compared with the others [8&9].

The main objectives and aims identify the prevalence and variation of the aberrant renal arteries of the kidney donors' cases, as they had been regarded as perfect normal cases for the study. Determine the anatomy and topography of the aberrant artery and main renal artery donor kidneys and preserving total donated kidney function.

Material and methods This study was designed to determine the main RAs and aRAs including its morphometric and location on each side in kidney

donors. A double-blinded prospective and retrospective angiographic and morphometric imaging study performed on one hundred and eighty-four (184) potential prospective living kidney donors. This study was conducted from December 2018 to May 2019 at the radiology and imaging centre in Zheen International Hospital in Erbil city, Iraq. Only the cases that fit for the donation were selected medico-legally. This study was approved by the research ethics committee of the college of medicine, and written consent was received from the cases. Regarding the contrast media allergy cases, no hypersensitivity cases were recorded, other than some simple cutaneous rashes in two cases which need no interferences.

Computed Tomographic Angiographic (CT-Angiographic) imaging for the cases performed according to the four-phase protocol; which include an initial non-contrast image acquisitionphase, followed by arterial phase, nephrographic phase, and excretory phase (delayed phase). The perfect anatomic coverage for CT-Angiography was extended between the dome of the diaphragm and the distal portion of the common iliac arteries or pelvic region. The dynamic CT-Angiography examination was performed with a 128-slice CT scanner (Somatom Definition AS+ 2012, SIEMENS Healthcare, and Germany) such that four phases were covered.

Finally, all images were reconstructed andanalyzed by Syngo program (Siemens Healthcare, Germany). In 3D CT-Angiography, the VRT, MRP and MIP images were used for evaluation of the renal arteries. Regarding the RAs morphometry (diameter); retrospective analysis of the RAs of all the kidney donors was carried out in a double blinded fashion and measured at the aortic origin. To carry out the analysis of renal arteries variations and diameter based on the type of arterial blood supply present, the total number of the kidneys and arteries were established and then divided into different groups according to the following criteria; Gender, Side, Anatomic eRA – early branch renal artery, a single renal artery originating from the aorta and giving branch before reaching renal hilum and supplying poles, hilum of the kidney, and extra renal organs.

Statistical Analysis

Statistical analysis of the current study data was done by using social package for scientific statistic (SPSS) software version 24 (IBM Corporation, Armonk, New York, USA).

Result: The CT-Angiographic findings analyzed in one hundred and eighty-four (184) cases i.e. three hundred and sixty-eight (368) bilateral kidneys were enrolled in this study. Cases are distributed between 128 males and 56 females. Participants' age ranged between 19-61 years for males and 19-58 years for females. The mean age of males is (24.05 ±6.4) years, and the females mean age is (28.12 ±7.9) years; which there are no statistically significant differences between these two groups (t-test, P= 0.961).

The incidence of only single RA is identified in 271 (73.64%) out of 368 kidneys. Whereas the renal arterial variations i.e. aRA were identified in 97 (26.3%) out of 368 kidneys.

Accordingly, the incidence of aRA on the right side is 38 (20.7%) out of 184 kidneys. While on the left side, the incidence is 59 (32.1%) out of 184 kidneys. There are statistically significant differences between the left and right side, as the incidence of aRAs were more frequent in the left than the right side (Chi-square test, P= 0.001)

The distribution of RAs that are supplying the right kidney classified as follow:

a) Only one

b) single renal artery is identified in 149 (79.3%) kidneys.

c) Double renal arteries (one main RA and one aRA) were identified in 37 (20.1 %) kidneys.

d) Triple renal arteries (one main RA and two aRA) were identified in 1 (0.5 %) kidney which considered one of the rare cases.

Regarding the left side, number of RAs that supplying the kidney distributed as follow:

a) One single renal artery was identified in 125 (67.9 %) kidneys.

b) Double renal arteries (one main RA and one aRA) were identified in 53 (28.8 %) kidneys.

c) Triple renal arteries (one main RA and two aRA) were identified in 5 (2.7 %) kidneys.

Importantly, one rare case recorded in this study in which the left kidney supplied by quaternary renal arteries (one main RA and three aRA) were identified in 1 (0.5%) kidney. Therefore, a statistically significant

difference between the left and right side, as the incidence of multiple RAs were more frequent in the left than the right side (Chi-square test, P= 0.001). demonstrating the RA and aRAs. Regarding the prevalence of the renal arteries among genders. Although there were large differences in aRAs frequency between males and females (more frequent in males) in the current study but these differences were not statistically significant (Chi-square, P= 0.663)

Among the 80 cases with aRAs, 63 (78.7 %) cases presented unilaterally. The remaining cases 17 (21.2 %) were the aRAs presented bilaterally. There is a statistically significant difference between groups with respect to the symmetricity were unilateral incidence was higher than bilateral incidence (Chi-square test, P< 0.001).

Regarding the origin of renal arteries, the total arteries that are originating from the aorta and other sources supplying the right kidney are 223 renal arteries distributed as 184 of single main RAs and 39 aRAs. In the left side, the total number of the renal arteries were studied are 249 renal arteries originating from the aorta and other sources distributed as 184 of single main RAs and 65 aRAs. Regarding the rare cases, only one (2.5 %) aRA was originated from the superior mesenteric artery was identified in the right side. In the left side, only one (1.5 %) aRA was identified to originate from the left common iliac artery. Table 1 summarizing the origin of renal arteries from the aorta and other sources.

Table 1: Distribution of renal arteries origin.

	Single RA (n.)	(%)	aRAs (n.)	(%)
Right side origin				
Aorta	184	100	38	97.4
Superior mesentericartery	0	0	1	2.5
Right common iliac artery	0	0	0	0
Total	184	100	39	100
Left side origin				
Aorta	184	100	62	95.3
Trunk of inferior mesenteric artery	0	0	2	3
Superior mesenteric artery	0	0	0	0
Left common iliac artery	0	0	1	1.5
Total	184	100	65	100

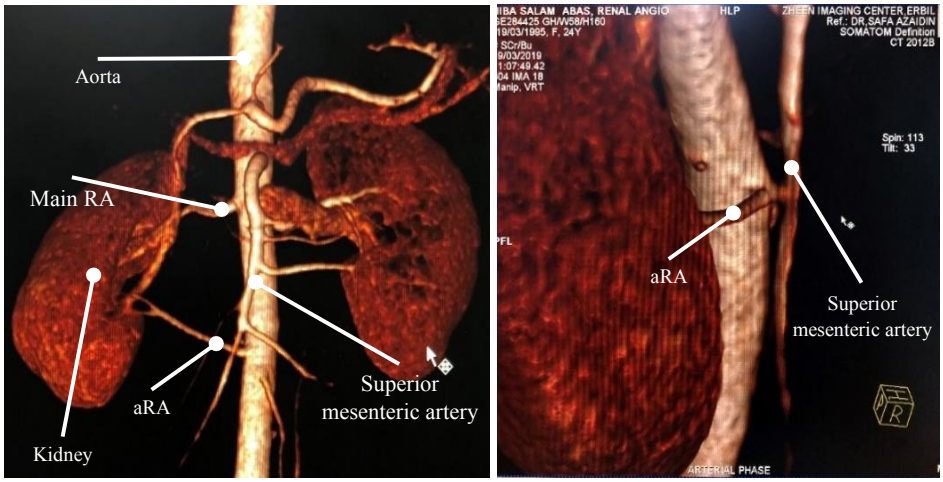

Figure 1: One of the rare cases demonstrating the aRA arising from superior mesenteric artery and merge with aorta and forming one artery supplying the lower pole of the right kidney.

The entrance of the main renal artery and aberrant renal arteries to the kidney:

The RAs that's entering the kidney either to the kidney hilum or lower and upper poles calculated based on the total number of RAs supplying kidneys. In the right side, the number of arteries supplying the kidney is 223 RAs. All the single main RAs 184 (100%) was found to enter the kidney through the hilum. In the left kidney, the number of arteries supplying kidney is 249 RAs. All the 184 single main RAs enter the kidney through the hilum. Regarding the aRAs, Table 2 summarizing the distribution of aRAs position to the kidneys:

Table 2: distribution of aberrant renal arteries Entrance to the kidneys.

Position to the right kidney	n.	(%)
Lower pole	23	58.9
Upper pole	6	15.3
Hilum	9	23
Upper pole and hilum	1	2.5
Total	39	100
Position to the left kidney		
Lower pole	36	55.3
Upper pole	19	29.2
Hilum	9	13.8
Upper pole and hilum	1	1.5
Total	65	100

No stable course observed in aRA as out of 59 cases with aRAs in the left side, a group of variations in the course of aRAs was seen in this study:

1) The left aRA originating from the aorta (just above main RA) and crossing the main RA to supply lower pole of the kidney instead of the upper pole that's demonstrated as nine cases (15.2 %) (Figure 2).

2) Regarding the right side, only one case (2.6 %) out of the 38 cases with aRA presented the crossing pattern; in which the aRA originating from the aorta and above the main RA then crossing it and enter the kidney hilum.

3) Two cases (3.3 %) demonstrating the aRA originating from the same trunk of the inferior mesenteric artery (Figure 3)

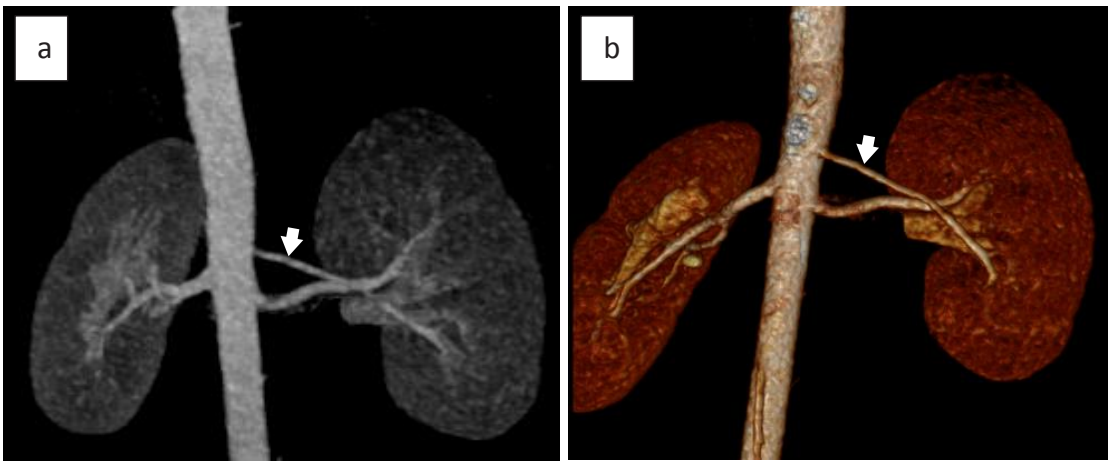

Figure 2: (a) and (b) showing the aRA (white arrow) arising just up to the main RAand crossing it to supply the lower pole of the left kidney.

Figure 3: The left aRA (white arrow) arising from aorta at the same trunk of inferior mesenteric artery (red arrow).

Regarding the eRA, in the right side, one tiny artery demonstrated in two (7.4 %) cases that were branching into two branches, first one reaching the pericardial area and the second one reaching the right diaphragm area supplying the right dome of the diaphragm. In the left side, a one (5.2 %) tiny eRA was reaching the left diaphragmatic curs.

Relationship between the aberrant renal artery and the diameter of the main renal artery.

Table 3 summarizing the diameter of main RA in case the presence of aRAs or not. However, on the right side, the diameter of the main RA in kidney presenting the aRA was significantly less than that of the kidney with single main RA (t-test, P= 0.006). Regarding the left side, the diameter of the main RA in kidney presenting the aRA was significantly less than that of the kidney with single main RA (t-test, P= 0.01).

Table 3: Summarizing the diameter of main RA in case presence of aRAs or not.

Symmetry	No.	Mean diameter of main RA (mm)	SD	P
Right side				
aRAs present	38	5.60	1.12571	0.006
aRAs not present	146	6.12	0.99664	
Total	184	11.73	2.12235	
Left side				
aRAs present	59	5.87	1.00817	0.01
aRAs not present	125	6.27	1.01004	
Total	184	12.1514	2.01821	

No.: number of cases, aRAs: aberrant renal arteries, (mm): millimeter, SD: standard deviation.

Discussion

Variations in the renal arteries are common, this explained by the development of the mesonephric arteries that's degenerates leaving only one mesonephric artery. Failure in degeneration of other mesonephric arteries results in an aberrant renal artery. This study is the first work in Iraq deals with aberrant renal arterial variation in details covering 184 kidneys donors' cases with medicolegal acceptance in Erbil city. This study showed the prevalence of aRAs of 26.3% compared with 20.2% in another angiographic study in Iraq [12] and within established ranges (20-30%) by most authors and textbooks [2&5]. The frequency of multiple renal arteries varies widely with ethnicity [13]. According to Gulas et al., the range of the frequency of multiple renal arteries depending on ethnicity is between 4% (Malaysians) and 61.5% (Indians) [14]. In the Polish population, the variability of multiple renal arteries seems to be between 11.2% and 38.3% [15]. This difference can be explained by the wide range of the prevalence of aRAs as showed by Satyapal et al (9-76%, average 28%) [13].

In this study, the entrance of aRAs to the kidneys was also studied. In the right side, 58.9% of the aRAs enters the lower pole of the kidneys, 23% of aRAs enters through the hilum, 15.3% enters the upper pole of the kidney. Regarding the left side, 55.3% of the aRAs directed toward the upper pole, and 29.2% passing to the upper pole, and 13.8% of the aRAsenters through the hilum. These findings matched with Alghizzi findings but the percentage lower than current study; Alghizzi reported the most of the aRAs was an inferior polar artery in 63.6% [12]. But present study findings disagreed with findings reported by Mustafa et al in which the results showed most of aRAs are pass through the hilum 53.62%, about 32.85% of the arteries enter the upper pole, and only 13.53% of the arteries enterthe lower pole of the kidney [20]. Rarely, in the right side, only 2.5% aRA gives branch to upper pole and hilum of the kidney. Regarding the left side, only 1.5% of aRA gives branch to upper pole and hilum of the kidney.

There were variations in the course of aRA as interestingly, the leftaRA originating from the aorta just above main RA and crossing it to supply lower pole of the kidney instead of the upper pole that's demonstrated as cases 15.2 % . Regarding the right side, only 2.6 % of the cases with aRA presented the crossing pattern; in which the aRA originating from the aorta and above the main RA then crossing it and enter the kidney hilum. About 3.3 % of the cases demonstrating the aRA originating from the same trunk of the inferior mesenteric artery (Figure 3).

Conclusion: This study concluded that:

1. The prevalence of aberrant renal arteries is 26.3% of Iraqi population.

2. Regarding the incidence side, it's more common in the left side (32.1%) than the right side (20.7%).

3. Presence of aberrant renal artery will affect the diameter of main renal artery; in which the diameter of the main RA in kidney presenting the aRA was significantly less than that of the kidney with single main RA.

4. No stable course observed in aRA in which the aRA originating from the aorta just above main RA and crossing the main RA to supply lower pole of the kidney instead of the upper pole.

5. Regarding the early branch renal artery, in the right side a one tiny eRA branching into two branches, one tiny artery reaching the pericardial area; and another artery that's reaching the right diaphragm area Source of Funding- Self

Ethical Clearance – Not required

Conflict of Interest: None.

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Evaluation of the Level of CXCL10 in Patients with Parainfluenza Virus Type III (HPIV3)

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Abstract

Acute respiratory illness could be caused by Human parainfluenza viruses (PIVs) in children, the old, and immunocompromised patients. The frequent and common cause of pneumonia and bronchiolitis is PIV3, whilst the upper respiratory tract illness and croup are frequently caused by PIV1 and PIV2. However, this study aimed to highlight a group of patients with type III influenza virus. 29 sample took from people suffering from upper and lower respiratory diseases as well as 27 conrol sample to assessing the level of a type of cellular motility in their catheter, cimclin cxcl10 by the use of ELISA. The study also showed that there were no significant differences between Male Injury Rate Now as well as an inverse relationship between the level of cimokin cxcl10 and age. Moreovere, this study showed an increase in the level of cimcin cxcl10 in the case of HPIV infection compared with the control group.

Keywords: *CXCL10; HPIV3; Respiratory infection*

Introduction

Human parainfluenza viruses (HPIVs) are among the most common cause of acute airway illness in children, the elderly and immunocompromised patients [(1)]the elderly, and immunocompromised patients. PIV3 is a common cause of bronchiolitis and pneumonia, whereas PIV1 and 2 are frequent causes of upper respiratory tract illness and croup. To assess how PIV1, 2, and 3 differ with regard to replication and induction of type I interferons, interleukin-6, and relevant chemokines, we infected primary human airway epithelium (HAE. HPIV consists of four major serotypes, PIV4 is a common respiratory pathogen which is alike to PIV3 in clinical demonstration [(2)], whilst the upper respiratory tract illness and croup are frequently caused by PIV1 and PIV2 [(1)]the elderly, and immunocompromised patients. PIV3 is a common cause of bronchiolitis and pneumonia, whereas PIV1 and 2 are frequent causes of upper respiratory tract illness and croup. To assess how PIV1, 2, and 3 differ with regard to replication and induction of type I interferons, interleukin-6, and relevant chemokines, we infected primary human airway epithelium (HAE. Due to optimal viral replication inside epithelial cells of the respiratory tract.HPIV3 is the common frequent in the worldwide amongst the four familiar serotypes that cause of hospitalization respiratory illness. Notwithstanding this high difficulty of illness,there is not any authorized vaccine or therapy to handle or even limiting this high difficulty of illness [(3)].

HPIV-3 is a virus which belongs to the Paramyxoviridae group, non-segmented, an enveloped as well as negative sense RNA virus [(4)]non-segmented, negative sense RNA virus that belongs to the Paramyxoviridae family. HPIV-3 is a common cause of bronchiolitis and pneumoniae in children less than 1 year of age and one of the leading causes of acute lower respiratory tract infections in children under five years of age. In Israel, the epidemiology of HPIV-3 infections is not well characterized. In this study, epidemiology and molecular characterization of HPIV-3 was performed on patient samples collected between January 2012 and September 2015. Nasopharyngeal swabs (N= 15,946. HPIV-3 infecting lower airway epithelial cells induces bronchiolitis and pneumonia in children less than one year of age and one of age and it is considered as the leading cause of acute lower respiratory tract infections in children under five years of age [(4)]non-segmented, negative sense RNA virus that belongs to the Paramyxoviridae family. HPIV-3 is a common cause

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of bronchiolitis and pneumoniae in children less than 1 year of age and one of the leading causes of acute lower respiratory tract infections in children under five years of age. In Israel, the epidemiology of HPIV-3 infections is not well characterized. In this study, epidemiology and molecular characterization of HPIV-3 was performed on patient samples collected between January 2012 and September 2015. Nasopharyngeal swabs (N= 15,946. [5] reported that HPIV3 may lead to asthma exacerbations in children and adults. Respiratory viruses contributing to the pathophysiology of lower and upper respiratory disorders by invading the epithelium activate innate immune response and induce inflammatory cytokines release. However, the effects of HPIV3 infection have not been well-defined on nasal epithelial cells (HNECs).

Chemokines are small, structurally related proteins, one of them is the chemokine interferon-γ (IFN-γ) inducible protein CXCL10 and also called IP-10 is a 10 kDa protein, which is functionally categorized as a T helper 1- (Th1) chemokine. Furthermore, CXCL□10 is a member of the CXC chemokine which is the ligand of CXCR3, and regulates immune responses through the activation and recruitment of leukocytes, such as, T cells, eosinophils, and monocytes to induce chemotaxis, apoptosis, cell growth and angiostasis (6).

Alterations in CXCL10 expression levels have been associated with inflammatory diseases such as immune dysfunction, infectious diseases, and tumor development. According to (7), the severity of various diseases can be predicted as well as recognized through CXCL10. Th1 orientated immune response is the special case of the host immune response that can be determined through CXCL10 level in blood. According to (8), CXCL10 is stimulated through Tumor necrosis factor-α and IFN-γ which is produced by Th1 lymphocytes in inflamed tissues recruited. The common abundant chemokines induced are The T cell chemoattractants CXCL11 and CXCL10. Differences in replication and cytokine secretion might explain some of the differences in PIV epidemiology and serotype-specific pathogenesis(1)the elderly, and immunocompromised patients. PIV3 is a common cause of bronchiolitis and pneumonia, whereas PIV1 and 2 are frequent causes of upper respiratory tract illness and croup. To assess how PIV1, 2, and 3 differ with regard to replication and induction of type I interferons, interleukin-6, and relevant chemokines, we infected primary human airway epithelium (HAE. In order to adequately recognize determinants of variability of immune responses to respiratory virus infections of airway epithelial cells in patients, this study focused mainly on understanding of the role of CXCL10 in HPIV3 infected patients.

Materials and Method

Blood samples

Blood samples were collected from people suffering from upper and lower respiratory diseases. The samples were collected from Al-Sadr Teaching Hospital and Al-Hakim General Hospital in Najaf Governorate. 5 mL of venous blood was withdrawn using a sterile syringe placed in a test tube containing no anticoagulant. The blood was then left at room temperature for half an hour; the serum was then removed from the rest of the blood components using a sterile microbial. It was distributed in five Pendrove tubes and 200 microliters per tube for the purpose of avoiding melting. , And was frozen (20-m) until the test (9).

Method

The antibodies against Paranfluenza (IgM) was determined in patients' and control urine, using ELISA technique , the test was performed according to the method reported and supplied with the VIRCELL test kit.

The number of tests used by Ray Biotech was used to measure the quantitative level of the public health laboratory in Najaf Governorate, as follows: The double-check factor and the human control factor (CXCL10) are studied in serum samples and by the associated immunosorbent system (Sandwich ELISA)

Statistical Analysis

The data were analyzed statistically using computerized SPSS v.24. Non- parametric tests has been used for variables that were n't followed the normal distribution such as Kruskal-Wallis Test (Multiple Comparisons) and Mann-Whitney Test, and Chi-square Test. ANOVA has been employed for variables that followed the normal distribution. Also, Microsoft Excel 2016 has been adopted to build histograms and figures of the correlation test. Data were expressed as (mean ± SE), statistically significant at p-value 0.05.

Result and Discussion

Many studies have focused on HPIV-3 infection in children because of high positive rate and morbidity of

HPIV infection in kids [10], therefore less is known about HPIV2 and HPIV-3 infection in adults.

In this study, 88 blood samples were collected from hospitalized HPIV-3 patient suffered from upper and lower respiratory airway after clinical diagnosis by the specialist doctor. The samples were collected from Al-Sadr Teaching Hospital and Al-Hakim General Hospital in Najaf Governorate, compared to 27 healthy people who did not suffer from respiratory or other chronic diseases, they were considered a control group.

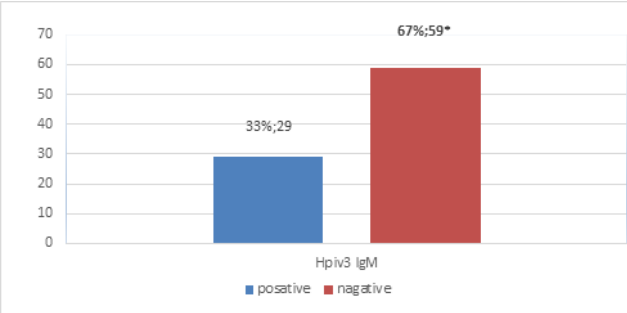

Figure (1) Spread of Parainfluenza virus type III HPIV3 in patient samples

The study demonstrated that the majority of HPIV3 patients had chronic infection negative IgM 59 (67%) while, 29 (33%) of HPIV3 patients had acute infection (positive IgM) as depicted in figure(1).This result somewhat compatible with previous studies have reported that, the majority of antibody levels of specific immunoglobulin G (IgG)-class antibodies in serum samples collected during the convalescent phase of the disease over the detection of specific IgM present during the acute phase of infection. [11]

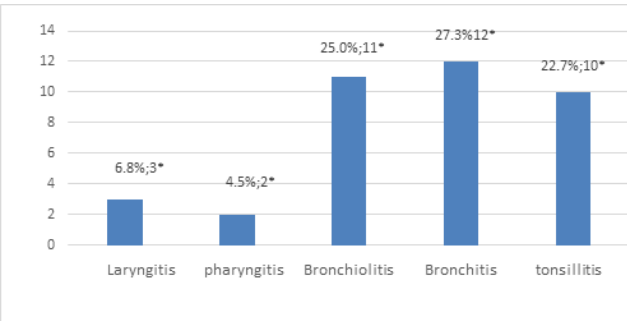

Figure (2): Percentage of clinical signs of patients infected with parainfluenza hpiv3

In figure (2) HPIV3 infections cause significant morbidity and burden of hospitalization. Clinical diagnosis revealed, 27.3% had bronchitis, bronchiolitis 25%, tonsillitis 22.7%, laryngitis 6.8%, and 2.4% had pharyngitis, these finding agree with the study noted that laryngeal trachea bronchitis (LTB) was most often caused by HPIV 1 and HPIV 2 with only a single case caused by PIV 3, cases of bronchiolitis occurred during infection were 26%, while 70% cases of pneumonia were caused by PIV 3 [12].

PIV in children accounted for 6.8% of all hospitalizations for fever and/or acute respiratory illnesses. HPIV3 is the most frequent cause of hospitalization, followed by HPIV1 and HPIV2 [13]. Some study reported that, the most common presenting symptom was cough 78% but other signs and symptoms of acute respiratory tract infection were common. 62% of patients had a documented fever upon presentation, and 40% of patients presented with gastrointestinal complaints of vomiting, diarrhea [12], and our findings agree with the previous clinical reports that, almost all patients had some respiratory symptoms, the majority had cough 63.6%, and a large proportion also had documented breathlessness was 59.1%, fever 54.5%, vomiting 29.5% and rhinorrhea was 25%, Both of clinical diagnosis and symptoms have significant differences at P <0.05 figure (3).

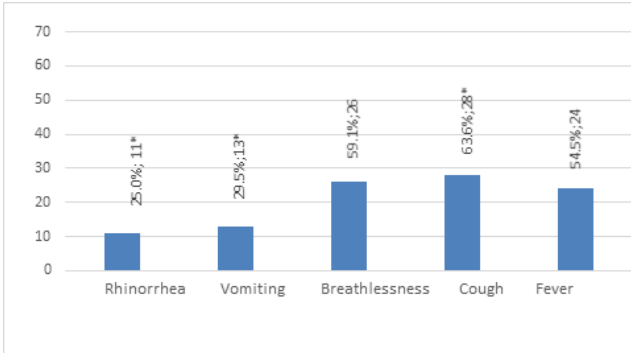

Figure (3): Percentage of clinical symptoms of patients with HPV3

HPIVs are common community acquired respiratory pathogens responsible for respiratory infections throughout the world without any gender, ethnic, age, or geographic boundaries [10]; however, morbidity and mortality rates are higher in patients living in poor countries such as rural areas without access to supportive care as compared to developed countries [14].

This study shown that HPIVs were more commonly isolated from females patients than male in patient with acute infection (positive IgM), they were 10 males and 19 females, their ages ranged from 20 to more than 50 years with (Mean ± SD) (39.97±2.83), a control group with mean age 40.85± 2.79, the Patients from cities were 14 and rural areas were 15 Patients. Therefore, no significant differences were found in the result of age, sex and

housing between patient and control group. While other studies founded that, the median age (77.5 years; range, 19.0 - 84.0 years) of the 24 HPIV-3-infected patients was higher than the median age (63.0 years; range, 18.0-99.0 years) of 961 patients who tested HPIV-3-negative and also agree with us no significant differences found (P 5. 04) [15]we tested acute- and convalescent-phase serum specimens from hospitalized adults participating in a population-based prospective study of lower respiratory tract infection during 1991–1992. We tested all available specimens from the epidemic seasons for each virus and ~300 randomly selected specimens from the corresponding off-seasons for antibodies to HPIV-1, HPIV-2, or HPIV-3. During the respective epidemic season, HPIV-1 infection was detected in 18 (2.5\\\\%, which was older than our findings it can be because the result of the past decades.

PIV infectio n causes a spectrum of diseases associated with the expression and release of proinflammatory mediators, PIV-infected patients had higher nasal wash concentrations of chemokines such as CXCL8, CXCL9 and CXCL10 as compared to uninfected control patients [12]. A specific diagnosis of a lower and upper respiratory viral infection is often difficult despite frequent clinical suspicion, may be improved the diagnostic tools by use of sensitive detection methods and biomarkers. The previous study demonstrated, that the T cell chemo attractants CXCL10 one of the most abundant chemokines induced in HPIV3 [1]the elderly, and immunocompromised patients. PIV3 is a common cause of bronchiolitis and pneumonia, whereas PIV1 and 2 are frequent causes of upper respiratory tract illness and croup. To assess how PIV1, 2, and 3 differ with regard to replication and induction of type I interferons, interleukin-6, and relevant chemokines, we infected primary human airway epithelium (HAE. Human airway epithelial cells may generate CXCL10 in response to virus infection with high concentrations. In particular, have been correlated with more severe HPIV disease [16].

This study findings support previous research, where this study found a significant difference in CXCL10 concentrations in patients when compared to control cases (P< 0.001), whereas CXCL10 concentrations levels were higher in patients than control group, the mean was 2023.98±273.13 and 776.62±86.79, respectively. Moreover other study suggested that, respiratory viruses can be found in patients with serious acute airway illness CXCL10 may be a useful biomarker for viral ARI (as opposed to non-viral) by use of PCR assays more frequently than previously appreciated [17] clinical predictors and inflammatory mediator profile of respiratory viral infection in serious acute respiratory illness were investigated. Sequential bronchoalveolar lavage (BAL.

A study conducted by [18] on 164 healthy subjects with age ranges from 10 to 79 year-old. They reported that serum levels of CXCL10 and sCCL2 were significantly increased in older individuals (r = 0.32, P < 0.001). This finding is in contrary with our finding, where, a significant negative correlation between was found between the serum level of CXCL10 and older HPIV3 patients (r=-0.428, P=0.020). This might be due to the small number of patients examined in the current study as compared to that of their study.

To compare the result associated with this study, we classified HPIV3 patient with acute infection (positive IgM group) into three different age groups. Group I consisted of 13 patients with age range from 20-34, group II consisted of 6 patients with age range 35-49 and group III 10 patients with age more than 50 year and were each group appears normally distributed using descriptive statistics.

Table (1): The effect of age in the concentration of CXCL10 among the group of Hpiv patients (Hpiv3 IgM) compared to control group

Age group	Control (SD ± mean)	CXCL10 (SD ± mean)	Number of patients
20-34	966.62 ± 145.07	2304.42 ± 435.4	13
35-49	966.62 ± 145.07	2148.63 ± 820.91	6
≥ 50	696.52 ± 110.22	1584.62 ± 293.29	10

In Table (1), figure (4) A significant relationship between CXCL10 among different age group of HPIV3 patients compared to control group was founded in this study with $P < 0.05$, the concentration of CXCL10 for the age group I was 2304.42±435 compared to control group (145.07 ±966.62) pg / ml, whereas the concentration of CXCL10 for the age group II was 2148.63±820.91 compared to the control group (145.07±966.62) pg / ml. In the group III CXCL10 concentration 1584.62±293.29was compared with control group (110.22±696.52) pg / m. This is agreeing with the study which mentioned that, decrease in pro-inflammatory cytokine expression by aging in bronchoalveolar lavage patients [19]the natural variation of cytokine expression in healthy horses has yet to be described. The objectives of this study were to: (1. This finding is in contrary with [20] study, increase of pro-inflammatory cytokines production by age-associated may be a co-factor for the pathogenesis of airway diseases, where pro-inflammatory cytokines in humans such as CXCL10 are increase with age, the phenomenon known as 'inflamm-aging'.

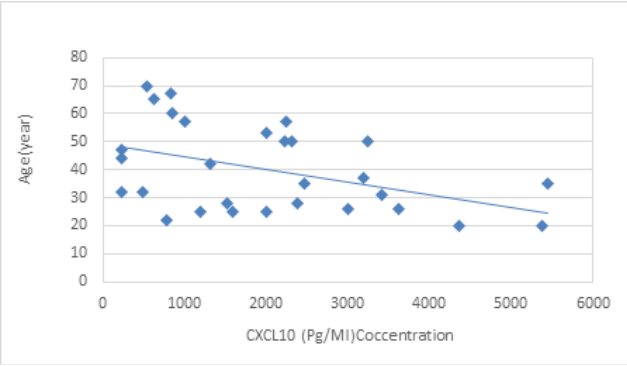

Figure (4):An inverse (negative) relationship between CXCL10 concentration and age in patients with HPV3 (r = -0.428 *, p = 0.020

Table (2) the effect of sex in the concentration of CXCL10 among the group of HPIV patients (Hpiv3 IgM) compared to control group

Group of study	Gender	SD ± mean	Total
Patient	Male	*385.72± 2001.66	29
	Female	*327.73 ± 2066.38	
Control	Male	134.50 ± 771.04	27
	Female	116.38 ± 781.81	

On the other hand, according to gender in table (2), the observed association of HPIV3 with over expression of CXCL10 were more pronounced in female than males in both patient and control group, the concentration of CXCL10 for males patients was (2001.66±385.72 *) pg / ml compared with the control group (771.04±134.50) pg / ml, while the CXCL10 concentration for females patients was (2066.38±327.73*) pg / ml compared to the control group (781.81±116.38) pg / ml. This finding is in agreement with the result of (18) reported that CXCL10 was slightly higher in healthy females than that of males. This may be due to estrogen enhanced CXCL10 expression (21). But this study finding is contrary what mentioned before, over expression of CXCL10 were more pronounced in males than in female patients with Cerebral malaria (22)CXCL10, is a strong predictor of both human and experimental cerebral malaria. Increased plasma and cerebrospinal fluid levels of CXCL10 were tightly associated with fatal CM in Indian and Ghanaian patients. In the present study, we hypothesized that in a subset of malaria patients, CM susceptibility is associated with variation in CXCL10 expression. We determined whether polymorphisms in the CXCL10 gene promoter region played a role in the clinical status of malaria patients and addressed the genetic basis of CXCL10 expression during malaria infection. Following extensive bioinformatics analyses, two reported single nucleotide polymorphisms in the CXCL10 promoter (−135G>A [rs56061981] and −1447A>G [rs4508917]. Further study involving larger sample size is recommended for evaluating usefulness of these markers in HPIV3 patients.

Ethical Clearance- Obtained from University of Kufa from Faculty of Education for women, Department of biology.

Source of Funding- Self

Conflict of Interest - Nil

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Association between the Characteristics of Pre-Surgery Vascular Ultrasonography and the Successful Arterio-Venous Fistula Shunt Surgery as Hemodialysis Access to Patients with Kidney Disease Stage V

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Abstract

Background: The failure rate of arterio-venous fistula shunt for hemodialysis access in patients with chronic kidney disease is high. Vascular characteristic is one of the important indicators in successful operation.

Objective: To determine the association between pre-surgery vascular ultrasonography characteristics and the successful arterio-venous fistula shunt surgery as hemodialysis access in patients with chronic kidney disease stage V.

Methods: This study used descriptive analytic studies to determine the relationship between pre-operative vascular ultrasonography characteristics, such as arteries (diameter, velocity, thickness of intima-media tunica) and vein (diameter, velocity, depth) with successful arterio-venous fistula shunt surgery that evaluated in four week post-surgery in patients with chronic kidney disease stage V.

Results: Eighteen samples that consisted of 27.8% female and 72.2% male were enrolled in this study. The most surgery location was vascular radio-cephalic of 13 samples (72.2%). Statistical analysis used was Mann-Whitney U Test. There was no significant correlation between arterial diameter (p=0.127), venous diameter of AVFs (p=1.000), arterial velocity (p=0.721), velocity vein (p=0.721), intima media tunica thickness (p=0.721), and venous depth (p=0.192) with successful arterio-venous fistula shunt surgery.

Conclusion: There was no significant correlation between diameter, thickness of intima media tunica, and arterial velocity as well as diameter, velocity and venous depth with successful arterio-venous fistula shunt surgery.

Keywords: *Vascular, Ultrasonography Examination, Arterio-venous Fistula*

Introduction

In patients with catheterization or patients requiring venous access, doppler ultrasonography of upper limb venous was required for identifying suitable vascular to access. This vascular was screened for the possibility of central stenosis or occlusion. It was recommended to use pre-surgery ultrasonography in patients who had experienced previous central venous access associated with deep venous thrombosis in order to assess central or occlusion stenosis[1]. SG of vascular mapping prior to placement of hemodialysis access was a prescribed procedure. Pre-surgery sonographic mapping prior to placement of hemodialysis access may change surgical management, with an increasing number of AVF and the

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possibility of an increase in the most functional vascular selection [2].

Chronic Kidney Disease (CKD) is the final stage of renal failure with the Glomerular Filtration Rate (GFR) of <15 ml/minute [3]. Causes of CKD are diabetes mellitus, hypertension, renal ischemia, toxic substances, obstruction, autoimmune disease and renal infiltration[4]. Data from The United States Renal Data System (USRDS 2013) showed the prevalence rate of people with chronic kidney disease in the United States (2011) of 1,901 every a million population. Treatment of End-Stage Organ Failure in Canada (2000-2009) reported that nearly 38,000 Canadians lived with chronic renal failure and has increased almost three times from 1990. 59% of those number (22,300) have undergone hemodialysis and 3000 patients have been waiting kidney transplant[5]. Meanwhile, Indonesia reached 200-250 cases every a million populations every year.

Hemodialysis requires a well-functioning vascular access. The best type of vascular access is the Arterio-Vena Fistula (AVF). Making AVF is associated with failure rates and complications. The most common causes of AVF dysfunction are non-maturation, thrombosis, and stenosis, thus most cases of previous stenosis lead to disruption of blood flow. Function and morphological of USG was used for AVF dialysis that affects the risk of vascular access dysfunction [6].

Vascular access is ideal if it can be used for long periods of time as access to blood circulation with minimal complications. Arterio-venous fistula (AVF shunt) is the most ideal access of all available hemodialysis access. If AVF shunt is successful then it can be used for long periods with low thrombosis and infection rates, without much interventions and low costs[7]. AVF-shunt cubiti operation is an AVF operation technique. AVF shunt operation is the first choice in AVF technique whereas; AVF cubiti is the second choice. However, the failure rate of both techniques remains high[8]. There remains a high failure rate of fistula arterio-venous shunt installation in patients with chronic kidney disease. Complications of vascular access are the common cause of failure and hospitalization in dialysis patients. Limited data to comprehend the complications of arterio-venous fistula especially stenosis and thrombosis also inhibit the success of arterio-venous fistula techniques.

Based on the research described above, it is necessary to examine the association between the characteristics of arterial ultrasonography (USG) and pre-surgery vein with the successful AVF shunt surgery, thus the characteristic factor of arteries and veins that affect the success of shunt AVF operation could be recognized. Therefore, the authors aimed to determine the association between pre-surgery vascular ultrasonography characteristics and the successful arterio-venous fistula shunt surgery as hemodialysis access in patients with chronic kidney disease stage V.

Method

This study was a retro-prospective longitudinal with the observational design. We used a consecutive sampling technique to the patients of chronic kidney disease stage V. The inclusion criteria were patients aged >18 years, undergoing routine hemodialysis or will be planned of routine hemodialysis, had been performed operation of AVF shunt in Dr. Soetomo General Hospital Surabaya from January to March 2015.

Patients with chronic kidney disease stage V, which will be operated with AVF shunt as a hemodialysis vascular access, were performed USG examination using high-resolution USG Hitachi equipped with linear probes (7-10 MHz) in Radio Diagnostic Unit of Dr. Soetomo General Hospital Surabaya. The scan was performed in recumbent position with the arm down of 45 degrees from the body.

The USG results were recorded and then analyzed statistically. Descriptive analysis was conducted to obtain the characteristic of the sample. Normality test using Kolmogorov-Smirnov test was performed prior to analyze the correlation between pre-surgery vascular ultrasonography characteristics and the successful arterio-venous fistula shunt surgery as hemodialysis access. Mann-Whitney U Test was used with confidence interval of 95% and p value <0.05. The analysis was conducted with SPSS software. The study protocol was approved by Dr. Soetomo Teaching Hospital.

Results

Characteristics of Subjects

This study obtained 16 CKD patients who will be performed AVFs surgery. Patients consisted of 12 male and 4 female with age ranged from 33 to 62 years. All patients had a vascular duplex USG examination at the location that will be performed, however two patients performed two surgeries at different sites due to the

failure on the first surgery thus we obtained 18 samples. The youngest age of the patient was 33 years old and the oldest was 62 years.

Distribution of Subjects by Age

The most age group in eighteen samples of study that will be performed AVFs surgery was 51-60 years of 9 patients (50%). The age group of <40 years was 2 patients (11.1%), 41-50 years old of 6 patients (33.3%), and >60 years of 5 patients (5.6%) (Table 1).

Table 1. Distribution of Samples by Age

Age	Frequency	Percentage (%)
< 40 years	2	11.1
41-50 years	6	33.3
51-60 years	9	50.0
> 60 years	1	5.6
Total	18	100.0

Distribution of Subjects by Sex

We found 13 male (72.2%) and 5 female (27.8%). CGK patients who had pre-surgery vascular USG of AVFs were more prevalent in males than females with a ratio of 3:1

Distribution of Subjects by AVFs Location

Subjects who had performed AVFs surgery were 5 patients (27.8%) in the inferior fossa cubiti brachio-cephalic blood vessels and 13 (72.2%) subjects who had performed surgery on arterial radio-cephalic arteries.

Characteristics of Vascular Samples

Table 2. Characteristics of Vascular Samples

		Mean	Median	SD	Minimum	Maximum
Diameter (mm)	a.Brachialis	3.2	3.2	0.2	2.9	3.5
	a.Radialis	2.1	2.2	0.5	1.2	2.8
	v.Cephalica	1.6	1.5	0.8	0.6	3.5
Tunica intima Media (mm)	a.Brachialis	0.4	0.4	0.07	0.3	0.5
	a.Radialis	0.4	0.3	0.1	0.2	0.6
Velocity (cm/s)	a.Brachialis	51.8	50.5	16.3	28.4	74.0
	a.Radialis	52.1	47.3	15.4	27.8	73.8
	v.Cephalica	7.1	7.2	2.7	3.2	12.3
Depth (mm)	v.Cephalica	2.8	2.6	0.9	1.8	4.4

Table 2 showed the vascular characteristics of the sample consisted of the brachial artery, radial artery, and cephalica vein as the vascular access of the AVFs surgery. We found five samples of brachial artery, 13 samples of radial artery, and 18 samples of venous cephalica.

Distribution of Subjects Based on Successful AVFs Surgery

Eight-teen samples have been performed AVFs operation which resulted in 14 (77.8%) successful operations and 4 (22.2%) failed operations.

Results Analysis

The Correlation of Artery Diameter with AVFs Operating Results

Successful AVFs surgery was 14 (77.2%) samples with a minimum diameter of 1.5 mm, a maximum of 3.5 mm, a median of 2.4 mm and a mean diameter of 2.5 mm and a standard deviation of 0.6. Meanwhile, failure surgery was 4 (22.8%) samples with a minimum diameter of 1.2 mm, maximum of 2.6 mm, a median of 1.8 mm and mean diameter of 1.8 mm and standard deviation of 0.7. The statistical test showed no association between arterial diameter with AVFs surgery results (p=0.127).

The Correlation of Vena Diameter with AVFs Operating Results

From 18 samples of the study, 14 (77.2%) of cephalica vein samples from successful AVFs surgery had a minimum diameter of 0.6 mm, a maximum of 3.5 mm, a median of 1.6 mm and a mean diameter of 1.6 mm and a standard deviation of 0.7. Whereas, 4 (22.8%) of cephalica vein samples from failed surgery had a minimum diameter of 1 mm, a maximum of 3.4 mm, a median of 1.4 mm, a mean of 1.8 mm diameter and a standard deviation of 1.1. The statistical test showed no association between vein diameter and result of AVFs surgery (p=1.000).

The Correlation of Artery Velocity with AVFs Operating Results

From 14 samples of brachial artery and radial artery from successful AVF operation had a minimum velocity of 27.8 cm/s, a maximum of 74 cm/s, a median of 50 cm/s and a mean of 50.9. Meanwhile, 4 samples from failed operation had a minimum velocity of 40 cm/s, maximum of 71.3 cm/s, median of 56.7 mm, mean of 56.2 cm/s and standard deviation of 15.5. Statistical tests showed that there was no association between arterial velocity and successful AVFs surgery (p=0.721).

The Correlation of Vena Cephalica Velocity with AVFs Operating Results

From 14 venous cephalica samples had a minimum velocity of 3.6 cm/s, a maximum of 12.3 cm/s, median of 7.2 cm/s and mean of 7.3 cm/s and standard deviation of 2.6. While the 4 sample venous cephalica from the failed operation had a minimum velocity of 3.2 cm/s, maximum of 10 cm/s, median of 6.1 cm/s, mean of 6.4 cm/s and standard deviation of 3.3. The statistical test showed no association between velocity of venous and successful operation of AVFs (p=0.721)

The Correlation of Thickness of Tunica Intima Media Artery with AVFs Operating Results

The intima media tunica thickness of 14 (77.2%) successful surgeries had a minimum diameter of 0.2 mm, a maximum of 0.6 mm, a median of 0.4 mm, a mean of 0.4 mm and a standard deviation of 0.15. Whereas, 4 (22.8%) samples from failed operation had a minimum intima media tunica thickness of 0.3 mm, maximum of 0.4 mm, median of 0.4 mm, mean of 0.4 mm and standard deviation of 0.06. The statistical test showed no correlation between intima media tunica thickness with successful AVFs surgery (p=0.721).

The Correlation of Depth of Vein with AVFs Operating Results

We found 14 (77.2%) samples from successful AVFs operations with a minimum depth of 1.8 mm, a maximum of 4.1 mm, a median of 2.5 mm and a mean of 2.6 mm. While 4 (22.8%) failed samples had a minimum depth of 2.2 mm, maximum 4.4 mm, median 3.4 mm and mean of 3.4 mm. The statistical test showed no association between cephalica vein depth with successful AVFs surgery (p=0.192).

Discussion

Doppler USG of vascular provided qualitative and quantitative data on arterial and venous systems prior to the establishment of AVFs. Doppler USG techniques could identify which veins are often missed on clinical examination. This approach was particularly useful in patients with DM and the elderly due to arterial narrowing and a relatively common classification in patients with CKD. Therefore, arterial evaluation should be performed to determine patency, morphology of capillary walls, diameter and anatomy, while venous evaluation was performed to determine patency, diameter, and depth[9].

The study obtained 18 samples consisted of 5 females (27.8%) and 13 males (72.2%). The most common age

group was 51-60 years by 8 people (50%) followed by age group of 41-50 years by 5 people (31.25%). This was similar the epidemiological data of CKD patients who underwent hemodialysis that it happened more in male than female and often occurred at the age of 40-60 years old [10].

The diameter of the artery in this study was smaller than the mean diameter of the brachial artery in other studies by 4.31±0.77 mm and the mean of radial artery by 2.325±0.4 mm[11, 12]. The velocity of the brachial and radial arteries in this study was lower than the normal value of Brachial artery by 50-100 cm/s and Radial artery by 40-90 cm/s [13].

There was no significant correlation between arterial diameter and AVFs operation results (p=0.127); the mean diameter of successful surgery was 2.5 mm and the failed surgery had a mean of 1.8 mm. The successful AVFs surgery were linked to a minimum diameter of artery by 2 mm [9].

The statistical test showed no correlation between the thickness of tunica intima-media with the successful operation of AVFs (p=0.721). Moreover, there was no association between arterial velocity and AVFs surgery results (p=0.721); a mean velocity of successful surgery was 50.9 cm/s and a mean velocity of failed surgery was 56.2 cm/s.

Statistics tests showed no association between vein diameter and AVFs operation results (p=1,000). The mean of venous diameter on successful AVFs was 1.6 mm and failed AVFs had a mean diameter of 1.8 mm. The results were different to a study by Silva who reported successful surgery of AVFs had a minimum diameter of 2.5 mm and she recommended a diameter of at least 3 mm for AVFs surgery[9].

The larger diameter of the cephalic vein was associated with lower resistance of blood flow and increased blood flow rate in the fistulas. The larger diameter of the cephalic vein was also associated with low risk of thrombotic, post-inflammatory lesions, and morphological vein wall abnormalities. This results confirmed the importance of pre-surgery vascular assessment prior to the establishment of AVFs[6]. Previous studies recommended to use an artery diameter of at least 2.5 mm and a vein diameter of at least 2 mm to establish the AVFs. However, there was no agreement on the threshold value of minimal diameter of the artery or vein that should not be used to form anastomosis. Therefore, there was also a medical center which required an AVFs procedure with a diameter of less than 1.5 mm[6].

This study also showed that there was no association between venocity of venous and successful AVFs surgery (p=0.721); the mean velocity of the successful operation was 7.3 m/s and the mean velocity of failed operation was 6.4 cm/s. Moreover, there was no association between cephalica vein depth with successful AVFs surgery (p=0.192). The mean venous depth of successful operation was 2.6 mm and the failed operation had a mean vein depth of 3.3 mm. This study showed that successful AVFs surgery had higher velocity rates and a shallower cephalic vein depth.

Conclusion

There was no significant correlation between diameter, thickness of intima media tunica, and arterial velocity as well as diameter, velocity and venous depth with successful arterio-venous fistula shunt surgery.

Ethical Clearance: The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

Conflict of Interest: There is no report of conflict of interest involved with this study so far.

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Prevalence of Childhood Disability in Basrah City Using the Ten Questions Too

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Abstract

Background: The neglect of disabled children in the developing countries and less information about its types and prevalence in addition to the low level of health services they had.

Aim: To identify the prevalence of handicapping disabilities among children up to 9 years of age and their epidemiological pattern in the Basrah city center.

Methods: In a population-base, cross-sectional household survey was carried out inBasrah city. A total sample (1734)children, males were 56% and females were 44 % of the sample using the ten questions survey tool.

Results: The survey team detected 73 disability cases 48 were males and 25 were females. The detected prevalence of disability in our sample was 37.7%.Disabled children, on average, were in the fourth or fifth birth order among their brothers and sisters and 47 % of them were counted as the second or third disability inthe same family. The number of disabling conditions in the same child varied widely: 31.5% of cases had a single disability, 24.6% had two conditions and 43.8% had three or more conditions. The questionnaire children up to 9 years of age in thesefamilies formed 56.2% of the population.

Conclusion: The survey managed successfully to document a comparable estimate of the prevalence rate for childhood disabilities in the community and provided a general picture of their types and suspected causes. It also reported a low service delivery for these disabilities and identified some risk factors related to their occurrence.To apply this survey method in center Basra city is justified in an extended field study with certain modifications to suit the local culture. This study also should be complemented with a second stage referral for follow up examination of detected cases of and evaluation of the disability and its required services.

Keywards*:Prevalence, Childhood disability, Basra, Ten questions tool*

Introduction

In countries with reasonably well-developed services for children with Disabilities, administrative data and registries provide a useful source of population-based Information on childhood disabilities[1]. In developing countries, where services for children with disabilities are not universally available, administrative data, if available at all, provide an incomplete account. Census Data provide an alternative source of population data on disability, but it is likely that[2]these data under-identify disabilities in children and especially in girls and children of low socioeconomic status[11]. A third approach is the so-called 'key informant 'approach, which relies on interviews with teachers, health care providers and other key persons in a community to identify children in the population with disabilities. This approach, however, has been shown to miss disabilities that are least publicly

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evident, such as cognitive and hearing disabilities[12]. A fourth approach consists of household surveys[3].

However, information on the validity of single-phase survey data on disabilities in children is lacking, especially in developing countries. In addition, instruments that have been used to survey childhood disabilities in developed countries are unlikely to be cross-culturally valid in developing countries. The above considerations led to the development of a two-phase methodology for surveying childhood disabilities in populations where professional resources are extremely limited. This paper describes the methodology, presents data on its reliability and validity across culture, discusses its uses and limitations, and identifies areas for future research[4]. The need for information on the frequency of childhood disabilities inpopulations, as well as the status and characteristics of children with disabilities, has been emphasized repeatedly[1-7]. For example, information on the number and status of children with mental retardation, learning, vision, hearing and seizure disabilities[5].Behavioral disorders is needed to monitor on a population level the impacts of: improvements in survival; exposures to nutritional deficiencies, environmental toxins, serious diseases and trauma; and interventions designed to improved child health and development. In addition, population-based, epidemiologic studies of childhood[6].Disabilities are needed for identifying risk factors and causes, and for needs assessments to facilitate planning of services for children and families with special needs[7].

In countries with reasonably well-developed services for children with disabilities, administrative data and registries provide a useful source of population-based information on childhood disabilities[8-10]. However, in most low income or developing countries, where services for children with disabilities are not universally[8].Available, administrative data, if available at all, provide an incomplete account. Census data provide an alternative source of population data on disability, but it is likely that these data under-identify disabilities in children and especially in girls and children of low socioeconomic status[11]a third approach is the so-called 'key informant'[9]. Approach, which relies on interviews with teachers, health care providers and other key persons in a community to identify children in the withdisabilities. This approach, however, has been shown to miss disabilities that are least publicly evident[10].

Such as cognitive and hearing disabilities and to include children from outside the population of interest[11].A fourth approach consists of household surveys. Howeverinformation on the validity of single-phase survey data on disabilities in children islacking, especially in developing countries. In addition, instruments that have been used to survey childhood disabilities in developed countries are unlikely to be cross-culturallyvalid in developing countries[12]. The above considerations led to the development of a two-phase methodology forsurveying childhood disabilities in populations where professional resources are future researchextremely limited. This paper describes the methodology, presents data on its reliability and validity across culture, discusses its uses and limitations, and identifies areas forHandicapping disabilities in both developing and developedcountries are important public health issues[13].

Basic data on their frequencies, underlying risk factorsand associations are necessaryin order to plan healthprograms and to provide social services for them. Screening in community surveys is a good strategy for providing a quick insight for this problem among children[14]. Many community surveys have been conducted for handicapped children in various parts of the world." the ten-questions survey tool was proposed for this purpose and has been validated for its sensitivity and specie city in many developing countries. This paper is reporting a led survey in Jeddah, Saudi Arabia[15]. In which a population-based study has applied the ten-questions tool to estimate the prevalence of handicapping disabilities among children up to 9 years of age and describe the epidemiological pattern of disabilities in the eastern part of the city. The study was part of the training led survey for male medical students in the Medical College. All children have a right to care by a parent or trusted adult[16].In countries with reasonably well-developed services for children with disabilities, administrative data and registries provide a useful source of population-based information on childhood disabilities.

Material and Method

Study setting and population

A Population based cross-sectional study carried out in Basra cityfrom October 2016 to February 2017. The estimated population of Basra city center is 38,989 and about 28.7% of that population being female in

child bearing age.Interviews at the selected houses wereconducted primarily by gathering the basic socio-demographic information of the family from the head of the family,usually the father. Variables included in this part were family income, number of family members, age of thechild, ages of parents, occupation of parents, educational level of parents, history of disabilities in either parents families and consanguinity between parents. The tenquestions tool with their probes was explained to thehead of the household to detect any disabled member ofthe family in the age 9 or below. Houses with nochildren at the required age were skipped and replacedby the next one fulfilling the research criteria. Thedetailed questionnaire was filled for any child with apositive answer on one or more of the disability questions.

The study sample and data collection

Total 1734 sampleswere collected to study the prevalence rate of childhood disabilities in Basra center from November 2016 to March 2017.The percentage of under nine years children from the total population is about 11.8% (WHO).

Statistical analysis

The data were fed to the (SPSS) program (version 16) for interpretation of result, through the application of the descriptive date analysis frequency and percentage.

Results

In the present study, we have screened 4601population and about 1734 samples were collected for further studies. So the prevalence can be calculated as follows

Prevalence = 1734/4601* 100 = 37.7 %

In the collected samples (1734), about 984 (56%) were males and 750 (43.2%) were females.It shows the high percentage male (56%) and the lower percentage female (43.2%) from the total sample.

Table 1. Characteristics of number of disable children in the family

Number of children	No.	%
1	5	6.8
2	30	41
3	37	52
Total	73	4.2

The high percentage (52%) of the studied families had three disabled child while the lowest percentage (6.8%) of families had only one disabled child's.

Table 2. Characteristics of order of birth of disable child among their sibling in the family

Order	No.	%
1	2	2.7
2	3	4.1
3	3	4.1
4	40	54.7
5	25	34.2
Total	73	4.2

This table show that the high percentage (54.7%) of disabled children had the fourth order of birth among their siblings in the family while the lower percentage (2.7%) have had the first order.

Table 3: Age frequencies of all children and the disabled group

	Disabled group		All children	
Age	No	%	No	%
1year>	1	1.3	85	4.9
1 – 2	8	10.9	121	6.9
2- 6	23	31.6	619	35.6
6-	41	56.2	909	52.4
Total	73	42	1734	43.3

This table show disabled group in age (6 -9) has 56.2% the high percentage this age group high percentage in total sample.

Table 4. Frequency of various types of disabling conditions in Basra city

Condition	Previously diagnosed	Newly identified
Speech	23	3
Motor	12	2
Mental	9	3
Fits	7	1
Learning difficulty	6	2
Hearing	7	1
Vision	4	1
Emotional problem	3	1
Chronic or hereditary	2	1

Conditions have not been totaled due to multiple disabilities in some cases.

Table 5. Causes of disabilities as perceived by parents

Suspected cause of disability	No	%
Hereditary causes	37	50.6
Non hereditary illnesses	10	13.6
Accidental/traumatic causes	4	5.4
Other causes	2	2.7
Unknown cause	20	27.3

This table show high percentage 50.6 the parents believes hereditary causes and the lower percentage 2.7 has other causes.

Discussion

Disabled children in developing countries are estimated to form 85% of the world's disabled children). Accurate determination of the prevalence is hindered by a group of inherent problems in these countries including under reporting, late identification, poor registration and lack of infrastructure for their monitoring and service provision.To overcome these shortcomings, a variety of methods were tried in order to identify disabilities at the community level.These include the addition of questions to the national census and the interviewing of key informants in the community such as community leaders and teachers. The previous methods were found to produce serious faults of under-enumeration of handicapping conditions and under representation of children and women. "Researchers in developing countries have proposed the use of the ten question tool in a house survey to overcome these drawbacks. The questionnaire Children up to 9years of age in these families formed56.2% of the population (1734)with male forming 56% of them.Household. Using the ten questions survey tool, the survey team detected 73`disability cases in the age group males.. Disabled children, on average, were in the fourth or fifth birth order among their brothers and sisters and 47 % of them were counted as the second or third disability inthe same family. The number of disabling conditions in the same child varied widely: 31.5% of cases had a single disability, 24.6% had two conditions and 43.8% had three or more conditions. These conditions were categorized according to the answers on the ten questions (table 6). Disabled children. The survey tool managed to document, successfully, a prevalence rate of childhood disabilities in the community with a general picture of their types and suspected causes which is quite compare to that found in Saudi Arabia, a multistage sampling method was applied to screen children in 875 houses using the ten questions survey tool for identification of disabilities. Further information collected for detected disabilities possible risk factors, cause of the disability as perceived by the family and services previously provided to the child. Results: A total of 137 cases of disability were detected giving a point prevalence rate of 36.7 per 1000 children. Twenty-nine children (21.2%) were discovered for the rest time during the survey. The majority of cases were male (57.7%) and the mean age for all cases were 10 years (SD^{-} 5.5) with no case detected under one year of age. Disabled children were in the fourth or fifth birth order among their brothers and sisters and 47 (34%)of them were recorded as a second or third disability in the same family. Number of disabilities in the same child varied widely: 59% of cases had a single disability, 22% had two conditions and 19% had three or more conditions.[17] [18][19]

Conclusions

The survey managed successfully to document a comparable estimate of the prevalence rate for childhood disabilities in the community and provided a general picture of their types and suspected causes. It also reported a low service delivery for these disabilities and

identified some risk factors related to their occurrence. To apply this survey method in Basra city is justified in an extended field study with certain modifications to suit the local culture. This study also should be complemented with a second stage referral for follow up examination of detected cases of and evaluation of the disability and its required services.

Conflict of Interest - Nil

Source of Funding- Self

Ethical Clearance- Not required

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Expression of e-NOS in Human Placentas of Idiopathic Intra Uterine Growth Restriction at Term

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Abstract

Endothelial nitric oxide synthase (e-NOS) is a chemical agent that involves in placental angiogenesis. It is conveyed through embryogenesis. In the contemporary study, we intended to examine the character of placental angiogenesis in the progress of intrauterine growth restriction (IUGR) by means of matching the levels of expression of e-NOS in normal-term pregnancy and IUGR placentas. Angiogenesis is one dominant factor in normal embryogenesis and hence the wellbeing of the newborn. The expression of e-NOS was planned using the streptavidin-biotin-peroxidase technique in placental tissues identified as normal (n = 33) and IUGR (n = 33) cases from mothers looking apparently normal. All were chosen at term pregnancy and obtained between 2nd of February of 2018 and 31th of January 2019 at selected hospitals. Outcomes were appraised semi-quantitatively. The expression of e-NOS marker as an immunohistochemistry total score and staining percentage were significantly greater ($p < 0.05$) in epithelial surface, smooth muscle cells of fetal vessels and the connective tissue terminal villous core of the IUGR placentas when matched with placentas collected from normal pregnancies at term. Encountered placental histochemical changes regarding the expression of the e-NOS angiogenic factor for idiopathic IUGR newborns raised the suspicion of that, it was caused by pure placental factors and could represent further requirement for Nitric Oxide to dilate fetal vessels to optimize functional requirement during placental insufficiency. The noticed Increased expression of e-NOS may be the result of inadequate uteroplacental perfusion supporting the proposal that abnormal angiogenesis plays a role in the pathophysiology of IUGR.

Keywords: *Idiopathic Iugr, E-Nos, Placenta, Terminal Villi, Immunohistochemistry.*

Introduction

Intrauterine growth restriction/retardation (IUGR) is a complex placental vascular disorder resulting in limited weight at birth, preterm delivery, with highly expected perinatal morbidity and mortality [1-4]. IUGR could be triggered by different fetal, maternal, and placental influences [1-3,5]. Angiogenesis is regarded as a placental factor acting essentially in the development of IUGR [2-4,6,7]. Angiogenesis involves the branching of new microvessels from present larger blood vessels. It is an imperative aspect in normal embryogenesis [8-10]. Angiogenesis affects greatly the development of the villous vascular tree and the development of chorionic terminal villi. Placental vascular branching early in embryonic life and continues all over gestation [9,11]. Precise angiogenesis up regulators and down regulators adjust this processe [4,8,9]. Fetal growth restriction happens because of the failure of elongation, branching, or dilitation of the capillary circulation of terminal villi [4]. e-NOS has been acknowledged as stimulator for angiogenesis [8]. It is highly expressed throughout embryogenesis and fetal development [1,6,10,12]. It synthesizes nitric oxide (NO) solely in the placenta [13,14], signifying that e-NOS is in charge for the increased NO in the placenta. e-NOS is chiefly accountable for the propagation of NO in the vascular endothelial layer of fetal vessels in the placenta [15,16 17]. The e-NOS-derived NO is likewise a strong vasodilator compound in the perfused feto-placental vasculature which is empowered by smooth muscles [18], that is essential for the upkeep of decreased resistance at the fetoplacental interface [19]. Initial revisions have demonstrated that drug conveyed e-NOS suppression results in signs resembling preeclampsia and growth restricted fetuses in rats [20-25].

Patients and Methods: This work was carried out through Department of Anatomy, Medical Faculty,

University of Al-Mustansiriyah, in assistance of Gynecology and Obstetrics department at Fatima Al-Zahraa Administrative Hospital, Al- Khadhraa Private Hospital and Al- Yarmook Teaching Hospital in Baghdad, Iraq. Permitted by the scientific committee of each of these medical institutes. A total of 66 mothers, along with their newborns and placentas were incorporated in this study. All were chosen at term pregnancy (of 38-40 weeks), they were non-smoker, non-diabetic, normotensive apparently healthy and normal women according to their history, clinical assessments, laboratory tests and ultrasound check. Fetal state as IUGR likewise was verified by Doppler ultrasonic exam without apparent fetal anomalies. Any mother suffered from difficult or delayed labor been ruled out from this work. A consent was gained verbally from each mother to be a component of this work. All fetal IUGR was considered to be idiopathic, given that there were no apparent maternal nor fetal basis. Placentas were assigned into two groups. The 1st embraced 33 placentas of newborns having normal average body weight (Control), whereas the 2nd group holds 33 placentas of newborns having idiopathic IUGR based on the basis of a valued fetal weight of less than the gestational tenth percentile, decreased amnion, and proved diminshed end diastolic flow velocity of the umbilical artery by Doppler study confirmed by successive obstetric ultrasound examination [26] . Every tissue block measured roughly 1×1×1 centimeter to be fixed to be stained by Immunohistochemistry e-NOS marker. Then, from every tissue block five sequential sections of about 4 μm thicknesses were obtained and were collected on positive charged slides. After de-waxing, the sections were rehydrated gradually. Immunohistochemical staining achieved by the avidin-biotin-peroxidase technique. 3% hydrogen peroxide blocking agent was used to block Endogenous peroxidase. Kept in oven for 15 minutes in sodium citrate buffer solution (pH=6.0) for antigen retrieval. Rabbit polyclonal antibodies reactive with e-NOS (Abnova) was added to the sections and incubated for one hour at room temperature. Sections were washed with phosphate-buffered saline with Tween, then a secondary antibody added for 20 minutes and after that with a biotin-streptavidin complex for half an hour at room temperature. 3,3-diaminobenzidine tetrahydrochloride (DAB) was used to visualize reaction. Finally, the sections were counterstained with hematoxylin and mounted. The intensity and localization of the staining reaction in Syncytiotrophoblasts (epithelial cells), vascular smooth muscle cells (vessels) and chorionic villous stromal connective tissue cells (C.T.) were calculated by two pathologists blinded to the drive of the study. Immunohistochemical reaction of e-NOS antibody scored semiquantitatively for intensity of staining: 0 / negative = no staining, 1+ = weak positive, 2+ = moderately positive; 3+ strongly positive. The total score of immunohistochemistry staining obtained from multiplying the staining intensity with staining percentage for each region in high power field. Statistical analysis achieved by using SPSS v24. Continuous variables were illustrated as mean ± SD and the categorical variables were demonstrated in percentages. Chi-square test was used to compare groups for immunohistochemical outcomes. P - values < 0.05 were considered statistically significant.

Results

e-NOS immunohistochemistry cytoplasmic staining was perceived from both control and idiopathic IUGR tissues (Table 1, and Figures 1,2 and 3). A highly significant increase in the expression of e-NOS in IUGR placentas seen in syncytiotrophoblasts (epithelial cells) when compared to control group with p value = 0.003, Also, increase in staining percentage in the IUGR group but no statistical significance with p value = 0.077 difference when compared to the control groups. Regardless, the total score of e-NOS marker in epithelium was significantly higher in IUGR group than that in control groups (p = 0.004). regarding the fetal vessels, the staining intensities of e-NOS marker were significantly higher in IUGR group with p value = 0.02 when compared to the control group. Staining percentage of e-NOS marker in vessels was also significantly higher in IUGR group than that in control groups with p value =0.016. The foregone total score of e-NOS marker in vessels was significantly higher in IUGR group than that in control groups with p = 0.011. Regarding the expression of e-NOS in the connective tissue of the villus core of terminal chorionic villi revealed statistically significant increase in intensity in IUGR group when compared to the control group with p value = 0.001. The staining percentage was also significantly higher (p=0.001) and the staining score is also significantly higher in IUGR when compared to control group (p=0.007).

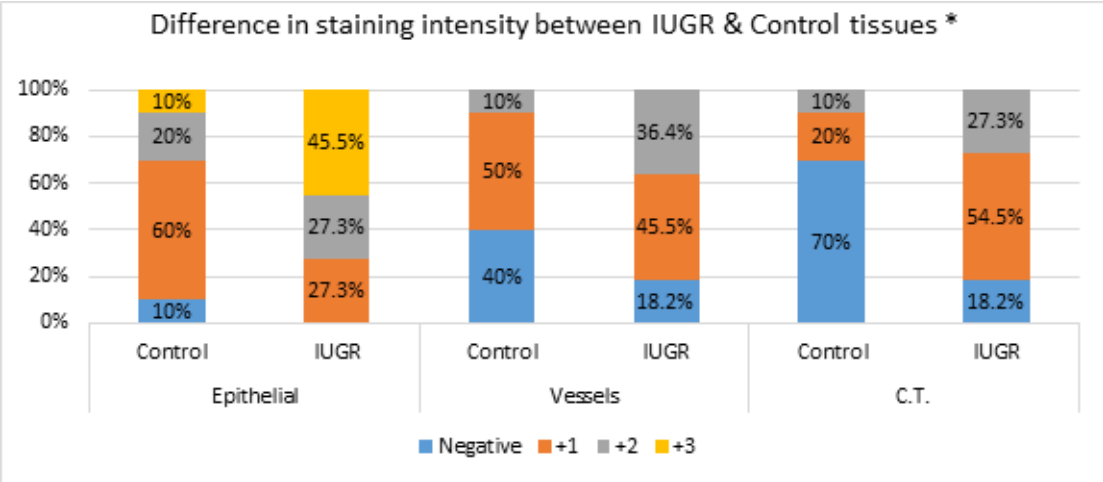

Figure (1): Difference in staining intensity of e-NOS between IUGR & Control tissues (* p value <0.05)

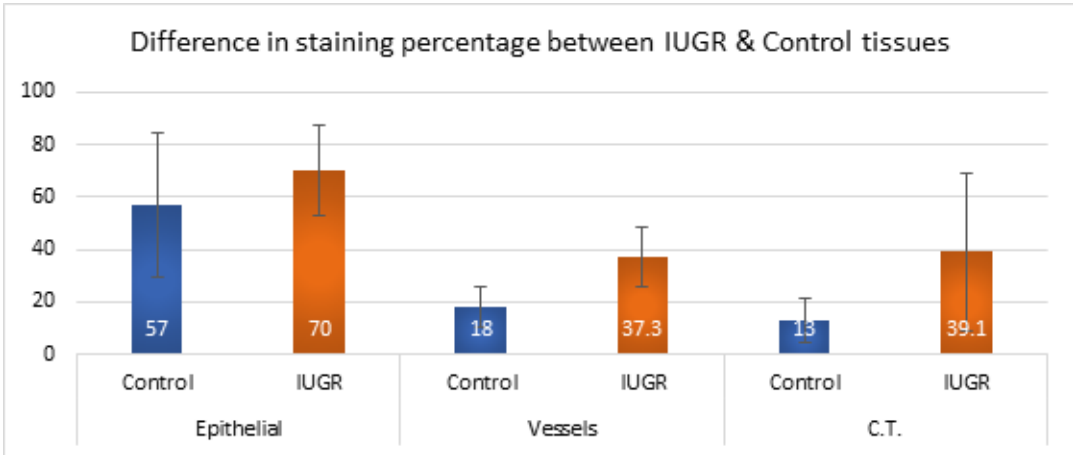

Figure (2): Difference in staining percentage of e-NOS between IUGR & Control tissues.

Table (1): Difference in staining total score of e-NOS between IUGR & Control tissues.

localization		Mean	Std. Deviation	p Value
Epithelial	Control	1.0	± 0.82	0.004
	IUGR	1.62	± 0.86	
Vessels	Control	0.27	± 0.5	0.011
	IUGR	0.61	± 0.62	
C.T.	Control	0.21	± 0.51	0.007
	IUGR	0.58	± 0.55	

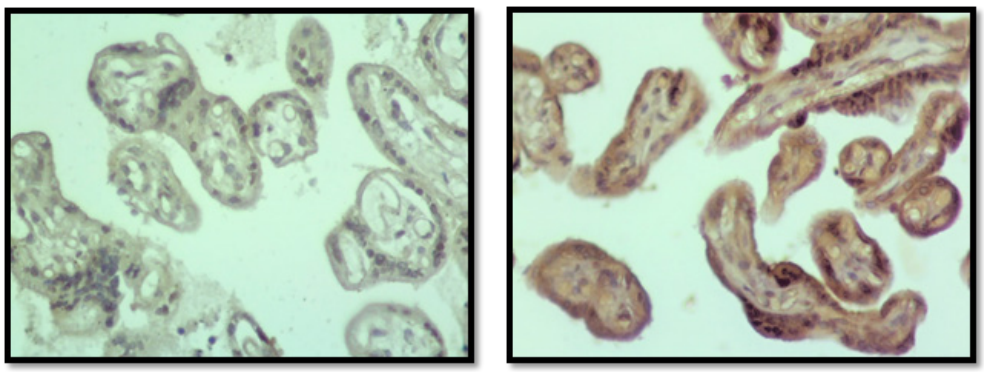

Figure (3): e-NOS staining reaction expressed in terminal villi of placenta, A; showing weak e-NOS staining in control group, B; showing e-NOS stronger expression in idiopathic IUGR group (DAB, A; B; × 400).

Discussion

Many previous studies had documented that a pure placental factor was the solitary cause in some IUGR cases [27]. IUGR incidence was noticed 3% regarding developed countries, though, it scopes 15-20% in developing countries, it partakes in more than 26% stillbirths and congenital anomaly [1,4,5]. Not to forget it yields many lasting health issues for grownups [28]. Therefore, the precise diagnosis, and proper management of IUGR-complicated pregnancies are important. Regarding e-NOS expression in the epithelial cells, our results differ from the study of Myatt et al (1997) [29] as they had found no differences in the intensity or type of staining. Regarding the fetal vessels, our results agree with Myatt et al (1997)[29] and Rutherford and colleagues (1995)[30]. However, other authors have found decreased levels of e-NOS in vessels in these condition like Giannubilo et al. (2008)[31] and Noris et al. (2004)[32]. We hypothesize that the increased e-NOS expression in their vessels is not a disease-specific feature, but perhaps an adaptive response to increased resistance and poor perfusion. It seemed to be no previous works measured the expression of e-NOS in the villus core of terminal chorionic villi of term placenta neither in normal nor in pathological cases like IUGR. The significant elevation in expression of this enzyme, e-NOS, in this region particularly could be explained by its rapid diffusion from endothelial cells to the core, it had been found that it down regulates fibroblast production to decrease villus fibrosis decreasing the fibroblastic activity[33] which in turn reducing the density of the villus core to enhance feto-maternal exchange. It is possible that increased expression of e-NOS seen in our results in idiopathic IUGR placentas at term in all mentioned regions impinge upon regulation of placental vascular reactivity by the smooth muscle of fetal vessels and hence blood flow regulation. In support of this, it had been shown that concentrations of nitrate, a breakdown product of NO, were significantly higher at delivery in umbilical venous blood from idiopathic IUGR pregnancies compared with controls [34]. The observed increased expression of e-NOS that we saw in placentas of Idiopathic IUGR could encourage pathological angiogenesis[35].

Conclusion

Toward elucidate the pathogenesis of idiopathic IUGR and the associated placental vascular insufficiency, it is needed to clarify the regulatory mechanism of placental vascular development. The noted increased staining expression of e-NOS designates pathological angiogenic action, due to lacking proper uteroplacental perfusion, resulting in the formation of idiopathic IUGR.

Ethical Clearance: was approved and granted from Al-Mustansiryia University, Al-Rusafa and Al-Karkh health Directorate application forms as a requirement to obtain PhD degree in Anatomy, Histology and Embryology. Research projects are reviewed and conducted ethically. This research is not published yet.

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Conflict of Interest: Nil

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Characteristics of Multidrug Resistant Tuberculosis in Minia, Egypt

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Background

Tuberculosis (TB) is a disease of great antiquity and has almost certainly caused more suffering and death than any other infection. A total of 40 patients were registered with a diagnosis of MDR-TB during 2010–2018 in Minia. The majority of patients were from rural areas (27; 67.5%) and male (27; 67.5%). Emergence of MDR-TB has the potential to be a serious public health problem in that necessitates strengthened TB control and improved continuous monitoring of therapy.

Keywords: *TB; MDR-TB; Therapy*

Introduction

TB is a major public health problem owing to its high risk of person-to-person transmission, morbidity, and mortality. TB still remains one of the major health miseries facing humans, particularly in developing countries (1).

Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat. World Health Organization estimates that there have been 558 000 new cases with resistance to rifampicin – the foremost effective first-line drug, of that – 82% had MDR-TB.

According to the latest WHO estimation the incidence of tuberculosis (per 100,000 people) in Egypt was reported at 13 in 2017 (2). Screening, diagnosis, notification, and registration of TB cases were implemented all over Egypt according to the National TB Strategy of the National Tuberculosis Control Program (NTP). One of the registration sites is Minia Chest Hospital, Minia, Egypt where the current study was conducted.

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Method

This was a hospital-based retrospective study conducted to detect the pattern of prevalence, risk factors and treatment outcomes among patients with multidrug-resistant tuberculosis (MDR-TB) in Minia, Egypt and involving a record review of patients with TB notified and registered in Minia Chest Hospital. Data of 40 MDR-TB cases reported from January 1, 2010 to December 31, 2018 were analyzed. The diagnosis of MDR-TB in Minia Chest Hospital is made in line with the National Egyptian TB Control Program Guidelines of the Ministry of Health (NTP). Sociodemographic characteristics, associated co-morbidities, fate of outcome and regimens of previous antituberculous treatments received either category I (CAT1) (Isoniazid "H", rifampicin "R", pyrazinamide "Z", ethambutol "E" with or without streptomycin "S" for 2 months followed by isoniazid and rifampicin for 4 months {2HRZE(S)/4HR}), category II (CAT2) (2HRZES/1HRZE/5HRE) were collected. Cured patient was defined as a patient who is smear-negative in the last month of treatment and on at least one previous occasion. Treatment completed was defined as a patient who has completed treatment but who does not meet the criteria to be classified as a cure or a failure. Death was defined as a patient who dies for any reason during the course of treatment. Treatment default was defined as a patient whose treatment was interrupted for two months

or more. Treatment failure was defined as a patient who remained or became again smear-positive at five months or later during treatment or defined as a patient who was initially smear-negative before starting treatment and become smear-positive after completing the initial phase of treatment. Trend curve of prevalence rate of MDR-TB cases in Minia, Egypt from 2003 to 2018 was performed.

Limitation of the study

By using retrospective data there were unavailability of clinical data, radiologic and other laboratory investigations.

Statistical Analysis

Data analysis was performed using SPSS version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. US). Crude and adjusted odds ratios (ORs) and their 95% confidence intervals (CI95) were calculated. A binary logistic multivariate model was applied to determine the independent predictors of the treatment outcome out of those factors that demonstrated significant association by bivariate analysis at a level of significance of $p \leq 0.05$.

Results

A total of 40 patients were registered with a diagnosis of MDR-TB during 2010–2018 in Minia. The majority of patients were from rural areas (27; 67.5%) and male (27; 67.5%). The mean age of the patients was 38 years (SD ±14.3 years). Smokers represented 55% of patients. Married patients were 60% of patients (Table1).

Majority of patients received CAT1 treatment at home and about half of them were regular in treatment. The commonest side effects of drugs used in treatment of MDR-TB patients were GIT symptoms and peripheral neuritis as shown in (Table 1).

The adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between the combined effect of independent variables and the outcome variable (MDR-TB). These estimates were obtained by logistic regression analysis. Marital status, place of previous treatment, compliance and associated comorbidities were statistically associated with. Compliance to treatment was found to be the most important determinant (Table 2).

Regarding the associated co-morbidities, 2.5% of the included patients were positive for HIV,17.5% of them had cardiac diseases, 25% were diabetics, 10% had HCV and 10% had chronic chest diseases (Fig. 1).

Patients completed treatment performed 37.5% and cases with favorable outcome were 22.5% while failure of treatment were only 5% (Fig.2).

The trend curves of MDR-TB and TB cases in Minia were decreasing in the years 2004, 2010, 2012 and 2018. The trend curves were increasing in the years 2005and 2011 (Fig 3).

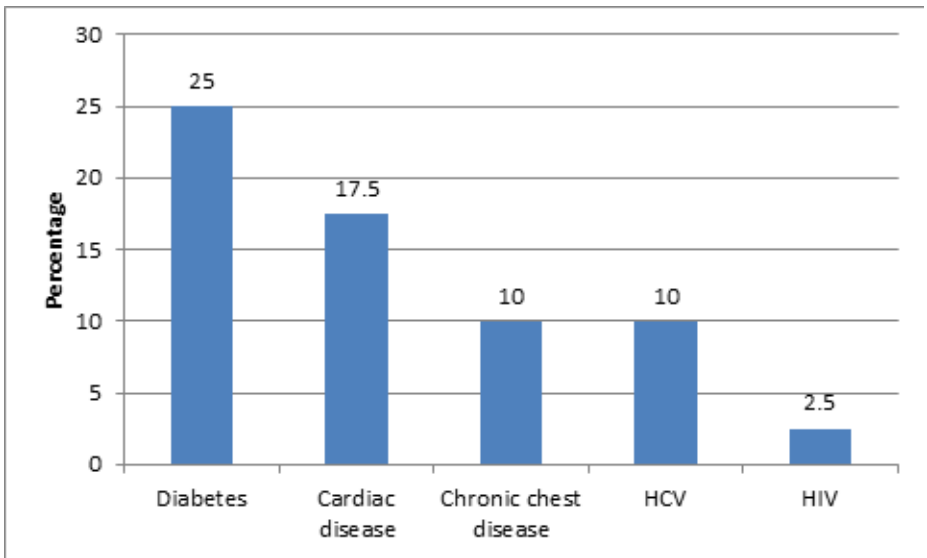

Fig (1) Associated co morbidities among the studied cases

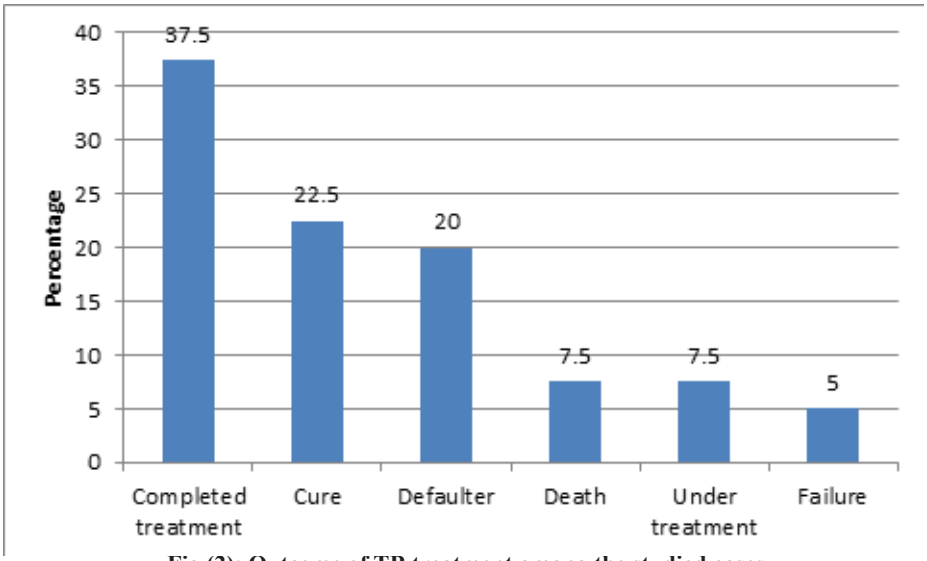

Fig (2): Outcome of TB treatment among the studied cases

Fig (3): Trend in prevalence of MDR- TB among TB patients in the period from 2003 to 2018

Table (1): Characteristics of MDR-TB cases in the period 2010-2018, Minia, Egypt

Age (years)	**Mean ± SD**	**38.6±14.3**	
		No.	%
Sex	Male	27	67.5
	Female	13	32.5
Smoking status	Smoker	22	55
	Non smoker	18	45
Residence	Rural	27	67.5
	Urban	13	32.5
Occupation	Non-worker	13	32.5
	Manual	6	15
	Farmer	13	32.5
	Housewife	8	20
Marital status	Single	5	12.5
	Married	24	60
	Widow	7	17.5
	Divorced	4	10
Place of treatment	Home	36	90
	Hospital	4	10
Regimen of treatment	CAT1	37	92.5
	CAT2	3	7.5

Cont... **Table (1): Characteristics of MDR-TB cases in the period 2010-2018, Minia, Egypt**

Compliance	Regular	21	52.5
	Irregular	7	17.5
	Uncertain	12	30
Side effects	Nothing	16	40
	GIT symptoms	18	45
	Peripheral neuritis	2	5
	Arthritis	4	10
Total		40	100

Table (2): Logistic regression analysis of factors affecting MDR-TB among the studied cases, Minia, Egypt

Variables	**OR**	**CI**	**P-value**
Age	0.95	0.86-1.04	0.26
Sex	1.1	0.075-16.5	0.9
Occupation	1.9	0.7-5.04	0.18
Residence	2.006	0.28-14.6	0.5
Marital status	5.6	1.1-28.7	0.03*
Smoking	4.5	0.4-51.5	0.2
Place of previous treatment	4.6	3.08-26.2	0.04*
Regimen of previous treatment	3.02	0.006-1509.8	0.7
Compliance to treatment	2.03	1.3-3.9	0.02*
Side effects of drugs	1.3	0.65-2.5	0.84
Associated comorbidities	1.95	1.57-2.6	0.04*

Discussion

Multi Drug Resistance Tuberculosis MDR-TB is a rapidly increasing public health problem with major socio-economic and individual consequences. The spread of MDR-TB can only be prevented by rapid identification of these cases and treatment with a combination of effective drugs [(5)].

In the present study, males constituted 67.5% while females represented 32.5%. This coincides with the epidemiological picture of tuberculosis where males spend more number of hours outdoor exposure and more challenging and hazardous working environments. Also this came in accordance with [(6)] who reported that the percentage of MDR-TB among males was 75.9% and that of females was 24.1% in three different governorates in Egypt.

In this study, we found that 55% of MDR-TB patients were smokers and this coincided with that of [(7)] in El-Abbasia Chest Hospital who revealed that in MDR TB, smokers were 61.53% and non-smokers were 38.47% among MDR-TB cases.

In many countries, differences in MDR-TB prevalence rates between urban and rural areas have been described. In the current study, 62.5% of patients were from rural areas and 32.5% were from urban areas. A study by [(8)]has reported similar findings that 90% were

from rural areas and only 10% were from urban area in the Dakahlia governorate from 2006 to 2011. Increased MDR-TB cases in rural areas could be explained by poverty, bad social conditions, milk sanitation, and occupational exposure to infected animals.

In this study, the most common co-morbidity associated with MDR TB was diabetes. This result agreed with those of [9] who reported that the highest co-morbidity among MDR-TB patients was DM 29.9%. Also, it was matched with those of [10], who reported that the highest co-morbidity among MDR-TB patients was DM (18.3%) of the patients.

In the present study, cured patients were (22.5%), dead patients were (7.5%) and defaulters were (20%). This result was matched with those of [11], who reported that (19.4%) were successfully treated, (20.9%) died, defaulted (13.4%). On the other hand, the result didn't agree with[12], who studied the outcome of treatment of MDR TB patients in Russia that 76.0% was cured. Again, the result didn't match with those of [13] who reported that 70.6% were cured in United Kingdom from 2004-2007. This difference might be due to patient compliance with treatment and regular drug in developed countries.

In the present study, 45% of patients suffered from gastrointestinal disorders. This result coincided with those of (14), who stated that the most frequent side effect of Anti TB drugs was gastrointestinal manifestations (64%). Also, this result agreed with those of (10), who reported that dominant adverse effect was gastrointestinal disorders (55%). Oppositely, the result did not coincide with those of (15) who reported that the highest adverse effects were ototoxicity 41.8%, psychological 21.3% and gastrointestinal 14%. This difference might be due to the fact that Törün study included 263 MDR TB patients who received individualized treatment for MDR-TB between April 1992 and June 2004 at Istanbul, Turkey and also the author said that the frequent and early occurrence of ototoxicity may be due to the extended exposure to amino glycosides and Capreomycin during or prior to MDR-TB treatment.

In the current study, the studied cases were resistant to CAT1 (rifampicin, isoniazid, ethambutol and streptomycin) represented 92.5%. This result agreed with the findings reported by (16) that 65% of patients were resistant to CAT1. Contrary to our results (17) in Bulgaria founded that only 52% of cases were resistant to CAT1 this high percent of acquired resistance may be due to alcoholism, number of previous treatments and irregular treatments. A study by (18) reported that only 42.8% of patients in a tertiary hospital in India were resistant to CAT1.Such variation may be due to varied geographical distribution, circulating strain patterns, demographic, ethnic, and epidemiological differences.

In this study comorbidities increased risk of MDR-TB ($p=0.04$) and this was observed by (19) in South Korea who found that relapse with resistant strains and poor treatment outcome of MDR-TB was documented among comorbid patients. Active screening for diabetes and HIV among TB patients is suggested as a cost-effective measure to be incorporated within the TB control program.

Our study revealed that MDR-TB infection had a statistically significant association with patients place of previous treatment ($p=0.04$) and this is in agreement with study done in Addis Ababa, Ethiopia by (20) on patients who visited health facilities ($p<0.005$). Compliance to treatment appeared to be a predictor for MDR-TB ($p=0.02$) and this finding was in agreement with a study by (21) in China ($p<0.005$), which requires strong commitment and collaboration among health organizations and greater compliance with TB treatment guidelines by service providers and patients.

In the present study, the trend curve of MDR-TB cases in Minia was decreasing in the years 2004, 2010, 2012 and this may be due to that the program of MDR-TB was approved and implemented in Egypt in the year 2003, by the Green Light Committee (GLC) (22). The trend curve was increasing in the years 2005 and 2011with more detection rate of drug resistant TB cases with drug susceptibility tests and increasing slum areas, low socioeconomic status and poor nutrition and political disturbance and 25th January revolution.

Conclusion

Emergence of MDR-TB has the potential to be a serious public health problem in that necessitates strengthened TB control and improved continuous monitoring of therapy. Emphasis on the need to complete medical records of patients in hospitals.

Declarations:

- There is no conflicts of interest and/or funding

- The paper has been read and approved by all authors.

- Ethical considerations: The study was approved by the ethical committee of the Faculty of Medicine, Minia University. Prior to data collection, official permissions were obtained from the authorities of Minia University Hospital. Following the ethical guidelines of epidemiological research, a written informed consent was taken from each participant.

- Data is available on request.

Ethical approval: Ethics approval to use, report, and publish the collected data was obtained from the administrator of Minia Chest Hospital. Patient information was anonymized and deidentified prior to the analysis. Research Ethics Committe at the Faculty of Medicine, Minia University, approved this study protocol.

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Prevalence of Uncorrected Refractive Errors Among the Internally Displaced Schoolchildren in Iraq

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Abstract

Purpose: The aim of this study was to estimate the prevalence of uncorrected refractive errors among the internally displaced schoolchildren in Iraq.

Method: A school-based cross-sectional study in the academic year of 2016-2017. Schoolchildren were selected from 8 primary schools for the internally displaced children in Kurdistan region, Iraq. All children underwent full ophthalmic examination, uncorrected visions were tested and cycloplegic refractions were done for children with visual impairment. Refractive errors in this study were determined by the results of the cycloplegic refraction.

Results: From 592 selected children, 94.7% had participated in our study, and 553 were eligible (age ranged from 6 to 12 years). The prevalence of refractive errors was 34.5%; 32.2% of them were uncorrected. The prevalence rates of myopia, hyperopia and astigmatism were 3.8%, 13.9% and 14.9%, respectively.

Conclusion: This study has found a high prevalence of uncorrected refractive errors among the internally displaced children in Iraq. This result represents an important health issue among those children, requiring major actions to tackle and resolve it.

Key-words: *Internally displaced children, Cross-sectional study, Refractive errors, Prevalence, Iraq.*

Introduction

Refractive errors (RE) affect a large proportion of the world's population, regardless of their age, gender and ethnicity [1]. According to the World Health Organization (WHO), uncorrected refractive error is considered as the leading cause of avoidable blindness and visual impairment worldwide, with estimated number of 12 million of schoolchildren aged 5 to 15 years worldwide affected [1, 2, 3]. Uncorrected refractive error has great impediment on the individual's education, personality development and career opportunities, along with its economic burden on society [1]. Therefore, the need for an effective screening programs to detect individuals with refractive error is rising in the recent years. VISION 2020- The Right to Sight, is the global initiative set by WHO to prevent the visual impairment resulting from the uncorrected RE. One of its strategies is to include a simple visual acuity test into the school health programs, and to perform spectacles provision to children with significant refractive errors [4, 5]. Provision of appropriate spectacles is considered as a simple, cost-effective strategy in order to improve vision.

In Iraq, as a result of the violence and conflicts in the past decade, more than 1.7 million persons, including over 900,000 children, had fled their homes and are living in camps or homes in the Kurdistan region of the country [6]. To our best knowledge, there is no available published data or information regarding the prevalence of visual impairment among these internally displaced children. Therefore, this study aimed to draw attention to the on-going health problems of the internally displaced people by determining the prevalence of the uncorrected refractive errors among the displaced schoolchildren.

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Material and Method

Study area and sampling

A cross-sectional school-based study was carried out among primary schoolchildren in the academic year of 2016 - 2017. The students were recruited from 8 primary schools for internally displaced children located in Kurdistan region, North Iraq. The study included all the cooperative students of both genders from these schools after obtaining informed consents from their parents or guardians. The age of students included in the study ranged from 6 to12 years; since in Iraq, children enroll in the elementary schools at age of 6 years, and the duration of study is six years.

Ophthalmic Examination

All the medical procedures were performed by trained ophthalmologists and optometrists. Examination included full ophthalmic history, slit lamp examination, and uncorrected visual acuity (UCVA) using Snellen's (E letter) chart at 6 meters' distance. Those with UCVA of 6/12 or worse in the better eye were declared to have defective vision and were examined by cycloplegic autorefraction using (Topcon KR 8000), and appropriate spectacle corrections were given. Children already wearing spectacles at time of examination were also examined and any changes in refractive errors were noted. The visual acuity, types of refractive error and correction were noted down in a self-designed proforma.

Definitions

Normal vision was defined an UCVA of 6/9 or better in at least one eye. Visual impairment was defined as an UCVA of 6/12 or worse in the better eye. Refractive errors were classified into myopia, hyperopia and astigmatism. Myopia was defined as a spherical equivalent (SE) of ≤ -0.50 diopter (D), hyperopia as SE $\geq +2.0$ D, and astigmatism as a cylinder of ≥ 0.50 D. Astigmatism was further classified into: simple myopic, simple hyperopic, compound myopic, compound hyperopic and mixed astigmatism.

Statistical Analysis

The prevalence of RE was calculated as the ratio of the number of subjects with RE to the total number of the children studied. The prevalence of different types of RE (myopia, hyperopia, and astigmatism) was presented as percentage. Microsoft excel package and SPSS software (IBM Version 23) were used for the data analysis.

Results

Study population

A total of 592 students from 8 primary schools for internally displaced children in Kurdistan region, Iraq, were recruited for this study. Of whom, 561 had participated in the study with a response rate of 94.7%. We excluded 8 cases from the data analysis because of poor cooperation and missed information. The remaining 553 subjects consisted of 287 males (51.9%) and 266 females (48.1%),Errors! Refrence source not found. The age of the students ranged from 6 to12 years. Mean age $\pm$ SD of the participants was 7.31 ± 0.59 years, **Table 1.**

Vision and VA

Uncorrected visual acuity of 6/9 or better in at least one eye was found in 362 out of 553 students (65.5%). The prevalence of visual impairment (UCVA of 6/12 or worse) in the better eye was 34.5% (191 of 553). The results of the vision screening according to gender and age of the participants are shown in **Tables 1 and 2.**

Of the 34.5 per cent of this sample found to have UCVA of 6/12 or worse in the better eye, 32.2 per cent of them had refractive errors (178 of 553). The remaining 2.3 per cent (13 of 553) had UCVA of $\leqslant$ 6/12 due to other ocular causes, see **Table 3.**

Table 1: Overall results of vision screening in schools of internally displaced students in Iraq by gender.

Gender	Screened students	Visual acuity 6/9 or better Number (%)	Visual acuity 6/12 or worse Number (%)
Male	287 (51.9%)	173 (31.3%)	88 (16%)
Female	266 (48.1%)	189 (34.2%)	112 (20.2%)
Total	553 (!00%)	362 (65.5%)	191 (34.5%)

Table 2: Overall results of vision screening in schools of internally displaced students in Iraq by age.

Age group (years)	Visual acuity 6/9 or better	Visual acuity 6/12 or worse	Total Number (%)
	Number (%)	Number (%)	
6 -7	161 (29.1%)	64 (11.57%)	225 (40.7%)
7 -8	70 (12.7%)	45 (8.14%)	115 (20.8%)
8 -9	56 (10.1%)	30 (5.4%)	86 (15.6%)
9 -10	57 (10.3%)	28 (5.06%)	85 (15.4%)
10 – 11	13 (2.4%)	18 (3.25%)	31 (5.6%)
11 - 12	5 (0.9%)	6 (1.08%)	11 (1.9%)
Total	362 (65.5%)	191 (34.5%)	553 (100%)

Table 3: Causes of visual impairment in the internally displaced schoolchildren in Iraq.

Causes	Number (%) of students with visual impairment
Refractive error	178 (32.2%)
Amblyopia	10 (1.8%)
Keratoconus	2 (0.3%)
Retinal disease	1 (0.2%)
Total	191 (34.5%)

Refractive errors

The overall prevalence of refractive error in the better eye was 32.2% of the studied sample (n = 178), **Table 3.** Of those 178 students with RE, only 18 (3.3%) wore spectacles during the time of the examination, and this making the prevalence of uncorrected RE was 28.9% in the examined schoolchildren (160 of 553). **Tables 4 and 5** are showing the prevalence of refractive error types distribution according to gender and age of the examined schoolchildren.

The overall prevalence of hyperopia was 13.9% (n = 77)**.** Myopia was detected in only 3.8% of the studied sample (n = 21), and the prevalence of astigmatism was 14.5% (n = 80). In case of astigmatism, mixed astigmatism was the most prevalent type (6.7%) of the total studied cases, followed by compound hyperopic astigmatism (3.6%) and compound myopic astigmatism (2.4%), **Table 6.**

Table 4: The prevalence of myopia, hyperopia and astigmatism by gender.

Type of refractive error	Gender		Total
	Male Number (%)	Female Number (%)	Number (%)
Myopia	9 (1.6%)	12 (2.2%)	21 (3.8%)
Hyperopia	25 (4.5%)	52 (9.4%)	77 (13.9%)
Astigmatism	41 (7.4%)	39 (7.1%)	80 (14.5%)
Total	75 (13.5%)	103 (18.7%)	178 (32.2%)

Table 5: The prevalence of myopia, hyperopia and astigmatism by age.

Age (years)	Type of RE			Total Number (%)
	Myopia Number (%)	Hyperopia Number (%)	Astigmatism Number (%)	
6 – 7	1 (0.2%)	32 (5.7)	27 (4.9%)	60 (10.8%)
7 -8	5 (0.9%)	17 (3.1)	20 (3.6%)	42 (7.6%)
8 -9	2 (0.4%)	14 (2.5)	12 (2.2%)	28 (5.1%)
9 -10	5 (0.9%)	7 (13)	14 (2.5%)	26 (4.7%)
10 -11	5 (0.9%)	5 (0.9)	5 (0.9%)	15 (2.7%)
11 -12	3 (0.5%)	2 (0.4)	2 (0.4%)	7 (1.3%)
Total	21 (3.8%)	77 (13.9%)	80 (14.5%)	178 (32.2%)

Table 6: Type and distribution of astigmatism by gender.

Type of astigmatism	Gender		Total
	Male Number (%)	Female Number (%)	Number (%)
Simple myopic astigmatism	5 (0.9%)	3 (0.5%)	8 (1.4%)
Simple hyperopic astigmatism	0	2 (0.4%)	2 (0.4%)
Compound myopic astigmatism	8 (1.4%)	5 (0.9%)	13 (2.4%)
Compound hyperopic astigmatism	9 (1.6%)	11 (2%)	20 (3.6%)
Mixed	16 (2.9%)	21 (3.8%)	37 (6.7%)
Total	41 (7.4%)	39 (7.1%)	80 (14.5%)

Discussion

This study was conducted to test the visual performance and estimate the prevalence of refractive errors in the internally displaced schoolchildren in Iraq.

In general, in this study, refractive errors were found in 32.2 per cent of the examined students. Results of refractive errors prevalence studies throughout the worlds vary widely. In Iraq, the prevalence of refractive errors among schoolchildren has been studied in a limited number of studies; in Amara city was (47%) [7], in Thiqar governorate was (35%) [8], in Erbil governorate was (25%) [9], and in Massif Kurdistan was (23.33%) [10]. In other studies around the world, refractive errors prevalence among children were; (26.67%) in Iran [11], (18.6%) in Saudi Arabia [12], (22.1%) in Egypt [13], (20.9%) in India [14], (6.3%) in Ethiopia [1], (12.8%) in China [15], (46.8%) in Australia[16], and (79 %) in the Unites States [17]. This wide variation in the results could be explained by; the racial and genetic factors of the different countries [18], the difference in the sampling methods and by the sample size. Also, the age range of the children is different in each study. While in our study it was (6 - 12) years, other studies had older or younger age groups included.

Furthermore, 28.9 per cent of the internally displaced children with refractive errors were uncorrected, as only a small number of them wore corrective glasses. This can be explained by the fact that; as a result of the conflicts in their cities, many of these children never had a previous visual examination. Also, for those who had previously examined and prescribed spectacles, the low

compliance for wearing glasses came from social stigma and financial causes.

In this study, astigmatism was found as the more prevalent type of refractive error (14.5%) followed by hyperopia (13.9%), while the prevalence of myopia was (3.8%) among the examined schoolchildren. An explanation to these results could be by the fact that the majority of the studied children were at younger age (mean age around 7 years). Also, the prevalence of astigmatism could be explained by the high prevalence of dry eye and allergic conjunctivitis among the Iraqis children [10, 19].

In conclusion, uncorrected refractive error was found to be an important health problem among the internally displaced children in Iraq. Major actions need to be taken by performing regular school-screening programs that provide corrective glasses at low costs or free to those who need them.

Conflict of Interest: Nil

Source of Funding: self-funded

Ethical Clearance: Ethical approval for this study was obtained from the Scientific Committee of University of Fallujah / College of Medicine before any measurements were carried out. The school principals also approved this study. Written informed consents were obtained from the children's parents or guardians.

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The Influence of Additional Food Hotong Porridge with Moringa Leaves Mixture on Improvement Nutritional Status of Children Nutritional Children

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Abstract

Malnutrition is one of the main nutritional problems in Indonesia. Toddler is one of the age groups that are vulnerable to nutritional problems. One of the causes immediate nutritional problem is a lack of energy. This study aims to analyze the effect of supplementary feeding (PMT) hotong porridge mixed with moringa leaves and government biscuits on weight gain and underweight nutritional status in the working area of the Five Public Health Center. This study used Randomized Controlled Trial Design, with a quasi-experimental approach. The distribution of PMT hotong porridge with Moringa leaf mixture as a treatment group and the administration of PMT biscuits by the government program as a control group. The sample size was 32 toddlers. Data were analyzed using paired-sample T test. There was a significant effect on body weight and nutritional status after administration of PMT hotong porridge with moringa leaf mixture and PMT biscuits of government programs with p-value of 0.001 in the treatment group and the control group. Hotong slurry feeding and biscuit mix Moringa leaves government programs throughout a period of 30 days to take effect on the increase in body weight and nutritional status of children on the mark with most of the value of z-score infants already stood at -2SD s/d +2SD, which means that most of the children under five are already in the nutritional status of the mall.

***Keywords**: Toddler, Weight, PMT, Nutritional status*

Introduction

Malnutrition is a condition of body weight according to age (weight/age) not in accordance with the age that should be[(1)]. Malnutrition at the age of 2-5 years can affect mental development (ability to think). The brain reaches its maximum shape at the age of two years, malnutrition at this age can result in disruption of brain function permanently and malnutrition in general causes disruption in the processes of growth, energy production,body defense, structural behavior and function[(2)]

Based on the results of Riskesdas, the prevalence of underweight by province and national is 2018 by 17.7% including poor nutrition by 3.9% and under nutrition by 13.8%. When compared with the national prevalence rate in 2007 (18.4%), in 2010 (17.9%) and in 2013 it was 19.6%. Maluku Province is one of nine provinces with a high prevalence of severe under nutrition (24.9%)[(3)]. In 2016 the incidence of malnutrition in Central Maluku district showed a figure of 5.1% in 2017 (4.5%) and in 2018 (4.5%)[(4)]

One direct cause of malnutrition is low nutrient intake[(1)]. Nutrient intake can be obtained from macro nutrients such as energy, protein and fat needed in large quantities by the body and plays a role in providing energy[(2)]. One ingredient food of high nutritional value, especially energy and protein is hotong. Hotong (*Setaria Italica (L) Beauv*) is a type of cereal crops sorghum. This plant is not widely known by the people of Indonesia and its processing is still limited.

Hotong plants grow widely in the Buru island area of Maluku and are used as staples with cassava,sago,corn,

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and rice. Hotong content high enough seed nutrients are carbohydrates (84.02%), protein (14.05%), fat (3.3%), 100 grams of seeds contain energy hotong huge 359 calories[5]. Moringa leaves also have a main source of several nutrients and theraupetic elements including antibiotics that stimulate the immune system. Moringa leaves have a high content of protein, vitamins and minerals that are good to be used as additional food for malnourished toddlers[6].

Method

This research was a quasi experimental study with Randomized Control Trial Design. The sampling technique was simple random sampling. The sample were stunting as many as 32 people consisting of 16 infants in the treatment group and 16 infants in the control group with the inclusion criteria, namely children aged 24-59 months who suffered less nutrition with weight/age. z-score -3SD up to <-2SD, no disease, infants who are not breastfed and are willing to be respondents. This research was conducted for 30 days from 10 May to 10 June 2019 in the working area of the Public Health Center "Lima", Central Maluku. The independent variable was the administration of PMT hotong porridge mixed with moringa leaves and biscuits, the dependent variable was weight and nutritional status. PMT hotong porridge was given to the treatment group as much as 1 20 grams with 324 kcal energy and 10 grams protein and biscuits PMT were given to the control group as much as 120 grams (12 pieces/day) with 300 kcal energy and 8.45 grams protein.

Toddler characteristics in this study consisted of age and gender. Family characteristics include the number of family members and family income. Primary data were obtained from body weight measurements using digital scales with 0.01 kg-accuracy, interviews with questionnaires and food recall form 1x24 hours. Secondary data were the number of malnourished children under the age of 24-59 months registered in the working area of the public health center "Lima".

The results of measuring under-fives weight use the weight/age index and compared with the z-score and are classified into 2 categories: malnutrition if included in the category of under nutrition and good nutrition and over nutrition if included in the category of good nutrition and over nutrition. Energy, protein and Fe intake compared with the cut of point Estimated Average Requirements (EAR), namely the deficit category if <70% RDA, less if 70-80% RDA, while if 81-90% RDA and good category if 91-100% RDA .

To see the effect of energy, protein, and Fe intake on body weight and nutritional status of toddlers the paired-sample T test.

Findings

Characteristics of toddlers and families in table 1 shows that most of the toddlers in this study were aged 24-36 months in both the treatment group of 12 toddlers (75%) and the control group of 11 toddlers (68.75%). This age group is usually vulnerable to nutritional problems such as underweight, stunting and wasting. Toddlers aged 2 years have also entered the weaning period and have adopted the same diet with other families so that food intake is very important to support the growth process of infants[7].

Table 1. Distribution of Toddler and Family Characteristics

Characteristics	Treatment		Control	
	n	%	n	%
Toddler Age (month)				
24-36	12	75	11	68.8
37-48	4	25	2	12.5
49-59	0	0	3	18.8
Total	16	100	16	100

Cont... **Table 1. Distribution of Toddler and Family Characteristics**

Toddler Sex				
Male	8	50	5	31.2
Girl	8	50	11	68.7
Total	16	100	16	100
Number of family members				
Solid (>4 people)	7	43.8	5	31.2
Rarely (≤4 people)	9	56.2	11	68.8
Total	16	100	16	100
Family Income				
<UMK	16	100	12	75
≥UMK	0	0	4	25
Total	16	100	16	100

The sex in this study were mostly women, both in the treatment group of 8 toddlers (50%) and the control group of 11 toddlers (68.75%). Research in Ghana states that girls are more at risk of nutritional problems compared to boys. That is because in parenting, diet and care, boys take precedence over girls[8] .

The number of family members in this study mostly had sparse family members (≤4 people), namely in the treatment group of 9 toddlers (56.25%) and the control group of 11 toddlers (68.75%). This shows that the number of members in a family can influence the less optimal distribution and level of food consumption. Families with a large number of family members tend to get less food available. Lack of food availability in a long time can affect the low level of food consumption and have an impact on malnutrition[9]

The level of family income in the treatment group (100%) and the control group (75%) <UMR. Family income is one of the socio-economic indicators that play a role in providing food and family eating patterns. Low income can be one of the factors causing underweight nutritional status in infants, if the amount of income is low, then the level of food expenditure is also low so that it can cause low purchasing power and availability of food that contributes to the level of suboptimal nutritional consumption[10]**.**

Prior to PMT administration,most toddlers experience a deficit in food intake. In the treatment group namely 14 toddlers (87.50%) deficits in energy intake, 5 toddlers (31.25%) deficits in protein intake and 9 toddlers (56.25%) had deficits in Fe intake.

The control group most of the toddlers experienced energy intake deficits namely 12 toddlers (75.0%), 4 toddlers (25.0%) had a protein intake deficit and 12 toddlers (75.0%) had a deficit in Fe intake.

In the treatment group, the level of energy intake deficit was reduced to 2 toddlers (12.50%), the level of protein and Fe intake decreased deficits so that there was no deficit intake of toddlers.

In the control group, there was a decrease in the deficit energy intake to 6 children under five (37.50%). The majority experienced an increase in the level of protein intake, namely 6 toddlers (37.50%) and Fe deficit intake increased to less intake, namely 9 children under five (56.25%).

The test results differ in levels of energy , protein and Fe consumption between the treatment group and the control group after PMT administration with p-value = 0.007. Previous research stated that there was a change in the level of energy consumption in the treatment and control groups after the administration of PMT by p=0.008[11]**.** This study is other states that people with a less risky energy consumption 6.73 malnutrition was comparable to the n that the energy consumption enough[12].

The effects of energy,protein and Fe intake on body weight and nutritional status before and after PMT administration are presented in table 4.

Table 2. Effects of Energy, Protein and Fe Intake on Body Weight and Nutritional Status Before and After Giving PMT

Average	Treatment		Control	
	Before	After	Before	After
Energy (%)	65.49	80.03	66.60	71.00
Protein (%)	79.53	95.88	85.27	86.61
Fe (%)	72.00	81.32	69.37	76.40
BW (kg)	7.93	10.06	8.05	8.95
Z-Score	2.58	3.86	2.02	2.93

Table 2 showed that the treatment group or the control group increased intake of energy, protein and Fe. In the treatment group, the intake of energy, protein, Fe, body weight and nutritional status increased after administration of hot porridge mixed with moringa leaf.

The results of different tests of energy intake,protein intake and Fe intake on body weight and nutritional status before and after PMT administration showed significant differences, namely in the treatment group p=0.001 and the control group p=0.039 and p= 0.001.

Discussion

Effects of Moringa Leaf Hotong Slurry Supplement Supplementation for Weight Loss

PMT hotong porridge with moringa leaf mixture is a snack made from several specific ingredients, as the main ingredient of hotong flour and Moringa flour with a comparison of certain nutritional levels in order to obtain high nutritional value. Consumption hotong flour and flour of Moringa is alternative way to promote weight loss nutritional status of stunting.

Hotong has several advantages compared with rice,nutrients are protein on hotong 3 times greater (13.05g) of protein in rice (5.0 g), carbohydrates at hotong of 84.02 cal and at 70-80 cal rice and fat in hotong 3.37 grams greater than fat in rice that is 1.0-2.0 g[(5)] .

Moringa leaves also have several advantages including vitamin C in moringa is equivalent to 7 times vitamin C in citrus fruits,4 times calcium in milk, 3 times potassium in bananas, 2 times protein in yogurt, 4 times vitamins in carrots and 25 times iron in spinach[(6)]. Moringa leaves also contain 46 antioxidant compounds that protect the body against the damaging effects of free radicals by neutralizing them before they can cause cell damage and disease.

The results of the study are known before giving hotong porridge the mixture of Moringa leaves the average value of body weight is 7.93 kg and after administration of hotong porridge the mixture of Moringa leaves the average value of a toddler's body is 10.06 kg, meaning that there is an increase in toddler body weight after the administration of hotong pulp mixed with moringa leaves in the treatment group was 2.13 kg (8.50%)

Hotong porridge mixed with moringa leaves can be used as an alternative intake of morning uptake with complete nutritional content. by consuming 1 plate or 120grams of hotong porridge mixed with moringa leaves can be fulfilled energy by 27.07% of RDA and protein 33.35% of RDA with the fulfillment of energy and protein intake in a day can increase weight gain in infants.

Earlier research stated that the more increased administration of Moringa leaves were added, the higher the content of protein,sugar,calcium,fiber content and β-carotene.This allows good absorption of nutrients contained in hotong and Moringa leaves[**(13)**].Other studies also stated that the body weight of children who received average Moringa leaf powder was higher (8.9±4.30 g/kg/day compared to 5.7±2.72g/kg/day in group II) and the level recovery is faster with an average stay time of 36±16.54 g/kg/day compared to 57±19.20 g/kg/day for those who do not receive Moringa supplements[(14)].

Other research states there is an effect of energy intake and protein intake on the addition of BB and TB under five after giving Moringa BMC PMT for 30days. The results of the analysis of energy intake and protein intake on BB and TB before and after giving BMC Moringa get the average value of BB before 10.29 kg and BB after 11kg. The average TB value before giving BMC Moringa PMT 88.12 cm and TB after an average of 89.16 cm[11].

The results of the difference between body weight before and after PMT administration showed a significant difference with a value of p= 0.001.

The Effect of Supplementation of Moringa Leaf Hotong Porridge Supplement Against Nutritional Status

The results showed that the nutritional status of the treatment group prior to the administration of hotong porridge with Moringa leaf mixture was lacking nutritional status-the average nutritional status of children under five was 2.58kg and after administration of hotong porridge with Moringa leaf mixture the average value of nutritional status was 3.86kg. meaning that an increase in nutritional status after the administration of hotong porridge mixed with Moringa leaves by 1.28 kg

In the control group before administration of government program biscuits the average nutritional status was 2,02 whereas after administration of government program biscuits the average nutritional status was 2.93. This means that an increase in BB of 0,91.

Statistical test results showed that there were significant differences before and after the administration of hotong porridge mixed with moringa leaves (p=0.001) and government program biscuits with a value (p=0.001) on nutritional status. From the results of the study, samples that were given supplementary food both hot moringa mixed porridge with leaves and biscuits during the government program for 30days increased nutritional status, and the most effective improvement in nutritional status was supplementary feeding of hotong porridge mixed with moringa leaves.

Increasing the nutritional status of children under five is influenced by good food intake. Meeting the nutritional needs of children under five is the fulfillment of food intake that contains important compounds needed by the body of a toddler, including calories, protein, vitamins and minerals in accordance with their age. So at the age of five, balanced nutrition is needed so that the growth and development of toddlers can run well.

Conclusion

There is an effect of the administration of PMT hotong porridge with moringa leaf mixture and PMT biscuit of government programs to increase body weight and nutritional status of under-fives nutrition.

Suggestion is that the hotong porridge mixed with moringa leaves can be considered as an alternative for breakfast as a substitute for rice porridge,so as to reduce the incidence of malnutrition in infants.

Ethical Clearance: Taken from Ethical Commission of Health Research, No.125/HRECC.FODM/IV/2019, Faculty of Dentistry-UNAIR.

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The Stiffness Comparison of Four-Screw versus Six-Screw Short Segment Pedicle Posterior Stabilization Instrumentation in Cyclic Axial Compression

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Abstract

Background: Thoracolumbar burst fracture required to conduct short segment posterior stabilization as the operative therapy. The short segment fixation (above and under fracture level (four-screw pedicle)) encounters loss of correction and failure including progressive kyphosis, screw pedicles and rods that are bent or fractured. Rod around fracture site receives bigger loads than other parts due to its function as cantilever. To overcome this, it uses short-segment posterior stabilization instrumentation in which pedicle screw is installed above, under, and on fracture site level (six-screw pedicle) to increase stiffness.

Objective: To analyze the comparison stiffness of four-screw versus six-crew short segment pedicle posterior stabilization instrumentation in cyclic axial compression.

Method: This study used in vitro mechanical test in simulation of vertebrae from UHMWPE (Ultra High Molecular Weight Polyethilene) that was fixated with four-screw and six-screw pedicle. Each construction is given cyclic axial compression. Afterwards, the stiffness of each construction was measured.

Results: Six-screw short segment pedicle posterior vertebrae stabilization instrumentation had higher stiffness of 43.38% than four-screw short segment pedicle posterior vertebrae stabilization instrumentation in cyclic axial compression. There was a significant difference in both groups ($p<0.05$)

Conclusion: Six-screw short segment pedicle posterior vertebrae stabilization instrumentation was better than four-screw short segment pedicle posterior vertebrae stabilization instrumentation in cyclic axial compression.

Keywords: *In vitro mechanical test,thoracolumbar burst fracture, six-screw short segment pedicle, four-screw short segment pedicle, cyclic axial compression, stiffness*

Introduction

In Indonesia, the case of spinal cord injury has commonly occurred. Spine fracture therapy can be performed either operatively or nonoperatively. The goal of spinal fracture therapy is to restore stability, reduce pain and correct deformity. The variety of therapy instrumentations that are currently used are SRSSI, PSP, and PSR (Pedicle Screw and Rod). Fixation with transpedicle screw is often used as a spinal instrumentation for the stabilization and correction of deformity caused by trauma, degenerative processes, tumors, and congenital abnormalities[1, 2]. The pedicle screw system can provide instability treatment because of a burst fracture or tumor resection in the thoracolumbar[3].

In the previous study on the short segment of four-screw pedicle, there was a change in kifotic angle post op of 2.0625 to 5.312 after 6 months, whereas in the short segment of six-screw pedicle, there was a change in kifotic angle post op of 1.000 to 1.333 after 6 months (4). The short segment fixation (above and under fracture level (four-screw pedicle)) encounters loss of correction and failure including progressive kyphosis, screw pedicles and rods that are bent or fractured. In

this case, if the construction is mounted a pedicle screw on the fracture site, it will overcome the problems (5). Several studies reported a 25% failure on lumbar or thoracolumbar fractures treated with instrumentation with short-segment pedicle screw(6). This is due to the short segment caused by the occurrence of axial instability that reflects the anterior and medial mechanical incompetence.

Several biomechanical studies are aimed to indicate the character of the spine in a physiological (non-injury) state, which includes a stiffness test or flexibility. The results of the study are described in the load displacement curve. Clinically, this series of instrumentation requires in vitro testing either in a plugged in synthetic material or cadaveric spine. The results of this biomechanical test provide information on tool stiffness, fatigue life and failure mechanisms to improve the instrumentation [(7)].

The researchers are intended to compare the stiffness by assigning a load to a cyclic axial compression force between the construction of four screw short-segment pedicles with six screw short segment pedicles on a vertebral simulation using polyethylene material.

Method

This research is an in vitro biomechanical test with true experimental. This method used Post test only control design, which distinguished two types of short-segment pedicle construction mounted on polyethylene (UHMWPE) material that resembles human spine with unstable fracture or (anterior and medial damage) and cyclic axial compression load. The first construction used four pedicle screw (pedicle screw mounted above and below fracture level) while the other type of construction used six pedicle screw (pedicle screw mounted above, below fracture level and fracture level).

The tool used was material testing machine servohidraulic type Schenck, 1990, made in Germany. Biomechanical tests on the vertebral simulation of polyethylene material was performed to compare the stiffness of a six-screw pedicle short-segment construction with four-screw pedicle short-segment construction in cyclic axial compression. The specimens were subjected to repeated loading on the top and downward direction in the sinusoidal function with cross head displacement of ± 1 mm. The frequency of displacement was 2 Hz in sinusoidal wave. The magnitude of this displacement was determined based on previous studies showing that displacement of 1 mm provided a load to the pedicle screw equivalent to the load received during walking [(8)]. The amount of load received is calculated; thus, the load displacement curve can be obtained to calculate the stiffness coefficient.

The measurement of the slope stiffness of the load displacement curve was carried out in the range of 10-90% from the 1000 cycles. It was intended to remove the pre-stress conditions received during insertion or tightening of the screw [(8)]. This measurement was conducted four times; thus, each specimen has a total of 3000 cycles to recognize changes in the entire cycle. This test used a servohydraulic testing machine (ESH) composed of displacement transducer and compressive load-cell. The data collection of load displacement was conducted through analog to digital interface. Three groups of data obtained were time (s), displacement (mm), and load (N).

Two holes were made on polyethylene on the posterior side with 40 mm distances. Both holes had the same distance from the bottom surface of the block, which was 9 mm. The direction of the screw hole was perpendicular to the cut posterior surface; thus, it angles 15° anteromedial. Both holes were tapered 6.0 and mounted two monoaxial pedicle screw in each polyethylene block. The screw had 35 mm long from 316L stainless steel made by PT Marthys Indonesia. This study used two types construction: the first construction used two blocks of polyethylene by spacing between two blocks and the second construction used three blocks of polyethylene. Once tested, the polyethylene specimen can be reused only if visual inspection was not cracked, ebris due to the usage, or permanent shaping. Furthermore, the reduced torque strength can cause the polyethylene cannot be used.

Screw pedicle used had a head structure that can accommodate longitudinal rod, perform closure mechanism, and deformity correction. Closure mechanism is an important part because it has an effect on the rigidity of a construction. To see the actual strength of the construction, a scenario condition was created, in which the construction must support axial compression loads on both polyethylene blocks above and below. The statistical analysis was conducted by using ANOVA (Analysis of Variance) with $p < 0.05$ as the degree of significance. This study compared the stiffness between devices as well as between cycles; therefore, it was not possible to use t test.

Results

Based on the statistical test descriptively, the highest average stiffness of 6 screw pedicle after giving axial compression style of 500 N was 528.75±32.50 N/mm in initial cycle (cycle 0). On the other hand, the lowest stiffness value in 3000 cycle was 431.25±37.50 N/mm. In the results of axial compression of 900 N, the highest stiffness in initial cycle was 356.25±71.80 while the lowest stiffness in cycle 3000 was 137.50±43.30 N/mm. The highest average stiffness of 4 screw pedicle after giving axial compression style of 500 N was 276.25±16.52 N/mm in initial cycle (cycle 0). On the other hand, the lowest stiffness value in 3000 cycle was 243.75±23.93 N/mm. In the results of axial compression of 900 N, the highest stiffness in initial cycle was 175±95.74 while the lowest stiffness in cycle 3000 was 87.50±25.0 N/mm.

Table 1. The multi-cyclic stiffness post compression force in pedicle screw installation

Treatment	Sample	Compression 500N				Compression 900N			
		Cycle	Cycle	Cycle	Cycle	Cycle	Cycle	Cycle	Cycle
		0	1000	2000	3000	0	1000	2000	3000
		(N/mm)	(N/mm)	(N/mm)	(N/mm)	(N/mm)	(N/mm)	(N/mm)	(N/mm)
6 pedicle screw	1	525.0	475.0	450.0	475.0	375.0	350.0	250.0	100.0
	2	515.0	485.0	475.0	450.0	400.0	225.0	200.0	175.0
	3	500.0	435.0	400.0	400.0	400.0	150.0	100.0	100.0
	4	575.0	450.0	410.0	400.0	250.0	175.0	150.0	175.0
4 pedicle screw	1	285.0	275.0	260.0	225.0	300.0	200.0	200.0	125.0
	2	295.0	285.5	275.5	260.0	100.0	75.0	75.0	75.0
	3	275.0	265.0	250.0	225.0	100.0	175.0	100.0	75.0
	4	300.0	270.0	250.0	250.0	200.0	175.0	100.0	75.0

The analytical statistic used Independent t-test. Mann-Whitney was performed to measure the average stiffness after giving the axial compression 500 N and 900 N in 6 screw pedicles and 4 screw pedicles. It was also repeated ANOVA test to understand the stiffness change along with cycle change (1000, 2000, dan 3000 N/mm) in both ways of installations.

Table 2. The comparison of stiffness mean in 6 pedicle screw and 4 pedicle screw (independent t-test)

Compression	Pedicle screw treatment	Stiffness mean (N/mm)	Difference percentage (%)	(p)
The comparison of stiffness mean in 6 pedicle screw and 4 pedicle screw post compression 500 N and 900 N (independent t-test)				
500 N	6 screw	463.75 ± 49.91	43.38	0.001
	4 screw	262.56 ± 19.11		
900 N	6 screw	223.44 ± 67.15	39.85	0.008
	4 screw	134.38 ± 66.38		
The comparison of stiffness mean each cycle in 6 pedicle screw and 4 pedicle screw post compression 500 N (independent t-test)				
0	6 screw	528.75 ± 32. 50	47.75	0.001a
	4 screw	276.25 ± 16. 52		
1000	6 screw	461.25 ± 22. 86	42.52	0.029b
	4 screw	265.12 ± 10. 95		
2000	6 screw	433.75 ± 37. 50	38.87	0.001a
	4 screw	265.12 ± 10. 95		
3000	6 screw	431.25 ± 37. 50	43.38	0.001a
	4 screw	243.75 ± 23. 93		
The comparison of stiffness mean each cycle in 6 pedicle screw and 4 pedicle screw post compression 900 N (independent t-test)				
0	6 screw	356.25 ± 71. 80	50.88	0.023
	4 screw	185 ± 95. 74		
1000	6 screw	225 ± 88. 97	30.56	0.238
	4 screw	156.25 ± 55. 43		
2000	6 screw	175 ± 64. 54	32.14	0.234
	4 screw	118.75 ± 55. 43		
3000	6 screw	137.50 ± 43. 30	36.36	0.104
	4 screw	87.5 ± 25. 00		

* Homogeneity test was performed using Levene test

a Analytical test using independent t-test parametric test

b Analytical test using non-parametric test Mann-Whitney test

It has been revealed that the average stiffness of 6 screw pedicles was higher than 4 screw pedicles with the percentage of 43.38% post compression of 500 N and 39.85% post compression of 900 N. It was a significant difference ($p<0.05$). The stiffness in 6 screw pedicles post giving axial compression of 500 N was higher than 4 screw pedicles of each cycle with the initial cycle

percentage of (47.75%) and it was considered significant ($p<0.05$). The stiffness in 6 screw pedicles post giving axial compression of 900 N was higher than 4 screw pedicles of each cycle in the initial cycle and it was considered significant ($p<0.05$). However, there was no significant difference in other cycles ($p>0.05$).

The average stiffness of 6 screw pedicles had tendency to decrease along with increased cycle yet it was not considered significant ($p>0.05$). The post hoc test of 4 screw pedicles showed that there was significant decreased stiffness from cycle 0 to 1000, as well as from cycle 1000 to 2000 ($p<0.05$). However, there was an insignificant decrease of average stiffness in cycle 2000 to 3000 ($p>0.05$). In general, there was decreased stiffness along with insignificant increased cycle with axial compression of 500 N in 3-level pedicle screw ($p>0.05$). In addition, there was a decreased stiffness along with insignificant increased cycle in 4 pedicle screws ($p>0.05$). Based on post hoc test in 4 pedicle screws, there was no statistically significant average stiffness in each cycle change ($p>0.05$). It can be concluded that there was no significant decreased stiffness along with increased cycle with axial compression of 500 N in 4 pedicle screws ($p>0.05$).

The average stiffness of 6 screw pedicle showed a tendency to encounter insignificant decreased along with increased cycle ($p>0.05$). Based on post hoc test in 6 pedicle screws, there was no statistically significant average stiffness in each cycle change ($p>0.05$). It can be concluded that there was no significant decreased stiffness along with increased cycle with axial compression of 900 N in 6 pedicle screws ($p>0.05$).

The average stiffness of 4 screw pedicle showed a tendency to encounter insignificant decreased along with increased cycle ($p>0.05$). Based on post hoc test in 4 pedicle screws, there was no statistically significant average stiffness in each cycle change ($p>0.05$). It can be concluded that there was no significant decreased stiffness along with increased cycle with axial compression of 900 N in 4 pedicle screws ($p>0.05$).

Discussion

To ensure the ability of instrumentation construction as a fixation of the spine, the implant construction must have genuine stiffness. Therefore, the stiffness should be measured to support and stabilize the spine until bone healing occurs in an instrumentation construction . Basically, the instrumentation construction has varying stiffness due to the difference in load and the amount of cyclic received. It is due to the interspecimen slippage and microcrack in the implantable material. By using UHMWPE as a consistent fixation medium with the right size, the depth and placement of the implants can be made constantly [(9)].

This study protocol is a consistent and logical technique for the posterior spinal instrumentation biomechanical test. This method also provides evaluation base and biomechanical comparison of both constructions. In implant construction, the construction material and types affect the stiffness from the implant construction (10). Theoretically, the posterior stabilization of the short segment of six screw pedicles can increase the resistance and stabilization of the spine (13). In this study, in addition to the stiffness between the two constructions, we also observed the change of stiffness between cycles, so it can be found how strong the construction after being given increased cyclic axial compression cycle. This is in accordance with studies stating that the stiffness of an implant will vary according to the size of the load cycle received as a result of the intraspicemen slippage and microcrack occurring in the implant material (10, 12).

In this study, there was no broken construction on screw pedals and rods when the device was given cyclic axial load or upon inspection after the tool was removed. From the results of research using synthetic spine of UHMWPE material, it is found how big the difference of stiffness in both constructions is. The use of UHMWPE has the advantage of being a stable specimen in a research because it can be reduced to biological deterioration; therefore, it can be used as a consideration for deciding which type of construction will be used to deal with cases of spinal trauma. However, the value obtained does not mean exactly the same as if it is implemented on the patient directly. In the use to the patient, it still must be considered because there are many biological factors such as the age of patients, osteoporosis, the patient's nutrition, postoperative rehabilitation of the patient or the presence of comorbid diseases such as malignancy, and infection.

Conclusion

The six-screw short segment pedicle posterior vertebrae stabilization instrumentation was better than four-screw short segment pedicle posterior vertebrae stabilization instrumentation in cyclic axial compression.

Ethical Clearance: This study received an ethical test from Dr. Soetomo General Hospital and faculty of medicine Universitas Airlangga.

Source of Funding: This research was carried out through individual funding.

Conflict of Interest: There was no conflict of interest from this study.

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Correlation of Aggressivity Papilloma Recurrent Respiratory Tract With Human Papillomavirus Types 6 And 11

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Abstract

Background : Recurrent respiratory tract papilloma (PSPB) is a viral disease that correlated with an airway exophthalmia lesion. Papilloma is primarily caused by human papillomavirus (HPV) types 6 and 11 which are classified as low-risk HPV. Reports indicate that patients with the HPV-11 experience more aggressive course of the disease than HPV-6. Patients are often diagnosed at a younger age, the longer duration, require more frequent surgery and rare diseases of the disease when compared with those caused by HPV-6.

Objectives : To investigate the correlation of aggressiveness of PSPB with HPV-6 and 11.

Methods : Fifteen samples were conducted in the Outpatient Unit of Otorhinolaryngology and the Lotus Inpatient Installation of Dr. Soetomo General Hospital from December 2012 to March 2013 then determine the disease aggressiveness. PCR examination was done at the Tropical Disease Institute of Universitas Airlangga. Aggressive correlation of disease with HPV type was tested using Chi-Square.

Results : Fifteen patients were enrolled that divided; the male was 10 and female was 5. The mean annual number of operations was 2.8 ± 1.27 and 9 patients reported more than 3 surgeries each year. 10 patients had distal papilloma and 11 patients had a history of tracheostomy. PSPB was found in 12 patients, HPV-11 was 9 patients and HPV-6 was 6 patients. Chi-square test results showed no significant results with p = 0.525.

Conclusion : There was no correlation between PSPB aggression with HPV-6 and 11

Keywords : *Aggressive Papilloma Respiratory Channels, HPV types 6 and 11, Chi Square.*

Introduction

Recurrent respiratory tract papilloma (PSPB) is divided into child-onset PSPB or juvenile type and adult or adult-onset PSPB[1,2]. Recurrent respiratory tract pneumonia is a disease caused by HPV in particular types 6 and 11[3,4]. The manifestation of this viral infection is an esophageal lesion in the respiratory tract and has repetitive growth properties. This disease might undergo remission after obtaining multiple microscopic laryngeal surgeries, but in other conditions, it continues to grow and spread along the respiratory tract making it more difficult to cure[5,6].

There are 76.47% of patients with implantation of the distal papilloma, even 21.57% has expanded to the trachea and bronchi. The tracheotomy was performed in 60.78% and 10% were tracheotomized more than once. A history of microscopic laryngeal surgery three times or more was obtained in 14.58% of patients. The transition sites include the inferior surface of the vocal cord, the vestibule of the rice, the nasopharyngeal mole palate, the trachea, the carina, and the bronchi, as well as the area around the tracheotomy stoma[7]. Virus type examination could predict the prognosis of disease travel[8].

Alternative therapies began to be considered based on the course of the disease. In-study therapy was included interferon-α, indole-3-carbinol, retinoid, bevacizumab, and cidofovir [9]. Human papillomavirus

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(HPV) part of the cellular-mediated immune-response[10], a virus belonging to the Papoviridae group that has no capsule, icosahedral, composed of double chains by 8000 pairs of DNA molecular bases[11,12]. There were 35 types that infect the genital mucosa, including high-risk types correlated with malignancy and low risk with condyloma or low-grade cervical intraepithelial neoplasia[12]. Human papillomavirus is considered an etiologic factor in PSPB with the discovery of an antigen or genome of HPV in papilloma tissue[13].

Mounts, et al (1982) examined capsid antigen in the surface epithelial nucleus that using immunoperoxidase techniques and the HPV genome in papillary tumor tissue was using hybridization analysis techniques. Papillary viral antigens were found in four of the 20 PSPB specimens, while the HPV genome was found in all specimens[13]. HPV transmission is suspected to be multifactorial, but in children, the most common risk factor is vertical transmission when the delivery from an HPV-infected mother. There was an increased risk of juvenile PSPB up to 200 times in women with genital condylomata accuminata. Transmission of adult type PSPB occurs through oral sexual activity with multiple partners[14]. Cesarean section operation is estimated to decrease the incidence of vertical HPV transmission[15].

Recurrent respiratory pneumonia is a problem for the THTKL expert because of the unknown aggressiveness correlation with HPV-6 and 11. This study intends to identify HPV types 6 and 11 in patients with PSPB is expected to be a factor in predicting the aggressiveness of the disease.

Method

An observational cross-sectional analytic was used by observing how HPV-6 and 11 in recurrent respiratory tract could lead to aggressive or non-aggressive disease[16]. Aggressive PSPB criteria were diseases that require surgical removal of papilloma three or more times within a year, have undergone a tracheotomy, or have implanted distal laryngeal papilloma on rigid or flexible laryngoscopy examination[17]. The non-aggressive PSPB criterion was a disease requiring surgical removal of papilloma less than three times within a year, never undergoing a tracheotomy and no implantation of the distal laryngeal papilloma on rigid or flexible laryngoscopy[17].

The PSPB patient the Outpatient Otolaryngology Dr. Soetomo General Hospital and undergoing Microscopic Laryngeal Surgery (BLM) at Surgical Installation Center from December 2012 to March 2013. The sample of this study came from the tissue of patients with PSPB who underwent BLM. The inclusion criteria were respiratory papilloma recurrence of all ages, medical records of patients during the complete treatment, and willing to participate the research by signing an informed consent. Patients will be determined by the group aggressive or not aggressive. The tissue of BLM surgery results conducted at Surgical Installation Center of Dr. Soetomo General Hospital was as a research sample. The sample was taken at the Universitas Airlangga Tropical Disease Institute for HPV-6 and 11 examinations using PCR (Polymerase chain reaction) method. The research data was tabulated in the data collection sheet and then processed statistically using the x2 test.

This research requires several tools in the implementation, that were Microscopic microscopic surgical devices include Optomic OP C-12 optical microscopes, Kleinsasser laryngoscope brand Storz 8590C, Aesculap straight or upturn forceps, cotton, lidocaine-ephedrine 8%, and suction set. The operative specimens were stored in a glass bottle filled with 0.9% normal saline liquor. Polymerase chain reaction checking tool is with PCR Thermal Cycler engine from Bioneer, HPV Intron 2x primer reagent PCR master mix. Primary HPV-6 in LCR region along 258-361 base pairs in the 5'-TAG GGG ACG GTC CTC TAT TC-3 'or 5'-GCA ACA GCC TCT GAG TCA CA-3' sequence. While the HPV-11 primer at the L1 region along 356 base pairs with the base sequence of 5'-GAA TAC ATG CGC CAT GTG GA-3 'or 5'-AGC AGA CGT CCG TCC TCG AT-3'.

Results

There were 15 patients that all of them was a child of PSPB type. All patients had complete data during the first diagnosis up to the control so that all were included in the study

Table 1. Distribution of distal laryngeal papilloma and history of tracheotomy of PSPB patients in Dr. Soetomo General Hospital Surabaya in December 2012 to March 2013

	Amount	Percentase
Distal laryngeal lymphoma		
Yes	10	66.67
No	5	33.33
History of tracheotomy		
Yes	11	73.33
No	4	26.67

Distal laryngeal lymphoma was found in 10 patients (66.67%), while 5 patients (33.33%) had a history of papilloma in the distal larynx. Tracheotomy history was found for 11 patients (73.33%), while 4 patients (26.67%) did not have a history of tracheotomy.

Table 2. Distribution of PSPB aggressiveness of PSPB patients in Dr. Soetomo General Hospital Surabaya in December 2012 to March 2013

Aggressiveness	Amount	Percentase
Aggressive	12	80
Non-aggressive	3	20

Aggressive papilloma was obtained from 12 patients (80%), while 3 patients (20%) were classified as non-aggressive PSPB.

Table 3 Distribution of HPV-6 and 11 patients with PSPB in Dr. Soetomo General Hospital Surabaya in December 2012 to March 2013

HPV Type	Amount	Percentase
HPV-6	6	40
HPV-11	9	60

Human papillomavirus type 6 was obtained in 6 patients (40%) and 9 (60%) were infected with HPV-11.

Table 4. Distribution of annual BLM history, distal laryngeal palsy, and history of tracheotomy on HPV-6 and 11 patients with PSPB in Dr. Soetomo General Hospital Surabaya in December 2012 to March 2013

	HPV Types		Total
	6	11	
History of BLM every year			
< 3x	3 (50%)	3 (50%)	6 (100%)
≥ 3x	3 (33.33%)	6 (66.67%)	9 (100%)
Distal laryngeal lymphoma			
Yes	3 (30%)	7 (70%)	10 (100%)
No	3 (60%)	2 (40%)	5 (100%)
History of tracheotomy			
Yes	4 (36.36%)	7 (63.63%)	11 (100%)
No	2 (50%)	2 (50%)	4 (100%)

The history of BLM <3x was obtained in three patients (50%) with HPV-6 and HPV-11. Whereas the history of BLM ≥ 3x was obtained by 3 patients (33.33%) with HPV-6 and 6 samples (66.67%) with HPV-11. The history of distal laryngeal papilloma was obtained in 3 patients (30%) with HPV-6 and 7 patients (70%) with HPV-11. Patients without the history of distal laryngeal papilloma consisted of 3 patients (60%) with HPV-6 and 2 patients (40%) with HPV-11. The history of tracheotomy was found in 4 patients (36.36%) in HPV-6 and 7 patients (63.64%) in HPV-11. Patients without a history of tracheotomy have the same amount. i.e., 2 patients (50%) in both HPV-6 and HPV-11 (Table 10).

Table 5 Aggressive correlation between PSPB and HPV-6 and 11 patients with PSPB at Dr. Soetomo General Hospital Surabaya in December 2012 to March 2013

	HPV types		**Fisher**
	6	**11**	
PSPB agrressivity Aggressive Non-aggressive	4 (33.33%) 2 (66.67%)	8 (66.67%) 1 (33.33%)	0.525

Aggressive recurrent respiratory febrile respiration consists of 4 patients with HPV-6 (33.33% of all HPV-6 patients) and 8 patients with HPV-11 (66.67% of all HPV-11 sufferers). Recurrent non-aggressive respiratory nasal passages consisted of 2 HPV-6 patients (66.67% of all HPV-6 patients) and 1 patient (33.33% of all HPV-11 sufferers). One-Sample Kolmogorov-Smirnov test results obtained p = 0.002. Fisher test results obtained p = 0.525 (Table 11).

Discussion

Table 1 shows the percentage of papilloma growing at the distal site of the larynx was 66.7% and the tracheotomy history was 73.3%. It shows that most sufferers have aggressive disease. The figure was proportional to Doyle. et al. (1994) that reported 60% had a history of tracheotomy and 55% had distal papilloma[18]. Wiatrak et al. (2004) reported 11% had the tracheotomy and 23.3% experienced distal papilloma growth[19]. The more aggressive course of the disease was seen from the many incidences of distal papilloma growth and the history of tracheotomy.

Table 2 presents an aggressive disease was 80%. Some of the aggressive PSPB findings was performed by Doyle. et al. (1994) with the amount 58.8% and Buchinsky. et al. (2008) was 81%. The results were in accordance with the above findings. The high findings of the aggressive PSPB were consistent with the high incidence of aggressive factors in the above discussion. All patients were classified as juvenile type PSPB[17,18].

Table 3 shows the percentage of HPV-6 infections by 40% and HPV-11 by 60%. No samples with double infection or HPV infection other than types 6 and 11. Wiatrak. et al. (2004) stated that patients with HPV-6 by 53.5%, HPV-11 was 39.7% and 6.9% had both types. Buchinsky. et al. (2008) get the percentage of HPV-6 infection by 60% and HPV-11 by 40%. The results of this study were in accordance with Buchinsky that the incidence of HPV-11 infection was more dominant than HPV-6[17].

Table 4 was the distribution of annual BLM history correlation, distal papilloma and tracheotomy history of HPV-6 and 11. The annual BLM history was grouped into two categories i.e., BLM history of more or equal to 3x (≥ 3x) within one year and BLM history less than 3x within one year period provided that the BLM history ≥ 3x was an aggressive type of PSPB category.

Patients with a history of BLM every year <3x consisted of 3 persons each with HPV-6 and HPV-11 infections, while patients with annual BLM category ≥ 3x consisted of 3 people with HPV-6 and 6 people with HPV-11. Human papillomavirus type 11 causes the patient to experience BLM every year ≥ 3x more often than BLM each year <3x (6 samples vs. 3 samples), while HPV-6 balanced in causing BLM every year ≥ 3x or <3x. Human papillomavirus type 11 tends to cause BLM surgery more frequently.

Papilloma with spread to the distal larynx was one indicator of disease aggressiveness. Seven patients with distal laryngeal palsy are known to be infected with

HPV-11, while a number of 3 patients infected with HPV-6. Patients with no history of distal laryngeal papilloma accompanied by HPV-6 were 3 patients and 2 patients with HPV-11. Wiatrak. et al. (2004) stated that 12 samples for papilloma in the trachea with HPV-11 infection and 11 patients with HPV-6. Patients without a history of papilloma in the trachea consisted of 28 patients with HPV-6 and 11 patients with HPV-11[19]. This study was in accordance with the literature that HPV-11 tends to cause patients to have distal laryngeal palsy compared with HPV-6.

Seven patients with a history of tracheotomy had the HPV-11 infection and 4 HPV-6 infected persons, while patients without tracheotomy obtained 2 patients with HPV-6 infection and HPV-11. Wiatrak. et al. (2004) stated that 21.7% of patients with a history of tracheotomy having HPV-11 of 5 samples and 1 sample with HPV-6. Patients without a history of tracheotomy consisted of 30 samples with HPV-6 and 18 samples with HPV-11[19]. The results were similar to the literature findings because the trends in HPV-11 cause the patient to require tracheotomy compared with HPV-6.

Table 5 shows the correlation between aggressiveness and HPV types 6 and 11. The assessment aspect of the correlation was whether there was an association between aggressive PSPB and HPV-11 versus non-aggressive PSPB with HPV-6. Aggressive respiratory febrile respiratory infections were 8 HPV-11 infected and 4 with HPV-6, while the non-aggressive PSPB consisted of 2 HPV-6 and 1 patients with HPV-6.

The results of the One-Sample Kolmogorov-Smirnov test was $p = 0.002$ which mean the data has abnormal distribution because of the value of $p < 0.05$. Fisher test got result $p = 0.525$ meaning that there was no correlation between aggressiveness PSPB and HPV types 6 and 11 because of $p > 0.05$.

Buchinsky. et al. (2008) obtained 43 aggressive PSPB samples with HPV-11 infection and 52 patients with HPV-6, while PSPB was not aggressive with HPV-6 infection of 19 patients and 4 patients with HPV-11. These results indicate that HPV-11 tends to cause more aggressive disease than HPV-6 and the correlation was statistically significant with $p = 0.02$ ($p < 0.05$).

Conclusion

There was no corrrelation between recurrent respiratory tract aggressiveness with HPV-6 and 11. More aggressive recurrent respiratory papillomas more infected with HPV-11 and HPV-6 than recurrent non-aggressive respiratory tract papillomas.

Ethical Clearance: The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

Conflict of Interest: None declared

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Investigation of IL-17F (rs763780) gene Polymorphisms in cases with Iraqi renal failure patients

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Abstract

Renal failure (RF) is common disease, it is decrease of kidney function, glomerular filtration rate (GFR) this use to estimate the kidney function reduction usually is less than 60 mL / min, depending on global Burden of Disease, and the disease is ninth cause of death in the world, According to the global Burden of Disease. Our study is investigated in the relationship between IL-17F (rs763780) genes polymorphic variants in renal failure patients in Iraq. This study was accompanied around Iraqi renal failure patients, the period from February to November 2018. This study included 70 patients from Abn-Sina Center for dialysis and kidney disease, and 30 healthy controls. Tetra-primer ARMS-PCR is rapid, effective and developed assay used our study for studying in genotyping polymorphism of IL-17F (rs763780) genes. Result of our report including the correlation between the genotypes of IL-17F (rs763780) and the incidence of renal failure, as the results show a non-marked difference between patients and controls when genotype T/T (OR = 0.75). Also, no significant difference between patients and control when genotype C/C (OR = 0.85), while the genotype T/C (OR = 2.23) show important difference between control and patients more than two times. In conclusion, IL-17F play an essential role as pro-inflammatory cytokine in renal failure patients and development disease.

Keyword: *- renal failure patients, rs763780 , ARMS-PCR and IL-17F gene.*

Introduction

Kidney is important organ, its function is regulation of the blood composition and volume, filtration the urine by eliminating the metabolic wastes, secretion of the end metabolism products and maintenance the acid or balance in the body via regulate fluids and electrolytes (1). Renal failure (RF) is common disease , usually is defined as the decrease of kidney function, glomerular filtration rate (GFR) this use to estimate the kidney function reduction usually is less than 60 mL / min (2) .

Interleukins -17F (IL-17F) types of cytokine produced from Th 17 cells. act on Stimulate inflammation and causing organ injury , also act on enhancing produce of pro-inflammatory cytokines by resident cells , that lead to increase penetration of neutrophils to affected organ and induce inflammation and damage in this organ such as kidney (3,4) . All these interleukins are similar in structure protein, but have in the N termini large sequence divergence.

Both IL-17A and IL-17F genes are located on chromosome 6p12.3-q13, and each consists of three exons and two intron the transcribed occurs in opposite direction, cytokine genes resulting together during gene replication also use same regulatory elements. In human, about of 50 % sequence identity between IL17A and IL17F, both of these types produce homodimer and heterodimer proteins (5). Cytokine production may be effected by cytokine gene Polymorphisms through effect this gene on transcription. Single nucleotide polymorphisms (SNPs), acts on induce susceptibility or resistance to any infection, also may be used to diagnose some factors that contribute to cancers or inflammatory disease because SNP occurs in regulatory regions for cytokine gene [(6)]. Tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR), these methods is very important to detect (SNP) genotyping because have several features rapid, ease to perform, an inexpensive, and accurate method [(7)].

This method is ARMS-PCR modification; four primers are used. Two inner primers were allele specific and lead to produce two bands are different in size (lower molecular weight), this band appears depend on wild or mutant allele is present. And two external or outer primers produce non–allelic was control band. And the location of mismatch is middle for the specific allele primers and use four primers in these methods (8). While in ARMS-PCR the location of mismatch in 3′ end for the specific allele primers and utilize five primers. T-ARMS-PCR assay represent successfully methods to detect SNPs and others genetic or biological markers that associated with different disease such as effect TNF-α gene polymorphism in chronic pancreatitis patients (9).

Aim of our study is to determining the association and relationship between IL-17A (rs2275913) genes and Iraqi renal failure patients .

Material and Method

This study was accompanied around Iraqi renal failure patients, the period from February to November 2018. This study included 70 patients from Abn-Sina Center for dialysis and kidney disease and 30 healthy controls.

1-Collection of samples:-

Study groups Samples were Collected 2 ml from whole blood in EDTE tube until extraction human genome. And use to investigate about IL-17F (rs763780) genes in study groups.

2-Genomic DNA Extraction:

The DNA was extracted by use special kit (Geneaid, Korea) according to the company directions.

3-T-ARMS-PCR and Primer Design:

Batchprimer3 program was used for designing, several types of primers are used included generic primers (including SSR primers with SSR detection) and SNP genotyping primers (including allele-specific primers, single-base extension primers, and tetra-primers for tetra-primer ARMS PCR), furthermore, DNA sequencing primers [(10)]. Show table 1. T-ARMS-PCR Analysis occurs by using Accupower PCR Premix (Bioneer, Korea) whole volume of 20 µL included DNA (4 µL), outer primers (1 µL), inner primer (1 µL) and add D.W (12 µL). PCR process is start at (95) ºC for five minutes, then the second step is start at (95) ºC for twenty second, then annealing is start for twenty sec , annealing was carried out at 72 ºC for twenty seconds and finally the process is finished at (72) ºC for three minute. Then electrophoresis are used for emigration of all PCR products after stained it by ethidium bromide.

Statistical Analysis

Our study used SAS program for statistical Analysis for differentiates between in parameters wherever LSD test was trying to compassion between groups [(11)]. Whereas odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using MedCalc for Windows, version 18.10 [(12)].

Table 1 the used primers used in ARMS–PCR technique

SNP	System	Primer	Allele	Tm	Amplicon
rs763780 IL17F	Forward inner primer	GAGTGGATATGCACCTCTTACTGCAAAC	C	62	158
	Reverse inner primer	CGTCACCCCTGTCATCCAACA	T	62	106
	Forward outer primer	AGACAGGACTTGTTGCAGAGCACTG		62	215
	Reverse outer primer	ATGAATTCCGTTCCCATCCAGC		62	

*Tm Temperature of melting

Result

The renal failure patients group consisted of 70 patients, 40 males (57%) and 30 females (43%), with a mean age of 49.5 ± 5.91 years. The healthy control group consisted of 30 subjects, with a mean age of 30.3 ± 3.62 years.

In IL-17F (rs763780), the fragment lengths for the specific amplicons were as follows; 158 bp for the C allele and 106 bp for the T allele. The presence of two specific amplicons for an SNP indicates heterozygosity. In addition, non-indicative amplicons resulting from the common primer pairs are always present (215 bp for the two outer primers) show figure 1.

The IL-17F (rs763780) TC, CC and TT genotypes figure (1) were detected in 5 (7%), 4 (6%) and 61 (87%) patients, and in 1 (3%), 2(7%) and 27 (90%) controls, respectively. Frequency of genotype TC, CC and TT significantly increase in the groups of patients compared with control.

The genotype TT recorded highly ratio in both groups patients and control (57-90%) which made it the common genotype for this locus in Iraqi population. both homozygous genotype showed preventive fraction according odds ratio (0.85, 0.75), while TC genotype according odds ratio consider as etiological fraction. The analysis of the allele impact, including both genotypes that containing C allele showed no significant association in patient group comparing with control (p= 0.829). According OR, allele C tend to be risky allele, while allele T tend to be a preventive allele (Table 2).

According to the sex the results in Table (3) the genotype TT showed as preventive fraction according odds ratio (0.34), while TC and CC genotype according odds ratio consider as etiological fraction (3.22, 2.35) respectively. Also according OR, allele C tend to be risky allele, while allele T tend to be a preventive allele.

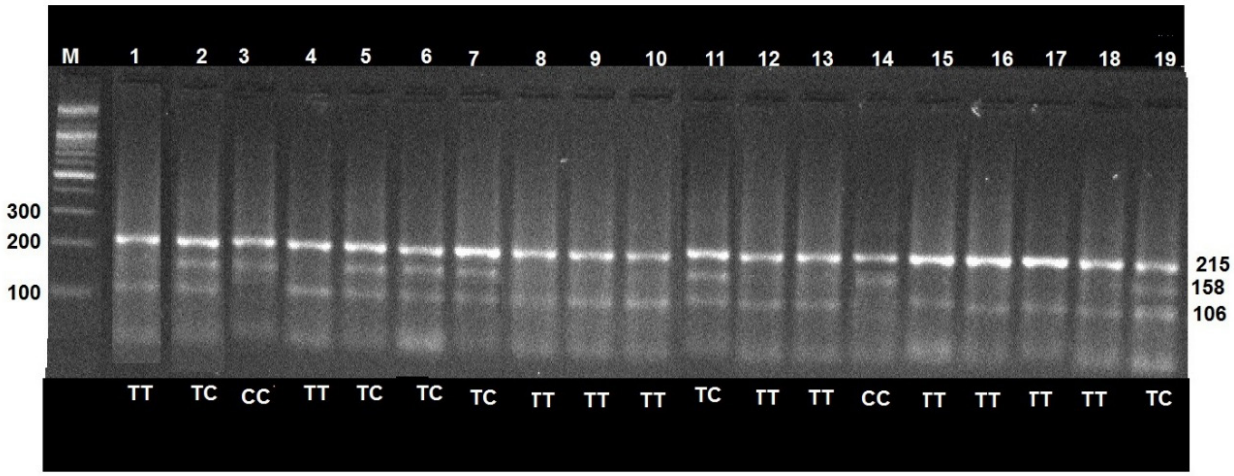

Figure (1): electrophoresis of samples on agarose gel by using T-ARMS-PCR assay: The IL-17F (rs763780) locus genotyping by T-ARMS-PCR resolved on a 2% agarose gel stained with ethidium bromide (45 min, 150V/cm, 1X Tris-acetic buffer). 215 bp, the bands are amplicon, and T and C allele-specific bands are 106 bp and 158bp amplicons respectively (Bioneer, Korea).

Table (2) Distribution of genotypes for the IL-17F (RS763780) gene samples of patients and healthy controls.

SNP	Genotype	Patients* n=70 n (%)	Control n=30 N (%)	OR(95%CI)	P.value
Rs763780 IL17F	T/C*	5 (7%)	1 (3%)	(0.26-19.30) 2.23	0.415
	C/C	4(6%)	2 (7%)	(0.15-4.79) 0.85	0.585
	T/T	61(87%)	27 (90%)	(0.19-2.94) 0.75	0.488
	Allele frequencies				
	T	127(91%)	55(92%)	0.88(0.301-2.61)	0.829
	C	13(9%)	5(8%)	1.12(0.39-3.31)	0.829

*only present significant in TC genotype between two groups.

Table (3): prevalence of IL-17F (Rs763780) gene in patient samples according to sex.

SNP	**Genotype**	**Male* n=40 n(%)**	**Female n=30 N (%)**	**OR(95%CI)**	**P.value**
Rs763780 IL17F	T/C*	4(10%)	1(3.3%)	(0.35-29.36) 3.22	0.281
	C/C*	3 (7.5%)	1(3.3%)	(0.24-22.95) 2.35	0.423
	T/T	33 (82.5%)	28 (93.4%)	(0.07-1.71) 0.34	0.164
	Allele frequencies				
	T	70 (87.5%)	57(95%)	0.36(0.1-1.4)	0.143
	C	10 (12.5%)	3(5%)	2.71 (0.71 – 10.33)	0.143

*only present significant in TC and CC genotype between two genders.

Discussion

Recently , some studies were improving CD4+ differentiation to Th1 ,Th2 and Th17. Th17 able to producing IL-17 family such as IL-17A and IL-17F and these are playing essential role in autoimmune disorders by stimulation (acute and chronic) inflammation such as renal disorders. Also macrophages, mast cells and neutrophils are able to produce IL17A [13].

Renal failure occurs in male more than female and the mean age is 49.5 ± 5.91 years respectively , this result agree with [14] this study group consisted of 53 Renal failure patients and present 28 males and 25females , with a mean of age 51.0±13.6 years. Also agree with [15] in this study included 100 sample: males sample was 61 (61%) and females was 39 (39%), and age range about (44.7 ± 22.1 year).

Polymorphisms located for IL-17A and IL-17F within genes coding play a role as genetic factors, and that lead to associate with occurring disease.

In IL17F polymorphisms the genotype T/C (OR = 2.23) show a significant difference between patients and control more than two times. This result similarity with [16] this study show IL-17F frequencies allele A were significantly higher in immune thrombocytopenia for patients and controls : 31.85% and 18.98% respectively . Specifically, patients have higher frequencies in AA and AG genotypes compared with controls [AA: 17.12% vs. 9.49% and AG: 29.46% vs. 18.98%].

This result is leading to role of AA and AG genotypes for IL-17F as risk factors in renal failure disease. IL-17F polymorphism have association with several disease include : IBD [17], Development of asthma [18] , autoimmune thyroid diseases [19], acute myeloid leukaemia [20] and recently in MS [21].

Another study is improves the polymorphisms in IL-17A and IL-17F genes play a significant role in Rheumatoid Arthritis [22]. In Southern Brazil, [23] were show the relationship between IL17A rs2275913 polymorphisms and chronic periodontitis, while IL17F polymorphism was not associated with same disease. IL-17A and IL-17F play main role in development of inflammation and others act on host defense against infection by prompting gene expression that encoding for several pro-inflammatory cytokines, chemokine and antimicrobial [24, 25] . IL-17F and IL-17A have same sequence in amino acid ant both have same function. The cytokine have able for activation of neutrophil [26] .

Conclusion

IL-17F play an essential role as pro-inflammatory cytokine in renal failure patients and development disease. And renal failure disease associated with heterozygote allele.

Conflict of Interest:- No there any conflict of the interests that related with this paper.

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Ethical Clearance:- Local ethics committee in Diyala University is approve results of our study.

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A Descriptive Study to Assess the Problem Faced by the Adolecents of Alcoholic Parent

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Abstract

A descriptive study to assess problems faced by the adolescents of alcoholic parent in selected community area, Kancheepuram district, Tamil Nadu. Objectives of the study to assess the problems faced by the adolescents of alcoholic parents and to find out association between the problems faced by the adolescents of alcoholic parent with their selected demographic variables. Non experimental approach and descriptive design used for this study. Samples were selected by using purposive sampling technique. The sample consisted of 50 adolescents. Self-structured questionnaires for assessment of demographic variables and used to assess the problem faced by adolescents of alcoholic parents. The data collected from 50 adolescents (Grils and boys) in the age group between15 to 18 years. Study show that 9(18%) severe problems, 31(62%) moderate problems and 10(20%) mild problems. Study shows that there was significant association between the levels of problem faced by the adolescents of alcoholic parent with age and occupation of alcoholic parent (p value =< 0.05).

Key words: *assess, problems, alcoholic parent, adolescents.*

Introduction

Alcoholism today clearly seen as a family disease ravaging not just the individual who drinks excessively but also the entire family. The primary victim is the individual who drinks excessively, no doubt, but the family members are also affected with just the same intensity, if not more. Families complicated by alcoholism can be considered as families in a never ending series of crisis. Alcoholism disrupts even normal routine family tasks and function, increase conflicts and demands adaptive responses from family members who simply do not know 'how' to respond appropriately. The social stigma associated with alcoholism, the economic drain on the family's resources, the threat to physical well-being job insecurity all compound to the problem[4]

An estimated 6.6 million children under the age of 18yrs live in household with at least one alcoholic parent. Current research findings suggest that these children are at a risk for a range of cognitive, emotional and behavioural problems.[1] Between 15 and 20 per cent of Indian people consume alcohol and, over the past twenty years, the number of drinkers has increased from one in 300 to one in 20. In globally today in there are an estimated 28 million children who have alcoholic parents. The adolescents world wide 10 to 24 years is 356 million,[3] Tamil Nadu 1.24 crore, India 253 million. According to 2012 in India report approximately 1.28 Million Parents have a alcohol addiction problem.11 million of those children under the age of 18. In 2012 about 3.3 million death or 5.9% of all global death due to alcohol consumption.[3]

Method

Adolescents (Girls and Boys) who were in the selected community area (pooncheri & payanoor) in the age group between 15 to 18 years residing in a selected community area, Kanchipuram district, Tamil Nadu,, 50 samples were choosen for the present study.

Design

In this study non experimental research approach and Descriptive research design adopted, Purposive sampling technique adopted. Self-structured questionnaires consist of 20 questions used and self -structured questionnaires to assess demographic variables. Each sample will take 30 minute to completion of the data.

Instrument/material

Self-structured questionnaires consist of 20 questions used and self -structured questionnaires to assess demographic variables. Each sample will take 30 minute to completion of the data. There are Two Section (A&B) Section A consist of self-Structured Questionnaires to assess demographic variable & Section B Self-Structured Questionnaires to assess the problem faced by the adolescents of alcoholic parent consist of 20 questions.

SCORING INTERPRETATION	
SCORING	**INTERPRETATION**
27-40	Severe
26-14	Moderate
13-1	Mild

Procedure

The written informed consent was obtained from the parent and guardian as well as from the study participants. Permission letter was obtained from the HOD of Mental Health Nursing department, HOD of Community Health Nursing department, Community area, UG Committee and Human Ethical Committee.

Statistical Analysis

Descriptive statistics like frequency distribution, percentage, mean, standard deviation and inferential statistics like chi square was used to analyse the data.

Result:

The study finding revealed that,

❖ Our study results shows that assessing the levels of problems faced by the adolescent of alcoholic parent 31(62%) moderate problems, 10(20%) mild problem and 9(18%) severe problem.

Table 1 Frequency and percentage distribution of problems faced by the adolescents of alcoholic parents.

S.no	Problems Level	Frequency	Percen-Tage
1.	Mild problems	10	20%
2.	Moderate problems	31	62%
3.	Severe problems	9	18%

(N=50)

Discussion

The study was conducted to assess H1 there is a significant association between the levels of problems faced by the adolescents of alcoholic parent with selected demographic variable and to assess H_2 There is significant association between the problems faced by the alcoholic parent and selected Demographic data. The hypothesis H_1 and H_2 was accepted suggestion that there will be significant association between the level of problems faced by the adolescents of alcoholic parent and the selected demographic characteristics of adolescents. The finding revealed that the problems has statistically significant with age of adolescent and occupation of alcoholic parent p value < 0.05 level and other variables are not significant. The limitation of the study was the data collection period was limited for one week. Large number of sample could not be obtained due to difficulty found the samples in the age group of 15 to 18 years of adolescent of alcoholic parent for data collection. Based on the findings of the study, following recommendation are put forth the study can be done on a large sample thereby findings can be generalized. a similar study can be conducted using teaching strategies like structured teaching programme, video assisted teaching. The nurse researcher will help us to know the adolescents with severe problems and give them awareness and psychological support and helpline numbers to prevent or to reduce the problem. On the basis of conducted research it can be concluded the assessment of problem faced adolescent by the adolescent of alcoholic parent has a important determination of early prevention from complication. Problems faced by adolescent of alcoholic parent and associated factor in previous study shows that the study to assess the problem faced by the adolescents of alcoholic father. The study is consisted of 60 adolescents . Study finding data shows that most (61.66%) of the children were females and 38.3% were males. The data presented was shows no association between physical health status and selected demographic variables like sex, age, duration of father's alcoholism, treatment received for alcoholism except to income of family per months at significance of 0.05 level. **Deepa shaji Thomas et al (2012).**

Ethical Clearance: Chettinad Academy of Research & Education – Institution Human Ethics Committee.

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Confilct of Interest: Nil

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Demographic, Clinical and Hormonal Characteristics as Predicting Factors Affecting the Outcome of Laparoscopic Ovarian Drilling in Women with Poly Cystic Ovary Syndrome

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Abstract

Background: Laparoscopic ovarian drilling as a second line of treatment modality for those PCOS patients failed to respond to clomophine citrate they may have the benefit of avoiding the side effects of long term drug therapy, lower costs and less time for successful ovulation and conceprtion compared to medical treatment. **Objectives:** Before doing LOD recognize factors that affect the outcome of the procedure for selecting patients to avoid unnecessary or unbeneficial intervention. **Method:** 143 anovulatory infertile PCOS patients followed up for 6 months after LOD for ovulation with pre-operative assessment for demographic characters and serum hormone levels (LH, FSH, free testosterone, AMH and prolactin). **Results:** Ovulation was demonstrated in 59.4%. Regression analysis showed significantly higher rates of ovulation among women < 5 years duration of infertility (OR = 3.35, 95% CI = 1.47-7.65), LH > 12 mIU/ml (OR = 4.18, 95% CI = 1.28-13.63) and free testosterone <4.5 ng/ml (OR = 3.22, 95% CI = 1.08-9.59). No significant (p = 0.078) association was detected between the ovulation rate and the AMH level, but it is evident that the OR was around 2 among women with AMH level of less than 7.7 ng/ml.

Conclusion: preoperative LH, free testosterone and duration of infertility regarded as 3 important predicting factor for success of LOD

Keywords: *PCOS:polycystic ovary syndrome; LOD: laparoscopic ovarian drlling, Ovulation.*

Introduction

Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrinopathies affecting about 5-10 % women of reproductive age group. There is a great controversies regarding the diagnosis and mode of treatment of this syndrome[1], although experts have been succeeded to develop a universally accepted criteria for diagnosis of PCOS[2] but adoption of optimal treatment had not yet reached. Wedge resection of ovary which is first described by Stein and Leventhal [3] for treating anovulation by laparotomy has been developed to less traumatic ovarian surgery through the laparoscope by Gjonnaess [4]. LOD is currently recommended as an alternative second line approach, safe and cost effective to gonadotropins for those with anovulatory infertility, without the risk of multiple pregnancy and ovarian hyperstimulation[5], still there is no well-established mechanism of action of LOD and therefore we can not exactly answer the question why some PCOS patients not responding to this treatment[6], and it is not known whether it will exerts it is effect directly on the ovary or through systemic endocrine pathway [7], it may act by destroying ovarian androgen-producing tissue and reducing the peripheral conversion of androgens to estrogens. However, others believe that ovarian diathermy works by increasing the sensitivity of the ovaries to endogenous FSH, and that only a minimal amount of thermal injury is required [8]. A fall in the serum levels of androgens and LH and an increase in FSH levels have been demonstrated after ovarian drilling [9],

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The aim of the present study was to identify some of the factors that affect the outcome of LOD for improvement of success in women with PCOS for better selection of the patients that get benefit from this intervention and avoidance of unbeneficial surgery.

Materials and Method

The current study followed 143 subfertile women with anovulation failed to respond to oral medical therapy (persistant anovulation despite maximum dose of oral therapy for up to 6 cycles) consulting the outpatient gynaecology and fertility clinic in Maternity Teaching Hospital, public maternity hospital in Erbil, Iraq from September 2016 to July 2018, All included patients in the study diagnosed to have PCOS based on Rotterdam criteria include at least two of the three criteria: oligo-anovulation ,clinical and /or biochemical evidence of hyperandrogenism, and sonographic features of polycystic ovaries[2]. Written informed consent was obtained from each participant after explaining the study purpose, procedures and follow up time. Those associated with tubal and male factors infertility, endocrine disorders (thyroid disease, cushing s syndrome and androgen secreting tumor) AMH < 4ng/ml and FSH >12.5mIU/ml are all excluded from the study. All enrolled women underwent transvaginal ultrasound preoperatively.

Preoperatively baseline hormones were measured at the day 2-4 of cycle for serum levels of LH, FSH, testosterone ,SHBG, AMH and prolactin, the LH:FSH ratio and FAI calculated. Serum hormone levels were measured by Elecsys machine (Roche Diagnostics, Hitachi, Switzerland) for determination of human LH, FSH, testosterone, prolactin and SHBG levels. AMH was measured by Enzyme linked immunosorbent assay (ELISA) kit.

After cycle in the proliferative phase (progesterone induced withdrawal bleed or spontaneous) LOD performed by or in the presence of first author to ensure that the same technique was used for each patient by a laparoscope (Karl Storz, Germany) with assessment of tubal patency with methylene blue and macroscopically uterus, tubes and ovaries were examined. The technique described by[9] four punctures performed per ovary, each for 4 s to a depth of 4-6 mm, a monopolar coagulating electrical current at 40 W power setting used, at the end of the procedure pelvis was irrigated with lactated Ringer's solution and 500 cc was left intraperitoneally. Ovulation and pregnancy followed up to 6 months(ovulation defined as the presence of at least one mature graffian follicle measuring ≥18mm by transvaginal ultrasound and serum progesterone level at the day of 21 as indicators of ovulation (serum progesterone level ≥13nmol/l was considered a strong indication for ovulation[10].

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 22). Student's t test of two independent samples was used to compare means of two groups. Chi square test of association was used to compare between proportions. When the expected count of more than 20% of the cells of the table was <5, Fisher's exact test was used. Logistic regression analysis was used where the dependent variable was binary categorical (ovulation whether yes or no). Variables found (by univariate analysis) to be significantly associated with the dependent variable were entered into the regression model as independent variables (covariates). A 'p' value of ≤ 0.05 was considered as statistically significant.

Results

The study included 143 infertile women with polycystic ovary syndrome. Their mean age ± SD was 29.52 ± 4.58 years, ranging from 20 to 39 years. The median was 29 years. Table 1 shows that around half (45%) of the students aged 25-29 years, and the duration of infertility of 64.3% of them was ≥ 5 years. The type of infertility of the majority of the women (76.2%) was primary. Regarding the menstrual pattern, it was irregular in the majority (88.8%) of the women. It is evident that only 9.8% of the women were of normal weight, 30.1% were over-weight, and 60.1% were obese.

Table 2 shows that the rate of ovulation was significantly high among women with less than five years of infertility (76.5%) compared with 50% among women with ≥ 5 years of infertility (p = 0.002). No significant association was detected between ovulation with age (p = 0.165), type of infertility (p = 0.264), menstrual pattern (p = 0.421), BMI (p = 0.138), hirsute (p = 0.377), and acne (p = 0.688).

The rate of infertility was significantly high (79.2%) among women with LH level of more than 12mIU/ml compared with 55.5% among women with LH level of ≤ 12mIU/ml (p = 0.031). The rate of ovulation was also high (63.4%) among women with free testosterone level of less than 4.5ng/ml compared with 35% among

women with free testosterone of ≥ 4.5ng/ml. Ovulation occurred in 66.7% of women with AMH level of less than 7.7ng/ml compared with 50% among women with AMH level of ≥ 7.7ng/ml (p = 0.044). No significant association was detected between the rate of ovulation with FAI (p = 0.096) and LH FSH ratio (p = 0.166).

Table 4 shows no significant differences between the means of hormones (SHBG, prolactin, and FSH) of women who have ovulated and the means of hormones of women who didn't ovulate (p = 0.173, p = 0.598, p = 0.0.058 respectively).

Regression analysis (Table 5) showed significantly higher rates of ovulation among women with less than five years duration of infertility (OR = 3.35, 95% CI = 1.47-7.65), high LH of more than 12mIU/ml (OR = 4.18, 95% CI = 1.28-13.63), low free testosterone of less than 4.5ng/ml (OR = 3.22, 95% CI = 1.08-9.59). No significant (p = 0.078) association was detected between the ovulation rate and the AMH level, but it is evident that the OR was around 2 among women with AMH level of less than 7.7ng/ml.

Table 1. Basic characteristics of the studied sample.

Age (years)	No.	(%)
20-24	19	(13.3)
25-29	65	(45.5)
30-34	33	(23.1)
35-39	26	(18.2)
Duration of infertility (years)		
< 5	51	(35.7)
≥ 5	92	(64.3)
Type of infertility		
Primary	109	(76.2)
Secondary	34	(23.8)
Menstrual pattern		
Regular	16	(11.2)
Irregular	127	(88.8)
Body mass index (Kg/m2)		
< 25	14	(9.8)
25-29	43	(30.1)
≥ 30	86	(60.1)
Total	143	(100.0)

Table 2. Ovulation rate by the studied factors after LOD.

	Ovulated		**Non ovulated**		**Total**		
	No.	**(%)**	**No.**	**(%)**	**No.**	**(%)**	**p**
Age (years)							
20-24	15	(78.9)	4	(21.1)	19	(100.0)	
25-29	40	(61.5)	25	(38.5)	65	(100.0)	
30-34	16	(48.5)	17	(51.5)	33	(100.0)	
35-39	14	(53.8)	12	(46.2)	26	(100.0)	0.165
Duration of infertility (years)							
< 5	39	(76.5)	12	(23.5)	51	(100.0)	
≥ 5	46	(50.0)	46	(50.0)	92	(100.0)	0.002
Type of infertility							
Primary	62	(56.9)	47	(43.1)	109	(100.0)	
Secondary	23	(67.6)	11	(32.4)	34	(100.0)	0.264
Menstrual pattern							
Regular	11	(68.8)	5	(31.3)	16	(100.0)	
Irregular	74	(58.3)	53	(41.7)	127	(100.0)	0.421
BMI							
< 25	11	(78.6)	3	(21.4)	14	(100.0)	
25-29	28	(65.1)	15	(34.9)	43	(100.0)	
≥ 30	46	(53.5)	40	(46.5)	86	(100.0)	0.138
Hirsute							
Yes	67	(61.5)	42	(38.5)	109	(100.0)	
No	18	(52.9)	16	(47.1)	34	(100.0)	0.377
Acne							
Yes	44	(57.9)	32	(42.1)	76	(100.0)	
No	41	(61.2)	26	(38.8)	67	(100.0)	0.688
Total	85	(59.4)	58	(40.6)	143	(100.0)	

Table 3. Ovulation rate by the hormone level after LOD.

	Ovulation						
	Yes		No		Total		
	No.	(%)	No.	(%)	No.	(%)	p
LH mIU/ml							
≤ 12	66	(55.5)	53	(44.5)	119	(100.0)	
> 12	19	(79.2)	5	(20.8)	24	(100.0)	0.031
Free testosterone ng/ml							
< 4.5	78	(63.4)	45	(36.6)	123	(100.0)	
≥ 4.5	7	(35.0)	13	(65.0)	20	(100.0)	0.016
AMH ng/ml							
< 7.7	54	(66.7)	27	(33.3)	81	(100.0)	
≥ 7.7	31	(50.0)	31	(50.0)	62	(100.0)	0.044
FAI							
< 5	8	(57.1)	6	(42.9)	14	(100.0)	
5-14.9	46	(68.7)	21	(31.3)	67	(100.0)	
≥ 15	31	(50.0)	31	(50.0)	62	(100.0)	0.096
LH FSH ratio							
< 2	27	(51.9)	25	(48.1)	52	(100.0)	
≥ 2	58	(63.7)	33	(36.3)	91	(100.0)	0.166
Total	85	(59.4)	58.0	(40.6)	143.0	(100.0)	

Table 4. Means of hormones by ovulation status after LOD.

	Ovulation		No ovulation		
Hormones	Mean	(+SD)	Mean	(+SD)	P
SHBG nmol/l	19.59	(+8.04)	21.68	(+10.18)	0.173
Prolactin ng/ml	19.43	(+9.73)	20.27	(+8.83)	0.598
Serum FSH mIU/ml	4.70	(+1.00)	4.39	(+0.94)	0.058

Table 5. SPSS output of binary logistic regression analysis where ovulation was the dependent variable.

	B	p	OR	95% C.I.for OR	
Duration of infertility (years)				**Lower**	**Upper**
< 5	1.21	< 0.001	3.35	1.47	7.65
≥ 5 (reference)			1.00		
LH					
> 12	1.43	0.018	4.18	1.28	13.63
≤ 12 (reference)			1.00		
AMH					
< 7.7	0.69	0.078	1.99	0.93	4.28
≥ 7.7 (reference)			1.00		
Free testosterone					
< 4.5	1.17	0.036	3.22	1.08	9.59
≥ 4.5 (reference)			1.00		
Constant	-1.75	0.003	0.17		

Discussion

Ovulation rate reported in our study was 59.4% and pregnancy rate 32.9% many studies recorded higher rates in their results[4,9,11]. For prediction of response we focused on ovulation being the primary outcome after treatment.

In the current study, there was no association between the age group, type of infertility, menstrual pattern, presence or absence of hirsute or acne and rates of ovulation after drilling, the same results recorded by other studies[6,12] while in another study age < 30 years considered as a significant predictor for success [13] this difference may be due to that their studied population more in younger age. BMI exhibited a great variation regarding it is effect on the success of LOD, some authors found that lean women respond more than obese [13,14], on the contrary an author correlated high BMI with the success [12], although in our results and previous studies reported that BMI had no influence on overall ovulation rate [11,15]. Shorter duration of infertility < 5 years appear to be the best determinant in the present study and before [6,13,16], this is not in agreement with the a study of [12] found no association, this can be explained that longer duration of subfertility may be associated with other causative factors and chronic anovulation needs more time for cure.

It was also noted that preoperative serum LH level were significantly higher among those who achieved ovulation after surgery with a " cut-off " of 12mIU/ml, this is in agreement with several other studies [11,15] using the same value and same results, Logistic regression in the study of [6,16] using 10 IU/L "cut-off " value also recognized high LH as a predicting factor for success.

Many researches considered AMH as a predicting factor for diagnosis and response to LOD, High AMH associated with poor response may be due to severity of the PCOS condition in these women. Although association of AMH and ovulation revealed significant ($p = 0.044$) in our data, but by regression analysis AMH is not regarded as a predicting factor. In contrary another author [7] reported that AMH affect the outcome after LOD using the same "cut-off" value (7.7ng/ml), a possible explanation is that number of the holes by electrocautery and degree of tissue destruction may affect the success.

Favorable outcome was recorded in those with low free testosterone level <4.5 ng/m, this result was consistent with [6]. Although this is in disagreement with earlier results [15,16] can be explained by smaller sample size in their study or inadequate destruction of androgen producing ovarian tissue, so an adjusted thermal dose based on ovarian volume [17] to be fully elucidated.

On the other hand LH/FSH ratio, FSH, SHBG, FAI and prolactin have no impact on the outcome after LOD, AL-Ojaimi[12] also recorded no significant association with FSH and prolactin level while LH:FSH ratio showed significant while Dubela et al[14] found that high SHBG is significant for success. Inability to follow-up patients for longer periods to record pregnancy and live-birth rates was the main limitation of our study.

Conclusions

Identifying factors that affect outcome of LOD helps in increasing success rate, it is an important issue not only to improve outcome but also to avoid unnecessary surgery and it is complications. Higher rate of ovulation found among those with less than 5 years duration of infertility, LH > 12mIU/ml and free testosterone < 4.5ng/ml.

Conflicts of Interest: No conflicts of interest

Ethical Clearance: The Research Ethics Committee of HMU, College of Medicine, approved the study proposal**.**

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Stress Perception and Stress Response against Internet Psychological Addiction (IPA) on Adolescents (Psychoneuroimmunology Approach)

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Abstract

Background: Phenomenon of IPA in long-term effect is dominated by negative effect that has risk in undergoing stress. Stress perception will activate the change of physiology system in the body in form of stress response through activating *Hypothalamic-Pituitary Adrenocortical* (HPA) that will increase cortisol production. **Purpose**: investigate how the correlation between stress perception and stress response against IPA for adolescents based on psychoneuroimmunology (PNI) concept. **Method**: This research used cross-sectional design. There were 39 Senior High School students, who were indicated to undergo internet psychological addiction and they were as the sample of this research. **Result**: There were 39 students in six Senior High Schools in Makassar, Indonesia, who were identified to undergo IPA. Stress perception in most of adolescents who underwent IPA was high (61.5%), particularly for adolescent who underwent high internet addiction, and so as the stress response on IPA, which was also high (53.8%), which described health quality for IPA. **Conclusion**: there was a significant correlation between stress perception and stress response on adolescent who underwent IPA based on psychoneuroimmunology approach.

Keywords*: internet psychological addiction, adolescents, psychoneuroimmunology, stress perception, stress response*

Introduction

Psychoneuroimmunology is an integrated concept regarding function of immune regulation for defending homeostasis. PNI concept views on the correlation among stress, immune system, and health. Stress is consisted of stress-perception and stress response[1]. Stressor is a cause for stress-perception and stress response[2]. Ader in Putra[3] showed that regulation of immune system or immunoregulation, which firstly was believed as autonomous process, was proven to be influenced by nervous system performance through learning proses that was occurred in central nervous system. Thus, it was proven unautonomous[3].

Limitation of PNI according to Ader-Cohen in Putra[3], related with beviour-neuroendocrine-imune system. Definition of stress that was stated was closer with Selye in Putra[3] and it was as non-specific response of the body to any demand with biological concept. However, definition of stress in PNI also used Eric Linderman-Gerald Caplan concept as psychological state involving cognition and emotion, which gave limitation of stress concept in psychological side, whether, psychic stress was defined as cognition that was learning process result or stress perception which could be observed based on modulation of immune response (stress response)[3].

The criteria of IPA are: excessive using internet (>6 months); making internet as a dominant activity (salience); undergoing internal and external conflict; appearing euphoria while being able to access internet (mood modification); increasing needs to access internet (tolerance), uncomfortable feeling when the use of internet was limited (withdrawal)[4-7].

Phenomenon of IPA on adolescents has become global epidemic. Research result regarding IPA in long-term effect is dominated by negative effects which have risk to undergo depression, anxiety, aggressive behavior,

social isolation, psychiatric symptoms, interpersonal problems and stress[8-10]. Even, negative effect of IPA is as dangerous as alcohol and narcotics addiction[11,12]

Phenomenon of internet addiction will influence other activities and this will impact to adolescent's physical, cognitive, socio-emotional, and spiritual development disorders that contribute to personal distress (dangerous and destructive stress)[13,14]

According to conducted observation, it was found low productivity on adolescent who underwent IPA in Makassar. Interview result with students in several Senior High Schools and IPTEK-KOM research[15] showed that they used internet excessively and they made the internet as dominant activity in daily life (Salience) until their time was wasting more only for accessing internet which was not for learning process.

Nevertheless, if the internet was used for searching information for completing homeworks or school tasks, the advantages of internet itself could raise enthusiasm for learning process (eustress), but if it was used excessively, it could cause distress. In other words, internet could cause the students did not want to learn anymore because they could not concentrate well, they were easy to forget (low memory), difficult to understand, and as the impact, they could have poor score at school, and also could have poor sleep quality. This would impact to their learning achievements at school, which caused high academic stress[16].

Internet could not be separated from adolescent's life. Almost all their needs could be fulfilled by internet[17]. There were pleasant experiences while accessing internet (mood modification) that was triggered by dopamine spending which caused them would repeat the experience until their time intensity that was needed to access the internet added (tolerance). Although the time intensity to access the internet was controlled, they would try to repeat it with longer intensity (relapse) because they had felt pleasant experience before. However, when they was hindered to access internet, they would feel unpleasant such as annoying, angry, and restless (withdrawal). When the internet had dominated life activity, it would cause imbalance among biological, psychological, and social needs, thus, it would cause conflict either internal or external conflict, even, it could cause aggressiveness to the adolescents[18].

Adolescent's characteristic which was in identity crisis stage, tended to have high curiosity[19,20] and this was really supported by socio-cultural condition in urban area that facilitated ease to access internet in several public facilities. Besides, it was also supported by booming smartphone market. Even, rapid information and telecommunication systems development also had the potency to facilitate the occurrence of IPA.

In addition, referring to psychoneuroimmunology paradigm, the stressor (impact of internet addiction on physical, cognitive, socioemotional and spiritual development aspects) would be responded by brain cognitively in stress perception (how coping or adolescent's self-adjustment cognitively and behavior in facing the impact of internet addiction). As we know that, stress perception is an ability to understand or conceptualize accepted stressor that results a cognition (understanding) which can cause stress response in modulation of immune response[3]. A condition that is stated as stressor by the brain will cause a stress and the change of physiological, psychological, and behavioral aspects.

Lazarus & Cohen assumed that stress involved transaction between the individual and other worlds from him/herself[21]. Primary appraisal is individual first assessment against the occurrences either in positive side, negative side, or netral side which can cause stress (such as incomplete homework due to they are too busy to access internet, school task is assumed as difficult thing that can cause stress). Secondary appraisal is an assessment against resources or self-ability in confronting an occurrence or intimidation. The adolescents will respond stress in different way, they will search information directly, they will not do nothing or will use their owned coping style as their defense efforts.

Perception process is consisted of selection, organization, and stimulus interpretations which are accepted by human sense until they become meaningful things[22]. Every individual will perceive a stress differently. If it is valued as negative thing by repeating the behavior without trying to correct it well, it will cause a problem for them.

Then, stress perception will activate the change of physiological systems in stress response through activating HPA that can increase production of cortisol. Hence, it impacts to development of health problem. In long-term effect, cortisol can cause the decrease of immune function and nerve damage in hippocampus. This change will increase infection, psychiatric problem,

and loss of concentration and memory[23].

This reseach was conducted in order to investigate how the correlation between stress perception and stress response against IPA on adolescent based on psychoneuroimmunology concept.

Method

This research utilized cross-sectional design. Locations of sample collection in this research were in six Senior High Schools in Makassar from August 2018 until February 2019. The population was all of twelfth grade students in Senior High School who were detected to undergo internet psychological addiction and there were 244 students. The sample size was 39, selected by purposive sampling. Data were collected using IPA questionnaire[25], serum cortisol levels measurement (Elisa) and stress perception questionnaire. Afterwards, the research result was analyzed by using Chi-square in order to observe the comparison among variables, then, it was conducted correlation analysis in order to investigate the correlation between stress perception and stress response.

Findings

IPA level on most of adolescents was high (Figure-1).

Figure-1. Distribution of IPA Level

The most of stress perception level was high (Figure-2).

Figure-2. Distribution of Stress Perception Level

Table 1 showed that there was no significant difference between stress perception score and IPA (p=0.097).

Table 1. Comparison of Stress Perception Score based on IPA Level

IPA	Stress Perception Score			p-value
	Mean	SD	Median	
High	55.10	2.83	55	0.097
Low	53.26	3.88	53	

The Stress Response Level on most of adolescents was high (Figure-3).

Figure-2. Distribution of Stress Response Level

Table 2 showed that there was significant difference between stress response and IPA (p=0.000) and significant difference between stress response and stress perception (p=0.016).

Table 2. Comparison of Stress Response based on IPA Level and Stress Perception

IPA	Stress Response Score			p-value
	Mean	SD	Median	
High	23.98	1.29	21.93	0.000
Low	12.20	4.01	11.60	
Stress Perception				
High	21.54	1.26	16.29	0.016
Low	12.97	5.80	11.60	

Discussion

Stress Perception

Most of stress perception on IPA was high. This condition was occurred more on female adolescents who accessed social network by using cellphone. Although it was not proven that there was a significant correlation between stress perception and respondent's characteristic, the activity of accessing internet since in early age was used for accessing others which did not have any advantages for learning process.

Adolescents were in identity crisis stage. They tended to have high curiosity and they were easy to be influenced by their peer friends[20]. The adolescents

tended to have high confidence to access internet, but unstable, thus, they had not been able to select useful internet activities.

Phenomenon of internet addiction would influence other activities that impacted to imbalance in fulfilling other needs and contributing to personal distress or dangerous stress and could damage the adolescent development in the future[13,14].

Several researchers proved that internet addiction in long-term effect was dominated by negative impacts, such as depression, aggressive behavior, mental disorders, and stress[25-29]. In psychoneuroimmunology concept, stress is divided by two kinds, which are eustress and distress[3]. Eustress is stress that is needed to advance but if it is excessive, it will be distress.

Stress Response

Most of stress response on adolescent was high. The stress condition on IPA gave description that cortisol, product from hormonal reaction of the body due to stress, had spread into the body. Regulation of cortisol secretion was controlled by three organs, which were hypothalamus secreted Corticotrophin Releasing Hormone which would stimulate the anterior pituitary gland to secrete Adrenocorticotropic Releasing Hormone which afterwards, it would stimulate adrenal cortex to secrete cortisol. Cortisol had important role in metabolism of protein and fat through increasing gluconeogenesis and it also had role in the process of adaptation against stress. In gluconeogenensis, it was occurred the increase of glucose secretion in the liver and changes in non-carbohydrate sources (amino acids) to be carbohydrates. Cortisol also caused lipolysis, thus, releasing free fatty acid increased, and it would be occurred centripetal fat deposits[30]. Other functions of cortisol were regulating arteriolar tone and maintaining blood pressure (stimulating angiotensin II secretion), increasing glomerular filtration rate, water excretion, potassium excretion, sodium retention, and suppressing calcium uptake in renal tubules and intestines. This condition was caused by significant permissive effect of cortisol against other hormone activities. Epinephrine was one of hormone types which its activity was influenced by hormone level of cortisol. Moreover, cortisol must be in sufficient quantities for epinephrine in order to cause vasoconstriction. In stress condition, human body would increase epinephrine secretion until 300 times greater from normal level and it was depended on type and intensity of stress stimulus[30]. Cortisol increased epinephrine activity, thus, it was occurred the increase of heart rate and blood pressure.

The role of cortisol in helping the body to overcome anxiety or stress, and it was estimated that it related with its metabolic effect. Cortisol had metabolic effect which was increasing concentration of blood glucose by using protein and fat from the body. A logical assumption stated that the increase of glucose, amino acids, and fatty acids supply was available to be used when it was needed[30].

Correlation between Stress Perception and Stress Response

Stress perception in overcoming the impact of internet psychological addiction that was occurred would be responded by Hypothalamus-Pituitary-Adrenalin (HPA-axis), hence, it caused the cortisol level increased. If stressor that was accepted by hypothalamus was strong, the CRF that was secreted would increase more and more. Thus, stimulation that was accepted by pituitary also increased and cortisol secretion by the adrenal gland also increased. If the emotional condition had been stable, coping mechanism became positive, signal in the brain would hinder the release of CRF[31-32]. Due to stress, cortisol secretion could increase until 20 times greater. Stress was main factor in causing relapse in all addictions[33].

The use of internet excessively on IPA chronically would cause change of sensitivity in nervous system. Adolescents who were in condition of negative emotion or anxiety would increase cortisol level in the blood. Almost each response in the body, such as stress, could increase cortisol even in 20 times greater. The ability in regulating stressor until resulting different perception was really depended on their condition and perception against accepted stress[34]. If their stress coping was good although there was stressor, the susceptibility against health problem could be reduced[35].

Conclusion

When the adolescents who underwent IPA was in stress condition, they could result perception against stress that would stimulate HPA axis, thus, CRF secretion by hypothalamus would increase. Then, it would be followed by the increase of ACTH by pituitary and the increase of cortisol secretion by adrenal gland.

Conflict of Interest- No

Source of Funding-Authors

Ethical Clearance-Yes

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The Prevalence of Sperm Parameters for Infertile Males in Thi-Qar City

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Abstract

Objective Male infertility is a term in which the male is not capable to attain pregnancy in a female. It occurs due to a lack of semen and low-quality semen. Semen Quality is referred to as a surrogate measure of male fecundity. This study aims to assess the frequency of sperm factors in infertile men in Thi-Qar City. Materials and Methods: This study involved an infertile couple, seminal fluid analysis (SFA) was done to evaluate male factors. Males with normal seminal fluid parameters were excluded. Regarding male, SFA was examined according to WHO 2010. Information such as age, duration of infertility and type of infertility were taken Results Among the percentage of abnormal sperm parameters for infertile couples in which the largest percentage was for asthenozoospermia that represent 66.70% of all infertile males. The second sperm abnormality is 12.13% for oligoasthenozoospermia. Asthenoteratozoospermia and oligoasthenoteratozoospermia have the same percentage (4.4). Azoospermia represents 6.7% while the lower percentage for teratozoospermia. Conclusions largest percentage was for asthenozoospermia that represents 66.70% of all infertile males .

Keywords: *Seminal fluid analysis (SFA), sperm parameters*

Introduction

Infertility is a different type of medical care as it relies on both male and female. Infertility is a unique medical condition because it involves a couple, rather than a single individual. In this condition, a couple fails to conceive after 1 year of intercourse without the usage of contraception in females less than the age of 35 years; and after the duration of 6 months of intercourse without the usage of contraception in females less than the age of 35 years and older (clinical definition) [1]. This is a very disturbing medical condition as the couple cannot have children if they are suffering from infertility. This further causes depression, psychological distress, and low self-esteem in the couple [2,3].

Types of male infertility

1. Asthenozoospermia

This term is described as the total sperm motility (non-progressive and progressive), non-progressive morality is less than 40 percent and progressive motility is less than 32 percent. [4)]

2. Oligozoospermia

A few months ago, the World Health Organization reevaluated the sperm criteria and announced a lower reference point which is less than 15M sperm/Ml. (WHO 2010).[4]

3. Azoospermia

This term is described as the full absence of sperms from a minimum of 2 individual samples of centrifuged semen [4].

4. Teratozoospermia

Teratozoospermia; normal sperm morphology is <4% Kruger strict criteria [4]

Male infertility

There is a common misunderstanding that the female is responsible for infertility but, it has to be understood that male is equally responsible for this severe medical

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condition [5].

Materials and Method

The study was carried out at infertility unit in Thi Qar city patient. From February 2015 to July 2017. This study involved an infertile couple, SFA was done to evaluate malefactors. Males with normal seminal fluid parameters were excluded. Regarding male, SFA was examined according to WHO 2010. Information such as age, duration of infertility and type of infertility were taken

Seminal fluid analysis

The seminal fluid sample was taken in a clean, sterile, and dry disposable Petri-dish after three to five days of sexual absence. This was done by masturbation in a quiet room near the laboratory of semen analysis. The dish was a label with the name of the male, his age, sexual intercourse absence period, and the exact time of sperms collection. The sample was liquified using an incubator at the temperature of 37 ºC for half an hour. It was then mixed and analyzed by microscopic and macroscopic tests. To note the results of seminal fluid analysis, the standard form of (WHO 2010) was used (Table 1).

Table 1: Normal values of semen variables [4]

Sperm parameters		**WHO Criteria**
Sperm concentration millions/mL		15x106 spermatozoa/mL or more
Total sperm motility (%)		>40%
Sperm Grade Activity	Progressive motility (%)	>32%
	Non Progressive motility (%)	
	Immotile sperm(%)	
Normal sperm morphology (%)		>30%*

Results

Figure 1 shows the percentage of abnormal sperm parameters for infertile couples in which the largest percentage was for asthenozoospermia that represent 66.70% of all infertile males. The second sperm abnormality is 12.13% for oligoasthenozoospermia Asthenoteratozoospermia and oligoasthenoteratozoospermia have a same percentage (4.4). Azoospermia represents 6.7% while the lower percentage for teratozoospermia.

In our study table, 2 show the effect of male infertility duration on sperm parameters. Regarding sperm concentration there is a significant decline at 5-10years, also there is a significant decline in sperm morphology when duration more than 10 years.

Table 3 discuss the effect of male infertility type in sperm parameters whether primary or secondary. There is no significant difference between primary and secondary types for all sperm parameters (sperm concentration, motility, and morphology).

Table 4 shows the distribution into primary and secondary fertility among infertile couples, 60% of them with primary infertility and 40% with secondary infertility.

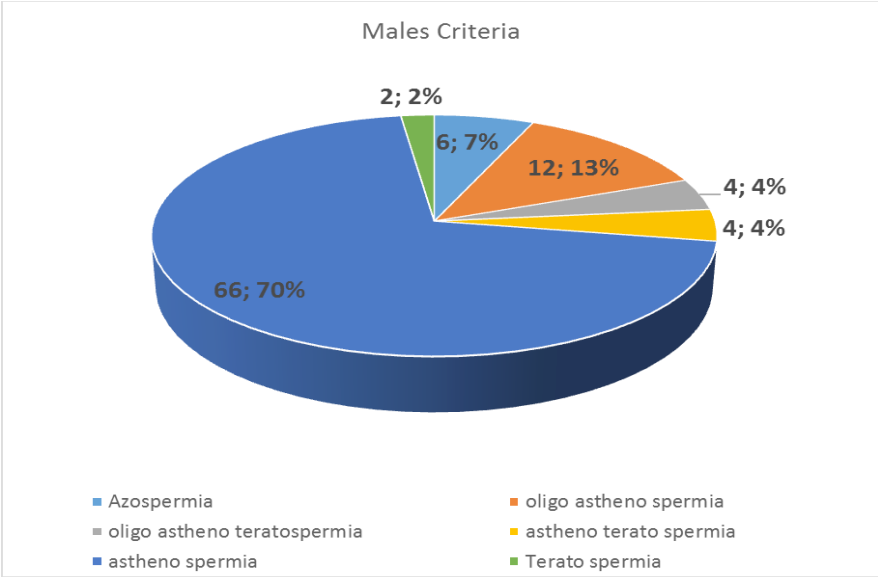

Figure 1. sperm parameters

Table2: Effect of male infertility duration on sperm parameters.

Infertility duration Parameters	**1-5 years**	**5-10 years**	**≥10 years**
Sperm concentration	44.417±4.10a	38.375±4.92b	46.944±5.74a
Progressive motility %	11.763±2.21a	8.081±2.58a	12.300±3.42a
Non-progressive motility %	28.383±2.69a	29.425±3.23a	32.178±4.17a
Immotile sperm	43.615±2.79b	44.813±4.38b	52.439±5.34a
sperm morphology %	52.944±3.67a	43.188±3.64b	45.468±2.47b

- Means with similar letters are nonsignificant different (P>0.05).

Means with different letters are significant deferments (P≤0.05).

Table3: Effect of male infertility type in sperm parameters.

Age groups Parameters	**Primary**	**Secondary**
Sperm concentration	42.459±3.59a	43.649±4.41a
Progressive motility %	10.298±1.80a	11.254±2.69a
Non progressive motility %	28.984±2.38a	30.141±2.93a
Immotile sperm	44.633±3.11a	47.265±2.84a
sperm morphology %	44.567±2.30a	48.595±3.05a

- Means with similar letters are non-significant different (P>0.05).

Means with different letters are significant deferments (P≤0.05).

Table 4: Distribution into primary and secondary fertility among infertile couples.

Infertility	Frequency	Percentage (%)
Primary	60	60%
Secondary	40	40%
Total	100	100%

Discussion

Moreover, the sperm parameters correlate negatively with increased period of infertility [7]. These findings are in agreement with the results of the present study that the male patients with duration of infertility (1-5) years were with the lowest percentage of sperm motility.

The results of this study showed that primary infertility (60%) was more than secondary infertility (40%) as it is obvious from table 4. But, in men having primary infertility, genetic and chromosomal factors have a vital role in the presence of primary infertility than secondary infertility [8].

A retrospective study, conducted between 1992 to 1999 highlights the occurrence of asthenozoospermia as 18.71% and for asthenozoospermia, it was 63.13%, linked with oligo- or teratozoospermia. So, 81.84 percent of the investigated samples indicated altered motility. [9]

In research conducted in Nepal, 20% of the couples indicated semen abnormality. 47% indicated oligospermia while 39% indicated azoospermia. Moreover, 14% of males had asthenormia.[10].

Socioeconomic, environmental, and nutritional aspects are responsible for compromising the health of the male reproductive system [11]. Chemotherapy, radiation, and surgery may have an impact on spermatogenesis [12]. A research conducted in Senegal to check this abnormality, oligoasthenoteratonecrozoospermia showed the highest percentage that is 20.2 percent while azoospermia showed 14.5 percent and asthenonecrozoospermia and astheno-necrozoospermia both showed the occurrence as 10.3% [13]. However, Pontonnier indicates that oligoasthenoteratozoospermia is the chief abnormality that is usually found in the general population and particularly in varicocele [14].

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Ethical Clearance: Not required.

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Awareness of Medico-Legal Issues among Medical College Health Professionals

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Abstract

Introduction: Medicine, a noble profession, aims at providing healthcare service to the people. But today medical practice is much beyond the concept of service. It deals with legal issues and it is necessary for doctors to know about the various medico-legal aspects of their practice. A doctor is solely responsible for the patient who comes to him for treatment. A patient has all rights to sue the doctor if the treatment and care provided is not satisfactory. Assessment of awareness about medico-legal issues and related aspects among the health professional population thus becomes necessary.

Methodology: A cross sectional study was conducted using a self administered questionnaire with clinical scenario based questions. Responses were tabulated and analyzed.

Result: Most of the doctors are aware about various medico-legal issues and precautions to be followed to prevent getting involved in such situations. Doctors have knowledge about informed consent, record keeping, confidentiality etc.

Conclusion: Medical practitioners are well aware about the consequences they will have to face if treatment and care provision becomes faulty. They also have knowledge about the steps to be followed in order to protect themselves from getting involved in such situations. There is a need to spread awareness about medico-legal issues to further raise the quality of the service they provide.

***Keywords**: Medico-legal cases, Informed consent, Confidentiality, Record keeping.*

Introduction

Medicine is a noble profession. It is considered as a pious profession all over the world. Its sole objective is improvement of quality of life and mitigation of sickness and suffering. It is not a mathematical process but a service oriented liberal profession having a self regulating code of ethics. The relationship between doctor and patient is based on hope and confidence (1).

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With commercialization spreading to all aspects, medical profession and services rendered are also affected. There is growing anxiety both within the medical profession and in the society regarding increasing trends of complaints and law suits against physicians (2). It is necessary that doctors be aware of the legal aspects linked or associated to their profession and take the needful measures to protect themselves and their patients from legal traps. Knowledge of medico-legal issues is as fundamental to practice of medicine as clinical skills.

Today, with the development of internet and other media, patients are much aware about their condition and treatment to be taken. Practicing medicine in the present times is not an easy task. A doctor is solely responsible for each and every step he takes in providing care and treatment to his patients.

Hence a doctor must be aware of the consequences he or she might have to face if something goes wrong in patient care. A doctor must know about the precautions to be taken when involved in a medico-legal issue.

MLC or medico-legal cases are an integral part of medical practice that is frequently encountered by medical officers. The occurrence of MLCs is on the increase, both in Civil as well as Armed Forces. Proper handling and documentation of these cases is very important to avoid legal complications [3]. Law suits for medico-legal issues can be minimized or avoided by taking steps to keep patients satisfied, adhering to policies and procedures, developing patient centre care and knowing ways of defending against malpractice judgments. Having comprehensive professional liability, insurance is a necessity in the present day litigious society [4].

This study focuses on medico-legal issues and aspects in daily clinical practice. Doctors face a lot of situations involving medico-legal issues in their daily practice [5]. It is necessary to have knowledge about these medico-legal aspects. Assessment of awareness about medico-legal issues and related aspects among the health professional population thus becomes useful. This study aims at analyzing and assessing the awareness of medico-legal issues among doctors.

Methodology

A cross sectional study was conducted using a self structured questionnaire with 20 questions that was distributed among 100 doctors of Saveetha Medical College. Each question was a clinical scenario with a medico-legal aspect. Questions about informed consent, patient confidentiality, important acts like MTP ACT, medico-legal cases, record keeping etc. were included in the survey. Response to each situation was recorded and analyzed.

Doctors willing to participate in the study were given the structured questionnaire after receiving written informed consent form. Out of the 100 doctors surveyed, 54 were men and the other 46 were women. The questions were of multiple choice type and participants had to tick the right option for each question. None of the participants left any questions unanswered. The response rate was 100%.All participant details were kept confidential and then analysis was done using SPS software. Response to each question was tabulated, analyzed and percentage for the same was also calculated.

Result

Most of them believed the purpose of informed consent was to protect both doctor and the patient while actually its main purpose was to protect the patient. 90% of the population were aware of the importance of informed consent (Fig: 1). They also take consent forms for various treatment procedures explaining to the patient about details, pros, cons and other treatment options available.

Regarding record keeping, 69% responded to maintaining records of MLC till the case is judged and 20% believed it to be 3 years (Fig: 2). 48% of the population feel that records of patients are their legal property while 37% don't so and the remaining 15% have no clear idea about this. In India it is a very common practice that the patient keeps all the records with him or her. In Government offered health services the records are with the respective hospitals, only the treatment summary is given to the patient during discharge or if need arises. In most of the corporate setups the patient's records are with the hospital and only copies in the form of treatment summary or photocopies of entire case file are given to the patient[6].

Among the doctors who were a part of this study, 95% feel it is wrong to reveal the sex of the fetus during scanning. Still, 5% are not aware that it is unethical and a criminal offence to reveal the fetus gender. 74% have the knowledge about the period up to which MTP can be performed while some doctors are still not sure (Fig:3).

A good proportion, 91%, know that they are obliged to treat any patient coming to them for help and care, at least provide first aid with the facilities available. A majority support to the fact that there is a necessity of informing the police about medico-legal cases like suicidal attempt, road traffic accidents etc. (Fig: 4). They stick on to their duty of providing service, care and treatment even if the patient behaves violently.

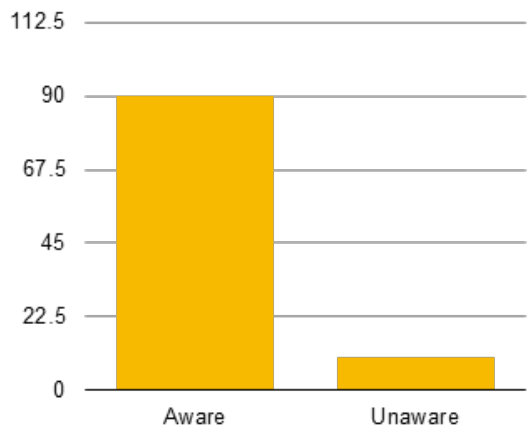
Keeping the patient details confidential signifies the strength of doctor-patient relationship. Most of the doctors believed that revealing patient's personal details with regard to illness is wrong. But there are situations where details with regard to the patient can be disclosed among the members of the treating team.

Question: The main purpose of consent form is to...

A: Protect doctor

B: Protect patient

C: Both



(Fig: 1) Awareness about informed consent

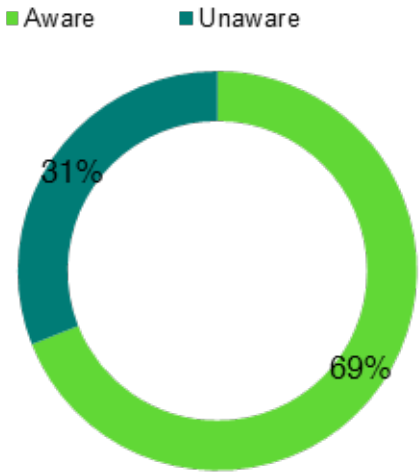
Question: How long do you have to keep the records of a patient if it is a medico-legal case?

A: 2 years

B: 3 years

C: 5 years

D: Till the case is judged



(Fig: 2) Awareness regarding record keeping

Question: The MTP act, 1971 permits termination of pregnancy for a broad range of conditions upto

A: 22 wks of gestation

B: 20 wks of gestation

C: 25 wks of gestation

D:18 wks of gestation

Question: A case of suicidal poisoning comes to Emergency Department. Patient insists he doesn't want a MLC. What will you do?

A: Agree to patient's request

B: Disagree to patient's request

C: Not sure

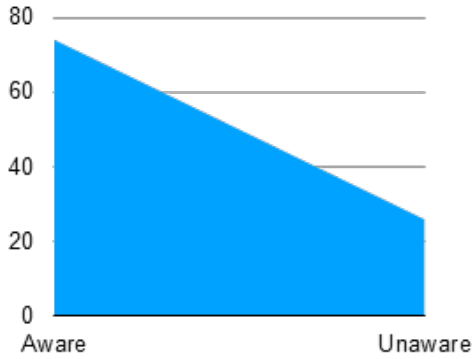
Discussion

The success of the health system depends on the medical personnel equipped with requisite knowledge, skills and attitudes towards patient rights [7, 8]. This study was an attempt to investigate and assess the awareness of medico-legal issues among health care professionals of Saveetha Medical College, Chennai. The participants of this study included both doctors working in the clinical and non-clinical departments of the college.

Most of them were aware about the legal aspects of their practice.

Informed consent is a process for getting permission before conducting a healthcare intervention on a person [9]. Consent signifies acceptance by a person of the consequences and complications of an act that is being carried out. Informed consent form aims at providing information to the patient about the procedure and consequences going to performed on him or her [10]. The main purpose of the consent form is to protect the patient. Consent is necessary for every medical examination and procedure [11].

Usually written consent is not taken for routine examination. But expressed consent is required for any procedure. In a study conducted in a medical college in Vadodara to assess knowledge and awareness of medical law and ethics among interns and residents, it was found that almost 90% of the respondents were aware of informed consent and 61% regarded informed consent with reasonable physician standard model as their choice [12]. This study also has a similar result whereby 90% are aware about informed consent.



(Fig: 3) Awareness about MTP Act

Written records, which include medical history, chart notes, radiographs, and photographs, must be meticulous, and it is necessary for the documents to be signed and dated. Legally, physician written records carry more weight than patient's recollections [13]. Regarding record keeping and maintenance almost 70% believed that records of medico-legal cases must be maintained till the judgment of the case. But these results vary slightly from the study on medico-legal awareness amongst health professionals in Sudan where 94.5% agreed that hospitals must maintain records until the case is judged.

It is difficult to decide if to treat a patient behaving violently. Article 21 of the constitution guarantees protection of life and liberty to all citizens. Every individual has the right to life which includes right to healthcare and medical assistance. There is still confusion among doctors to decide if to provide treatment to violently behaving patients. Majority of the doctors agree to provide treatment to such people. The healthcare worker should take reasonably practicable steps to maintain a safe environment for patients and staffs. This helps in providing better patient care. If violence or aggression cannot be controlled so as to provide a safe working environment, the health service's duty to staff takes precedence over its duty of care of patients.

Health care services and professionals must work together to develop strategies to prevent and manage the risk of occupational violence [14]. When compared to a study conducted to asses awareness and knowledge about medico-legal issues in a medical college in Vadodara, where more than half the respondents (55%) said that treatment should not be refused if the patient behaves violently, the response in this study differed by 78% of the population agreeing to treat the patient despite his violent behaviour.

According to Section 357 C Cr.P.C., all hospitals, public or private, whether run by the Central Government, the State Government, local bodies or any other person, shall immediately, provide the first-aid or medical treatment, free of cost, to the victims of any offence covered under section 326A, 376, 376A, 376B, 376C, 376D or section 376E of the Indian Penal Code, and shall immediately inform the police of such incident. Thus a doctor has no right to deny treatment to any person who comes to him for treatment, be it a criminal, victim or an ordinary man [15].

Medical practice is a service and not accepting to help one in need of treatment and care is a criminal offence. When facilities at a clinic or a healthcare centre are not sufficient to provide complete care or treatment, a doctor can refer to nearby hospitals with sufficient facilities. This does not give him rights refuse first aid to an emergency case like road traffic accident with head injury. Necessary first aid should be provided and then only can he refer or shift the patient to a hospital with better facilities needed to sustain the patient's life.

Confidentiality is an implied term of contract between the doctor and his patient [16]. The doctor is obliged to keep secret, everything he comes to know concerning the patient in the course of his professional work. There are situations where doctors of a treating team must know all details about the patient even if it is about HIV or Hepatitis [17]. If an HIV positive patient approaches for treatment involving a surgery, then the doctor can inform his colleagues about it if they are a part of the treating team. Confidentiality regarding the patient is maintained within the treating team. A study conducted in Ghana reveals that there is no consensus among health care workers on confidentiality matters regarding management of HIV [18]. There is a variation in this study where majority (91%) agrees to maintain confidentiality regarding matters like HIV and its treatment.

Conclusion

In general the doctor population of Saveetha Medical College is well aware of various medico-legal issues and its aspects in daily life practice. They do have knowledge and idea about various medico-legal issues and how to face them. They do have a general idea of how to approach medico-legal cases and knowledge about the things they should follow in order to protect

themselves from such issues and continue a successful career. Doctors must follow the guidelines provided in their practice. This will help them have a trouble free and a peaceful time while working. Guidance and awareness is necessary among health professionals to raise the quality of service they provide and also to protect themselves from the troubles and difficulties they might face in their practice.

Conflict of Interest: Nil

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Ethical Clearance: Obtained

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The Law and Tourism: The Policy of Health Service for Tourists in Lombok Island

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Abstract

The Province of West Nusa Tenggara is known as one of the Indonesian provinces which has become a tourism destination apart from Bali. This province has two main islands, which are Lombok Island and Sumbawa Island. They have a richness in the variation of nature and culture, so that they have a high potential for the development of tourism. The problem which must be taken into consideration in creating a better tourism destination is giving healthcare services which are agreed upon through the local government's policies. The scope of tourism health is very broad, as it includes the health of the tourists, the health of the society as the hosts, the health of the workers in the tourism industry, the health of the environment in the tourism area, the safety of food in the tourism area, including the different policies regarding health and tourism. Apart from that, the effort of chemoprophylaxis may be informed to the tourists who have the risk to be contracted of an illness, but may be avoided through the use of some medications. For example, the tourists who visit West Nusa Tenggara, or the other eastern parts of Indonesia, may be advised to consult to health workers to receive preventative medicine. Tourism consultants and tourism guides may also have the role to give suggestions regarding the general health situations which are present in the area on several occasions.

***Keywords**: Law, Health services, Lombok Island Tourism*

Introduction

Indonesia is a country which has the potential to become a global tourism destination. Tourism is an activity which provides the services of accommodation, transport, consumption, recreation and other services [(1)]. The marketing of tourism services involves some aspects, such as the economy, cultural, social, religious, environmental, safety aspects, as well as others. From those aspects, the aspect of economy has the largest attention for the development of tourism, so that the tourism can be said as a form of industry [(2)]. In the world of tourism, the protection of the tourists, both the international as well as the domestic tourists, is still very low. Moreover, the law which apply sometimes do not have enough protection for the tourists. [(3)]

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Indonesia is a developing country which has many tourism potentials, both for the tourism of nature as well as culture [(1)]. As a large territory, which is supported by the various natural resources which have the potentials to be processed and utilized, tourism becomes one of the things which may increase the foreign exchange rate in the country [(2)]. According to the World Tourism Organization, there are 702 million international tourists who underwent tourism journeys on 2002. The tourism sector created income as much as US $ 474 billion, and 214 million work opportnities worldwide. [(4)]

Tourism can also be used to increase the welfare of the people and also to encourage national development [(4)]. The importance of tourism's role in the economic development of a country cannot be denied. In the recent years, some countries choose to work on the tourism sector seriously, making tourism the primary sector in increasing the foreign exchange rate, to create job opportunities, and to decrease poverty [(5)]. Tourism is one of the largest foreign exchange earner in Indonesia, which is not from the oil and gas sector [(6)].

The province of West Nusa Tenggara is known as one of the provinces which become a tourist attraction destination in Indonesia after Bali [7]. This province has two main islands, which are Lombok Island and Sumbawa Island, which has various natural and cultural richnesses, thus they are very competent in becoming a potential for tourism development [7].

Lombok and Bali islands have been determined as the national tourism gateway in The Masterplan of the Indonesian Economic Development Acceleration and Expansion. Lombok Island becomes one of the national tourism destination with the nickname of Island of a Thousand Mosques.

In almost all coasts of Lombok Island, there are beaches which become tourism destinations, starting from East Lombok which is famous for its pink beach, Labuan Haji and Gua Jepang Beach; Central Lombok with Kuta Beach, Selong Belanak, Mataram City and Ampenan Beach; West Lombok with Senggigi Beach; also North Lombok with its three *gili*'s: Gili Trawangan, Gili Meno and Gili Air [8]. There are abundant choices of tourism destinations in Lombok Island, and almost all regencies along the coastlines offer beautiful beaches. With the potentials as described, there needs to be an understanding of the government's regulations regarding the health services in Lombok's tourism objects.

Research Method

The kind of approach used for analysis in this research is the doctrinal approach. In this stage, legal inventarisation is firstly done to find the legal norms which manage the health services of tourists [9]. The source may be from the constitutional regulations and policies. This activity is then continued with a normative analysis, to find the in-concreto law (which comes from the analysis between the existing secondary data and the constitutional regulations which apply) and the legal principles which come from the analysis of the existing secondary data which regulate the health services for the tourists.

Discussion

The Republic of Indonesia's Ministry of Tourism Regulation No. 14 Year 2016 regarding the Sustainable Tourism Destination Guidelines states that, in consideration of realizing the tourism development which focuses on cultural and natural diversity, uniqueness, and peculiarity without ignoring the future needs, so it is hoped that the tourism may encourage the economic development which may bring benefits for the welfare of the society [10]. To realize the development of tourism destinations, efforts needs to be done with integrity, sustainability, and responsibility, thus there needs to be a description regarding the sustainable tourism destination criteria [11].

Another problem which needs to be given attention for the sake of creating a better tourism destination is in the healthcare sector. There should be healthcare services, which are agreed upon through the local government's regulation. The scope of tourism health is very broad, as it includes the health of tourists, the health of the society as the hosts, the health of the workers in the tourism industry, the healthy condition of the tourism area's environment, the food safety in the tourism area including the many regulations regarding health and tourism. The tourism health service is a branch of the public health science which is unique and specific, related to the tourism journey and activity. The government's policies regarding the tourism health services will have impact towards the health of the population and the environment in the tourism destination. Apart from the health issues, another important aspect which is not seen here is the economic considerations. The tourists who are unhealthy will give impact towards the tourism industry as well as the society in that area. Contrarily, health tourism objects can be used as one of the ways to further promote that attraction.

Some other issues which actually urgently need more attention is the need of the tourism city's government to make some regulations regarding the health service in Lombok Island. They should be able to identify the potential threats and analyze the risk of the tourism health, which are related to the tourism journeys or the activities related to the offered tourism packages.

The relation between the tourism industry and the health professions will be crucial, and each party must know and understand their reponsibilities. Thoes who have such interests must also discuss how the ideal relationship will be in creating an effective healthcare service in Lombok Island.

Generally, the tourism industry has three components which must be fulfilled. They are suppliers of travel services, tour operators, and retail travel agents. Included in the suppliers of travel services category are the suppliers of transportation services (air, land, sea),

accomodation (hotels, motels, inns), and restaurants.

The Bureau of Tourism Trips have the role as the the operator as well as the agent of tourism trips, which must provide tourism guide services. As a profession which has direct relations with the activity of tourism, the tourguide workers have a crucial role. Tourguides are usually provided by the agent of the tourism trip services, which also coordinate freelance tourguides to fulfill the demand of independent tourists and the demand of the Bureau of Tourism Trips. In its business, the tourguide services must still be aware of the touirguides' requirement of professionalism. This business is obliged to continuously make efforts to increase the skills of the tourguides who will work, so that they fulfill the requirements which apply. These are the components which must be fulfilled to reach the ideal role of tourism.

From the description above, the chance to give health services as a form of health-risk prevention in the tourism areas is very large. Theoritically, the consultant of tourism trips may have a large role in the efforts to prevent the tourists' health problems. The first step which may be done is their role in informing the importance of vaccination, as it is regarding the legal aspect of visiting a certain place.

For instance, to visit the Kingdom of Saudi Arabia, visitors must provide a certificate of meningitis vaccination. The policy regarding the obligation to take actions to prevent and to protect oneself from meningitis is an operational principle which regulates the health services for the prospecting pilgrimage participants (for both *hajj* and *umra)*, and also for the Indonesian Migrant Workers. Regarding the reason of why this meningitis vaccination becomes obligatory, it is because the Kingdom of Saudi Arabia is one of the endemic places for the spreading of the virus causes the meningitis disease. The spreading of this virus is through the air. With this spreading manner, it is certainly very difficult to predict whether or not a pilgrimage participants might be free of that virus.

The government of Lombok Island must oblige the tourism workers to give recommendation regarding vaccinations which are needed to prevent some diseases. For example, when there was an outbreak of the rabies disease in Bali, the toursim trip consultants may inform the importance of rabies vaccination before the journey to the tourists before they visit. Or while in Bali, the tourism trip consultants or the tourguides may give information on what must be done if bitten or scratched by animals which have the chance to be infected by rabies.

Apart from that, the effort of chemoprophylaxis can also be informed to the tourists who have the risk to infected by some disease, yet can be prevented by the consumption of some medicines. For example, the tourists who visit the West Nusa Tenggara area, or other Eastern parts of Indonesia, may be advised to consult with medical workers to receive some preventive medicine. The tourism trip consultants and the tourguides may also be used to give advises regarding the general health situations in some areas during a certain period. In some cases where the dengue fever have increased, the tourism trip consultants may give information regarding the efforts to prevent being infected, like the protection needed while undergoing outdoor activities. The local government must always coordinate with the tourism trip agents and the tourguides, including giving information regarding the importance of trip insurance, repatriation information, and the medical service condition in the tourism destination area.

Epidemiologically, the Ideal Role of Tourist Health Profession is an important population group as it has high mobility in giving disease prevention services in tourism areas. Not only the issue of tourism areas, yet also regarding the tourists who often move from one tourism destination to the next. They have the risk of having infected by some diseases, and there may be some unwanted incidents outside of the original place, thus mild cases may not be reported, and they seldom seek help or medications. This may cause an import of diseases to the original place and vice versa, there might be exported diseases to the destination places. This may increase the risk of changing the disease's non-endemic area into an endemic area. This shows that the epidemiology of diseases related to tourism is one of the basic competences which should be posessed by medical staff and public health staff in the tourism destination area. Even though economically, the increase of tourists poses a positive impact, yet this trend may also be followed by the increasing risk of health.

Based on the research conducted by Reid, Keystone, & Cossar, international tourists who come to developing countries will experience health problems regarding tourism. There are many tourists who return to their original countries and seek medication. This shows that there are many health problems commonly experienced

by tourists.

The government's policies regarding health services also depend on the role of medical staff and the understanding of the society in the tourism area regarding the handling of health issues. The medical staff should have a good competence of understanding regarding tourism health, which refers to the tourism health basic competence which are set by the International Society of Travel Medicine (ISTM), starting from a good diagnosis and a correct way of handling the case.

The scope of tourism health is very broad, thus the government of Lombok Island must be able to predict the diseases present in that area to secure the health of the tourists, the health of the society as hosts, the health of the workers in the tourism industr, also the health of the environment in Lombok Island's tourism area. The government of Lombok Island is hoped to have a good understanding regarding the different kinds of vaccination an prophylaxis (the consumption of medications for preventive purposes) which are related to tourism. Last but not least, the government should give services of the health rights in the tourist destination places, and undergo an inter-professional interaction collaboration between the government of Lombok Island and the various medical staff, especially doctors, nurses, the society, also the travel bureaus, who have a big role in the realization of tourism health services which are according to the concept which has been described in this paper. If this goes well, the vision to realize the health service policies for tourists in Lombok Island will be a reality.

The principle of safety in preventing diseases in the tourism area is the government's policy principle which becomes the background in giving services regarding the healthcare rights. This principle of safety is a special principle which is based on the general principles, which are the principle of safety, the principle of protection, and the principle of benefit.

Conclusion

The service of tourism health is a unique and a specific branch of the public health science, which is related to tourism trips and activities. The government's policy regarding tourism health services will give impact towards the health of the population and the environment in the tourism destination area.

The above description shows that the chance to give healthcare services as a form of health risk prevention in the tourism destinations is opened very wide.

Apart from the government of Lombok Island and the medical workers, the society's role in the tourism area also has a large role when involved. They have a big role in understanding the important element in disease prevention and specific insidents toward the tourists, understanding the promotional aspect of tourism health, and to be able to make health impact assessment. They should also be able to identify threats of danger, make risk assessments, and making health risk-prevention efforts in the tourism areas.

The scope of tourism health is very wide, thus the government of Lombok Island must be able to predict the diseases in that area to secure the health of the visitors, the health of the people as the host, the health of the tourism industry workers, also the health of the tourism area in Lombok Island.

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Conflict of Interest: no

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Effect of Antenatal Exercise Teaching Program on Neonatal Outcomes During Delivery in Erbil, Iraq

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Abstract

Background: Antenatal exercises are safe and beneficial for the fetus, and neonatal health, wellbeing, were positive effect on neonatal outcomes during delivery, such as prevent fetal distress, remained the Apgar score and birth weight in normal range level. The aim of this study was to determine the effect of antenatal exercise classes on the neonatal outcomes during delivery. **Methods:** prospective -Interventional study was conducted in Maternal – Child Health Department in Najdi Heydari Health Care Center, Maternity and Razgary Teaching Hospitals / Erbil City / Kurdistan region / Iraq. One hundred and twelve healthy pregnant women. One half of the pregnant women were included in the intervention group (n = 56) and the other half in the control group (n= 56). The intervention group was educated about antenatal exercises. While the control group remained on standard maternal health care program (without exercise classes). Neonatal outcomes evaluated from documents of birth registry and mothers interview in the postpartum ward using a special check list prepared for that purposed.

Results: In the original data, there was a statistically significant difference between both groups. Regarding the fetal heart rate (P<0.01), Apgar score (P<0.004), neonatal complications (P<0.039), admission neonatal intensive care unit (P<0.003) through the progress of delivery. in other hand, not a statistically significant difference between intervention and control groups concerning neonatal weight**. Conclusion:** Antenatal exercise is recommended for healthy pregnant women as a safe and effective strategy, enhance the neonatal outcomes during the delivery process.

Key words: *antenatal exercise, teaching program , neonatal outcomes*

Introduction

The pregnancy phase is an interval of growth, development, and physiological change in mother and fetus[1]. In uncomplicated pregnancies, women with or without a previously sedentary lifestyle should be encouraged to take part in aerobic and strength class exercises as a part of a healthy lifestyle[1]. Both the American College of Obstetricians and Gynecologists and the Society of Obstetricians and Gynecologists of Canada currently recommend that in the absence of complications during pregnancy, pregnant women should exercise for half hour at a moderate intensity on most, if not all, days of the week [3,4,5]. Many evidences showed there was a significant effect of exercise training during the course of pregnancy on the fetal heart rate, fetal complications during labor and neonatal Apgar score [6,7,8], and the effect of exercise during pregnancy on the newborn's birth weight is unclear [9,10]. Moderate to high level of continues maternal exercise have been associated with reduced birth weight [11]. A few studies[12,13] have found reduced birth weight among women who continue vigorous exercise during pregnancy compared with those who discontinue the exercise or who are sedentary.

In this study, we amid to evaluate the effectiveness of antenatal exercise classes on the neonatal outcomes during delivery in Erbil City / Kurdistan Region / Iraq.

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Materials and Method

Tools for collecting data included the following:

assessment form was constructed by the researcher based on the literature and recommendations of antenatal exercises by the American College of Obstetrics and Gynecologists, 2015[14], that included identifying demographic data, obstetrical history, assessment contraindications during pregnancy of the participants and checklist form. Content validity and reliability was secured. The sample size was calculated as 112 subjects based on PS program (power and sample size calculation) version 3.0.43. Who was attending the Maternal – Child Health Department in Najdi Heydari Health Care Center, Maternity and Razgary Teaching Hospitals, Erbil City, Kurdistan Region , Iraq. The subjects were selected using accessible sampling. The inclusion criteria were the following: age 18 - 40 years old, gestational age 17- 36, single tone pregnancy. Exclusion criteria were: free from contraindications of performing an exercise during pregnancy based on recommended of the American College of Obstetricians and Gynecologists (ACOG),2015 [4], as an obstetrician assessed all study participant subjects who had any medical health problem or pregnancy complications were excluded from the study. Subjects were purposely divided into intervention group 56 and control group 56. The intervention group was divided into nine subgroups and required to attend exercise classes for one hour weekly for four weeks. The purpose of the study was explained to all subjects in both groups then informed consents were taken from them, and they completed the basic information forms which were prepared by researchers. The classes include the following:

1-Benefits, counterindications and discontinue of exercises during pregnancy.

2- Aerobic exercises and strength training of all the major muscle groups (walking or running and lower limbs relaxation exercise)

3- Pelvic floor exercises and birth ball exercise.

4- Coping in Labor (Helpful positions and breathing for labor).

During the classes demonstration of exercises and practical training, slides and pamphlets were used. All study participants were instructed to stopped exercises and reported immediately to the Maternity Teaching Hospital in the case of vaginal bleeding, abdominal contraction, vaginal fluid discharge. Participants in the control group received the routine care without attending classes. All participants who were unable to continue the program from both groups were excluded from the study finally subjects in the intervention group 48 and control group 49 Figure 1.

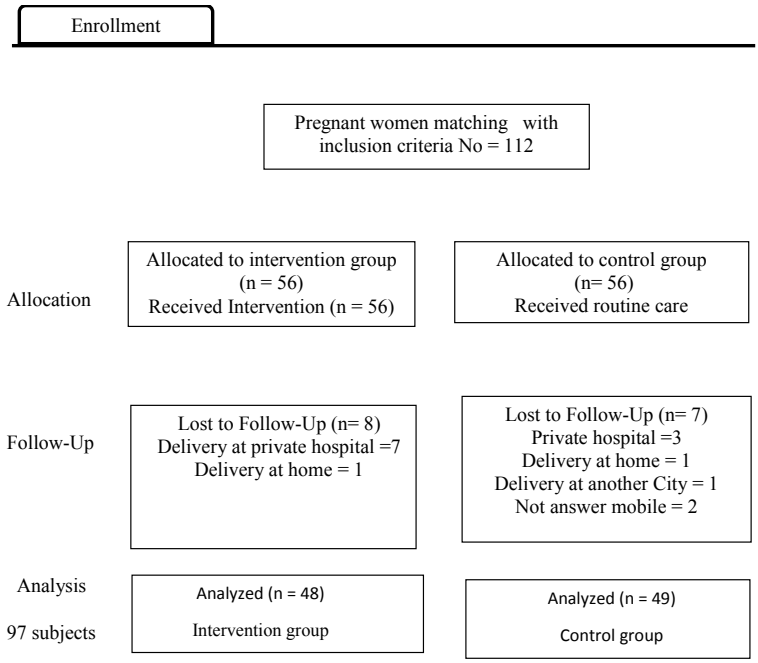

Fig 1. Flowchart of the participants through the study.

Outcomes and Statistical analysis

The delivery outcome of the fetus and neonatal (fetal heart rate, fetal complications during labor, Apgar score, birth weight, and admission neonatal intensive care unit) was evaluated from documents of birth registry and mothers interview in postpartum ward using a special checklist prepared for that purpose. Statistical analysis was performed using SPSS for Windows version 20.0 software. Chi-square tests, ANOVA test, and Fisher exact were used for comparisons of intervention and control groups data. A value of P values (< 0.05) was accepted as statistically significant.

Results

The present study findings that highest percentage (57.1%) (58.9%) of intervention and control groups respectively their age ranged between 20-29 years old and highest percentage (37%) of the intervention group had six years schooling were (32.1%) of the control group was Illiterate. The same table indicates that the highest percentage (91.1%) (92.9%) of both groups were housewives, respectively. Results also showed that demographic characteristic of both groups was homogeneous and no significant difference was observed between them regarding the age group, educational level and gravidity (P<0.978, P<0.738, P<0.849) respectively (Table 1).

Table 1: Demographic data of the study sample N= 112

Variables	IG =56 No. (%)	CG = 56 No. (%)	P- Value
Age (years) < 20 20 – 29 40 +	 6 (10.7) 32(57.1) 18(32.1)	 6 (10.7) 33(58.9) 17(30.4)	0.978
Education level Illiterate Primary Secondary Institute & College	 13(23.2) 21(37.5) 12(21.4) 10(17.9)	 18(32.1) 17(30.4) 11(19.6) 10(17.9)	0.738
Gravida 1 2 – 4 = 5 +	 27(48.2) 22(39.3) 7(12.5)	 25(44.6) 22(39.3) 9(16.1)	0.849

IG: Intervention group, CG: Control group

The highest percentage 97.9% of study sample their fetus heart rate was normal range rate of intervention group within compared to 81.6% of the control group. Which observed a statistically significant difference between intervention and control groups concerning pregnant women is fetal heart rate) P>0.01(, in favors of intervention as shown in (Table 2).

Table 2: Comparison of the fetal heart rate during delivery between the two groups

Variable	IG (48) No. (%)	CG (49) No. (%)	-P- Value
Fetal Heart Rate/ bpm			
>120	0(00)	7(14.3)	
120 -160	47(97.9)	40(81.6)	0.01
<160	1(2.1)	2(3.1)	

IG: Intervention group, CG: Control group, bpm: Beats per-minutes

Table 3 showed that 10.4% of the study sample in the intervention group had fetal distress within compared to 28.6% of control group, a statistically significant difference between intervention and control groups concerning neonatal complication) (P>0.039), in favors of the intervention group.

Table 3: Comparison of the fetal complications during labor between the two groups

Variables	IG (48) No. (%)	CG (49) No. (%)	-P- Value
Fetal distress	5(10.4)	14(28.6)	0.039
Non- complications	43(89.6)	35(71.4)	

IG: Intervention group, CG: Control group

Table 4 indicated that highest percentage (93.8%) of the neonatal their Apgar score higher than 7 scores in intervention group within compared to 71.4% of control group, a statistically significant difference between intervention and control groups concerning neonatal Apgar score (P>0.004), in favors of the intervention group. The same table showed that higher percentage (89.6%) of the neonatal in the intervention group their body weight between 2500 to < 4000 mg within compared to 89.8% the control group, while 89.8% of the neonatal their body weight between2500 to < 4000g, not a statistically significant difference between intervention and control groups concerning neonatal weight (P<1.00). In addition, only 8.3% of the neonatal admission to intensive care unit in the intervention group within compared to 32.7% of control group, a statistically significant difference between intervention and control groups concerning neonatal admitted care unit (P>0.003), in favors of the intervention group.

Table 4: Comparison of the neonatal Apgar score, birth weight and admission neonatal intensive care unit between the two groups

Variables	IG (48) No. (%)	CG (49) No. (%)	-P- Value
APGAR Score			
< 7Score	45(93.8)	35(71.4)	0.004
7 > Score	3(6.3	14(28.6)	
Birth weight / g			
<2500	1(2.1)	1(2.1)	
2500 to < 4000	43(89.6)	44(89.8)	1.000
≥4000	4(8.3)	4(8.2)	
ANICU			
Yes	4(8.3)	16(32.7)	0.003
No	44(91.7)	33(67.3)	

IG: Intervention group, CG: Control group, g: gram, ANICU: Admission neonatal intensive care unit

Discussion

This study was carried out to determine the effect of antenatal exercise on neonatal outcomes during the delivery process. In the present study, the novel program was studied, the lower limbs relaxed, sitting on the birth ball, Kegel exercises (pelvic floor exercise), and breathing exercise and relaxation during labor are new phenomena for the pregnant women in Kurdish culture. Through this program were oriented the pregnant women about the importance of antenatal exercise and the effect of antenatal exercise on the neonatal outcome and their health wellbeing during delivery. The studies related antenatal exercise is limited but suggests that moderate exercise during uncomplicated pregnancy does not lead to adverse outcomes for the fetus and improves overall neonatal health well-being [12,13,14,15].

The present study showed that the fetal heart normal range 120 -160 bpm in the intervention group were higher than the control group, which a statistically significant difference between two groups (P<0.01). Found a higher percentage of the fetal heart rate (less or above normal range 120-160 / bpm) in the control group (17.4% vs. 2.1%) respectively, the reason for abnormal fetal heart rate during delivery in the control group are due to prolonged labor, hypoxia and meconium [14]. Concerning the fetal complication, fetal distress was less reported during labor, also the rate of fetal distress in intervention group less than the control group. The reasons of fetal distress are due to prolonged labor, lack of fetal head descent into the pelvis, incomplete rotation of the fetal head hypoxia and meconium [14,16].

Another appealing finding in this study is that the Apgar score was higher in the intervention group compared to the control group at the first minute [2,8,15]. Miquelutti et al and Nascimen to disagrees with the result of the present study, found that exercise during pregnancy has no effect and no significant difference between intervention and control groups regarding the Apgar score at first minute [17, 18]. Regarding birth weight, in the present study, we did not find a statistically significant difference in birth weight between intervention and control groups, we observed that the prevalence of newborns with birth weight between 2500 to < 4000 g was 89.6% (n = 43) in the intervention group vs. 89.8% (n = 44) in the control group, also was no a statistically significant difference between groups in low birth weight (<2500 g) or macrosomia (< 4000 g). The results of some studies are agreement with the present study findings [2,7,14, 18,19,20,21,22, 23] there are found that performing the exercise during pregnancy has no effect on birth weight.

We also found that the pregnant women performed of antenatal exercises had a significant association with their newborn admission to neonatal intensive care unit. The rate of referring newborn to neonatal intensive care unit in the intervention group less than the rate of neonatal admission in intensive care unit in a control group. 8.3% (n=4) in the intervention group vs. 32.7% (n= 16) in the control group needed admission to the neonatal intensive care unit after birth. were due to meconium aspiration, asphyxia[17].

Conclusion

Antenatal exercise is recommended for healthy pregnant women as a safe and effective strategy for enhancing fetal and newborn outcomes (fetal heart rate, fetal complications during delivery, Apgar score and reduced birth weight and admission neonatal intensive care unit)

Conflict of Interests: None

Ethical Clearance: This perspective - intervention study, was approved by the Ethics Committee in the College of Nursing, Hawler Medical University (Registration No:8) and the Directorate of health in Erbil City, Kurdistan Region, Iraq.

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Study of Dyslipidemia in Iraqi Hypertension Patients in Tikrit City

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Abstract

Background: Hypertension is prevalent all over the world and represent a major health problem. Dyslipidemia is a major cause of cardiovascular morbidity and mortality. There is a correlation between hyperlipidemia and hypertension.

Aim of study: to find out the prevalence of dyslipidemia in Iraqi hypertension patients, and to find out how many of them on statin therapy, and how many reach target according to the last guidelines

Patients and methods: a cross-sectional study conducted in outpatient clinic in Salahaddin General Hospital in Tikrit City. About 344 hypertensive patients were included in this study. A questionnaires including general information about patients were filled and venous blood was drawn from every patient and sent for hospital lab to measure necessary investigations. ASCVD risk score was calculated for every patient by ASCVD Risk Estimator Plus. Then data were analyzed by application of Microsoft excel program and Statistical Package for Social Sciences (SPSS) version 23.

Results: (49%) of hypertension patients had total cholesterol of >200 mg/dl**,** (63.4%) had LDL of ≥115 mg/dl**,** (57.6%) had HDL of <40 mg/dl, and (54.1%) had triglycerides of ≥150mg/dl. Smoker patients in this study had highest ASCVD risk score (12.65±6.63%) followed by former smokers (7.36±3.56%) and non-smokers (6.02±4.16%).Only 76 (22.1%) of hypertension patients were on statin therapy for hyperlipidemia and only 18 (23.7%) of them reaching target LDL level according to 2016 ESC/EAS guidelines. While 268 (77.9%) of hypertension patients had no statin therapy and only 65 (24.3%) of them had LDL level within target.

Conclusion: Hyperlipidemia is very prevalent in hypertension patients in Tikrit City, despite that only one of five receiving treatment and only one of four from those who received treatment reaching the target of LDL level according to 2016 ESC/EAS guidelines, and three of four of those who are on no treatment are out of the target. This is a major health problem need attention.

Keywords: *Hypertension, Dyslipidemia, Cholesterol, HDL, LDL, Triglycerides*

Introduction

Hypertension can be defined as an abnormally high arterial blood pressure. It is still accepted that hypertension defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg [1-3]. Because of its prevalence, hypertension represent a major health problem [4-7]. Hypertension affect about 32.6% of US population above 20 years of age [8]. In Iraq , about 35.6% of population above 18 years of age have hypertension[9]. Worldwide, dyslipidemia is a major cause of cardiovascular morbidity and mortality [10, 11]. Atherosclerosis is a progressive pathological process that lead to heart and cerebrovascular diseases [12,13] an dyslipidemia is a major risk factor of atherosclerosis.

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About 39.6% of Iraqi population above 18 years of age have raised cholesterol or currently on statin treatment[9]. There is a correlation between hyperlipidemia , hypertension and pulse pressure [14].

The aim of this study: to find out the prevalence of dyslipidemia in Iraqi hypertension patients, and to find out how many of them on statin therapy, and how many reach target according to the last guidelines.

Patients and method

This is a cross-sectional study conducted in outpatient clinic in Salahaddin General Hospital in Tikrit City, from 1st of January 2019 to 31st of May 2019. All established hypertension patients above 25 years of age that agreed to participate in this study were included.

Exclusion criteria: Patients with: established coronary or valvular heart disease, chronic kidney disease, diabetes mellitus , stroke or peripheral vascular disease were excluded.

About 344 hypertensive patients were included in this study after an oral informed consent taken from every patient . 156 (45.3%) of them were males, and 188 (54.7%) were females. Age range from (30-80) years with mean age of (50.34±10.35) years. Every patient was reviewed individually by a questionnaire including general information about patients beside smoking state and history of : cardiovascular disease, diabetes mellitus, chronic kidney disease or peripheral vascular disease, and history of treatment of dyslipidemia. Weight (Wt) and height (Ht) of every patient was recorded and body mass index (BMI) was calculated. Systolic and diastolic blood pressure of each patient was also recorded. Then 5 ml of venous blood was drawn from every fasting (for 14 hours) patient in this study and sent directly to hospital lab for measuring : fasting blood sugar, blood urea and serum creatinine to exclude diabetic patients and patients with Chronic kidney disease. Lipid profile including: serum total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (vLDL) and serum triglycerides were also measured.

Then atherosclerosis cardiovascular disease (ASCVD) risk was calculated for every patient by ASCVD Risk Estimator Plus , an application provided by American College of Cardiology (ACC). Then patients were grouped into 4 groups: 1- low risk with ASCVD risk score of <1%, 2- moderate risk with ASCVD risk score of ≥1% and <5%, 3- high risk with ASCVD risk score of ≥5% and <10% and 4- very high risk with ASCVD risk score of ≥ 10% according to European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) 2016 guidelines for the management of dyslipidaemias [15].

Then data were analyzed by application of Microsoft excel program and Statistical Package for Social Sciences (SPSS) version 23. Outcomes of analysis were arranged into tables and figure. P-value was calculated by different probability tests.

Results

About 344 hypertensive patients were included in this study. 156 (45.3%) of them were males, and 188 (54.7%) were females. Age range from (30-80) years with mean age of (50.34±10.35) years. FB. sugar mean ± SD was (93.00±0.00 mg/dl) , B. urea mean ± SD was (33.79±6.82 mg/dl) ,and S. creatinine mean ± SD was (0.83±0.15 mg/dl).

About 171 patients (49%) had total cholesterol of >200 mg/dl (42.1% males and 57.9 females, and 218 of patients (63.4%) had LDL of ≥115 mg/dl (43.1% males and 56.9% females, and 198 of patients (57.6%) had HDL of <40 mg/dl (57.1% males and 42.9% females), and only 158 of patients (45.9%) had serum triglycerides of <150 mg/dl (38% males and 62% females. There was statistically significant increment of HDL and triglycerides in females as shown in table (1).

Table(1) Frequency of dyslipidemia according to gender distribution in Hypertension patients.

Variables	Subvaraibles	Male		Female		Total		P-value
		No.	%	No.	%	No.	%	
Total Cholesterol	>200 mg/dl	72	42.1	99	57.9	171	49.7	0.230
	≤200 mg/dl	84	48.6	89	51.4	173	50.3	
LDL	≥115 mg/dl	94	43.1	124	56.9	218	63.4	*0.621
	<115-100 mg/dl	32	51,6	30	48.4	62	18.0	
	<100-70 mg/dl	25	45.5	30	54.5	55	16.0	
	<70 mg/dl	5	55.6	4	44.4	9	2.6	

Cont... **Table(1) Frequency of dyslipidemia according to gender distribution in Hypertension patients.**

HDL	≥40 mg/dl	43	29.5	103	70.5	146	42.4	<0.0001
	<40 mg/dl	113	57.1	85	42.9	198	57.6	
Triglycerides	< 150 mg/dl	60	38.0	98	62.0	158	45.9	**0.039**
	150-199 mg/dl	48	52.7	43	47.3	91	26.5	
	≥ 200 mg/dl	48	50.5	47	49.5	95	27.6	

By Fisher Exact test.*

Patients with BMI of ≥ 30 Kg/m2 had higher total cholesterol (212.38±54.84) mg/dl and HDL level (38.25±11.86) mg/dl , while patients with BMI of 25-29.9 Kg/m2 had higher level of triglycerides (190.44±128.34) mg/dl and patients with BMI of 18.5-24.9 Kg/m2 had higher level of LDL (144.71±44.84) mg/dl as shown in table(2).The differences were statistically not significant.

Table(2) Lipid profiles (mean ±SD) among hypertension patients according to BMI.

Variables	**18.5-24.9 Kg/m2**	**25-29.9 Kg/m2**	**30≤ Kg/m2**	**P. value**
Total cholesterol (mg/dl)	210±46.21	200.37±41.57	212.38±54.84	0.132
HDL (mg/dl)	36.86±7.65	37.63±8.08	38.25±11.86	o.174
LDL (mg/dl)	144.71±44.84	127.46±33.03	134.06±41.22	0.815
Triglycerides (mg/dl)	123.71±32.34	190.44±128.34	179.88±114.87	0.133

Smoker patients in this study had highest ASCVD risk score (12.65±6.63%) followed by former smokers (7.36±3.56%) and non-smokers (6.02±4.16%) respectively as shown in table (3). The difference was statistically significant.

Table(3) ASCVD-score (Mean ±SD) among hypertension patients according to smoking status.

Variable	**Smokers**	**Former**	**Non-smoker**	**P-value**
ASCVD score (%)	12.65±6.63	7.36±3.56	6.02±4.16	<0.0001

In the present study there were 107 (31.1%) patients with hypertension had very high risk for developing ASCVD, most of them were males (65%), while 128 (37.2%) patients had moderate risk for developing ASCVD, most of them were females (71.9%) as shown in table (4). The difference was statistically significant.

Table (4) ASCVD risk according to 2016 ESC/EAS guidelines and gender distribution in hypertension patients.

Variables	**Male**		**Female**		**Total**		**P-value**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Low risk	7	63.6	4	36.4	11	3.2	<0.0001
Moderate risk	36	28.1	92	71.9	128	37.2	
High risk	48	49.0	50	51.0	98	28.5	
Very high risk	65	60.7	42	39.3	107	31.1	

Only 76 (22.1%) of hypertension patients were on statin therapy for hyperlipidemia and only 18 (23.7%) of them reaching target LDL level according to 2016 ESC/ EAS guidelines. While 268 (77.9%) of hypertension patients had no statin therapy and only 65 (24.3%) of them had LDL level within target as shown in figure (1). The difference between treated and non- treated patients in reaching targeted LDL level was statistically not signicant.

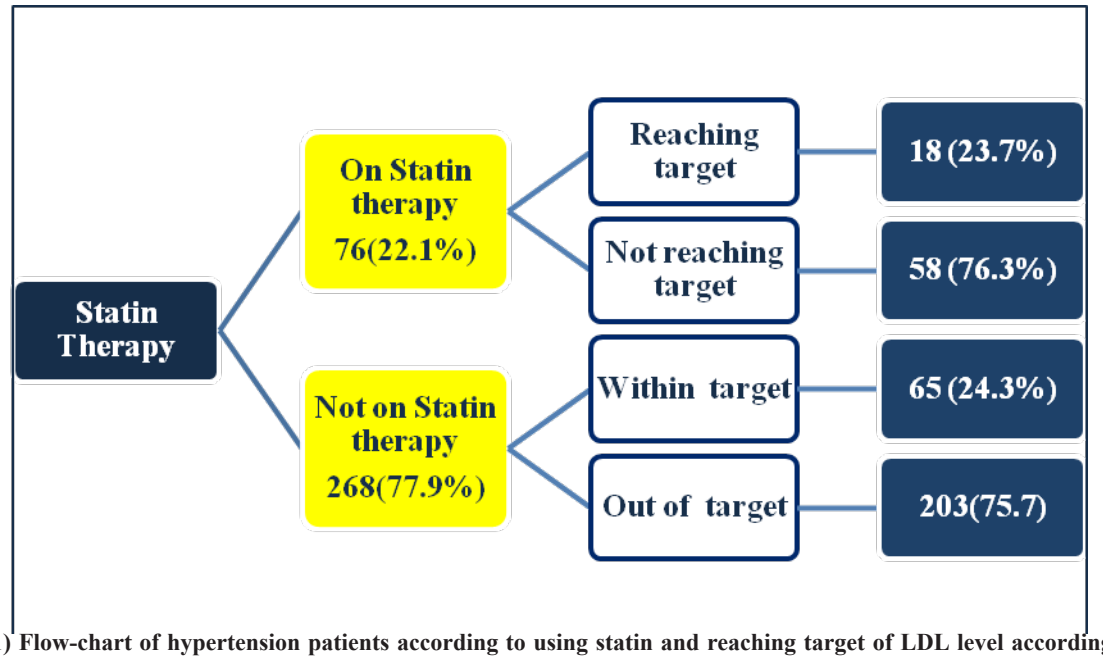

Figure (1) Flow-chart of hypertension patients according to using statin and reaching target of LDL level according to 2016 ESC/EAS guidelines.

Discussion

In the present study, females had levels of HDL and triglycerides significantly higher than that in males , while there was no significant difference in regard to levels of total cholesterol and LDL. Previous study [16,17] reported that sex -related differences in triglycerides, total cholesterol , HDL and LDL levels, other study [18] found no sex -related differences in TC and LDLC in subjects aged 50 years and more but females had lower level of triglycerides and HDL than males. In another study [19] females aged 18 years and more had lower levels of triglycerides and LDL and higher level of HDL than males.

In the present study there was no significant differences in levels of total cholesterol, triglycerides, LDL and HDL among different BMI groups. Associations of adiposity at baseline and dyslipidemia have been shown in large prospective studies[20-24], however there is inverse relationship between smoking and body weight [25], and smoking associated with low HDL [26], for this reason and perhaps using of statin therapy by some patients in this study may lead to these results.

The present study revealed the effect of smoking on raising ASCVD risk and this is in agreement with other studies [27-30]. Also this study revealed that most of very high risk group for developing cardiovascular disease were men and this is in agreement of other studies [20-24].

The present study revealed that only 22.1% of Iraqi hypertension patients in Tikrit City on statin therapy and only 23.7% of them reaching target of LDL level , while 77.9% of them are not on any treatment for hyperlipidemia, although 75.7% of them are out of target of LDL level according to 2016 ESC/EAS guidelines. This is comparable to a study done in Russia [31].

Conclusion

Hyperlipidemia is very prevalent in hypertension patients in Tikrit City, despite that only one of five receiving treatment and only one of four from those who received treatment reaching the target of LDL level according to 2016 ESC/EAS guidelines, and three of four of those who are on no treatment are out of the target. This is a major health problem need attention.

❖ **Conflict of Interest -** (nil – There are "NO CONFLICT OF INTEREST")**.**

❖ **Source of Funding -** By all researchers **(self).**

❖ **Ethical Clearance:** Committee members are approved to perform a study about

"Study of dyslipidemia in Iraqi Hypertension patients in Tikrit city"

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Isolating Some Pathogenic Bacteria from Packed Milk and Detecting of Formalin in its Components

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Abstract

Aims: The main aims of thisstudyis to isolate pathogenic bacteria from packed milk in Basrah city, and to detect the presence of formalin in milk samples.

Methodology:A total of 9types of packed milk were collected from the market in different period. The samples were transported to the laboratory in cooling conditions. They were stored in a refrigerator and analyzed within 24 hours. One ml from each sample was diluted in 9ml sterile distilled water, the diluted sample was a streak inoculated on chromogenic media**.** Three ml of milk sample was taken in a test tube and diluted with equal size of water. 5ml of sulfuric acid (90%) were added to the diluted milk slowly to the side of test tube which must handle with slant position in order to form separation layer. In case of formaldehyde presence a violet layer will form. This method detect of 1 part of formaldehyde in 200000 parts of milk

Result: The result showed only *Staphylococcus saprophyticus* and *Staphylococcus aureus* have been isolated from milk samples (23.4 %) and (10.6 %) respectively. The result showed that high temperature not very effective since two pathogenic bacterial species have been isolated and formalin have been detected in milk samples. The result showed no effect of addition of formalin in some sample and did not inhibit the growth of bacteria.

Conclusions: This study concludes that most of the packed milk under study contaminated with some pathogenic bacterial species, most of milk samples contained formalin.

Key words: *Bacteria, Milk, Formalin, Basrah*

Introduction

Milk and its products have been an important part of the human diet for some 8000 years and are part of the official nutritional recommendations in many countries worldwide. Milk products are rich in calcium, protein, potassium and phosphorus and it is very important for children and adolescents because it contains numerous essential nutrients so itprovides around 52–65 % of the dietary reference intake of calcium and 20–28 % of the protein requirement[1,2].Some of the bacteria contained in milk (such as *Lactobacillus spp* or *Bifidobacterium spp*.) are also present in the healthy human gastrointestinal tract, aiding in digestion and protection from other infections, while other bacteria can be extremely harmful to human health.Milk can be polluted by *Mycobacterium bovis*, *Brucella* species, *Streptococci* and *Coxiellaburnetti*from infected cattle. Agents from human sources such as *Salmonella* species, *Shigella* species, *Corynebacterium diphtheria* and *Streptococcus* species can also be presented in milk[3].

Many preservatives such as nitrate, boric acid, salicylic acid, hydrogen peroxide, formalin, carbonate and bicarbonate are adding to milk to improve keeping quality of milk and to delay spoilage is a problem for regulatory bodies from the early history of dairying. The toxic effect, hypersensitivity, teratogenic effect

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and carcinogenic effect are the most common serious public health hazard appears as a result of accumulation of preservatives[4].The addition of Formalinas adulterant in milk affects on the health of consumers; it causes vomiting, diarrhea and abdominal pain. Larger doses may cause decreased body temp, shallow respiration, weak irregular pulse and unconscious. It also affects the optic nerve and cause blindness. It is one of the potent carcinogens[5]. Therefore the present study was performed to throw out a light on the microbiological examination for detecting pathogenic bacteria and formalin detection in packed milk.

Methodology

Collection of milk products

Nine types of packed milk were collected from the market in different period. The samples weretransported to the laboratory in cooling conditions. They werestored in a refrigerator and analyzed within 24hours[6].

Microbiological analysis

One ml of each sample was diluted in 9ml sterile distilled water.The diluted sample was a streak inoculated on chromogenic media as given below:

**Staphylococcus* chromagar (Paris, France) used to isolate *Staphylococcusaureus*and *Staph. Saprophyticus*.

**E. coli* chromagar (Paris, France) used to isolate *Escherichia coli* and other gram negative bacteria.

*Salmonellchromagar (Paris, France) used to isolate *Salmonella* spp.

**Pseudomonas*chromagar (Paris, France) used to isolate *Pseudomonasaeruginosa*

Detection of formaldehyde presence

Three ml of milk sample was taken in a test tube and diluted with equal size of water. 5 ml of sulfuric acid (90%) were added to the diluted milk slowly to the side of test tube which must handle with slant position in order to form separation layer. In case of formaldehyde presence a violet layer will form. This method detect of 1 part of formaldehyde in 200000 part of milk[7].

Results

The recent study was detected of pathogenic bacteria(*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*,*Staphylococcus saprophyticus* and *Pseudomonas aeroginosa*) in packed milk samples from different origin of products in more than one date of production (November 2018 to February 2019). Only,*S. saprophyticus* and *S. aureus* have been isolated from milk samples (23.4%) and (10.6%) respectively.The CFU number of *S. aureus* and *S. saprophyticus* isolated from milk samples was documented in Table 1.

Table 1: number of bacterial colony forming unit (CFU) on chromo agar media

Type of sample/origin of product	No. of samples	Number of CFU	
		S. aureus	S. saprophyticus
Safio /KSA	1		Uncountable
Almarai/ KSA	1	1	
	2		1
	3		1
KDD/ Kuait	1	2	6
	2	Uncountable	
	3		1
	4		3
	5		Uncountable
	6		1
Kalleh /Iran	1	1	
	2		1
	3		1

All samples under study have been written on its packet "sterilized by high temperature" and no added preservative was mentioned. However, the result showed that high temperature not very effective since two pathogenic bacterial species have been isolated and formalin have been detected in milk samples (Table 2).In some samples, the addition of formalin to milk also did not inhibit the growth of bacteria.

The lack of mention of the ingredients of any particular food products, especially the presence of preservatives is a commercial fraud.

Table 2: Number of milk samples contain formalin

Sr.No.	Type of sample / origin of product	Total number of samples	No. of the samples contain formalin
1	Safio /KSA	8	2
2	Almarai/ KSA	8	8
3	KDD/ Kuait	11	11
4	Kalleh /Iran	6	_
5	Farms alsaba/Syria	3	_
6	Tiffany /UAE	6	3
7	Nesquik/ Turky	2	2
8	Alis / Iran	2	2
9	Nada/ KSA	1	1

Discussion

Iraq relies heavily on imported products, so that there was no Iraqi milk available during the collection of samples from the market. There are Iraqi products of yogurt and cheese, but not packaged milk. The packaged milk found in the Iraqi markets is a Saudi, Kuwaiti, Turkish, Iranian, UAE and few Syrian ones. Since, the war in Syria have been started, Syrian products decreased in Iraqi markets. Formaldehyde is quickly absorbed from the gastrointestinal tract following ingestion and quickly diffuses into many tissues, including the brain, testis, and liver[8]which makes it a dangerous chemical to be used as preservative[9,10].Toxicological effects including histopathological alteration in the stomach (i.e., gastrointestinal lesions (such as papillomata's hyperplasia and hyperkeratosis), allergy, asthma[11], abdominal pain and vomiting.

A Bangladesh study showedsome severe histological alterationsin liver and kidney in treated mice, which werefed with formalin as 30µl, 3µl and 0.3µlfor 30 days[12].Formalin is significantly related with cancer, particularly nasopharyngeal cancer in humans through inhalation during occupational exposure[13].Sometimes caused gastrointestinal cancer when present withhigh concentration in drinking water[14].Consumption of foods contaminated with this dangerous chemical exposes humans, particularly kids and elderly to severe health problems in Iraq and some developing countries.We conclude that most of the packed milk under study contaminated with some pathogenic bacterial species, most of milk samples contained formalin.

Ethical Clearance: Ethical clearance taken from nursing college/university of Basrah.

Funding Source: Self

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Screening Study for Some Strains of E. Coli Collected from Five regions in Kurdistan-Iraq for its Sensitivity, Resistance and MDR against Thirteen Antibiotics

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Abstract

Background: New strains of bacteria, which are sensitive and resistant to antibiotics, has been selected due to the overuse of antibiotics. The profiles of *Escherichia coli* (*E. coli*), which is resistant to antibiotics, are taken from different samples of human fecal and urine.

Aims: This article investigates the occurrence and observing the sources of *E. coli*, which is sensitive and resistant to antibiotics. The study samples are collected from human urine and fecal by limited inhibition zone.

Results: The study revealed a 92% sensitivity to Chloramphenicol and Gentamycin, 77% to Clarithromycin and Neomycin and 54% to Doxycycline. While the lowest sensitivity level was documented for Erythromycin and Novobiocin represented by 6.8%. Furthermore, the lowest level of sensitivity was reported for Erythromycin, and represented by 38% in comparison to control. Moreover, the sensitivity to penicillin was low. Besides, this study revealed that the isolated samples of *E. coli* were highly resistant to the multiple antibiotic resistance of the three antibiotics, namely Ampicillin, Lincomycin and Rifampin during the same time.

Conclusion: All of the antibiotics used in this study had a clear impact against *E. coli* bacteria as antimicrobial drugs to inhibit its growth and infection.

Keywords: *E. coli, Antibiotics resistance, Antibiotics sensitivity.*

Introduction

E. coli is gram-negative bacilli, facultative anaerobic bacteria that belong to the *Enterobacteriaceae* family [1], and propelled by long flagella. It is found in the intestinal tracts of human and animal due to faecal contamination or food contamination during the slaughter of an animal. It frequently exists in food, water and soil [2]. Furthermore, *E. coli* causes lower urinary tract infection, septicaemia or coleocystis. Resistance to multiple drugs in human pathogenic bacteria has been established because of the random use of commercial antibacterial medicines commonly utilized in treating infectious diseases. In the literature, there are a number of factors impact of developing resistance to antibiotics. These factors include 1) the specific nature of the relationship between bacteria and antibiotics; 2) using an antimicrobial agent; 3) characteristics =of the host; and 4) ecological factors. Generally, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are used as therapeutic agents[3].

Antibiotic resistance

It is well known that the extended-spectrum β lactamases (ESBLs) are produced by multidrug-resistant Enterobacteriaceae, commonly *E. coli* (e.g., the enzymes of CTX-M). These enzymes are more active against cefotaxime than other oxyimino-beta-lactam substrates like ceftazidime, ceftriaxone or cefepime. They are defined within the setting of the community as a significant cause of urinary tract infections (UTIs). Recently, there are studies describing ESBL-producing *E. coli* as being a cause of bloodstream infections related to these community-onsets of UTI [4]. It is an organism that occurs widely in sewage because it is a

faecal coliform. It has a significant role in the analysis of water sanitary. Over the past fifty years, two studies conducted by Maaloe and his colleagues have described the growth rate regulation in bacteria for the first time. This has been a significant issue in bacterial physiology [5]. The virulence factor (toxicity) of *E. coli*. explains its pathogenicity. There are varied factors that define the pathogenicity of *E. coli*. Based on the nature of these factors, various infections may be caused by these strains, including skin wounds infections [6].

Pathogenic strains of this organism are distinguished from normal flora by the presence of virulence factors, such as exotoxins. *E. coli*, also known as UPEC, is the most prevalent extra digestive pathogen that causes UTI in children and accounts for about 80-90% of the community-acquired cases. Five pathogenic strains of *E. coli* are frequently isolated from humans and animals suffering from diarrhea. *E. coli* is the most commonly recognized bacterial pathogen in infantile gastroenteritis [7].

Recently, bacteria species resistant to antibiotics are formal in the environments. The negative effect of bacteria has considerably increased. Resistance to antibiotics in *E. coli* is totally defined in isolates from human, animal and environmental sources. Among the antibiotics, Ampicillin, that belongs to the penicillin family, is used to treat and study these bacteria. The standard Kirby-Bauer disk diffusion method has been employed in this study for determining the profiles of antibacterial sensitivity of the *E. coli* isolates with different antimicrobial agents [1].

Aim and Objectives

This article investigates the occurrence and observing the sources of *E. coli*. The objectives of this work are as follows:

Isolating pure culture of *E. coli* from human fecal samples.

Assessing the antibacterial effectiveness of antibiotics against isolates under study.

Describing resistant strains for the antibiotics used in this study.

Limited multi drug antibiotics (MDA) resistant.

Material and Method

Collection of Stool Specimens

This study included 250 male and female patients. The ages were ranged between 6 months and 5 years. Fifteen samples were selected from 5 years (i.e., positive sample) that included 2 control (not infected). *E. coli* was taken from human fecal. The samples were obtained from hospitals in areas of Soran, Qaladwza, Hawler, Rania and Koya in the Kurdistan region, Iraq. The period of sample collection was during the period from October 2014 to April 2015. The samples were transferred to the laboratories of the research center at the University of Soran. *E. coli* was isolated using the bacteriological and biochemical test of fecal sample collected from patients [1].

The gram-stained slides were obtained from the Central Drug House (P) Ltd. (CDH), which was tested microscopically. Samples were cultivated on Nutrient Broth (Lab M™, Bury, UK) to activate the bacterial isolation of the chemical test reactions (37 ºC, 24 h). After that, they were transferred on selective media (Mac Conkey agar, Lab. M. Limited UK) to isolate the rod to the negative gram bacteria and differentiate between fermented and non-fermented isolates of sugar Lactose, Eosin methylene blue (EMB) agar Himedia. The ionic dye contained two blue ones planted by E. coli, giving a bright color green. Concerning the final identification, tests of API 20E Biomerieux were used [8].

Culture media

The preparation of all media utilized in the current study was done in accordance with their manufacturers.

Isolation of bacteria (*E. coli*):

Culture media used were MaCconkey agar, EMB agar, Nutrient agar and Blood agar. The standard loop was sterilized and dived in a vertical way after cooling in fecal samples, filed and immediately transported to culture media by duplicate streaking. One of the streaked plates was incubated under aerobic conditions of growth, and the other one was incubated under partial anaerobic conditions of growth with 5% of CO2. Finally, all streaked plates were placed at 37 ℃ for 24 hours [9].

Identification of bacterial isolates

Culture media were prepared for the purpose of purification, conservation, revitalization, quality

appearance identification, cultural recipes and for making a biochemical test of the bacterial isolates. Also, the dyes, solvents and reagents were used to complete biochemical tests as clarified below:

Characteristics of the developed colonies:

The colonies observed on culture media were distinguished according to the following: 1) color; 2) the surface of the colony; 3) strength; 4) transparency; 5) smell; 6) lactose fermentation on Macconkey agar; and 7) the appearance of metallic sheen on EMB agar for *E. coli* [9].

Microscopic examination:

Smears of each type of appeared colonies were prepared. Gram stain was done to examine the microbial cells gram positivity and negativity as well as cells arrangement [9].

Biochemical tests:

In the literature, Biochemical tests were done to distinguish *E. coli and Staphylococcus epidermidis* from other bacterial isolates. They included Catalase, Oxidase, Coagulase Voges Proskauer, Methyl red, Motility test, Urease, Indol, H2S Production, Citrate utilization, Sugar fermentation Haemolysis [9].

Preparation of McFarland turbidity standard No. 0.5

In this study, 50 µl of a 1.175% (wt/vol) barium chloride dihydrate (BaCl2•2H2O) solution was added to 9.95 ml of 1% (vol/vol) sulfuric acid to prepare the 0.5 McFarland turbidity standard. Next, the McFarland standard tube was sealed with Parafilm for preventing evaporation. Then, it was stored in a dark place at room temperature. After that, the density accuracy of the prepared McFarland standard was examined through utilizing a spectrophotometer with a 1 -cm light path for the 0.5 McFarland standard, the absorbance at a wavelength of 625 nm and water as a blank standard was 0.08 to 0.13. The turbidity was vigorously agitated by the 0.5 McFarland standard on a vortex mixer before use. As with the barium 40 sulfate standards, a 0.5 McFarland Standard was analogous to a microbial suspension of 1.5 X 108 colony-forming units (CFU)/ml [10].

Antibiotics Tests

Antibiotic discs

High potency discs of the following 13 antibiotics were used against all E. coli: Streptomycin (S), Novobiocin (NV), Neomycin (N), Lincomycen (L), Norfloxacen (NOR), Chloramphenicol (C), Doxycycline (DO), Rifampen (RA), Ampecilline (Am), Erythromycin (E), Tetracycline (TE), Gentamycine (CN), Clarithromycen (CLR).

Antibiotic resistance screening test

The resistance of test strains to different antibiotics was determined using the disk diffusion method as described by the Clinical and Laboratory Standard Institute (CLSI) standards. The test strains were first enriched in nutrient broth for 24 h at 37 °C by picking off technique. By using sterile swab sticks, plates were seeded with 1 ml of a suspension of the test strains containing approximately 106 cells. Antibiotic discs were dispensed on the plates seeded with organisms. The plates were incubated at 37 °C for 24 h, and antibiotic resistance was interpreted by inhibition [11].

Results and Discussion:

Outcomes of isolation and identification of tested bacteria

There is a significant reason for mortality and morbidity throughout the world, represented by bacterial infectious diseases. Consequently, there is an increasing interest in developing new antibacterial agents for treating bacterial infections.

Table 1: Antibiotic disk.

Antibiotics	**Con.** Mg	**Antibiotics**	**Con** µg.
Ampicillin	10 µg	Rifampen	5µg
Streptomycin	10 µg	Gentamicin	10 µg
Novobiocin	30µg	Chloramphenicol	30 µg
Neomycin30	30 UI	Erythromycin	15µg
Lincomycen	15µg	Tetracycline	30µg
Norfloxacen	10µg	Clarithromycen	15µg
Doxycycline	30µg		

Note: Con.: Concentration

Table 2: Antibiogram of *E. coli* isolates from fecal of different provinces.

Average inhibition zone of diameter size (mm) of *E. coli* isolates																
Antibiotics	C1	C2	Ave	S 1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
S	1.8	2.8	2.3	1.7	1.7	1.7	1.6	2.4	2.7	1.9	1.5	1.9	1.9	1.7	1.5	1.6
NV	R	0.8	0.4	0.9	0.9	0.9	0.9	1.3	0.5	0.8	2.2	0.9	0.7	1.8	1.7	1.7
N	1.7	1.9	1.8	2	**R**	**R**	**R**	1.6	1.9	1.5	1.7	1.9	1.5	1.6	1.5	1.7
L	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
NOR	2.7	2.8	2.7	3.2	3.2	3.2	2.5	2.7	3	3	2.1	3	2.7	1.9	2.5	2.4
C	2.5	1.6	2	2.5	3	1.6	2	3.2	2	1.2	1.6	2.5	2.1	0.7	1.9	1.5
DO	1.3	**R**	0.6	3	2.5	**R**	2	**R**	**R**	**R**	2.5	**R**	**R**	1.7	1.9	1.9
RA	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Am	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
E	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	1.2	2.5	**R**	**R**	2.5	3	2.1
TE	1.2	R	0.6	**R**	**R**	**R**	**R**	**R**	**R**	**R**	1.4	1.7	**R**	**R**	1.3	1.4
CN	2.3	1.8	2	2.5	2.6	**R**	1.5	2.6	2.5	1.2	2.5	1.7	0.9	2.5	2.6	2.5
CLR	1.5	1.5	1.5	**R**	2	**R**	2	1.2	2	1.8	2.5	2.3	**R**	2.5	4.3	4.3

**C: Control, **S: Sample, **R: Resistant, Ave: Average

Table 3: The percentage of E. coli sensitive to antibiotics isolated from different samples of fecal.

Antibiotics													
Number	C	S	L	NOR	N	NV	TE	CN	CLR	AM	RA	DO	E
	12	13	0	13	10	5	4	12	10	0	0	7	5
Percentage	92	10	0	10	77	38	31	92	77	0	0	54	38

Table 4: The percentage of E. coli resistant to antibiotics isolated from different samples of fecal.

Antibiotics													
Number	**C**	**S**	**L**	**NOR**	**N**	**NV**	**TE**	**CN**	**CLR**	**AM**	**RA**	**DO**	**E**
	0	0	13	0	3	0	9	1	3	13	13	6	8
Percentage	0	0	0	0	23	0	70	8	23	100	100	46	62

Table 5: The percentage of intermediate resistance of E. coli isolated from different samples of fecal.

Antibiotics													
Number	**C**	**S**	**L**	**NOR**	**N**	**NV**	**TE**	**CN**	**CLR**	**AM**	**RA**	**DO**	**E**
	1	0	0	0	0	8	0	1	0	0	0	0	0
Percentage	8	0	0	0	0	62	0	8	0	0	0	0	0

Table 6: Multiple antibiotics resistance of E. coli isolated from different samples of fecal.

Source of isolates	**Number of isolates**	**Number (%) of isolates that showed resistance to multiple antibiotics** **3 antibiotics** L, RA , Am
Children	13	100%

Prevalence of ***E. coli*** that is sensitive and resistant to antibiotics isolated from faecal samples:

The results showed that all of the *E. coli* isolates from human fecal are resistant to all medicines at different levels to different antibiotics. As illustrated in Table 2, 53% of the isolates were sensitive to antibiotics, 41% resistant, and 6% intermediate resistant. Some isolates under study showed sensitivity to Erythromycin compared with the control, which gave a negative result. There was a possibility that these isolates were Rfactor. According to Table 3, 92% of Chloramphenicol and Gentamycin, 77% of Neomycin and clarithromycin, 54% of Doxycyclin, 38% Novobiocin and erythromycin, 10% of Streptomycen and Norfloxacin.

Nevertheless, equal amounts (100%) of isolates were found to be resistant to Ampicillin, Lincomycen and Rifampin, 70% of Tetracycline, 62% of erythromycin, 46% of Doxycycllin, 23% of clarithromycin and Neomycin (see Table 4). These results were consistent with those of studies of Masuder (2007), and Zinnah et al. (2008) for above 80% and 53% of the *E. coli* isolates from human fecal were sensitive to Gentamycin, Clarithromycin and Neomycin, respectively. Simultaneously, these studies differ from the current research, as only 8 % of isolates showed resistance to Gentamycin. Also, this study is consistent with that of Wolde Tenssay (2002), which revealed that 100% and 79% of isolates showed resistance to amoxicillin and tetracycline, respectively. Nevertheless, results of this study are similar to those of in terms of the isolates percentage (50%) that are resistant to tetracycline and due to the insignificant percentage of *E. coli* isolates from human fecal and urine that showed resistance to cotrimoxazole, respectively. Likewise, the current paper was consistent with that of [12] in that a very unimportant number of isolates reported resistance to norfloxacin. Table 5 shows that the isolates are intermediately resistant to antibiotics depending on the inhibition zone.

Bacteria that were resistant to multiple drugs were taken from fecal samples that were collected at Al-Iklem (see Table 6) represented by 13 E. coli isolates examined for revealing resistance to antibiotics. All of them (100%) demonstrated multiple resistance for antibiotics [13]. Finally, similar studies conducted in and nearby Jimma, Ethiopia indicated that nearly all E. coli isolates from environmental sources showed resistance to the frequently used antibacterial medicines including amoxicillin, tetracycline and cotrimoxazole.

Conclusion

From the above findings, we conclude the following:

All antibiotics used in this study had a clear impact against *E. coli* bacteria as antimicrobial drugs to inhibit their growth and infection.

The isolated strains of *E. coli* have a highly sensitive effect on the third-generation aminoglycosides, fourth-generation cephalosporins and carbapenem.

The method of disc diffusion is simple, reliable and low-cost for examining the *E. coli* susceptibility to Am, S, N, L, NOR, C, DO, RA, E, TE, CN, CLR.

Limited MDR depending on the resistant zone, the study found multiple resistance to three types of L, RA and Am.

Conflict of Interest: There is no Conflict of Interest..

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Ethical Approve: This study was conducted with approval from the research ethics committee at the Department of Biology, College of Science, the University of Kirkuk as well as Training and Human Development Division, Department of Health, Kirkuk Governorate.

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The Role of Silver (Ag) Nanoparticles synthesis by *Penicillium spp* against the Toxicity of *Echinococcus Granulosus* in Adult Albino Male Rats

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Abstract

The aim of the study was to synthesize Ag nanoparticles by using filamentous fungus

Penicillium sp. The fungal culture was isolated from the soil samples collected from agriculture fields in Kirkuk city. The synthesis of silver nanoparticles was investigated by X-ray difraction peaks were measured at (101), (200) and (202) respectively, and scanning electron microscopy .

The present study was designed to indicated the role of Ag nanoparticles synthesis by *Penicillium spp* against toxicity of *Echinococcus granulosus*. The present study used twenty adult albino male rats that distributed at random to following teams (each group consist five rats); management group received ad libidium, second group injected with 2,5 X 10^3 of *Echinococcus granulosus* protoscolices third group injected with protoscolices and treatd with 50 mg/kg AgNanoPs, fourth group injected with protoscolices and treatd with 100mg/kg AgNanoPs. The results show high important exaggerated ($P < 0.05$) in levels of MDA (malonedialdehyied) and important decrease ($P < 0.05$) in levels of glutathione (GSH) and catalasce compared with management group. While, after used AgNPs with *Echinococcus granulosus*, the results indicated non-significant changes ($P < 0.05$) in MDA, GSH and catalse also showed non-significant changes ($P < 0.05$) compared with control group. histological study show decrease in numbers of spermatogonia and spermatocytes with absent of spermatid. While, after using AgNPs the testis in third and fourth groups appear semi-normal. It had been ended that AgNanoPs has been potential role against tocxicity of *Echinococcus granulosus* in rats male.

Keywords: *Ag nanoparticles; Echinococcus granulosus; testis.*

Introduction

Cystic Echinococcosis (CE) is one of the most important zoonotic helminthic diseases throughout the world (1). The larval stage of the Echinococcus granulosus leads to hydatidosis (2). Adult worms live in the small intestine of canids as definitive hosts with a high prevalence in the world (3, 4). Intermediate hosts include humans as well as cows, sheep, camels, horses et al that acquire the infection by oral uptake of tapeworm eggs. once intake by appropriate host and sequent passage through abdomen and intestinal, the oncosphere brute become activated, penetrate the tissue layer, enter to the blood stream and body fluid vessels and area unit disseminated within the body. once associate indefinable time period, E. granulosus metacestodes area unit shaped (1, 5). Nano-biotechnology is presently one among the foremost dynamic disciplines of analysis in modern material science whereby plants and totally different plant merchandise ar finding an important use within the synthesis of nanoprticles (NPs) (6, 7). in nano-biotechnological analysis, AgNPs have received important attention owing to their distinctive physical chemical, biological properties, and since of their pertinence in natural philosophy, optics and drugs (8). Among diverse nanaoparticles, Ag nanoparticles because various properties like chemical change,, chemical science conduction and antimicrobial activity, are often employed in completely different applications like biomedicine, agriculture, icon chemicals and food chemistry (9, 10). The present study was designed to indicate the role of green Ag nanoparticles againsted toxicity of *Echinococcus granulosus*.

Materials & Method

Animal model

In this study twenty adult male albino rats, (wt 225-275 gm with age4-6 month) obtained from Technicals colleege/ North Technical Univerisity, and unbroken on customary pellete diet for week to insure its normal.

Isolation sample

Soil samples were obtained from town of city. For soil suspension preparation, CaCO3 was treated with one0: 1 weight / weight and incubated in brooder at thirty seven ° C for four days. For drying functions, a series of ten - so took 0.5 milliliter of dilutions 10-3.10.4 and placed in sterile Petri dishes and poured within the middle of the sterile PDA and fluid to (50-45) m and stirred the dish to homogenize the soil answer with the middle of the plant so incubated for 6-4 days when uninflected the isolates and diagnosing, looking on.

Preparation of fungal biomass and composition of silver nanoparticles

The fungal fungus was prepared by taking a tablet of the clean, pure fungal colony using a 7 mm sterile veneer hole, where the developing colony edges were punctured on the fungal growth plate and placed in a 100 mL conical flask of the liquid MYPG medium where it was quietly placed to the disc on the center surface After a period of time, the disk stabilized on the surface of the liquid medium, and then placed in the incubator at 26 ° C for 5-7 days to obtain a mat with different weights (11). After the fungus was nominated the fungal mass using the filter paper(Whatman filter paper NO 1) And then rinsed thoroughly with distilled water to remove the residue of the medium. The fungal flask was then placed in a conical flask containing 100 ml of distilled distilled water and left for 72-24 hours in the incubator. The fungal mass was again filtered using filter paper to ensure that all components of the medium were removed. Filtration in an electric oven of Memmert (Germany) at a temperature of 50 m. The dry weight of the fungus was measured by weight between the dried paper mass with its contents after filtration and the filter paper block. It was dried before filtration. 10 g of fungal mass and then placed in 100 ml of Ag NO3 prepared silver nitrate With a concentration of 1mM and placed in the incubator In completely dark conditions and after chromatography, AgNPs were examined x-ray shimadzu during a laboratory within the academic department - school of Science, University Bagdad. The scanning microscopy SEM (Scaning negatron Microscopy) (TESCAN-VEGA) was conjointly employed in the engineering center.

Echinococcus granulosus

hydatid cysts were collected and obtained from infected sheep livers. They were put in plastic bags, and transported to the Department of biological science , school Technical , North Technical University, wherever protoscolices were isolated from livers in keeping with (12) technique . Protoscolices indicates the fertility of hydatid cyst and it's were counted according to method cited by(13). cyst and it's were counted in keeping with technique cited by (13). The viable protoscolices for parasite were counted in oneml from supernatal supported the formula : Viability in 1 metric capacity unit = variety of protoscolices in (10 µl) × one hundred.

Experimental design

Twenty adult male albino rats were used and divided as follow (each groupe consist 5 rats):

management group: rats were received normal pellet diet just for seven days so killed..

Positive group rats injected with protoscolices, and so killed.

Third group rats injected with protoscolices and treated with 50mg/kg Ag NanoPs for month, and so killed.

Fourth group rats injected with protoscolices and treated with 100mg/kg Ag NPs for month, and then killed.

Prepare of blood solution

The blood collection from rats by internal organ puncher, below anaesthesia, and place in check tubes . After clotting, the tubes were activity for ten min to get sera. The bodily fluid was taken and hold on by deep phase transition till used.

Homogenization

Testis samples were removed immediately and the put in glass dish contents 0.9% NaCl buffer for washing and removed the blood. To oxidative stress factors determination, 10% from organ weight was dissolved with buffer (PH 7.4) and the organ tissue was crashed

by use ceramic mortar. Then mixture was centerfigation for 10 min. Supernated was taken and stored by deep freezing till used (14).

Measurements

Plasma Peroxidation levels (MDA), Glutathione (GSH) and Catalase

MDA (malonedialdehyied), was measured based on the quantitative chemical analysis reaction with thiobarbituric acid (TBA) exploitation photometer (15). GSH level calculable bymixed two.3 cubic centimeter buffer with zero.2ml of the sample and so side zero.5ml of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB).The mixture was analyzed by spectrophotometer(16). Catalase was measured by using the procedurce of Biovision-USA kits.

Histological study

testis biopsies were soft on 4mm punch and 2% xylocaine was used as an anesthetic. The biopsies were mounted in 10% formalin, habitually processed and embedded in paraffin sections that were stained with hematoxylin and fluorescent dye andand examined under the microscope.

Statistical Analysis

the info were analyzed employing a applied math Minitab program. A applied math distinction between the suggests that of the experimental teams was analyzed exploitation a way analysis of variance (ANOVA). Results

Isolation and identification of *Penicillium sp*.

Fungal cultures were isolated from the soil samples collected from varied agricultural lands in kirkuk city. The fungus isolates were characterised on the premise of colony characteristics and microscopic look (17). genus Penicillium sp colonies appeared as velvety and fissure with inexperienced color on personal organiser medium plates. Reverse aspect of the colony was yellow in color. Results are displayed in Figure1 . Microscopic identification of the plant isolates was performed by LPCB mounting. genus Penicillium sp. appeared as extremely branched mycilium part, septal hyphae. Conidiphores up on the mycelium part and conidiospores were unreal.

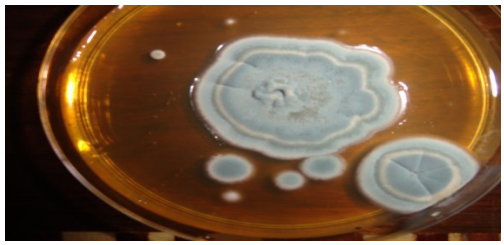

Figure 1: Colony morphology of the *Penicillium sp*. isolate

Characterization of silver nanoparticles Color amendment Cell free filtrate of *Penicillium sp*. was mixed with caustic resolution and incubated in dark in rotary shaker. Samples showed modified in color from virtually colourless to brown, this is often a transparent indication of the formation of silver nanoparticles within the reaction mixture. The intensity of the color was accumulated throughout the amount of incubation. the looks of brown color was because of the excitation of surface plasmon vibrations(18) .

X-Ray differentiation analysis

The XRD pattern therefore clearly shows that the conductorNO3 nanoparticles fashioned by the reduction of Ag ions by fungus genus sp are crystalline in nature (Figure 2). Our results similar output was obtained by (19, 20)

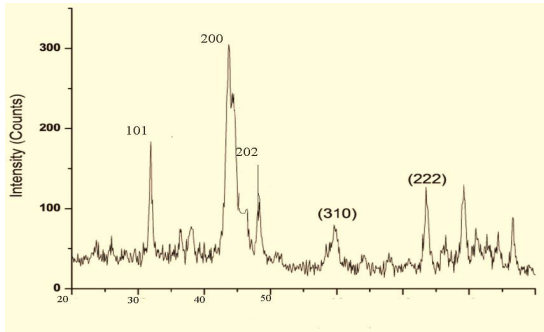

Figure (2) XRD of silver nanoparticles mistreatment genus Penicillium sp .

Scanning Electron Microscope

The dried silver nanoparticles were obtained by action at 10000 rev for twenty mts. the scale and form of the silver nanoparticles biosynthesized was studied by SEM as in figure 3.

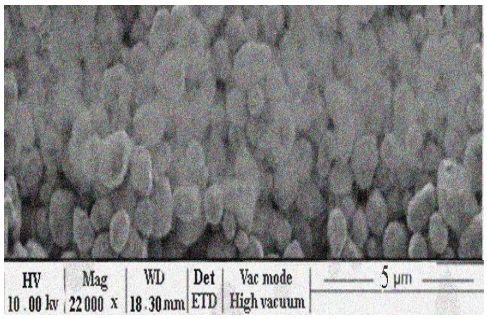

Figure (3)SEM image of silver nanoparticles.

Oxidative stress (MDA) & antioxidant parameters (GSH and catalase) in testis

The levels of MDA (2.18 ± 0.37), GSH (0.139 ± 0.025) and enzyme catalase (0.46 ± 0.07) in second group show high significant changes (P < 0.05) compared with management rats (1.24 ± 0.11; 0.276 ± 0.043 and 1.16 ± 0.02 respectively). The levels of MDA (1.38 ± 0.2; 1.27 ± 0.16 respectively), GSH (0.252 ± 0.031; 0.294 ± 0.052 respectively) and catalase (1.03 ± 0.04; 1.11 ± 0.03 respectively) in third and fourth groups show non-significant changes (P < 0.05) compared with management rats as shown in table (1).

Table (1): The levels of MDA, GSH and CAT in testis

Parameters Groups	MDA (mmol/l)	GSH (mol/l)	Cata (mmol/l)
Control group	1.24 ± 0.11 b	0.276 ± 0.043 a	1.16 ± 0.02 a
Second group	2.18 ± 0.37 a	0.139 ± 0.025 b	0.46 ± 0.07 b
Third group	1.38 ± 0.2 b	0.252 ± 0.031 a	1.03 ± 0.04 a
Fourth group	1.27 ± 0.16 b	0.294 ± 0.052 a	1.11 ± 0.03 a

Figure (4): testis of control group show spermatogonia (SPG), spermatocytes (SPC) and gamete (SPD). H&E X400.

Histological study

The sections that prepared with control group that show normal in normal structure of spermatogonia, spermatocytes and spermatids as shown in figure (4). The sections that prepared from third and fourth groups show a semi-normal structure spermatogonia, spermatocytes and spermatids as shown in figure (5).

Discussion

The results of present study increased in levels of MDA and decrease in levels of glutathione (GSH) and enzyme catalase in second group that infected with *E. granulosus* With decrese in numbers of spermatogonia and spermatocytes. The results is in agreement with (21) who referred that the infection with *Echinococcus granulosus* lead to eleveated the levels of MDA, where found the mean +/- SD of MDA levels of patients with *Echinococcus granulosu*(21). On the other hand, (22) referred that *E. granulosus* lead to decrese in numbers of spermatogonia and spermatocytes that is in agreement with present study (22). About the treatment and the role of AgNPs. many study show the ability of AgNPs as antimicrobial against different bacteria (*S.aureus*, *E.faecalis*, *Pseudomonas* and *E.coli*) (23). The bactericidal activity of nanoparticles is associated with alternative ways by direct react with microbial cells or effect on metabolic process lepton transport from biological process that inhibits respiratory chain enzymes or interferes through covering porousness to phosphate and protons(24) .Finally, the present study show after using AgNPs decrese in MDA and increase levels of GSH and catalase suggest the possibility of using Ag NPs as an anti-oxidant agent by inhibition the formation of free radicals and scavenging all species of (ROS) (25).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Characteristics of Lower Back Pain in Pregnant Ladies in Thiqar-Iraq

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Abstract

Objectives: to evaluate the frequency of back pain among pregnant female in Thiqar province in Iraq and its demographic characteristics.

Methodology: this is a case series descriptive study of one hundred pregnant female in Thiqar province in Iraq complaining from lower back pain in different stages of their pregnancies. the patients were evaluated firstly by history taking through questioner specially prepared for this study ;as well as; clinical and radiological assessment.

Results: in our study seventy one patients experience back pain in different stages of pregnancy giving a (71%) as frequency of back pain in the studded group. Most of the patients Seventy four patients (74%) developed the pain during the first trimester of pregnancy and (22%) experience the pain during the second trimester. Fifty eight (14) patients (11.96%) gave history of previous back problems before pregnancy; of these; nine patients (7.69%) diagnosed before pregnancy as herniated lumbar disc prolapse and kept on medical treatment and physiotherapy, 2 patients (1.7%) with previous laminectomy surgeries for herniated lumbar disc prolapse, 3 patients (2.56) with diagnosis of degerative spine. One patient underwent cesarean section for sever back pain dueto lumbar disc prolapse at the end of the pregnancy period (non-gynecological indication for cesarean section).

Conclusion: lower back pain is a common problem during pregnancy ,and it shows some common epidemiological characteristics.

Keywords: *Thiqar-Iraq ; back pain ; pregnant ladies*

Introduction

Low back pain is a common complaint among pregnant women. It is estimated that about 50% of pregnant women complain of some form of back pain at some point in pregnancy or during the postpartum period [(1)].

Pregnancy has a profound effect on the human body, particularly the musculoskeletal system. Hormonal changes cause ligamentous joint laxity, weight gain, and a shift in the center of gravity that leads to lumbar spine hyperlordosis and anterior tilting of the pelvis. In addition, vascular changes may lead to compromised metabolic supply in the low back. The most common musculoskeletal complaints in pregnancy are low back pain and/or pelvic girdle pain. They can be diagnosed and differentiated from each other by history taking, clinical examination, provocative test maneuvers, and imaging. Management ranges from conservative and pharmacologic measures to surgical treatment. Depending on the situation, and given the unique challenges pregnancy places on the human body and the special consideration that must be given to the fetus, an orthopedic surgeon and the obstetrician may have to develop a plan of care together regarding labor and delivery or when surgical interventions are indicated [(2)].

Risk factors that contribute to the development of low-back pain during pregnancy have been examined in several studies. The results consistently suggest that the major predictors of back pain during pregnancy are back

pain prior to pregnancy and multiparity.[Age, height, weight, race, baby weight, and socioeconomic status do not seem to correlate with risk for development of back pain during pregnancy [3].

Two major subtypes of back pain occurs during pregnancy ; (Lumbar and pelvic pain) lumbar pain located at and above the waist in the center of the back. But what is most common is the posterior pelvic pain which 5 times more common than lumbar pain and experience below the west on either sides [4].

Usually the treatment is conservative by rest, sleeping on the side, physiotherapy, lifting in proper way without bending, acetaminophen may be sufficient to relieve the pain. But ; the managing doctor should remember the fact that Bach pain during pregnancy may be a sign of premature labor or urinary tract infection. Sever back pain during pregnancy lasting more than one week is dangerous sign and need physician consultation [5].

Patients, material and method

This is case series of One hundred pregnant in Thiqar province in the south of Iraq were studied, all patients were selected randomly from the outpatients clinic of Bint Alhuda teaching hospital during their antenatal care visits and history of back pain were recorded; patients with positive history of lower back pain were evaluated by gynecologist firstly to exclude non-musculoskeletal back pain like preterm labor, urinary tract infection or other rare cause like ectopic pregnancy, such patients were excluded from the study, all patients then were referred to orthopedic out patient in Alhusein teaching hospital and evaluation done by orthopedic surgeon by history taking according to special questioner .clinical musculoskeletal examination of the back done by localizing the site of the pain, straight leg rising test, FABER test for sacroiliac joint pathology, in addition to full neurological examination of the lower limbs. Some patient were sent to x-ray examination for lumbosacral spine and the data from the were collected aiming to study the demographic and epidemiological characteristic of back pain problem in Thiqar province-Iraq.

Results

Our study include 100 pregnant women. 71 patients were found to have back pain giving frequency of (71%) .The mean age of the pregnant woman was 29.4 years. The mean gestational age were 28.3week . 29 patients (41%) were employer. Thirty nine patients out of 71 patients with back pain (54.92%) were not completed their secondary school. Fifty four patient out of 71 were multipara (76.05) . seventeen patients out of 71 with back pain patient ere primigravida(23.94) .forty one patients of multipara patients experience the same back pain during previous pregnancy(75.92). fifty seven patient out of 71 patient with back pain experienced the pain during second trimester (80.28%). The pain nature was burning sensation in lower back in 49 patients (69.01%). thirty patients with back pain have striae gravidarum in the lower abdomen and flanks (42.25%).

Discussion

Some studies considered back pain during pregnancy is a part of the pregnancy reflecting its common occurrence, Ng BK and his colleagues studied the frequency of back pain during pregnancy and it shows a frequency of 84.6% ; our result is consistent with that study in which a result of 71% indicating that this problem is common during pregnancy in addition to; this study is the one of the few studies which discussed the strong association between the problem of back pain during pregnancy in office worker, our study also shows some association between employment of pregnant female and the problem of back pain (41%) [6].

In our study 54.92% of pregnant female with back pain didn't completed their secondary education and this result is significantly differs from that of Carvalho MECC in his study which showed only 16.8% only and this difference may be dueto the cultural and educational facilities between our country and European population [1].

Our study show association between multiparity , striae gravidarum and the problem of back pain and this association is well studied by Kokanalı D which showed a an association between presence and severity of striae gravidarum and back pain [7].

Our study shows that most of pregnant female developed the back pain during second trimester of pregnancy (81.4%) and this finding is not consistent with result of Carvalho MECC in his study who show incidence of back pain during second trimester is only 43.7% a point which need further study and evaluation [1].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of

both environmental and health and higher education and scientific research ministries in Iraq

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Thyroid Dysfunction in Iraqi Patients with Acromegaly

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Abstract

Acromegaly is a chronic endocrine disorder caused by hypersecretion of growth hormone (GH) mostly because of pituitary adenoma. GH induces the formation of Insulin like Growth Factor1 (IGF1)from the liver. Both GH andIGF1 lead to sign and symptom of acromegaly.

Aim: This study designed to identify the prevalence of thyroid dysfunction in Iraqi patients with acromegaly ,to identify the factors responsible for thyroid dysfunction in patients with acromegaly.Is the treatment modalities for acromegaly has a role in thyroid dysfunction?

Study design: A cross sectional case control study

Patients and Method: seventy patients with acromegaly were enrolled from 2 different tertiary centers of endocrinology from Jan. -Dec. 2017 compared with 70 non acromeglic subjects. Their mean age 46 +/-11 years ;forty one (58.5%) males and 29(41.5%)females. Thyroid function test conducted to both patients and control groups including TSH, free T4 and total T3 in both centers. In addition to other variables.

Results: Out of 70 (100%) patients with acromegaly; (51.5%) were euthyroid, (20%) had primary hypothyroidism , (15.7%)had central hypothyroidism and (12.8%) had hyperthyroidism that is significantly higher than normal population ($p<0.001$).Patient with acromegaly had significant systolic and diastolic hypertension vs. control ($p=0.015$,0.004 respectively).Age of the patients, diastolic blood pressure ,first GH and history of hypophysectomy were significant risk factors for thyroid dysfunction ($p=0.02$,0.008,0.008 and 0.02 respectively)

Conclusion : Thyroid dysfunction is more prevalent in patient with acromegaly whether hypothyroidism (both primary and secondary) and hyperthyroidism. The Age and diastolic blood pressure are independent risk factors of thyroid dysfunction. There is significant role of hypophysectomy in thyroid dysfunction development while Sandostatin (Octreotide LAR) treatment had no role.

Key-words: *thyroid dysfunction ,acromegaly ,hypothyroidism ,central hypothyroidism, secondary hypothyroidism, hyperthyroidism.*

Introduction

Acromegaly is a chronic endocrine disorder caused by hypersecretion of growth hormone (GH) mostly because of pituitary adenoma (macroadenoma or less commonly microadenoma) .GH induces the formation of Insulin like Growth Factor1 (IGF1)from the liver [1]. GH and Insulin –like Growth Factor 1(IGF-1) act altogether to induce features of acral and visceral enlargement. Acromegaly is caused by tumors arising from anterior pituitary gland that secreting GH or very rarely by extrapituitary disorders[2]. GH secreted in a pulsatile fashion, while IGF1 levels are relatively stable with little diurnal variation [3]**.** It is evident through different studies that IGF1 play an important role in thyroidal cell growth .However it may be responsible for pathogenesis of different thyroid related diseases . One study shows that IGF-1 stimulated synthesis of protein and DNA as well as promoted thyroid cells proliferation and differentiation [4].

The thyroid gland is an endocrine organ present at the front and sides of the neck, anterior to the trachea. The thyroid gland produces thyroid hormones that are

responsible for regulation of metabolism as well as the development and differentiation of different body cells[5].

Acromegaly and thyroid hormonal abnormalities:

There are different factors responsible for thyroid stimulating hormone (TSH) secretion from anterior pituitary gland. The synthesis and secretion of TSH are under the inhibitory effect of T3 and T4 and stimulatory effect of TRH and to a lesser effect of other factors like ,leptin ,dopamine ,GH, IGF1 and somatostatin[6,7]. The thyroid gland sensitivity to TSH can be modulated by autonomous nervous system [8] .

TSH secretion has an important role for producing basic heat and energy homeostasis maintenance in the body [9].

Studies show that TSH secretion from thyrotrophic cells can be modulated directly or indirectly by GH and IGF-1[10]. In thyrotrophic cells; somatostatin inhibits TSH secretion through activation of two somatostatin receptor subtypes, SST2 and SST5[11]. GH under physiological conditions exerts a negative feedback on somatostatin secretion from hypothalamus and this lead to reduce the growth –hormone releasing hormone (GHRH) secretion **[12].** Thus, the increasing of somatostatin secretion has an impact on thyrotrophic cells that inhibit the TSH secretion**.** On the other hand, The GHRH has a synergistic effect on the TRH and further on TSH secretion both in healthy subjects and in patients with acromegaly [13]. As well as an increase in intrapituitary conversion of T3 to T4 in thyrotrophic cells because of GH excess can reduce TSH synthesis and secretion [14]. Moreover, because of the feedback mechanism, the GH excess with somatostatin participation has an inhibition effect on TSH secretion [6]. The TSH secretion can also be reduced by the presence of a pituitary tumor per se [10].

Also, acromegaly can cause panhypopituitarism because of compressing effect of the tumor mass on the surrounding tissue, therefore there may be secondary thyroid or adrenal failure hypogonadism or amenorrhea[15,16].

Patients and method

This is a cross sectional case control study in which seventy patients with acromegaly were enrolled in this study compared with 70 non-acromegalic subjects from January 2017- December 2017 from 2 endocrinology specialized centers.

An informed consent have been obtained from all patients and control counterparts ,signed and dated before participation in this study.

The enrolled subjects were already diagnosed as acromegaly clinically ,biochemically and radiologically.

They fulfill the definition of being acromegalic as their IGF1 exceed the cutoff value for their age and sex ,GH level above the reference range and GH fail to drop below 1ng/ml in response to glucose tolerance test. The diagnosis was fortified by detection of pituitary adenoma by MRI either microadenoma (<10mm in diameter) or macroadenoma(>10mm in diameter).

When the GH drop to <1ng/ml and the IGF1 level became within the reference range that is age and sex matched on serial follow up period considered as [inactive]; while those who not met these criteria considered as [active]. The information were written in prepared printed forum including the name, age, sex, height, weight, systolic and diastolic blood pressure ,duration of acromegaly, size of tumor (micro or macroadenoma), history of hypophysectomy, radiotherapy or gamma knife ,medical therapy (type, dose of somatostatin analogue Long Acting Repeatable (LAR), number of injections) GH level in 1st and last visits, IGF1 in 1st and last visits, disease activity (according to last GH,IGF1 clinical assessment), history of smoking ,and family history of thyroid disease.

Thyroid function tests were requested (TSH (reference range 0.4-4.2 mIU/L), free T4(reference rang of kit 0.7-1.9 ng/dl) and total T3(80-180ng/dl)[measured by electrochemiluminescence immunoassay]. GH level (obtained from the registered data at first and last visit(by chemiluminescent immunoassay)) and IGF1level(according to sex and age matched limits) (also from the registered data at first and last visit (measured by immunochemiluminomertic assay (ICMA)))

Continuous variables were expressed by mean +/- SD while categorical variables as percentage ,all these collected data and variables analyzed by using crosstab chi square test for categorical variables or Student's paired t-test for continuous variable and ANOVA test between categorical and continuous variables .The value of <0.05 considered as statistically significant.

Results

The subjects of this study were 70 patients with acromegaly with a mean age 46 +/-11 years .Forty one (58.5%) males and 29(41.5%)females disease duration from time of diagnosis differ with a range from 1year -30years (average 8+/-6). A growth hormone producing macroadenoma was the cause of acromegaly in 62 patients (88.5%) and in the remaining 8(11.5%) had microadenoma .Thirty six (51.4%) underwent neurosurgical treatment in form of hypophysectomy; 2 of them underwent hypophysectomy twice and another 2 underwent hypophysectomy thrice .Six patients (8.5%) exposed to gamma knife and another one to conventional radiotherapy. All of our patients (even who had history of hypophysectomy or gamma knife after the intervention if they didn't achieve a remission immediately) received medical treatment in form of monthly Octreotide (Sandostatin LAR) injection). Hypophysectomy, radiotherapy and/or medical therapy for acromegaly induced a significant reduction of mean GH and IGF-1 levels (27.83 +/- 25.69 vs. 4.73 +/- 6.72 ng/ml, p< 0.001 and 772.15+/- 521.9 vs. 404.2 +/- 278.6 ng/ml, p < 0.001 respectively) that is shown in figure (1), but both GH and IGF-1 values normalized only in 12 (17.2%) at time of collecting the data(5 of them had a remission after surgery and medical treatment , 2 patients had remission after both surgery and gamma knife, one had remission after gamma knife and medical treatment, and 4 patients had remission after medical treatment only) ;while the remainder 58(82.8%) had active disease (including those with partial response ;those who had elevated IGF1 with normal GH or those with elevated GH with normal IGF1).

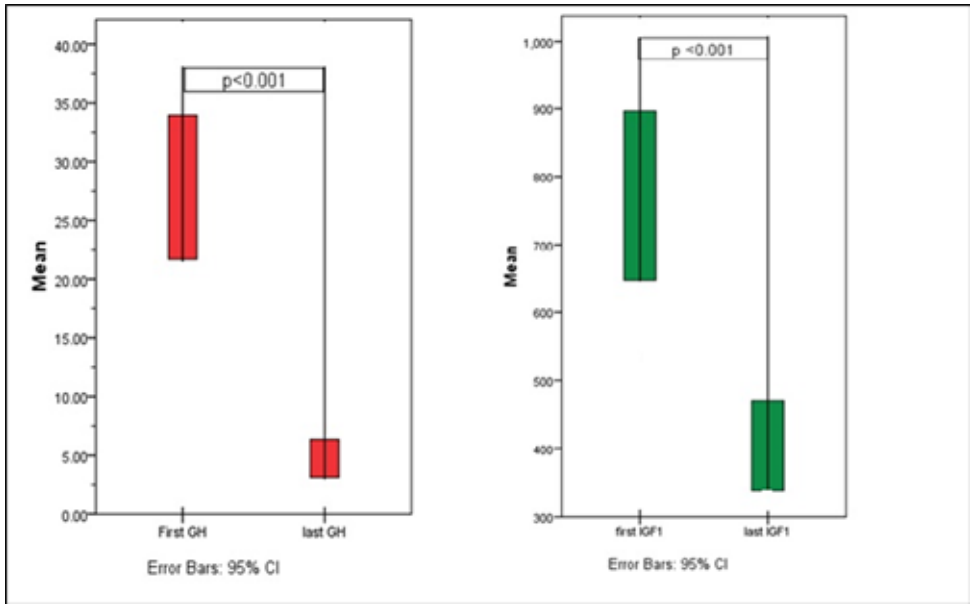

Figure (1) the comparison between the mean first and last result of both GH and IGF1.

The mean basal GH at time of diagnosis was (27.83+/-25.7) and the mean last GH was (4.73+/-6.72) with a percent of reduction were (65.2%). While the mean basal IGF1 was (772.15+/-522) and the mean last IGF1 was (404.26+/-278.62) with a percent of reduction were (35.9%). Twenty eight (40%) of patients were hypertensive, 13(18.5%) were smoker.

Family history revealed thyroid disease or goiter in 17(24.2%).

The control group was composed of 70 non-acromegalic subjects (50% males) with a mean age of 43.54+/-7.17 years.

Table (1):The clinical and analytical comparison between patients with acromegaly and control groups.

		Acromegaly	Control	*P* value
Number(%)		70(100)	70(100)	
Age mean +/- SD		46.21+/-10.9	43.54+/-7.17	0.07*
Gender	**Male**	41(58.5)	35(50)	0.46 †
	Female	29(41.5)	35(50)	
Body mass index mean +/- SD		29.03+/-4.92	28.30+/-4.05	0.38*
Systolic BP mean +/- SD		132.55+/-23.73	123.64+/-18.19	**0.015***
Diastolic BP mean +/- SD		86.64+/-17.1	79.07+/-14.2	**0.004***
Euthyroid		36 (51.4)	67(95.7)	**<0.001†**
hyperthyroid		9 (12.9)	1(1.4)	
primary hypothyroid		14(20.7)	2(2.9)	
central hypothyroid		11(15.7)	0(0)	
TSH mean +/- SD		12.7889+/-23.3	2.1721+/-1.90	**<0.001***
free T4 mean +/- SD		1.149 +/-0.75	1.3737+/-0.34	**0.028***
total T3 mean +/- SD		114.3+/-67.2	132.8571+/-22.67	**0.027***

* Paired sample T-test

† Cross tabulation Chi-square test

Table (2):the general clinical characteristics of patients with acromegaly subdivided according to thyroid function:

Variables		**Euthyroid**	**hyperthyroid**	**primary hypothyroid**	**central hypothyroid**	***total***	*P* value
Number (%)		36(51.5)	9(12.8)	14(20)	11(15.7)	70(100)	
Age mean+/-SD		46.9+/-11.3	54.6+/-8.2	41.2+/-10.3	43.2+/-8.9	46.2+/-11	**0.02***
Gender	Male No.(%)	25(61)	6(14.6)	5(12.2)	5(12.2)	41(100)	0.12†
	Female No.(%)	11(37.9)	3(10.3)	9(31)	6(20.6)	29(100)	
Family history of thyroid disease	Present No.(%)	5(29.4)	3(17.6)	5(29.4)	4(23.5)	17(100)	0.22 †
	Absent No.(%)	13(58.5%)	6(11.3%)	9(17%)	7(13.2%)	53(100%)	
BMI (mean+/-SD)		28.3+/-4.4	30.9+/-4.1	27.9+/-5.3	31.4+/-6	29.+/-5	0.13 *
Hypertension	Hypertensive No.(%)	12(42)	5(18)	7(25)	4(14)	28(100)	0.5 †
	Not hypertensive No.(%)	24(57)	4(9.5)	7(16.6)	7(16.6)	42(100)	
Systolic Blood Pressure (mean+/-SD)		129.4+/-24	140.5+/-24.3	136.42+/-24.6	131.36+/-22.26	132.55+/-23.73	0.57*
Diastolic Blood Pressure (mean+/-SD)		83.47+/-16.42	81.67+/-15	100.35+/-16.6	83.63+/-14	86.64+/-17	**0.008***
Smoking history	Smokers No.(%)	9(69.2)	2(12.4)	1(7.7)	1(7.7)	13(100)	0.4 †
	Not smoker No.(%)	27(47.4)	7(12.3)	13(22.8)	10(17.5)	57(100)	

* ANOVA test

† Cross tabulation Chi-square test

Table (3):The specific clinical characteristics of patients with acromegaly subdivided according to thyroid function:

Variables		**Euthyroid**	**hyperthyroid**	**primary hypothyroid**	**central hypothyroid**	***total***	***P* value**
Adenoma size	microadenoma No.(%)	4 (50)	2(25)	1(12.5)	1(12.5)	8(100)	0.7 *
	macroadenoma No.(%)	32 (51.6)	7(11.3)	13(20.9)	10(16.2)	62(100)	
Activity of Acromegaly	Active No.(%)	28(48.2)	8(13.8)	14(24.1)	8(13.8)	58(100)	0.2 *
	Inactive No.(%)	8(66.7)	1(8.30	0(0)	3(25)	12(100)	
Duration of acromegaly (Mean +/-SD)		7.5+/-6	8.0+/-7.5	7.1+/-7.3	11.9+/-8.6	8.+/-7	0.2 †
First GH (Mean +/-SD)		25.07+/-23.54	21.36+/-20.43	20.65+/-19.13	51.26+/-32.67	27.83+/-25.7	**0.008†**
Last GH (Mean +/-SD)		4.53+/-7.02	3.40+/-3.87	5.61+/-7.2	5.34+/-7.54	4.73+/-6.72	0.87†
First IGF1 (Mean +/-SD)		867.83+/-565.88	735.72+/-493.6	810.25+/-529.16	440.34+/-207.7	772.15+/-522	0.12†
Last IGF1 (Mean +/-SD)		432.9+/-285.51	261.34+/-145.82	502.67+/-319.65	302.26+/-232.74	404.26+/-278.62	0.11†
Dose of Sandostatin (Mean +/-SD)		742.77+/-446	677.77+/-512	549.28+/-536	736.36+/-496	694.71+/-476.5	0.63†
History of hypophysectomy	present No.(%)	13(36.1)	4(11.1)	10(27.8)	9(25)	36(100)	**0.02***
	absent No.(%)	23(67.6)	5(14.7)	4(11.8)	2(5.9)	34(100)	
History of Radiotherapy	Present No.(%)	4(57.1)	1(14.3)	2(28.6)	0(0)	7(100)	0.66*
	Absent No.(%)	32(50.8)	8(12.7)	12(19)	11(17.5)	63(100)	

* Cross tabulation Chi-square test

† ANOVA test

Table 1 shows the clinical and analytical characteristics between patients with acromegaly and control groups. The systolic blood pressure was higher in patients with acromegaly compared to control subjects (132.55+/-23.73 vs. 123.64+/-18.19 respectively P =0.015). The diastolic blood pressure was also higher in patients with acromegaly compared to control subjects (86.64+/-17.1 vs. 79.07+/-14.2 respectively P=0.004) .There is significant thyroid dysfunction in patients with acromegaly compared to control subjects (p value <0.001). As well as there is significant differences in mean TSH, free T4 ,total T3 of patients with acromegaly compared to control subjects (with P value <0.001, 0.028, 0.027 respectively)

In table 2 the acromegalic patients were subdivided into 4 main group according to their thyroid function into euthyroid 36(51.5%), hyperthyroidism 9(12.8%), primary hypothyroidism 14(20%) and central hypothyroidism 11(15.7%).Then the analysis of variance in between subgroups conducted to identify the differences in variables of general clinical characteristics which shows that the age is significant risk factor (p=0.02) and the diastolic blood pressure also significantly different between subgroups (p=0.008).

In table 3 the analysis of variance conducted to the subgroups of patients with acromegaly that were already classify according to their thyroid status to identify the

differences of specific acromegalic characteristics of variables which shows the significance of basal growth hormone level at time of diagnosis as well as the history of neurosurgical treatment (hypophysectomy) on the thyroid status (p=0.008, 0.02 respectively). There is no significant correlations between thyroid status from one side and pituitary adenoma size, activity of disease ,duration of disease ,history of radiotherapy treatment, last GH and basal or last IGF1on the other side.

Discussion

Most of our patients with acromegaly were in euthyroid status (51%) while there is 20% had primary hypothyroidism, in addition to 15.7% had central (secondary) hypothyroidism and only 12.8 %had hyperthyroidism; these findings are accepted if it is compared with Cannavo S. et al study that show the approximate levels of euthyroidism of about (67%) and about 25% have hypothyroidism [17]. As well as we can accept our result regarding hyperthyroidism if we compared it with other study that found in 3.5–26% of patients with acromegaly in previous studies [18]. Thyrotoxicosis is not frequent [18], but it increases cardiovascular risk, particularly when is coupled with high GH and IGF-1 levels, and for that reason it is important to gain euthyroidism as fast as possible. Hyperthyroidism should be taken into consideration in diagnosing a patient with acromegaly and weight loss, after excluding cancer [19].

Lastly we fail to found relationships between thyroid dysfunction from one side and duration of disease , last GH, first or last IGF1 on the other side which are similar to what is found in other studies like Cannavo et al [17].

Conclusion

Thyroid dysfunction is more prevalent in patient with acromegaly whether hypothyroidism (both primary and secondary) and hyperthyroidism. The Age and diastolic blood pressure are significant risk factors of thyroid dysfunction. There is significant role of hypophysectomy in thyroid dysfunction development while Sandostatin (Octreotide LAR) treatment had no role.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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20. Figure (1) the comparison between the mean first and last result of both GH and IGF1.
21. Table (1):The clinical and analytical comparison between patients with acromegaly and control groups.
22. Table (2):the general clinical characteristics of patients with acromegaly subdivided according to thyroid function:
23. Table (3):The specific clinical characteristics of patients with acromegaly subdivided according to thyroid function:

Simulation of poly (ADP-ribose) polymerase (PARP) Enzyme with BAI compound in the for Thyroid cells

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Abstract

In this study, the simulation for the preparation of pharmacological compound anti-cancer Baicalein (6,7-dihydroxy-2-phenyl-4H-chromen-4-one). These compounds have an importance of the known biologic and its wide medical requests. Our theoretical results dedicated to simulating that acquired for researchers (SE EUN HAN et. al) Practically against cancerous cells in the thyroid gland and matching them through a program (MOE) theoretically. From the illustrious effects, the compound (BAI) is behaving as a good legend when it interacted with amino acid for the enzyme (PARP).

Key words*: Docking, BAI, anticancer, FOR Thyroid cells*

Introduction

Flavonoids contain of a large set of polyphenolic compounds consuming a benzo-γ-pyrone configuration and are ubiquitously current in plants. They are synthesized by phenylpropanoid alleyway. Obtainable reports be likely to show that subordinate metabolites of phenolic environment counting flavonoids are responsible for the variety of pharmacological activities [1, 2]. Flavonoids are hydroxylated phenolic substances and are known to be combined by plants in reaction to microbial infection [3]. Their activities are structure reliant on. The chemical nature of flavonoids be contingent on their structural course, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization [4]. Topical interest in these substances has been motivated by the thinkable health assistances rising from the antioxidant actions of these polyphenolic compounds. Purposeful hydroxyl groups in flavonoids arbitrate their antioxidant effects by scavenging free radicals by chelating metal ions [5, 6]. The chelation of metals be able to vital in the avoidance of radical generation which damage impartial bio-molecules [7, 8]. As a dietary component, flavonoids are supposed to have health-stimulating properties due to their great antioxidant capacity both in vivo and in vitro systems [9, 10]. Flavonoids have capability to induce human protective enzyme organisms. The number of trainings has suggested protective effects of flavonoids in contradiction of frequent infectious (bacterial and biological diseases) and degenerative infections such as cardiovascular diseases, cancers, and further age-amalgamated diseases [2, 9, 10]. The mechanisms tangled in safety providing by flavonoids are termed separately. Flavonoids also performance as a secondary antioxidant defense system in plant tissues showing to different abiotic and biotic stresses. Flavonoids are situated in the nucleus of mesophyll cells and within centers of ROS generation. They also regulate growth factors in plants such as auxin [11]. Bio-synthetic genes have been accumulated in several bacteria and mushrooms for improved creation of flavonoids [12] Flavonoids are a set of natural compounds with variable phenolic assemblies and are established in plants. In 1930 a novel substance was quarantined from oranges. At that time, it was supposed to be a member of a new class of vitamins and was selected as vitamin P. Advanced on it converted clear that this substance was a flavonoid (ruin) and till now more than 4000 varieties of flavonoids have been identified [13]. The poly (ADP-ribose) polymerases (PARPs) are an emerging family of enzymes that share the ability to catalyze the transfer of ADP-ribose to target proteins (poly ADP-ribosylation) [14,15]. There are at least 18 members of the PARP family that are encoded by different genes, and stake homology in a preserved catalytic domain.1 Although some isoforms containing PARP1 and PARP2 are finest known for their involvement in DNA reparation routes, it is now clear that these and other PARPs have an important role in several cellular processes including cell proliferation and cell death. A number of cellular

substrates for PARP have been well-defined, and a mainstream of these proteins are nuclear proteins that are complicated in nucleic acid metabolism, modulation of chromatin structure, DNA synthesis, and DNA restoration. PARP also auto transforms itself in the appearance of DNA filament breakdowns, and is one of the chief acceptors of poly ADP ribose in vivo. PARP1 is the first and best described member of the PARP family. PARP2 is supreme cautiously related to PARP1 with 69% match in its catalytic domain, and was predictable on the foundation of the perseverance of PARP activity in PARP1deficient cells [14,16]. Thyroid tumors are present broadly classified as follicle-derived (thyroid epithelial) neoplasms, other epithelial tumors, non-epithelial tumors and secondary tumors assembled on pathological, clinical and genetic characteristics [17, 18]. These tumors can be benevolent, borderline or malignant, focus on their biological performance within the body. The follicular adenoma, hyalinizing trabecular tumor, encapsulated follicular-patterned thyroid tumors, papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, Hürthle cell tumour, poorly differentiated thyroid carcinoma, anaplastic thyroid carcinoma and squamous cell carcinoma comprise the major thyroid epithelial neoplasms. Some of the other epithelial tumors found in the thyroid gland have medullary carcinoma, salivary gland-type tumors, mucinous carcinoma and thymic tumors, at the same time as tumors like paraganglioma, peripheral nerve sheath, vascular, smooth muscle, solitary fibrous and histiocytic tumors, lymphoma and teratoma fall under the non-epithelial tumors of the thyroid [16-19]. The well separated thyroid cancer such as follicular and papillary carcinomas description for 95% of all thyroid cancer cases and are regularly interrelated with a good prognosis and existence rate when detected early [19]. On the other hand, the injured or undistinguishable anaplastic thyroid carcinoma, albeit a rare cancer, is nearly continually fatal [18].

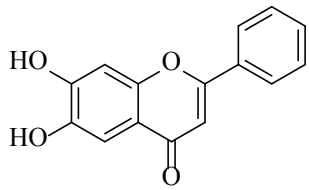
Materials and Method

These compound are generally used in pharmaceuticals and their effectiveness has been measured against cancer by advanced programs (MOE).

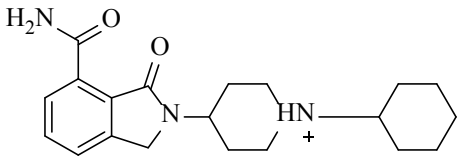
A-MOE program 2015.

Docking study Ligand: In the field of molecular forming, docking is a method which expects the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Molecular Network software packages provide Chem 3D, which was used for the generation of 3D coordinates from. Again, using Converter of these same server 2D structures of the drugs was converted into PDB format which is an acceptable form for any standard docking software. Finally using MOE were prepared by the addition of hydrogen atoms. Structures.

B-(BAI) Baicalein (6,7-dihydroxy-2-phenyl-4H-chromen-4-one).



C- 4-(4-carbamoyl-1-methyl-3-oxoisoindolin-2-yl)-1- C-cyclohexylpiperidi1-ium.



D- PARP poly (ADP-ribose) polymerases.

Results and Discussion

Docking Analysis, the docking scores were obtained from (Baicalein (6,7-dihydroxy-2-phenyl-4H-chromen-4-one) withe against (PARP) receptor. The output of all ligands was given by energy values in kcal/mol as shown in Table 1. Some ligands show good docking scores when compared to standard drug. Docking score of the compounds targeted was compared with the score of the drug Baicalein (6,7-dihydroxy-2-phenyl-4H-chromen-4-one) which is used as a potent drug for the treatment of tumor show higher docking scores in comparison with standard drug. The interactions were stronger for all the ligands which are used for docking simulation table (1-3) Validation of Ligands. The results showed in Tables (1) and (2) that the best correlation with protein and separated from Thyroid cancer was that the strongest correlation of the first compound with amino acidity was found to be strongly linked with the amino acid (GLY 863) was associated with the functional group (hydroxide) H-donor And H-acceptor of protein amino acids as shown in Figures ((1-8)) and the second amino acid is (SER 904) associated with the functional group

(hydroxide) acceptor

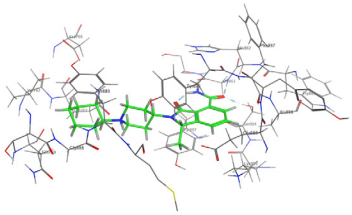


Figure 1 : (Ligand with receptor).

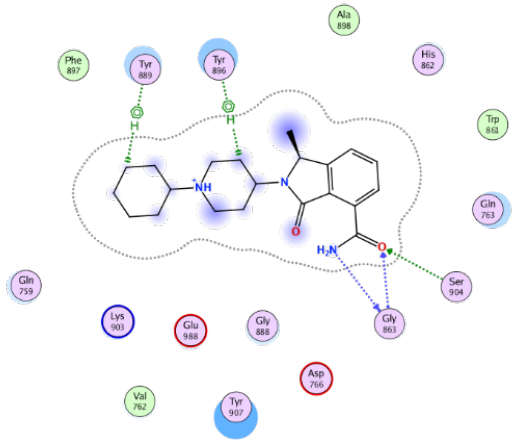


Figure 2: (Ligand with 618M enzyme)

Table. (1): Comparison of Ligand bond strength with BAI compound

Compound number	*S (Kcal/ mol)*	*Amino acid interaction*	*function group interaction*
Ligand	-7.9142	SER 904	Amin
Ligand	-7.9142	GLY 863	OG
BAI	-5.4426	GLY 863	Hydroxide
BAI	-5.4426	SER 904	Hydroxide

Table. (2): Ligand report

Receptor	*E (Kcal/ mol)*	*Amino acid interaction*	interaction	*Distance*
O	-3.0	GLY 863	H-donor	3.17
N	-3.9	GLY 863	H-acceptor	3.o7
OG	-1.3	SER 904	H-acceptor	3.10
6-ring	-1.2	TYR	H-pi	3.93
6-ring	-o.6	TYR	H-pi	3.83

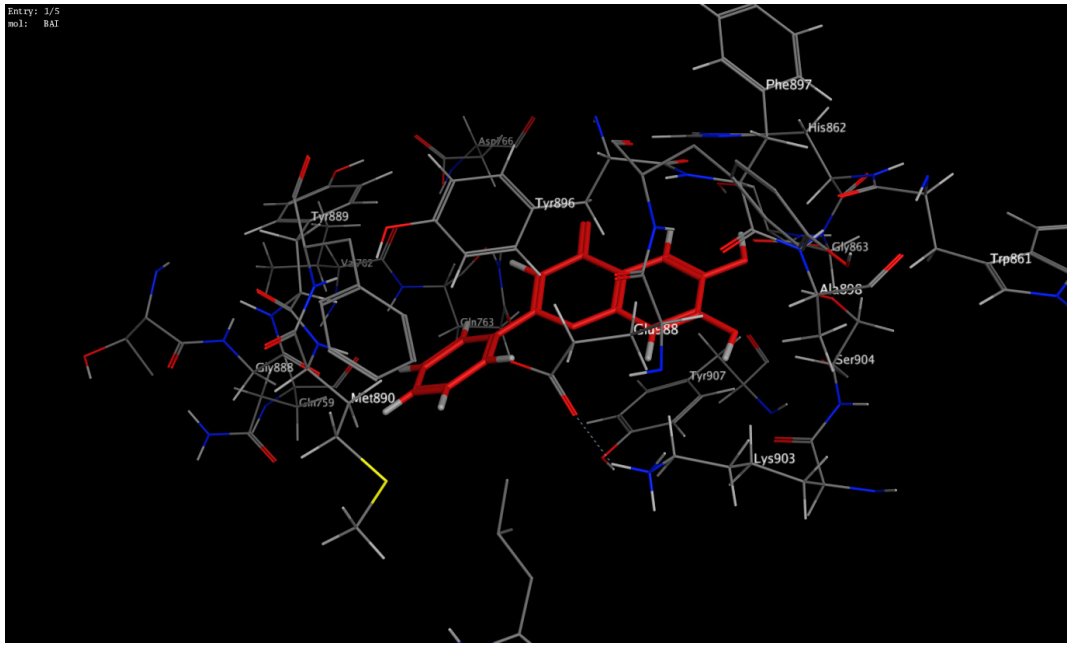


Figure 3: (compound configuration 1 with receptor).

Table. (3): BAI compound

Receptor	*E (Kcal/ mol)*	*Amino acid interaction*	interaction	*Distance*
O	-4.4	GLY 863	H-donor	2.90
N	-5.1	GLY 863	H-acceptor	2.87
OG	-2.7	SER 904	H-acceptor	2.72

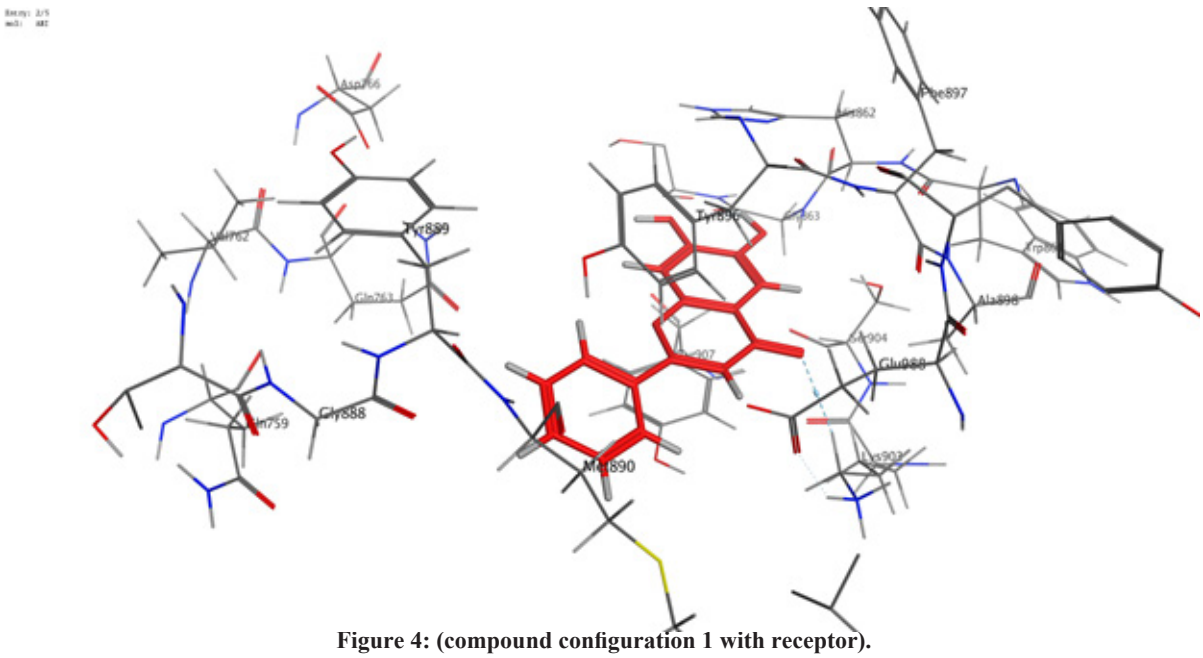


Figure 4: (compound configuration 1 with receptor).

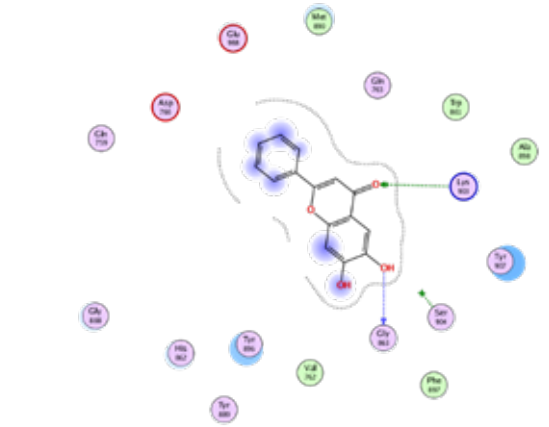


Figure 5:(interaction configuration2 with receptor)

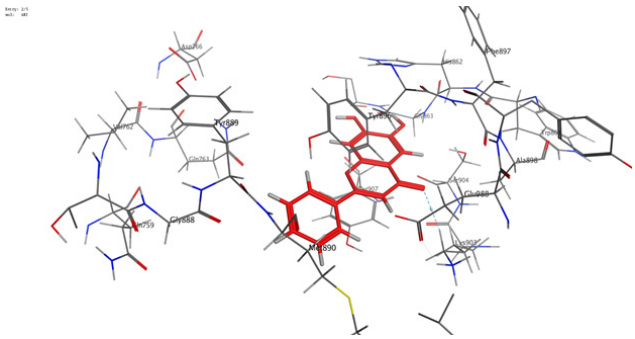


Figure 6: (compound configuration 3 with receptor).

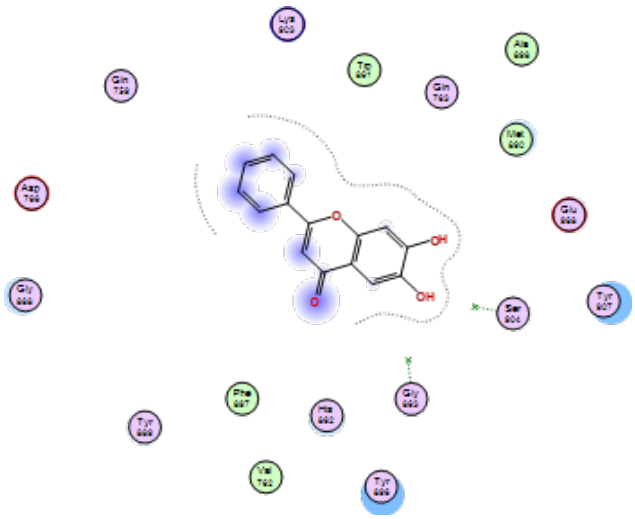


Figure 7 (interaction configuration3 with receptor)

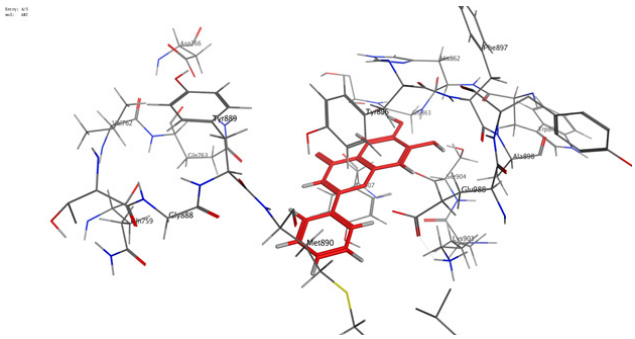


Figure 8: (compound configuration 4 with receptor).

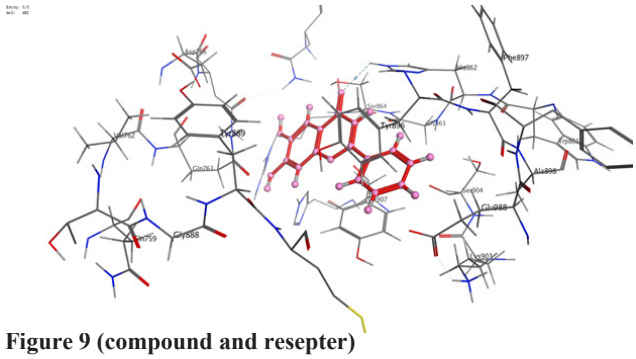


Figure 9 (compound and resepter)

Conclusion

We have computer-generated compound from the former researchers (SEUN HAN et. el) with Baicalein (6,7-dihydroxy-2-phenyl-4H-chromen-4-one) compound. Bendable docking of ligand to receptor molecules is an emerging method and is extensively used to reduce cost and time in drug innovation.

From the results, a process was showed to detect the strength of the link between the compound and the enzyme using the program (MOE), from the previous literature that showed efficacy as anti-fungal and some bacteria and the effect of these compound on thyroid cells cancer and inhibition of the causative enzyme (PARP)

Thus, this study showed that the compound (1) takes the best formula of is alignment more the association with the link with amino acids (GLY 836and SER 904) and gives the best consequences, thus clarifying the best inhibitor of the association with the enzyme.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Advantage of Application of Topical Hyaluronic Acid in Reducing Post Tonsillectomy Pain; Cross Sectional Comparative Study

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Abstract

Introduction: Tonsillectomy is common surgical operation performed in otolaryngology department. Various techniques could be used that include traditional cold dissection, powered instrumentation like electro cautery, harmonic scalpel, laser, and radiofrequency technique. As with any surgery, there are some risks can occur with tonsillectomy, these includes bleeding, pain at the site of surgery and/or referred otalgia, and infection. The aim of our study was to assess the postoperative use of topical hyaluronic acid with regards to safety, pain relief. **Method:** The research was considered a cross-sectional research made on 60 patients needing tonsillectomy from a period began from January to September 2018. The patients had been separated into two groups both from 30 patients, one group (group A) treated by using high molecular weight(HMW) Hyaluronic acid spray on tonsillar beds after operations , and the second group(group B) dealt as control. Early complications were registered in questionnaires and then the data were evaluated for each group. **Results:** The use of high molecular weight (HMW) Hyaluronic acid spray on tonsillar beds after surgery has reduces the pain significantly after 3 – 7 days (p-value = 0.0001) while has no implication on pain reduction within12 - 24 hours p-value (0.17 and 0.3) respectively . **Conclusion:** The use of (HMW) hyaluronic acid spray can be used safely in post tonsillectomy patients. Hyaluronic acid spray can minimize post tonsillectomy pain.

Keywords: *High molecular weight (HMW) hyaluronic acid (H.A.), tonsillectomy, pain*

Introduction

The history of surgical removal of tonsils has been returned back to three thousand years ago1, nowadays, Tonsillectomy is usual operation almost lead to open wound, so, connected with important postoperative pain that may last as long as 2 to 3 weeks period 2–5. This pain constitutes the major morbidity factor leading to poor nutrition, dysphagia, dehydration and delaying time for returning to work or school6,7. Many factors contributes to the post-tonsillectomy pain as mucosal damage, irritation of vagal , glossopharyngeal nerves ,tissue inflammation ,retained debris and spasm of the muscles of the pharynx 5,8,9.

Nonsteroidal anti-inflammatory drugs, codeine and paracetamol are frequently used to minimize the pain occurs following tonsillectomy 10,11.Opioids are associated with respiratory depression ,nausea, vomiting, depression of cough reflex and sedation5,10,12. So, more safe opioid and non-opioid analgesia are used as pain killer to alleviate pain and discomfort after removal of the tonsils. Ibuprofen now suggested by American Academy of Otolaryngology— Head and Neck Surgery for pain relief following tonsillectomy 4,8,13.

Optimal pain relief after tonsillectomy is challenging .Many researchers use different options for pain reduction whether systemic or topical like bupivacaine 14,15, dexamethasone , lignocaine15 , sucralfate 16, etc.

Hyaluronic acid is high molecular weights, hydrophilic glycosaminoglycan that is a naturally component of connective tissue particularly seen in the synovial fluid of joint and inside the extracellular matrix of skin 17–19. It provides a role in wound healing where

its secretion in proliferative phase stimulates fibroblast migration and proliferation 20,21. Finally it shows a suppressive effect on inflammatory mediators , tissue reactions to trauma so could be applied safely as an anti-inflammatory factor 21,22. Hyaluronic acid is a lubricant material with ability to stimulate growth so widely used to be the normally non-immunogenic, biocompatible and anti-inflammatory dressing biomaterial 22. Hyaluronic acid is reported to decrease pain, inflammation with stimulation of wound healing in pressure ulcers 19.It has a role in management of osteoarthritis and tendon problems 17, in burns, and chronic wounds 23,24 as well as in healing of vocal fold wounds 25 .

Materials and Method

A cross-sectional study carried out on 60 patients requiring tonsillectomy throughout the period began from January to September 2018. The study certified by hospital ethical committee with written informed consent was taken. The indications of surgery are recurrent attacks of acute tonsillitis, chronic tonsillitis and/or large obstructive tonsil with sleep apnea syndrome. Patients with quinsy, associated adenoiditis or adenoid hypertrophy, tonsillectomy for biopsy were excluded. Our study population was arising from those attended to Al-Sadiq teaching general hospital in Hilla city, Babylon, Iraq. The tonsillectomies have been done under general anesthesia, with placement of oral endotracheal intubation. The tonsils were removed by cold steel dissection method and hemostasis by ligatures, packs or cautery. The study sample were divided into 2 groups each from 30 patients ,first group (group A) dealt with postoperative use of high molecular weight(HMW) Hyaluronic acid spray (0.01% topical spray) on tonsillar beds three to five times per day starting 3 hours postoperatively and the second group (group B) considered as a control. Pain assessment was done 12 hours,24 hours ,3days and 7 days post tonsillectomy using visual analogue scale from 0-10 (0 represents no pain at all , 10 represents the severe incapacitating pain). Data collected were comprised of age, gender, all data collected for both groups were analyzed by SPSS 22 used Mann - Whitney test for calculate P-value and another statistical analysis.

Results

In our study we take 60 patients all undergone tonsillectomy, 30 patients were used H.A. after tonsillectomy as a (case) and 30 patients not used H.A. as a (control). 38(63.33%) of patients were female and 22(36.67%) were male as in figure 1.

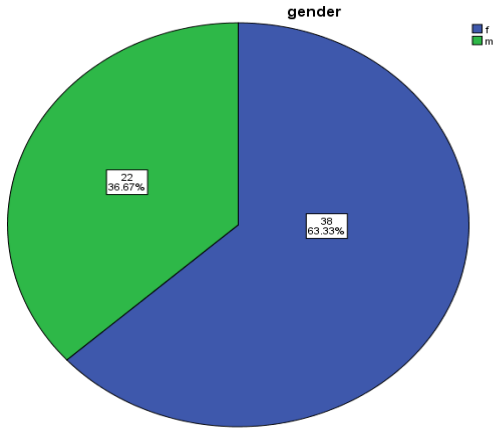


Figure1: gender distribution

Figure 2 shows gender distribution according to the type of sample.

Table 1 shows that the two groups (case and control) are similar regarding the age (p value > 0.05)

Table 1: Mean age of the study sample

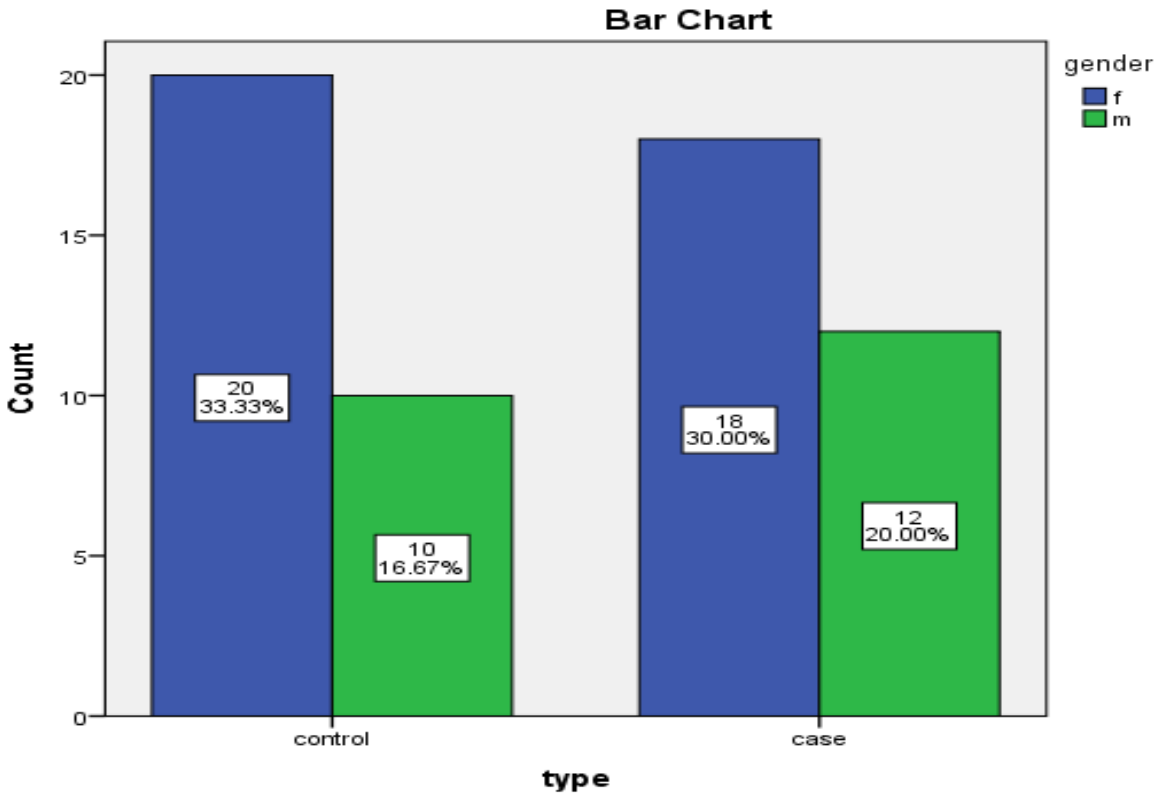
	Case Mean ±SD	Control Mean ±SD	Z- test	P value
Age (years)	18.7 ± 3.6	18.9 ± 3	0.3	0.53

Z: Man-Whitney test; P- value <0.05 =significant.

Gender Chi square = 0.3 with P-value= 0.79 no difference between gender in case and control sample. **Figure 2: gender distribution according to type of sample**

Table 2 shows that there is no difference in pain score at 12 hours and 24 hours postoperatively after using H.A. between the case and control group (p - value 0.17 and 0.3) respectively. However the use of H.A. spray post tonsillectomy in case group results in significant decrease in pain after 3 days and 7 days postoperatively (p value <0.05).

Table 2: Pain assessment by visual analogue scale post operatively



Time	Case Mean ±SD	Control Mean ±SD	Z- test	P-value
After 12 hours	7.20±1	7.60±1.1	1.37	0.17
After 24 hours	7.13±1	7.40±1.2	1.078	0.3
After 3 days	4.60±1.2	6.60±1.7	4.491	0.0001**
After 7 days	2.47±1.3	4.07±1.34	4.071	0.0001**

Z: Man-Whitney test; P- value <0.05 =significant.

Discussion

Pain management after tonsillectomy is a crucial factor in decreasing postoperative morbidity and complications like poor oral intake, dehydration, dysphagia and disturbed sleep 10,11,26. The pain and inflammatory reactions following oral surgery may reach its maximum level two days postoperatively and decrease through 7-10 days 27

Hyaluronic acid is a linear polysaccharide found in many tissues especially connective tissues where its responsible for tissue elasticity ,control of hydration of connective tissues and synovial fluid elastoviscosity .It is an important factor in the process of wound healing 28,29. In our study it was found that postoperative pain following tonsillectomy was dramatically reduced by using topical (HMW) Hyaluronic acid spray especially after 3 days. This may be explained by the fact that this substance has a role in repairing tissue and cover the exposed wound of tonsillectomy as well as its minimize the risk of post tonsillectomy infection .These effects were clearly described to induce healing for pressure ulcers and to increase the rate of healing in diabetic foot30. Hyaluronic acid also has anti-inflammatory effects; decreasing leukocyte infiltration and enhance the angiogenesis31. H.A. is effective in management of osteoarthritis pain17 and pain of temporomandibular joint disorder18, where it used for intra articular

injection21 . Researchers from turkey studied the effect of applying hyaluronic acid gel during tonsillectomy and found that the pain decrease significantly postoperatively with rapid wound healing. In our study the maximum benefit from applying the H.A. spray was between 3-7 days (p value <0.05) while no significant pain reduction was observed within 24 hours postoperatively. This may indicates that immediate effect of H.A. need time to act as anti-inflammatory, coating material and stimulatory for wound healing.

Several studies test the effect of hyaluronic acid following teeth extraction especially third molar teeth and found significant pain reduction with decrease in analgesics need 32,33. Little researches studied the effect of hyaluronic acid following tonsillectomy so researchers should be encouraged to explore its effects, adverse effects with a larger study samples.

Conclusion

The use of topical (HMW) Hyaluronic acid spray is recommended post tonsillectomy to decrease post tonsillectomy pain and decrease the need for analgesia. Its shows maximum effect after 3 days postoperatively which may help to speed recovery, decrease morbidity and ensure early return to school and work.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Atherogenic Index of Plasma among Type2 Diabetic Patients Cross-Sectional Study In Iraq

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Abstract

The study was carried out on (60) males previously diagnosed with diabetes mellitus type2 aged (40-55) years with mean ± SE of age (48.46 ±5.03), who visited the Specialist Center for Endocrine and Diabetes at Baghdad province.The total cholesterol(TC) mg/dl levels were (180.90±11.68 ,209.60±14.50 ,203.10±14.65) respectively showed non significant ($p>0.05$) variation in diabetic groups ,TG mg/dl levels in normal weight group (172.55±14.13) decreased significantly ($p<0.01$) in comparison with over weight and obese (273.95±22.83) ;(306.95±25.20) ,respectively. LDL mg/dl levels were (101.50±9.56; 110.00±12.71; 105.40±9.99) respectively showed non significant ($p>0.05$) variation in diabetic groups. VLDL mg/dl in normal weight group (35.75±3.31) decreased significantly ($p<0.01$) in comparison with over weight and obese (59.70±4.59); (61.30±5.03).HDL mg/dl levels were (43.20±2.01 ,42.85±3.05,40.05±3.32) respectively showed non significant ($p>0.05$) variation in diabetic groups. FBG showed non-significant correlation with TC(r=-0.13),LDL(r=-0.13),TG (r=-0.12),VLDL(r=-0.15),HDL(r= 0.24). HbA1C showed positive significant ($p<0.05$) correlation with TC(r= 0.25), while showed non significant correlation with TG (r= 0.10),LDL (r=0.18),VLDL (r=-0.01), HDL (r=0.16).BMI showed non-significant with TC(r=0.15),LDL(r=0.08),HDL,(r=-0.08).While positive significant ($p<0.01$) , correlation with TG (r=0.50), VLDL(r=0.39). The levels of AIP differ significantly ($p<0.01$) among diabetic groups (0.576 ± 0.04; 0.800 ± 0.06 and 0.911 ± 0.05) but over weight and obese(0.800 ± 0.06 ; 0.911 ± 0.05) were not significant ($p>0.05$). AIP was significantly correlated with body mass index(r = 0.25), visceral fat (r = 0.37), TC (r = 0.22), LDL (r=0.24), HDL (r = −0.72), TG (r = 0.84), glucose (r = 0.32). Positive correlation ($P<0.01$) with BMI(r=0.36) ;VLDL (r=0.28)($p<0.05$) and negative correlation ($p<0.01$) with HDL-C (r=-0.32)was seen .

Keywords:- *Diabetes mellitus ,Dyslipidemia,lipid profile,Atherogenic index of plasma(AIP).Isulin resistance(IR).*

Introduction

Dyslipidemia in T2DM is characterized by high level Of total cholestrol (TC), low density lipoprotein (LDL-C) ,triglyceride (TG) with low level of (HDL-C) [1].The early detection of lipid abnormalities with their treatment can reduce the risk of atherogenic cardiovascular and cerebrovascular disorders in T2DM patients[2].Atherosclerotic coronary artery disease and different form of cardiovascular disease (CVA) are the principle causing of morbidity and mortality in T2DM, that alterations in lipid profile lead to atherosclerosis in T2DM[3].The deffintion of atherogenic index of plasma AIP is used logaritham(log) of the ratio of plasma concentration of TG/HDL-C. Which was correlated with size of HDLand LDL molcules[4]. The AIP is regarded as astrong marker for plasma atherogenicity and correlated with (CVD) .The AIP value under 0.11 is associated with low risk of CVD but value between 0.11-0.21 and above than 0.21 causes intermediate and elevated risk respectively[5]. Diabetes (T2DM) and related cardiovascular complication are major public health challenges among world wide. Individuals with T2DM have two to four fold risk of coronary artery disease (CAD) causing death among people of Type2Diabetes mellitus [6]. Dyslipidemia participates in causing of atherosclerosis ,which pathological affects of CVD persons with metabolic syndrome or T2DM exhibit a characterstics pattern of serum lipid abnormalities ,consisting of low HDL-C and increased TG levels with increased LDL particles[7].Various mechanisms forming the atherogenic lipid disorders in Type2 patients. The increase in breakdown of

intracellular Triglycerides and release of Free fatty acids increased into the blood causing free fatty acids hepatic infiltration, muscle , pancreatic B-cell.predisposing to T2DM .Increased hepatic FFAS contribute to elevation of hepatic TG with subsequently resulting in increased VLDL particles. From other side cholesterol ester transfer protein (CETP) converts Triglycerides from Very low density lipoprotein to cholesterol found in HDL and LDL resulting to cholesterol rich atherogenic VLDL. particles.HDLparticles that under go these modification are cleared the renal function,resulting in decrease HDL-C levels [8]. Endothelial dysfunction (ED) is another major factor for the development of atherosclerosis.It might may be caused by dyslipidemia ,oxidative stress,inflammation,lipid abnormality regard the major cause of ED,espically LDL particles are more susceptible to oxidation , oxidized LDL particles reduces the availability of nitric oxide (NO) and impair endothelial function.This creates appositive feedback loop in which inflammatory factors promote monocyte and Tcell adhesion ,foam cell information,extracellular matix digestion and vascular smooth muscle migration and proliferation leading to atherosclerosis [9].The prostacyclin and Nitric oxide production by normal endothelial inhibition with platelet reactivation and relaxation vascular smooth muscle improving circulation influx normaly. Diabetic patients showed reduction in release of prostacyclin and Nitric oxide with chronic defects of endothelial Nitic oxide synthesis action, which is a predictor of dyslipidemia and explain partly the accelerated atherosclerosis in DM[10]. In diabetes long term hyperglycemia causes generalized vascular endothelial damage which reduced function of lipoprotein lipase leading to increase TG,TC,and LDL[11].

Material and Method

Collection of Information and Patients Selection

The study was carried out on (60) males previously diagnosed with diabetes mellitus type2 aged (40-55) years with mean ± SE of age (48.46 ±5.03), who visited the Specialist Center for Endocrine and Diabetes at Baghdad province.The study was begun from 1/ November 2018 to 30/January 2019. Informed consent was obtained from patients cross sectional study to fill the questionnaire form them before venipuncture which was used for recording the necessary information that concerning with subject groups which included name, age, length, body weight, and duration of diabetes onset.

Study design:-

The subject study included group (60) patients as diabetic group (cross sectional study).The diabetic group was divided into three subgroups according to BMI

Normal weight BMI (18.5-24.9) Kg/m^2.

Over weight (OV) BMI (25-29.9) Kg/m^2.

Obese BMI (≤ 30) Kg/m^2.

2-Collection of blood samples:-

Eight milliliters of blood were drawn from each patient after (12- 14) hours fasting via venipuncture, by using 10 ml disposable syringes between (8.00 –10.30 A.M). Blood specimen used in two aliquots; two and three ml. The first one was put in a tube have ethylene diamine tetra acetic acid (EDTA K3) as anticoagulant and stored at (2-8°C) for analyses of HbA1c, while the second aliquot was transferred into gel tubes without anti-coagulant; blood was left to clot for 20-30 minutes at (37°C) in an incubator. Serum were separated by centrifugation at 3000 rpm for 10 minutes used for the determination of FBG, lipids profile, serum was stored in Eppendorf tubes at (-20°C) for analyses later.

Lipid profile calculation:-

Measurement of serum total cholesterol (TC) Cholesterol was determined by using the Mindray Bs-200 [12]. Measurement of Triglycerides (TG) Concentration.Triglyceride was determined by using the Kit SPINREACT according to the method by mindary [12]. Measurement of Serum High Density Lipoprotein (HDL) Concentration. MINDRAY BS-120 / 200E Autoanalyzer use SPINREACT kit [13]. Measurement of Low Density Lipoprotein (LDL).Mindray BS-120 / 200E Autoanalyzer use SPINREACT kit [13]. VLDL(mg/dl)=

Atherogenic index of plasma was measured using base 10 logarithm of the ratio Triglycerides to HDL, formula = log (TG/HDL-C). (Dobiásová and Frohlich ,2001).**AIP =Log**.

Results and Discussion

Table (1) Effect of different groups of diabetic subjects in lipid profile.(Mean ± SE)

Parameters (mg/dl)	Groups			LSD value
	Normal weight	Over weight	Obese	
Total cholesterol	180.90 ± 11.68 a	209.60 ± 14.50 a	203.10 ± 14.65 a	38.747 NS
Triglyceride	172.55 ± 14.13 b	273.95 ± 22.83 a	306.95 ± 25.20 a	60.221 **
LDL	101.50 ± 9.56 a	110.00 ± 12.71 a	105.40 ± 9.99 a	30.722 NS
VLDL	35.75 ± 3.31 b	59.70 ± 4.59 a	61.30 ± 5.03 a	12.390 **
HDL	43.20 ± 2.01 a	42.85 ± 3.05 a	40.05 ± 3.32 a	8.081 NS
** (P<0.01), NS: Non-Significant. Means having with the different letters in same row differed significantly				

From the result table (1),the TC mg/dl levels were (1 80.90±11.68,209.60±14.50,203.10±14.65) respectively showed non significant (p>0.05) variation in diabetic groups ,TG mg/dl levels in normal weight group (172.55±14.13) decreased significantly (p<0.01) in comparison with over weight and obese (273.95±22.83) ;(306.95±25.20) .respectively. LDL mg/dl levels were (101.50±9.56; 110.00±12.71; 105.40±9.99) respectively showed non significant (p>0.05) variation in diabetic groups.VLDL mg/dl in normal weight group (35.75±3.31) decreased significantly (p<0.01) in comparison with over weight and obese (59.70±4.59); (61.30±5.03).HDL mg/dl levels were (43.20±2.01 ,42.85±3.05,40.05±3.32) respectively showed non significant (p>0.05) variation in diabetic groups.

Table (2) Correlation coefficient between lipid profile with FBG,

HbA1c and BMI in diabetic patients.

Parameters	Correlation coefficient-r		
	FBG	HbA1c	BMI
Total cholesterol	-0.13 NS	0.25 *	0.15 NS
Triglyceride	-0.12 NS	0.10 NS	0.50 **
LDL	-0.13 NS	0.18 NS	0.08 NS
VLDL	-0.15 NS	-0.01 NS	0.39 **
HDL	0.24 NS	0.16 NS	-0.08 NS
* (P<0.05), ** (P<0.01), NS: Non-Significant.			

From the result table (2), FBG showed non-significant link with TC(r=-0.13),LDL(r=-0.13),TG(r=-0.12),VLDL(r=-0.15),HDL(r=0.24).HbA1C showed positive significantly (p<0.05) correlated with TC(r=0.25), and showed non significant correlation with TG (r= 0.10),LDL (r=0.18) ,VLDL (r=-0.01) , HDL (r=0.16). BMI showed non-significant with TC (r=0.15) , LDL (r=0.08), HDL (r=-0.08).positive significantly(p<0.01)

correlated with TG (r=0.50) ,VLDL(r=0.39). The dyslipidemia means to change in lipid profile involving elevating serum TC , TG, decreased HDL, elevation LDL and VLDL. Dyslipidemia is present to be accompany with both, insulin resistance and coronary heart disease [8].Studies lipids profile in T2DM and control subjects in overweight and obese groups in Saudi Arabia was seen high incidence of dyslipidemia in T2DM patients (Our results showed non significant increase of TC in overweight and obese diabetic patient >200mg and significantly higher TG and VLDL levels; while LDL and HDL levels were non-significant this attributed to that excess acetyl CoA from lipolysis of fat to free fatty acids (FFA) gets converted to more and more cholesterol and its concentration in blood rises in T2DM.VLDL increased either because of increased hepatic production of VLDL or decreased removal of VLDL circulation. Serum concentration of TG also increases because of decreased removal from circulation.The increased TG in our study was agreed with (Taskinen,2003). who reported the increased serum TG levels in T2DM patients are widley because of an elevation VLDL, specially large VLDL1 molcules.Both rising production and late catabolism of VLDL are responsible for the rising VLDL.Insulin resistance is accompanied with reduction activity of hormone-sensitive lipase in adipose tissue by insulin, resulting lipolysis increasing and, portal flux of NEFA to hepatic cells to stimulate synthesis of TG in hepatic cellsThe role of increasing in TG levels in hyperglycemic patients also includes decrease of lipoprotein lipase (LPL) activity. It has been recorded that LPL activity is reduced in patients with T2DM.(Nikkila and Taskinen,1981).LPL hydrolyses TG of chylomicrons and (VLDL). The FFA flux into glycogen rich hepatocytes stimulates TG synthesis, which produced of VLDL-cholesterol..Obesity and T2DM are accompanied by elevation deposition of TG in non-adipose tissue, like heart, liver, pancreas, and skeletal muscle. Obesity, fat accumulated in the under skin, abdominal and visceral depots, which is have affinity in risk of metabolic and heart blood vessels complications; Obesity is positively affecting risk the developing of T2DM,dyslipidemia, IR and hypertensive. It characterized by adipose tissue defects growth resulting in increasing size of fat cell (hypertrophic obesity) or fat cell number (hyperplastic obesity), or both. Obesity is known as body mass index (BMI). As we observed mean serum TG levels higher in diabetics groups .As BMI showed high significant correlation with TG(r=0.50) and VLDL (r=0.39). Higher HbA1c concentrations in our study were associated significantly correlation with higher TC(r=0.25) (P<0.05 reported that significant linear positive correlation with HbA1c and lipid parameters except HDL in T2DM group.

Table (3) Effect of different groups in Atherogenic index of plasma

(AIP) in diabetic patients.

Groups	**Mean ± SE of AIP**
Normal weight	0.576 ± 0.04 b
Over weight	0.800 ± 0.06 a
Obese	0.911 ± 0.05 a
LSD value	0.154 **
** (P<0.01).	

The levels of AIP differ significantly (p<0.01) among diabetic groups (0.576 ± 0.04; 0.800 ± 0.06 and 0.911 ± 0.05) but over weight and obese(0.800 ± 0.06 ; 0.911 ± 0.05) were not significant (p>0.05), table (12-3), Figure 1.

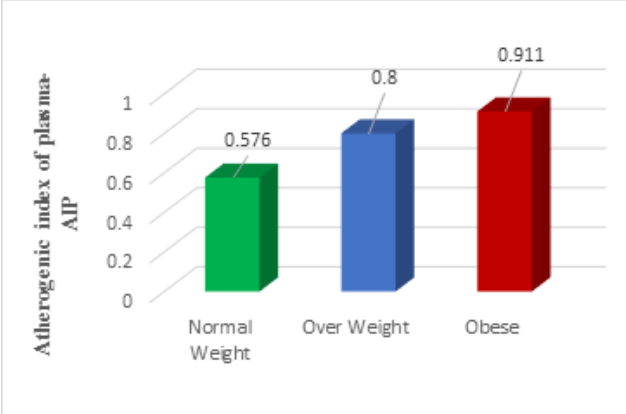

Figure 1 showing Atherogenic index among diabetic patients

AIP values were above normal values (under 0.11) while 0.11 and 0.21 values of AIP associated with intermediate and high risk of CVD ([5]). Previous researchers they concluded that AIP was elevated in diabetic and neuro diabetic and could be used a marker for predicting CVD in patients groups. AIP showed the presence of atherogenic minute LDL and HDL molcules, is a marker prediction of heart atherosclerosis and cardiovascular dangerous as an alternative for IR. [4,14]The AIP was significantly higher in 300 T2DM patients in Bangladesh and revealed dyslipidemia with increased TG and decreased serum HDL-C levels . AIP showed correlation BMI(r = 0.25), visceral fat (r = 0.37),

TC (r = 0.22), LDL (r=0.24), HDL (r = −0.72), TG (r = 0.84), glucose (r = 0.32).. Other Researchers the found TG to HDL was a strong prediction of heart infarction .That atherogenic index of plasma was an index of highest sensitivity for predicting the acute coronary events . That AIP correlation with BMI (r = 0.182, P < 0 001), (FBG (r = 0.153). AIP is used for detection of Type 2Diabetic Mellitus with high risk of complications [15].

Conclusions

AIP is used clinically for prediction of Type Diabetic Mellitus with higher risk of complications and this correlated with lipid profile,BMI .

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The relation between Serum Vitamin C and D, Calcium Intake With Obese Type 2 Diabetic Patients

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Abstract

This study took place at Al-kindy hospital on 30 men with type 2 diabetes mellitus aged between (35-52) years old and 30 control men aged between (37-48) years old.

In addition, the body mass index, vitamin D and C, serum calcium were measured.

It was noticed that the body mass index increases in diabetic group (28.9 kg/m^2) in comparison with the control group (24.2 kg/m^2).

Besides, serum calcium, vitamins C&D showed a significant decrease in diabetogenic patients (7.601mg/ml, 0.61ng/ml,16.48ng/ml respectively)

Compared with the control ones (9.871mg/dl, 1.21ng/ml, 28.104ng/ml respectively)

***Key Words**: Obese type 2 diabetic patients, antioxidant, vitamin C and D, Serum Calcium.*

Introduction

Gaining weight is a medical issue that has been expanding over the most recent couple of years because of increment of fast food causing cautions among numerous nations(1).

Vitamin C is viewed as an antioxidant agent, and auxiliary comparability among nutrient C and glucose makes it of enthusiasm for diabetes (2). Oxidation pressure can prompt aggravated glucose digestion and hyperglycemia (3). Along these lines, an advantage of cell reinforcements to counteract diabetes or to accomplish positive outcomes in type 2 diabetes mellitus (T2DM) is organically conceivable.

Vitamin D assumes fundamental job through entire body calcium homeostasis by applying exemplary impacts on the duodenum, bone and kidney (4) and modify intracellular calcium singles and in this manner assumes a job in pancreatic insulin discharge and insulin affectability, the two of which identify with Ca^{2+}levels. It has potential in the counteractive action of type 2 diabetes mellitus . A job of it in insulin opposition has been considered broadly and Vit.C is nutrient for whom researchers have shown relationship with high glucose level(5).

The blend of decreased serum 25-hydroxyvitamin D (25[OH]D) and lacking calcium admission has been related with heart and blood vessels hazard factors, for example, hypertension, overweight, metabolic disorder and type 2 diabetes mellitus (6). Vitamin D inadequacy and low calcium administration are accounted for, alittle is thought about the impact of those micronutrients in the avoidance of numerous infections, for example, heftiness (7). Be that as it may, a few examinations propose Ca and vit D admission don't avert nontransferable chronic ailments (8).

Point of this study is to know the impact of vit C and vit D as defensive measures in aversion of obesity and TDM2, and diminish its danger, additionally to think about the part of Ca admission take to diminish the danger of TDM2.

Material and Method

The study carried out on 30 men diabetic patients aged (35-52) years who visited the specialist center for endocrine and diabetes at Al-Kindy hospital in Baghdad,

and 30 men control aged (37-48) years were included in this study, the study was begun from January,2019 to march, 2019.

The body weights of individuals were measured by balanced and body heights of them were measured by paper tape. Body mass index was calculated as weight(w) in Kilograms divided by height (H) in meters squared [9] with following formula

BMI = W/H2 the values for BMI showed in table 1.

Table 1: measuring of Body weight

Risk of disease	Body mass index (Kg/m2)	obesity	Weight classification
Low	< 18.5		Under weight
Normal	18.5-24.9		Normal
Increased	25 -29.9		Over weight
High	30-34.9	Obese	Obesity
Very high 35 -39.9		Sever obese	
Extremely high	> 40	Morbidly obese	

Five milliliters of blood were drawn from each patient after (10-12) hours fasting via venipuncture , by syringes between (8-10 A.M), the blood sample was transferred into Gel tubes without anticoagulant serum were separated by centrifugation a 3000 rpm for 10 minutes used for the determination calcium, vitamin C and D.

The normal sufficiency of vitamin D is 10-44 ng/ml. However, the normal value of Ca is 8.5-10.2 mg/dI and to vitamin C is 0.4-1.8 mg/dL.

Table 1: weight classification by body mass index (WHO)

The statistical Analysis system SAS [13] program was implied for effect variation factors in study parameters. T-test was used to significant comparability between means.

Results and Discussion

The BMI showed highly significant increase (P<0.01) in adiabatic group (30.73) comparison with control group (24.2). Also, the age showed non-significant increase in diabetic group (45.09+2.86) in comparison with control group (44.89+3.21).

Table 2 showed that the diabetic patients BMI with overweight. [10].

Table 2. The means of Age and BMI in diabetic and control groups.

Groups	Mean + SD	
	Body mass index (Kg/m2)	Age group (year)
Control	44.89+3.21	24.2±0.87
Diabetic	45.09±2.86	28.9+1.21
T- test	0.29 NS	3.23
P-value	0.623	0.0005

NS=non significant **P<0.01

Overweight.][10] demonstrated that the most reduced danger of diabetes happens in people who have BMI under 21, expanding frequency of T2DM predominance was been found when obesity levels expanded . Nevertheless, abundant fat tissue adds to a chronic diseases, the use of unsaturated fats decreasing the utilization of glucose as a cellular vitality weight][11].

Despite the fact that age did not indicate noteworthy distinction among diabetic and control (Table 2). Expanded concentration of free fatty acids in blood builds glucose formation, and this will restrain insulin that takes apart in glucose take-up [12].

Obesity is viewed as a general medical issue that has been progressively arriving at disturbing extents in all districts of the world[1].

Table 3 shows the changes in calcium, Vitamin D and Vitamin C, Calcium level was increased significantly (P<0.01) in control group 9.871 mg/dI compared with diabetic group 7.601 mg/dI.

Furthermore, the data were showing significant (P<0.01) elevation of vitamin D in control group (28.104 ng/ml) with the diabetic group (16.48 ng/ml). On the other hand, vitamin C was increased significantly (P<0.01) in control group (1.21mg/dL) compared with diabetic group (0.61mg/dL).

Table 3. Levels of Calcium, Vitamin D and Vitamin C (Mean± SD) in control and Diabetic Groups.

Groups	Mean ± SD		
	Calcium(mg/dI)	**Vitamin D (ng/ml)**	**Vitamin C (ng/ml)**
Control	9.871+0.862	28.104±3.572	1.21±0.053
Diabetic	7.601±0.973	16.48±4.104	0.61±0.035
T- test	1.283**	6.218**	0.42**
P-value	0.001	0.0052	0.001

** P<0.01

It is accounted for that 1.25 (OH)2D3 can control insulin emission out of pancreatic β-cells [14]. Quick increment in intracellular calcium [Ca^{2+}], triggers insulin discharge. The job of 1.25(OH)2D2 in insulin emission gets from its impacts on Ca^{2+}deluge assembly and buffering in pancreatic β-cells [15].

Moreover 1.25(OH)D3 has a significant job in the guideline of cell Ca^{2+} signaling that is connected to cell reactions, signaling and secretion[16]. Supported Ca^{2+}sign activated by 1.25(OH)2D2 inquired about to the guideline of apoptosis a procedure that can decide cell passing in diseases , for example, obesity and type 2 diabetes [17].

Besides, 1.25(OH)2D3 induces Ca^{+2} signals (Ca^{+2} motions) can manage insulin discharge from pancreatic β-cells [18]. Vitamin D status has been connected to insulin resistance and T2DM in observational studies[19]

The complete calcium substance diminished in clear human lens between 18-55 years, it at that point expanded with age between 55-75 years [20].

Lack of 25-hydroxy vitamin 25(OH)D3 has been described as value of (20ng/ml) and has been perceived as a reason for childhood rickets and adulthood osteomalacia since the mid nineteenth century. Extreme insufficiency has been characterized as under 10ng/ml (25nmol/I) over the most recent 40 years, observational examinations have connected 25(OH)D lack to the advancement of chronic conditions including cardiovascular diseases and diabetes mellitus [21] . Vitamin D inadequacy is related with diabetes mellitus in elder working adults [22].

Vitamin D dissolve in fats actually, while vitamin C liquefy in water. Vitamin C is a cancer prevention agent and the auxiliary similitude between nutrient

C and glucose makes it of enthusiasm for diabetes [23]. Oxidative pressure can prompt disturbed glucose digestion and hyperglycemia [24;25]. Subsequently, an advantage of cancer prevention agents to counteract diabetes or to accomplish positive results in T2DM is organically conceivable. Inadequacy of vitamin C brings about blemished development of collagen and connective tissues in the skin, ligament, dentine, bone and blood, vessels, patients with diabetes should all get dietary guidance about good dieting and vitamin C dietary sources, including crisp leafy foods, the prescribed a dietary admission of the vitamin C is 45mg every day for grown-ups, vitamin C levels are lower among individuals with T2DM and not totally clarified by a distinction in dietary vitamin C consumption, various systems fundamental the abatement in vitamin C levels and expanded necessities in T2DM have been proposed expanding endogenous degree of vitamin C and D can be accomplished by dietary modifications[26].

Conclusion

Vitamin D and C along with calcium are essential protective micronutrients to reduce and control type 2 diabetes mellitus especially in those suffering from obesity. Doctors should recommend a diet rich with those elements.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Assessment of Anti-Mullerian Hormone and Anti Ovarian Antibody in the Sera of Patients with Polycystic Ovarian Syndrome in AL-Najaf Al-Ashraf Province

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Abstract

Polycystic ovarian syndrome(PCOS)is the most common endocrinopathy,that affects 5 to 20% of women in age of(12-45year)and cause a widespread of reproductive disorder in women and it is a major cause of hirsutism,acne,hormonal disturbance and infertility. Women with PCOS may associated with health conditions obesity,amenorrhea, oligomenorrhea,hyperandrogenemia and insulin resistance.The PCOS patients are with increased risk for type 2diabetes mellitus,diabetic complications,dyslipidemia and cardiovascular disease .

Objective: Aim of the study to investigate the pathogenicity,disease severity and early diagnosis roles anti-Mullerian hormone and anti-ovarian antibody in ladies with PCOS.

Conclusion: Polycystic ovarian syndrome was related to the increase level of AMH in different age of PCOS patients,which comprised 55.0% in PCOS patients that cause defect in ovulation and resulting fertility problem,while no relationship between anti-ovarian antibody and patients with PCOS.

***Key words**: AMH, AOA, PCOS and infertility.*

Introduction

Polycystic ovarian syndrome(PCOS)is a common and complex endocrine issue and hormonal abnormalities which influences whole body,that affected 5 to 20% of regenerative age ladies,occurred in age of(12-45)year,and it is a main reason for hirsutism and fertility problem[(1)]. PCOS is more typical than other conceptive infection; it is related with metabolic issue,for example,type 2 diabetes mellitus,dyslipidemia,hypertension and expanded risk of cardiovascular disease[(2)].

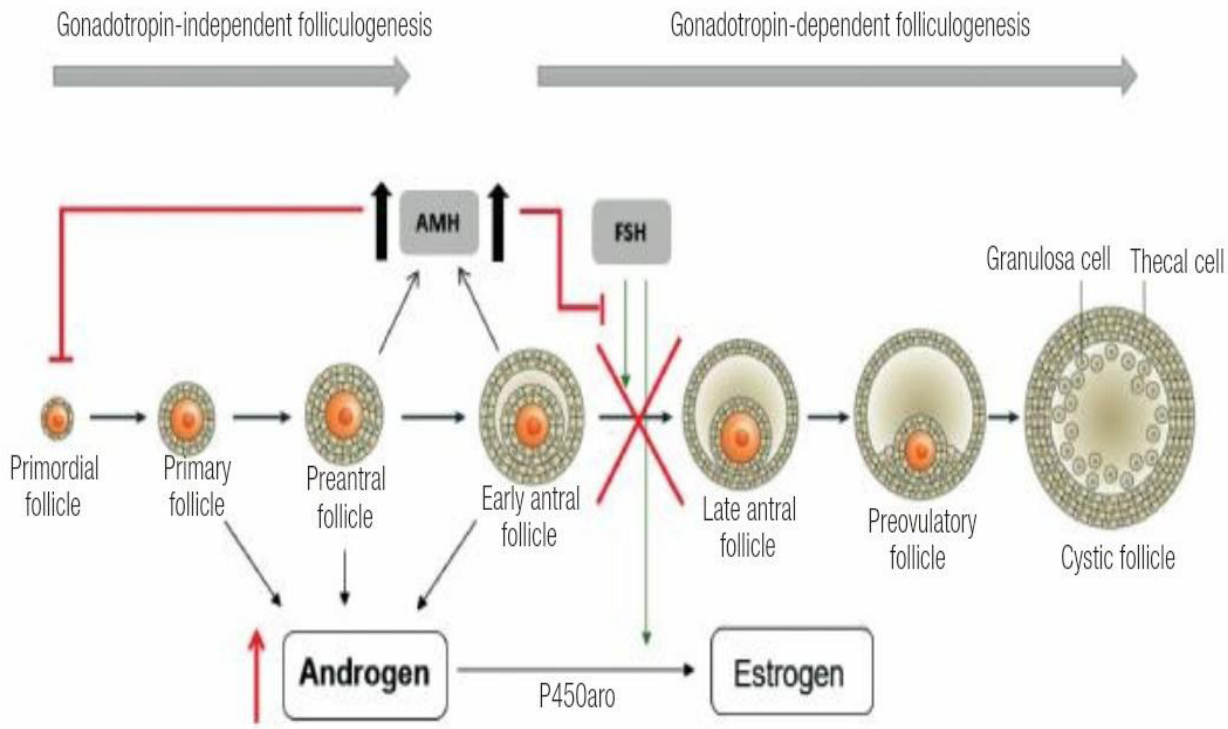
The investigators between young adult and adolescent patients with PCOS found a few phenotypic differences ,where the more severe component of the phenotype was in young adults was obesity,with the mean body mass index(BMI)and prevalence of obesity and morbid obesity being higher in young adult compared with adolescent patients[(3)]. The etiology of PCOS not clear,but rather numerous elements might be related with PCOS,which include obesity,hyperandrogenism, insulin resistance that lead to compensatory hyperinsulinemia , increment discharge of luteinizing hormone (LH) and disappointment ovarian follicular improvement because of expanded follicular advancement blocker paracrine factor , such as anti mullerian hormone (AMH) that produce from granulosa cell of follicles in size under 8 mm[(4)].

The diagnosis of PCOS in ladies is ordinarily as indicated by the European society of human reproduction and embryology/American society of reproductive medicine(ESRHE/ASRM) criteria known as Rotterdam criteria, which rely upon nearness at any rate two of three highlights of oligo-ovulation/anovulation(menstrual dysfunction), hyperandrogenism and appearance of polycyclic ovaries in ultrasound look like the pearl string[(5)].

Anti -Mullerian hormone:-

Anti-Mullerian hormone(AMH)also define as Mullerian-inhibiting substance,Mullerian inhibiting factor and Mullerian-inhibiting hormone.It is a glycoprotein dimer,it belongs to the member of the transforming growth factor beta superfamily,it is produced from the 36th week of gestation, by the granulosa cells of growing follicles of the ovary,primary,

secondary,preantral and antral,start produced at elevated values by the last two and has an a specific role in the evolution and maturation of follicles[6].It has two main mechanisms of ovarian dysfunction by disrupting folliculogenesis through:suppress the initial induction of primary follicles from primordial follicles, and suppress the responsiveness of antral follicles to (FSH) through period induction (Fig.1).



Figure(1): Anti-Mullerian hormone(AMH)and folliculogenesis[7].

The AMH stops the premature exhaust of follicle[7]. AMH level higher value at age 25 years,after that decreased to the menopause[6].Circulating AMH levels in serum is high two or three times in the patients with PCOS than healthy women,and not influenced by the menstrual cycle nor changed through the use of oral contraceptives,thus it can be applied as a potent parameter for detection PCOS .AMH measurement is valuable because, it can be useful in predicting ovarian hyperstimulation,multifollicular complications and may be a novel marker for ovarian aging as well as evaluating treatment efficacy in PCOS.The starting of folliculogenesis is suppressed because increased values of AMH, in women with PCOS,increased AMH value have harmful role in disruption of ovarian physiology and worse fertility outcomes[8].AMH also block the action of the aromatase enzyme,suppose that the AMH participate in the severity of PCOS,therefore can AMH may can be applied as a delegate parameter of classical hyperandrogenism.AMH has two receptors type 1 receptor(AMHR-I)is a non specific and type two receptor(AMHR-II)is specific by the AMHR2 gene present on the AMH target organs such as mullerian duct and granulosa cells of ovary,binding of AMH to AMHR2 inhibit follicle development by suppressing recruitment of primordial follicles into the pool of developing follicles[9].

AMH protein expression initiate at the primary follicle phase,very raise expression is determined in FSH-dependent preantral and small antral follicles of<4mm in diameter and AMH reduced and absent in follicles larger than 8mm.The distinctive feature of PCOS is failure of follicular development,causing anovulation and aggregation of preantral and small antral follicles, which may be contributed to high introduction of AMH[I(10)]. Hyperandrogenism quickens preantral and antral follicle growth in the ovary,and expanded LH causes in premature luteinization resulting follicular arrest leading elevated values of AMH.It is likewise proposed the obesity had an extra prohibitory impact on gonadotrophin discharge because of an expansion in convert androgens to estrogens in fat tissue bringing about the concealment of LH and the subsequent restraint

of the dominant follicle[11].

level of AMH in women benefit as a biomarker of ovarian storage and refer to the small follicles cannot growth,if low in the clinical state of decreased ovarian store,indicating that presence a little number of follicles in the ovary.AMH block the premature evolution of follicles and eggs,this happen by decreasing the number of FSH receptors on ovaries and preventing the premature egg maturation by FSH in each cycle[6].

Other importance of this hormone in various pathological conditions of ovaries not only the diagnosis and follow up of ovarian tumors of granulosa cell origin, but also prognosis of ovarian hyperstimulation syndrome and PCOS[12].

AMH prevent the secretion of FSH and impact on growth of follicle by suppress the expression of aromatase enzyme dependent FSH and luteinizing hormone (LH) receptor.In PCOS ladies,there is a partition that keep the follicles to turn into the predominance follicle.In addition to the reduced value of FSH,increase values of AMH slower the responsiveness of follicles to FSH,therefore the follicles stop development into a predominance follicle,that cause an aggregation of several small antral follicles less than (9) mm in diameter in the ovary[13].

Anti -ovarian Antibody: -

Polycystic ovarian syndrome is the endocrinopathy and disturbance that cause menstrual impairment and infertility through propagative age,genetic and hormonal status have important function in the pathogenesis of PCOS.Decrease secretion of progesterone in PCOS give rise to over-stimulation of immune system that increase oestrogen secretion that lead to different autoantibodies,have been reported in PCOS, for such as anti-nuclear antibodies(ANA),anti-thyroid,anti-histone,anti-islet cell and anti-ovarian antibodies (AOA)[14].AOA is a group of auto-antibodies against ovarian antigens, include anti-oocyte plasma,oocyte membrane(zona pellucida),different parts of the ovary granulosa,theca interna and lutein follicular cells.Many causes for AOA formation,may be include chronic inflammation of the ovaries, laparoscopic abdominal surgery,the collection of oocytes for assisted reproductive techniques(ART)and also the presence of auto-antibodies against human heat-shock protein 90-β(anti-HSP90β)in the sera of infertile women,this protein causes ovarian autoimmunity and is considered to be a marker of ovarian damage also associated with POF[15].AOA disrupt the development and function of oocytes and result a decline in the fertilization rate,thus they could likewise be an autonomous marker for possible ovarian failure.The AOA have antagonistically influence on egg quality or hinder the luteal stage and implantation. Presence of AOA either against tissues ovary or oocytes and presence of these AOA proposes detection of autoimmune process that might be the primary or secondary response to an ovarian combination,found high level of AOA in women after follicular puncture of unsuccessful in vitro fertilization(IVF) trials may be in charge of for the pathophysiology of unprompted miscarriages,additionally found with, endometriosis,other reproductive disorders such as adrenal failure,polyglandular autoimmune disorders,unexplained infertility and PCOS associated with antiovarian autoimmunity[16].Another cause in the production of anti-ovarian antibodies may be an autoimmune process of POF.The anti-ovarian antibody are reported in premature ovarian insufficiency(POI),more found of an AOA(30-67%) autoantibodies has been reported in POI patients.A few antibodies in the aggregation of AOA are thought to connect with an immediate activity on ovarian tissue, while others have no such impacts, comparably to autoantibodies in the other autoimmune diseases, therefore it is conceivable that few various antigens are include in the ovarian autoimmunity on the grounds that both ovarian cell and zona pellucida,oocyte antibodies have been documented.The production of AOA in some cases was activated by hormonal incitement of ovaries and coordinated against(LH)receptors,or human chorionic gonadotrophin(HCG)receptors,or both of them,the AOA appraisal,demonstrates that AOA are particular coordinated to theca interna cells.Ovarian antibodies have been appeared to be available in infertile ladies in spite of the fact that demonstrating ordinary level of FSH and inhibin-B,recommending that ovarian antibodies are free indicators of potential of autoimmune ovarian defeat,the pathological importance for presence ovarian antibodies to different cell parts of the ovary can (a)diminish fertilization rates (b)decline pregnancy rates (c)create a poor reaction to gonadotropin prompting (d) influence egg and embryo improvement (e)could be in charge of implantation defeat[17].

Materials and Method

Subjects

The present study consist of 120 women (80

PCOS patients and 40 control) attended from Al-Furat teaching hospital and fertility center of Al-Sadder medical city in Al-Najaf province.They were seen from(June/2018– October/2018).All these patients and healthy control were examined for PCOS diagnosis by ultrasound,measuring parameters including AMH and AOA. The AMH detected by (ELFA) technique by using minividas instrument (Biomerieux,France)and AOA measured by ELISA technique (Human,Germany).

Sample collection:

Venous blood samples(5mL)were collected from all patients and healthy controls by using disposable syringe, the specimens were taken during(2^{nd}_7^{th})day of the menstrual cycle from those of patients and healthy control.The samples were collected by venous puncture and pushed slowly into a jel tubes ,then centrifuged directly by centrifugation at (4000 rpm) for 10 minutes. Serum of AMH was measured immediately before stored and the remaining of the sera were stored at deep freeze in disposable plain plastic tubes,until the time of the analysis of AOA**.**

A. Inclusion criteria:

All patients must fulfill the international diagnostic criteria for PCOS.

All patients and control group should be more than (15 and less than 40) year.

The PCOS is diagnosed for not than one year ago.

B. Exclusion criteria:

Patients that do not fulfill the international diagnostic criteria for PCOS.

Any diagnosed case of PCOS under current medication.

Control with a previous history of PCOS and recovered

Patients of unexplained causes of infertility

Patients with other chronic disease .

Results and Discussion

Table (1) : Levels of AMH and AOA in sera of studied groups .

Parameters	Patients n=80	Control n=40	Statistics t test	p value	Sig.
	Mean ± SD	Mean ± SD			
AMH	7.75 ± 2.49	2.63 ± 1.21	t = 12.28, df = 118	0.001	HS
AOA	4.92 ± 2.52	4.52 ± 0.29	t= 4.875, df = 118	0.08	NS

The level of AMH in patients and healthy control.

Table(1)and figure(2)explained a highly significant elevation(P<0.01)in the mean concentration of AMH in sera of patients in comparison with the mean serum concentration of control [AMH(7.75 ± 2.49 ng/mL)and(2.63 ± 1.21 ng/mL)],respectively with a highly significant difference(P<0.01).At an optimal cut-off value of (1.19)ng/mL , AMH showed the sensitivity and specificity as 61.0 % and 85.0 % respectively,with a AUR(0.575) (95% Cl:0.471-0.679),as showed in table(2)below and figure(3)below. The current study appeared increased result of AMH in (55.0%) of PCOS patients more than normal value.

Table (2): ROC for studied parameters.

Parameters	Cutoff	AUR	95%CI	Sensitivity%	Specificity%
AMH	1.19	0.575	0.471- .679	61	85
AOA	1.5	0.651	0.552-0.750	88	85

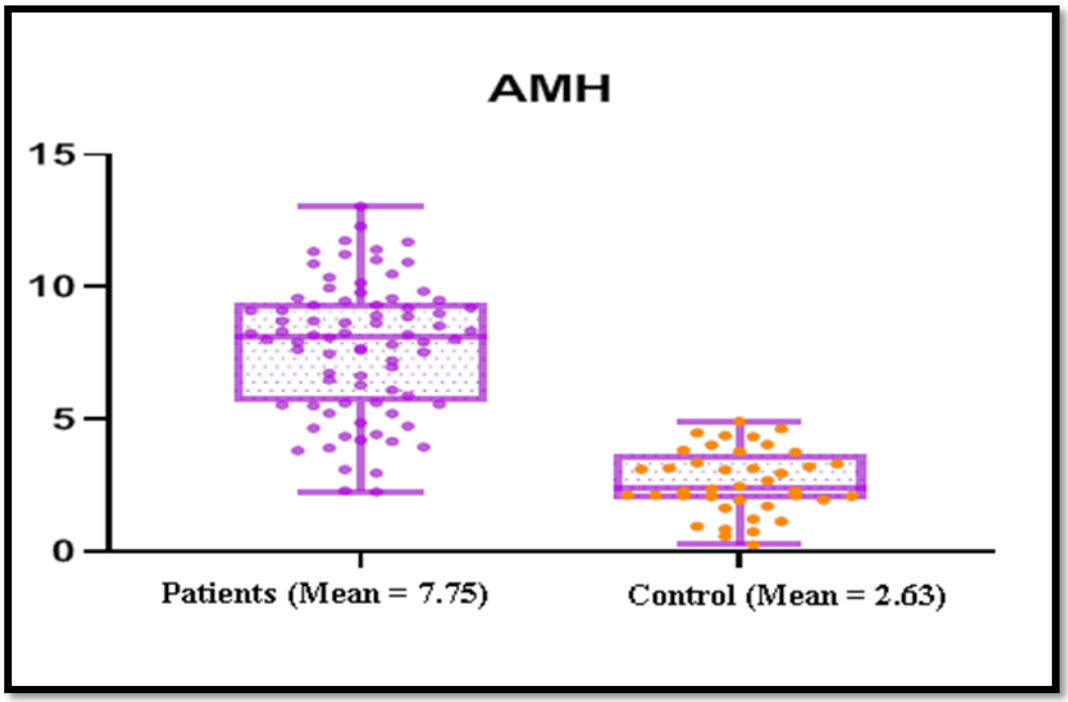


Figure (2): Boxplot of AMH levels in studied groups.

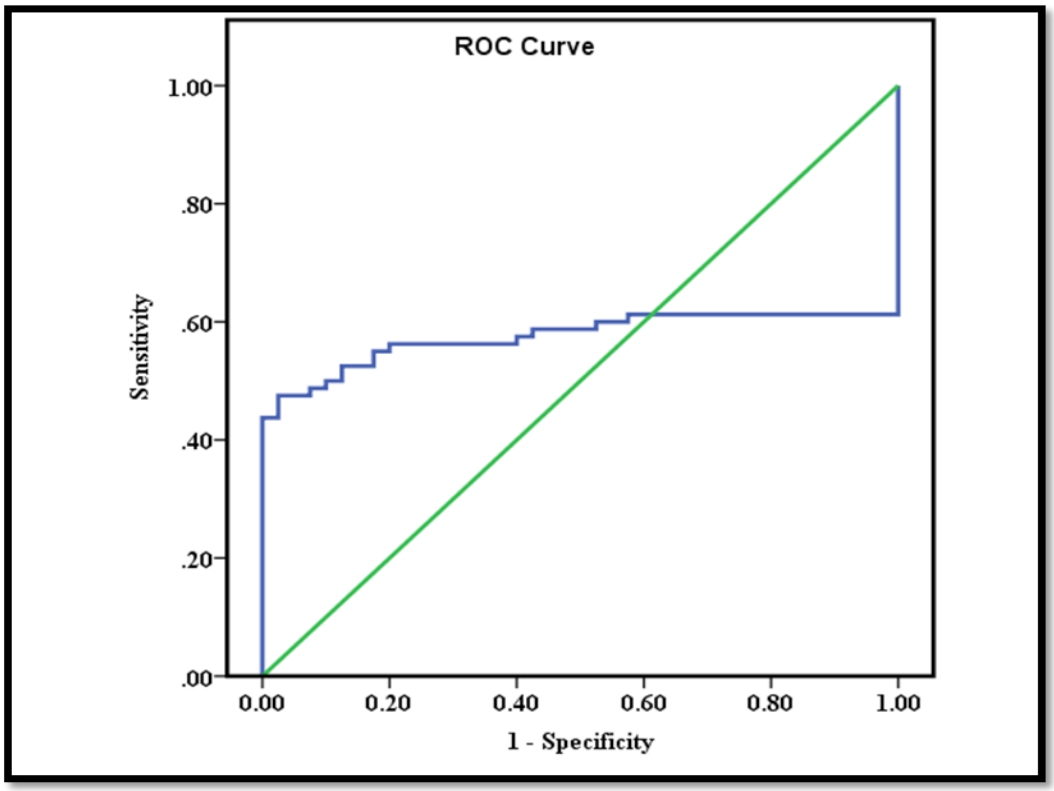


Figure (3): ROC curve analysis of AMH to discriminate the studied groups.

This study agreed with recent Iraqi study finished by(Mohaisen *et al*.,2019)[18]in Misan city.Several recent studies confirmed by(Deshmukh *et al*.,2019)[19] in England and by (Cela. *et al*.,2018)[20]in Italy,that reported increased AMH level in PCOS ladies than healthy ladies.The increase level of serum AMH in PCOS refer to the fact that AMH level indicate the count

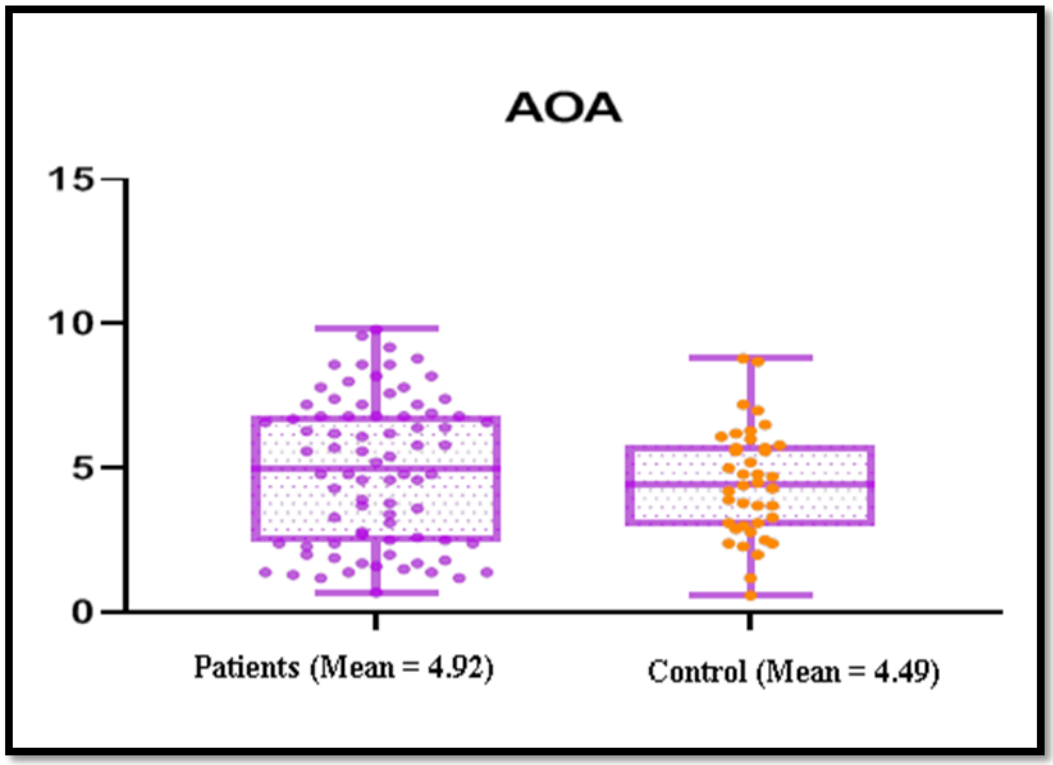
of small antral follicles and increased secretion of AMH per follicle and therefore the increased AMH expression has been indicated in the phase of preantral and small antral follicle size (4-8mm) and dissolve in follicle size more than 9 mm[21].

The increase of production of AMH prompt the decrease in the sensitivity of follicles to FSH at levels of receptors,which is very necessary for growing of this follicles,it leads to an increase of the numbers of antral follicles on the detriment of their size and the number of small antral follicles size(2-5mm)increases, therefore failure the selection of the dominant follicle and anovulation occur with oligo or amenorrhea[22].

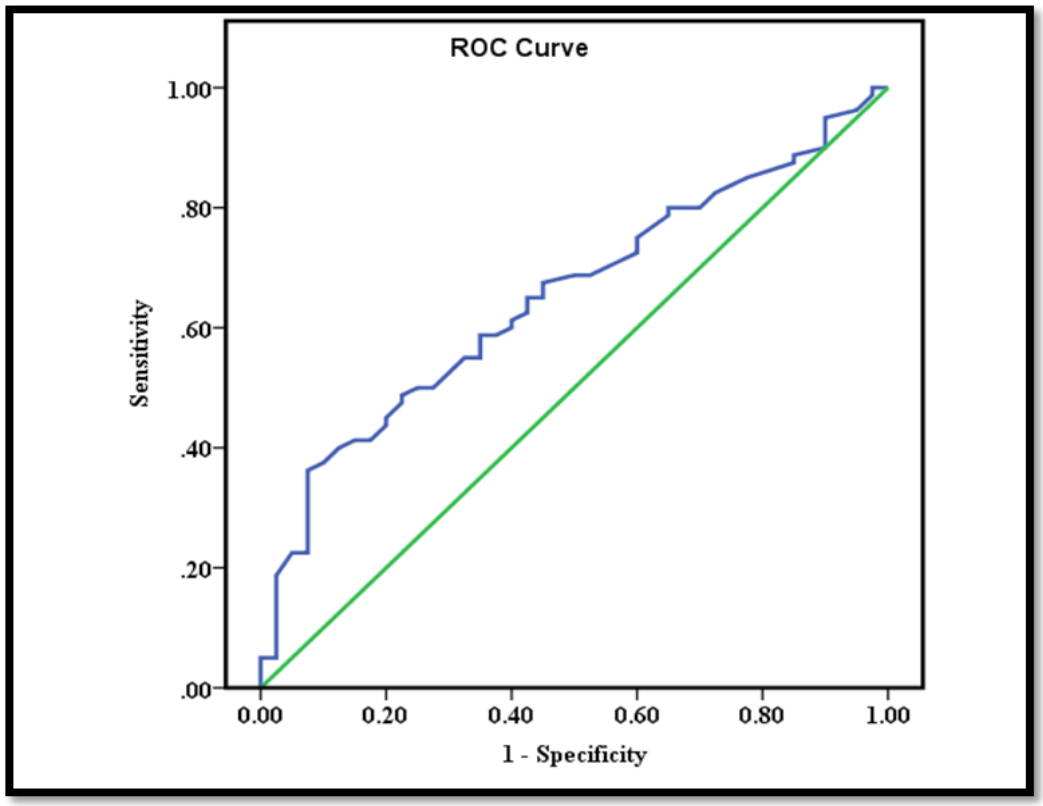
Other cause for elevation AMH associated with the raise insulin levels that participate to hyperandrogenemia in (PCOS) that result a defect in folliculogenesis,then participate of polycystic morphogenesis of the ovaries and increased AMH level than normal.An increase level of AMH is indicate either to inflexible anovulation or refer to ovarian hyperstimulation syndrome.Therefore,it is suggested that the apply of insulin sensitizers will decrease insulin resistance,hyperandrogenemia,and also lower AMH level in serum and will alter the anovulatory cycles to ovulatory[23].

Level of AOA in sera of patients and healthy control.

The serum AOA(U/mL)in table(1)and figure(4) illustrated a non significant difference (P>0.01)in the mean concentration of AOA in sera of patients[serum AOA(4.92 ± 2.52) U/mL]in comparison with mean sera concentration of control group[serum AOA(4.52 ± 0.29) U/mL].At an optimal cut-off value of (1.5) U/mL, AOA showed the sensitivity and specificity of 88 % and 85 % respectively,with a AUR(0.651) (95% Cl: 0.552-0.750),as showed in table(2)above and figure(5)below.



Figure(4): Boxplot of AOA level in studied groups.



Figure(5):ROC curve analysis of AOA to discriminate patients from control.

The result of this study explained no correlation between the AOA and PCOS,where all the concentration of the AOA within normal value in patients and healthy control and no significance difference between them.

The studies about the relationship between AOA and PCOS very little,although the AOA is very important analytical tool to detect and explain the mechanism of disease development, evolution and resultant disrupt in the ovary.

The current result of this study agreed with study in USA occurred by(Aritro *et al*.,2014)[24] which failed in different investigations to affirm presence of AOA in PCOS ladies.The study done by(Beata *et al*.,2016)[25] in Poland confirmed a high prevalence of anti ovarian antibodies(AOA)about (30-67%) has been observed in patients with premature ovarian failure(POF).

Other study accomplished by (Pires *et al*.,2015)[26] in USA reported presence of AOA as an auto immune antibody,after failure several attempts of in vitro fertilization (IVF) and also presence of it causes reduce IVF success.

The reason of not detect the presence of high level of AOA in PCOS patient s may be due to presence hyperandrogenemia,which considering a protective function, high levels of androgen play a role in PCOS cases and prevent the development of autoimmune diseases [27].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Cervical Spinal Stenosis and Risk of Pulmonary Dysfunction: Case Control Study

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Abstract

Background: Cervical spinal stenosis is well-defined as debility in the volume of the spinal canal. Consequently, distracting in the descending neural pathways at any level in the cervical spine lead to impaired smooth and effective breathing under normal conditions .

Objective: To assess the effect of cervical spinal stenosis (C5and above) on pulmonary function tests , to assess the sensitivity and specificity of pulmonary function tests in patient with cervical spinal stenosis(C5 and above).

Methods: This is a case-control study which included 40 patients divided into two groups 30 females and 10 males patients with cervical spinal stenosis (C5 and above) and 60 healthy volunteers with body mass index < 30 (Kg/m^2). Pulmonary function tests has been done for all subjects.

Results: The results demonstrate the comparison between all patients and controls show that VC EX% ,FEV1% ,FVC% ,PEF% ,MVV% , were significantly lower in patients in comparison to control groups as well as VC EX %,FEV1%,FVC % were significantly lower in male patients in comparison to controls and in female patients as compared to female controls and MVV% were significantly lower in female patients in comparison to male patients ,while no significant difference has been identified between patients and controls regarding age ,weight , height , BMI,VC IN% and FEV1\FVC %. Sensitivity and specificity and cut off value for pulmonary function tests parameters show FEV1% is the highest specificity and sensitivity.

Conclusion: The respiratory dysfunction that is established in patients with cervical spinal stenosis usually subclinical not overt defect that compromised respiration ,this pulmonary dysfunction is a type of restrictive lung disease since phrenic nerve is affected. . FEVI% is most essential among other parameters of spirometry in patients with cervical spinal stenosis.

Keywords: *Cervical spinal stenosis, pulmonary function tests, Spirometry*

Introduction

Stenosis of cervical spine is well-defined as a waning in the volume of the canal of spine.[1] Even though stenosis of the central canal can transpire everywhere alongside the length of the spine, this condition is deliberated more threatening with greater forthcoming for serious neurological sequelae when it occurs in the cervical spine.[2,3,4]

Degenerative cervical myelopathy DCM is judged to be the most common cord of spine illness, and is predictable to have an increasing prevalence with the aging population in the work world. [5]

Respiratory function has been well-ordered by the phrenic (C3–5) nerves, the intercostal (T2–10) nerves, and autonomic nerve, especially the sympathetic (T1–L3) nerves. Consequently, distracting in the descending neural pathways at any level in the cervical spine lead to impaired smooth and effective breathing under normal conditions. The effect might lead to the worsening of pulmonary function even if the impairment of neural function was subclinical. [6,7,8]

Method

This is case control, nonrandomized, single-center study evaluating pulmonary function tests in patients

with cervical spinal stenosis (C5 and above) who attend orthopedic and neurosurgical consultations in AL-Imamian AL-Kadhymiyian medical city during period from November 2018 to May 2019.

40 patients divided into two groups 30 females and 10 males patients with cervical spinal stenosis (C5 and above) with age range from (25 to 70) years and body mass index < 30 (Kg/m^2). The duration of their illness ranges from (1 to 24) months.

All patients were clinically examined and diagnosed by MRI with or without CT scanning and X ray of the cervical spine to determine the level of compression. The diagnosis was supported by trans cranial magnetic stimulation (TMS) technique. Smokers and (x) smokers for at least six months, systemic disease known to cause peripheral neuropathies (Diabetes Mellitus), Spinal tumor, chronic pulmonary disease. Central obesity (WC ≥94 cm for men and ≥80 cm for women), [9] or waist-to-hip ratio (WHR)≥0.90 in men and ≥0.85 in women and a WHTR of >0.50[9] ,BMI ≥ 30(Kg/m^2) had been excluded from the study. Sixty apparently healthy peoples aged and sex matched nonsmoker nor centrally obese as volunteers control group thirty males and thirty females .Their age ranges from (30-75) with BMI < 30 (Kg/m^2).

Pulmonary function tests have been done for all subjects, the result was presented on a discrete paper in a special form. The tests incorporated the following: Vital capacity in inspiration VC IN, Vital capacity in expiration VC EX, Forced expiratory volume in 1st second FEV1, Forced vital capacity FVC, Forced expiratory flow between 25-75 of the FVC or Forced mid –flow (FEF25-75%,FMF), Peak expiratory flow rate PEFR , Mean voluntary ventilation (MVV), ratio of FEV1 to FVC (FEV1/FVC). Predicted values of the Spiro metric parameters were based on the patient's height, age, and sex. The percentage of predicted values was used for comparison.

% predicted = measured ÷predicted [10]

The study approved by the Institutional Review Board (IRB) of the college of Medicine, Al-Nahrain University and informed consents were obtained from all the participants.

Statistical analysis

Statistical analysis was performed with SPSS V23. (statistical package for social sciences) and also Excel 2007 programs. Data analysis was done using t–test and the values were considered statistically significant when p-value < 0.05. For each parameter the sensitivity and specifity by ROC analysis.

Results

Table 1 shows that VC EX%, FEV1%, FVC%, PEF%, MVV%, were significantly lower in patients in comparison to control groups (P<0.001), (P<0.001), (P<0.001), (p=0.042), (P=0.037), respectively .While there were no significant difference has been identified between patients and controls regarding age ,weight , height , BMI,VC IN% and FEV1\FVC%.

Table (1): Comparison of data between all patients and all controls by unpaired t test

Parameter	All Patients N=40 Mean±SD	All Controls N=60 Mean±SD	P value
Age (yr)	50.05+12.5	49.92+11.04	0.955
Weight (kg)	67.05+8.04	70.42+9.36	0.066
Height (cm)	159.93+8.99	162.28+9.49	0.217
BMI (kg/m2)	26.21+2.29	26.71+2.29	0.282
VC IN%	90.48+14.35	94.33+13.58	0.177
VC EX%	79.23+11.24	90.86+11.8	**<0.001**
FEV1%	89.16+15.59	105.53+14.04	**<0.001**
FVC%	77.26+13.1	90.56+12.69	**<0.001**
PEF%	85.21+14.82	93.21+21.32	**0.042**
FEV1\FVC%	95.72+4.08	96.56+3.89	0.302
MVV%	98.42+16.77	106.14+18.55	**0.037**

Table (2) shows that VC EX %, FEV1%, FVC % were significantly lower in male patients in comparison to controls (P=0.001) (P=0.004), (P=0.002) respectively. While there were no significant difference has been identified between male patients and male controls regarding age, weight, height, BMI, VC IN %, PEF%, FEV1\FVC%, and MVV%.

Table (2): Comparison of data between male patients and male controls by unpaired t test

Parameter	Male Patients N=10 Mean±SD	Male Controls N=30 Mean±SD	P value
Age (yr)	55.5+12.07	52.9+11.38	0.541
Weight (kg)	75.0+6.38	73.87+10.46	0.749
Height (cm)	170.5+5.97	168.4+7.89	0.446
BMI (kg/m2)	25.78+1.44	25.94+2.43	0.850
VC IN%	84.7+14.11	89.94+12.36	0.269
VC EX%	74.71+7.51	88.32+11.71	0.001
FEV1%	88.18+12.5	105.81+16.36	0.004
FVC%	73.51+9.75	88.9+13.08	0.002
PEF%	89.59+14.02	92.1+16.41	0.667
FEV1\FVC%	95.36+3.11	95.9+4.33	0.715
MVV%	119.11+11.67	119.2+15.24	0.987

Table (3) shows that: VC EX%, FEV1%, FVC % were significantly lower in female patients in comparison to female controls ($p<0.001$), ($p<0.001$), ($p<0.001$) respectively. While there were no significant difference has been identified between female patients and female controls regarding age, weight, height, BMI, VC IN%, PEF%, FEV1\FVC%, MVV%.

Table (3): Comparison of data between female patients and female controls by unpaired t test

Parameter	Female Patients N=30 Mean±SD	Female Controls N=30 Mean±SD	P value
Age (yr)	48.23+12.3	46.93+10	0.655
Weight (kg)	64.4+6.73	66.97+6.65	0.143
Height (cm)	156.4+6.8	156.17+6.59	0.893
BMI (kg/m2)	26.35+2.52	27.48+1.86	0.051
VC IN%	92.4+14.14	98.73+13.49	0.082
VC EX%	80.74+11.96	93.4+11.53	<0.001
FEV1%	89.48+16.67	105.25+11.54	<0.001
FVC%	78.5+13.96	92.21+12.29	<0.001
PEF%	83.74+15.02	94.32+25.55	0.056
FEV1\FVC%	95.84+4.39	97.22+3.33	0.177
MVV%	91.52+11.79	93.08+10.72	0.594

Table (4) shows that : weight, height, MVV% were significantly lower in female patients in comparison to male patients, ($p< 0.001$) .While there were no significant difference has been identified between male and female patients regarding age, BMI, VC IN%,VC EX%, FEV1%, FVC %, PEF%, FEV1\FVC%.

Table (4): Comparison of data between male and female patients by unpaired t test

Parameter	Male Patients N=10 Mean±SD	Female Patients N=30 Mean±SD	P value
Age (yr)	55.5+12.07	48.23+12.3	0.112
Weight (kg)	75.0+6.38	64.4+6.73	<0.001
Height (cm)	170.5+5.97	156.4+6.8	<0.001
BMI (kg/m2)	25.78+1.44	26.35+2.52	0.507
VC IN%	84.7+14.11	92.4+14.14	0.144
VC EX%	74.71+7.51	80.74+11.96	0.144
FEV1%	88.18+12.5	89.48+16.67	0.823
FVC%	73.51+9.75	78.5+13.96	0.303
PEF%	89.59+14.02	83.74+15.02	0.286
FEV1\FVC%	95.36+3.11	95.84+4.39	0.747
MVV%	119.11+11.67	91.52+11.79	<0.001

Table (5) shows:

Cutoff value of VCIN%,VCEX%,FEV1%,FVC%,PEF%,FEV1\FVC%,MVV%

In ROC were estimated and consequently the sensitivity and specificity were evaluated FEV1% show the highest specificity and sensitivity.

Table (5): Area under curve, sensitivity, specificity and cutoff value for pulmonary function tests parameter

Parameters	AUC	Sensitivity	Specificity	Cut-off value
VC IN%	0.573	53.3%	55.0%	91.25
VCEX%	0.779	70.0%	70.0%	82.2
FEV1%	0.813	75.0%	75.0%	95.1
FVC%	0.784	66.7%	67.5%	82.6
PEF%	0.623	56.7%	60.0%	86.75
FEV1\FVC%	0.554	56.7%	57.5%	97.02
MVV%	0.624	61.7%	62.5%	100.06

Discussion

Patients with compressive cervical spinal stenosis designated by the spirometric data as mild but considerable impairment of respiratory function. Lesion in the cervical spinal cord can lead to respiratory insufficiency by disturbing the descending pathways.[11]

The present study show no significant differences between age ,height ,weight of patient and control groups and this resembling to study done by (Toyoda et al).[11]

Pulmonary functions endure the major biologic variable that is affected by aging. There has been great individual variation in the extent of the aging process in the lungs. Even in individuals with good health, there are quantifiable decrements in function of the respiratory system with age.[12]

The weight is another factor that have been with adverse effect on lung function which showed by various studies.[13,14] Congregation validation designates that the association between increase in weight and both lung volume and lung function , the lung volume and capacity that reduced in overweight subjects compared with healthy subjects.[13]

The present study showed that VC%, FVC%, PEF%, FEV1%, MVV% of patients were significantly lower than controls.

The probable explanation for the pathomechanism of this may embrace that the phrenic nerve has been partial injured, decrease in the strength of the respiratory muscle, and the intercostal muscle tone loss. In healthy individuals the parasympathetic activity has been prime, so in the mild respiratory dysfunction imbalance of the autonomic nervous system has been arise.[11] Consequently, sympathetic activity has been predominant in the chronic compression of the cervical spinal cord in addition to diminish in peripheral airway resistance.

The present study show no significant difference between FEV1\FVC % of patients and controls and this agreement with Bhagavatula et al,[10] since in our study the result of spirometric data involve normal and restrictive lung function values ,so according to study done by Pellegrino et al[15] which state that restrictive ventilation disorders are characterized by a reduced TLC with normal FEV1/FVC , Spirometry can confirm that FVC is reduced, while FEV1 may be reduced secondarily to a reduction in FVC, or relatively maintains as normal. As a result, FEV1/FVC is normal or may increase slightly.

The present study show MVV % was significantly lower in females in comparison to males' patients. This is in accordance with the study done by Budhiraja et al;[16] this can be attributed to the fact that the men have bigger lungs for the same height as compared to females. Another contributing factor could be the greater strength of respiratory muscles in males.[17]

The present study shows that FEV1% has the highest specificity and sensitivity among other parameters of spirometry in patients with cervical spinal stenosis since the classification of the severity of obstructive and restrictive impairments based upon the grade of reduction in FEV1 and VC respectively[18] otherwise the categorizing of restrictive impairment stated by ERS(European respiratory society) in 2005 has been established based on predicted FEV1% [15], the categorization of both obstructive and restrictive impairment has been recommended by ERS using FEV1 due to easiness compared to ATS method , the reasonable for such a suggestion, in disorders presenting with restrictive defects, is because the FEV1/VC ratio is conserved (normal or increased) and therefore the reduction in FEV1 has been correlated with the analogous reduction in VC .

Conclusion

The respiratory dysfunction that is established in patients with cervical spinal stenosis usually subclinical not overt defect that compromised respiration ,this pulmonary dysfunction is a type of restrictive lung disease since phrenic nerve is affected. . FEVI% is most essential among other parameters of spirometry in patients with cervical spinal stenosis.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Molecular Study of spy1258 gene in Streptococcus Pyogenes Isolated from Pharyngitis Patients in Fallujah City

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Abstract

Background : *Streptococcus pyogenes* (GAS) strict human pathogen that give rise to a wide range of diseases. GAS is the most current bacterial cause of pharyngitis and firstly effect school-age children 5-15years of age. SPY1258 gene is a specialized gene group in GAS and its considered specific for GAS and can be used for its detection as a markers.

Method : A total of 300 throat swab were collected from patients suffering from pharyngitis referred to Fallujah general teaching Hospital, Fallujah taeching Hospital for women and chiladren and Amerya general Hospital , Iraq during a peroid between 2018-2019.

Theses samples were submit for bacteriology and molecvular tests.

Results : Out of all samples screened 43 isolates (14.3)were identified as ß – hemolytic streptococci and only 13 isolates were detected as streptococcus pyogenes by bacteriology and biochemical test and also by PCR GAS isolates exposed in order to detect the particular gene (SPY1258). Antibiotic sensetivity test showed a high incidence of bacterial resistance to macrolids.

Conclusion : Bacitracin sensetive is non- specific test for the identification of *Streptococcus pyogenes*, and should be used further test, for best result should confirmed by SPY1258 gene. The SPY1258 gene can be used for accurate molecular diagnosis of *Streptococcus pyogenes* .

Key words: *Streptococcus pyogenes, SPY1258, pharyngitis*

Introduction

Streptococcus pyogenes , a group A streptococci (GAS), is strict human pathogen that give rise to a wide range of diseases , from moderate to acute invasive diseases . *Streptococcus pyogenes* is countable for over than 500000 death each year [(1)].

β - hemolytic *streptococci* produce a toxin that forms a clear zone of hemolysis on blood agar, confirmation its strength to devastate red blood cells. This hemolysis is referred to toxins created by *Streptococcus pyogenes* called “Streptolysins” which can destroy the red blood cells and also the white blood cells which is responsible for destroying pathogen [(2)]

The skin and mucus membranes of the human host are the only reservoirs for *Streptococcus pyogenes* in nature . The major reservoirs of *Streptococcus pyogenes* are the school aged children (5-15) years [(3)] .

Pharyngitis (sore throat) is one of the most widespread situation encountered by the family practitioner [(4)].

Streptococcus pyogenes is the most current bacterial cause of pharyngitis and firstly affect school-age children 5 – 15 years of age [(5)] .

The universal development of antibiotic resistance between GAS strains , and the economic crisis emerge out of it in healthcare industries has presupposed the need for an alternative agent with novel texture features[(6)]

SPY1258 gene is a specialized gene group in *Streptococcus pyogenes* which was encodes transcriptional regulators . As transcriptional regulators represent fundamental molecular combination in the adaptation and existence of *Streptococcus pyogenes* [7] .

Materials and Method

Three hundred swabs were collected from children with pharyngitis ranging in age from 4 to 14 years , who refeered to fallujah general teaching Hospital , fallujah teaching Hospital for women and children , The health center of the primary health care sector in fallujah , health centers in refugee camp and special medical clinics in the fallujah city , During the peroid from November 2018 to May 2019 .

Throat swab was taken from the pharynx of each patient , and immediately placed in a trypton soya broth with 5% blood and transferred to TSA, the plates were incubated at 37℃ for 18 to 24 hours under 5- 10% Co2 , in the candel Jar [8].

Identification of streptococcus pyogenes :

The identification of the colonies were confirmed based on morphological and growth features , including the phenotypically discrete colonies , beta- hemolysis on blood Agar plate. Biochemical test and Bacitracin discs susceptibility were used in order to confirmed streptococcus pyogenes. GAS diagnosis was confirmed by using vitek 2 compact.

Antimicrobial susceptibility testing :

the standard disc diffusion method on Muller – Hinton agar supplemented with5%blood was done in order to confirmed GAS isolates and Antimicrobial susceptibility . Incubated for at lest 24hours at 37℃ in air inrichment with 5-10% carbondioxide . The aproper disc with known concentration were used such as Penicillin G , Ceftriaxon , Vancomycin , Azithromycin , Erythromycin , clindamycin , Levofloxacin ,chloramphenicol , Ampicillin suleactum.

The interpretation of the results for the sensetivity testing were done depending on clinical and laboratory standard institute (CLSI).

Effect of Trans-cinnamic acid and 4-amino-2-hydroxybenzoic acid as antibacterial agent :

In our study we tested the effectiveness of some ligand (Trans- cinnamic acid and 4- Amino- 2-hydroxybenzoic acid) against the viable cells of different strains of *Streptococcus pyogenes* . The method of Agar diffusion by wells was followed to prove the inhibitory activity of ligands . In this study , four different concentrations (25mg/ml , 50 mg/ml , 75mg/ml , 100mg/ml) of each ligand were applied on five sensitive and resistance strain of *Streptococcus pyogenes* and the inhibitory activity of these ligands determined by measurement of the zone of inhibition .

DNA Extraction :

GAS isolates were inoculated on Brain heart infusion broth and incubated at 37℃ for 24 hours. After then genomic DNA extracted from a fresh brain heart infusion broth by using the instructions above .

SPY1258 gene polymerase chain reaction :

SPY1258gene were synthesized from Alpha DNA (Alpha DNA co , caued) Which were designed according to [7] Table(1) .

Table (1) : Sequence of primer sets used for PCR amplification of SPY1258 gene.

Gene	Primer name	Primer Sequence	Product length
SPY1258	SPY1258 (F)	5’ AAAGACCGCCTTAACCACCT3’	407bp
	SPY1258 (R)	5’ TGCCAAGGTAAACTTCTAAAGCA 3’	
Company		Alpha DNA / Monteral	

The PCR reaction kit (Gotaq gene master mix) was chosen from the promega and the PCR program which are used in amplification of the DNA target were included in a total volume of 25 µL in 0.5 mL eppendorf tube containing 5 µL templet DNA, 12.5 µL PCR master mix, 2.5 µL of each primer, 2.5 µL PCR water. The reaction for *eaeA* was included in a total volume of 25 µL in 0.5 mL eppendorf tube containing 2 µL templet DNA, 12.5 µL PCR master mix, 2 µL of each primer, 6.5 µL PCR water.

The PCR amplified products were detected and analyzed in 1.3gm% agarose gel electrophoresis with presence of (0.5mg/ml) ethidium bromide , and the detection of the specific band 407bp in UV transluminater.

Results

The results of our study showed that from 300 throat samples from children with pharyngitis , 43 (14.3%) samples were ß – hemolytic streptococci , 73 (24.4%) isolates of Alpha – hemolytic streptococci , 87 (29%) isolates of Staphylococcus spp. , 23(7.6%) isolates of candida and 74 (24.7%) throat swab were no growth , table (2) .

Table (2) :Number and percentage of isolated bacterial isolates .

Type of Microorganism	Number of isolate	%
ß – hemolytic streptococci	43	14.3%
α-hemolytic streptococci	73	24.4%
Staphylococcus.spp	87	29%
Candida.spp	23	7.6%
Negative throat swab	74	24.7%
Total isolates	300	100%

Out of 43 ß- hemolytic streptococci isolates , GAS were detected in 13(4.3%) . The isolates of *streptococcus pyogenes* were identified according to the phenotypic identification criteria . The streptococcus pyogenes isolates belonged to 10(3.3%) male and 3(1%) female patients .

Out of 43 ß – hemolytic streptococci there 30 isolates belonging to bacteria other than *Streptococcus pyogenes* were diagnosed , such as *streptococcus Salivarius* , *Streptococcus alactolyticus , Streptococcus agalactiae* and *Granulicatella adiacens* .

The results from susceptibility test of *streptococcus pyogenes* are presented in figure (1) , showing that chloramphenicol revealed high activity with 92% of the strain susceptible . In the present study observed the good activity of ß – lactams antibiotic against the streptococcus pyogenes with 84% and 70% of the isolates were susceptible to the Ampicillin sulbactam and Penicillin G respectively .

As for Ceftrixone, it showed greater activity than Penicillin were 76% of strain were sensetive. In this study of clindamycin and Vancomycin were also good active against *streptococcus pyogenes* with susceptibility rate 76% of isolates.

Levofloxacin also revealed good activity 69% of isolates were susceptibile.

In this present study *streptococcus pyogenes* revealed high resistance to macrolid antibiotic, the percentage of resistance for Erythromycin and Azithromycin of *streptococcus pyogenes* were 69% and 38% respectively .

The result depicted in figure (1) showed the percentage of bacterial resistant to antibiotic .

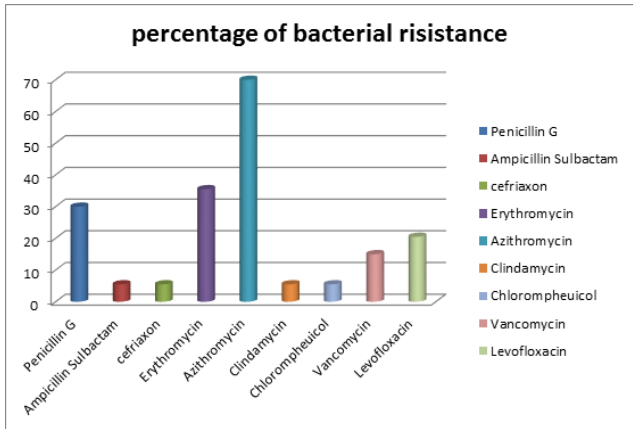

Figure (1) : percentage of bacterial resistant to Antibiotic .

The results revealed high effect of Trans – cinnamic acid and 4-amino-2-hydroxybenzoic acid. Trans-cinnamic acid showed good activity at concentration 75mg/ml and the concentration 100mg/ml . figure (2) .

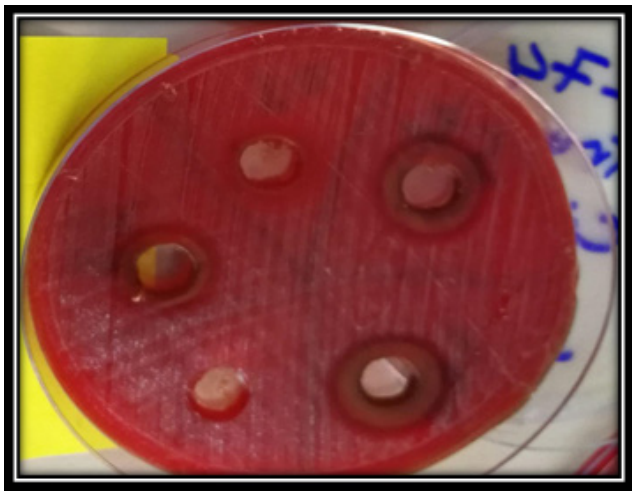

Figure (2) : The results described as high sensitive to the new ligands at concentration 75mg/ml and 100mg/ml .

While 4-amino-2-hydroxybenzoic acid revealed very good activity against *Streptococcus pyogenes* at all concentration figure (3) .

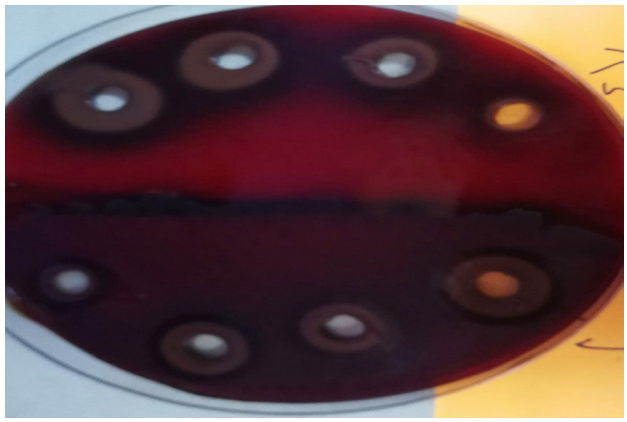

Figure (3) : Excellent activity of 4- amino -2-hydroxybenzoic acid .

The results in the current study were shown that the occurrence of *streptococcus pyogenes* 13from the total 300 isolates. In the present study, the diagnosis of *streptococcus pyogenes* isolates was confirmed by conventional PCR .

The results depicted in figure (4) reveals the presence of the diagnostic bands of the SPY1258 gene, which has a molecular weight of 407base pairs in all 13 isolate 100% , that gave positive result to vitek examination .

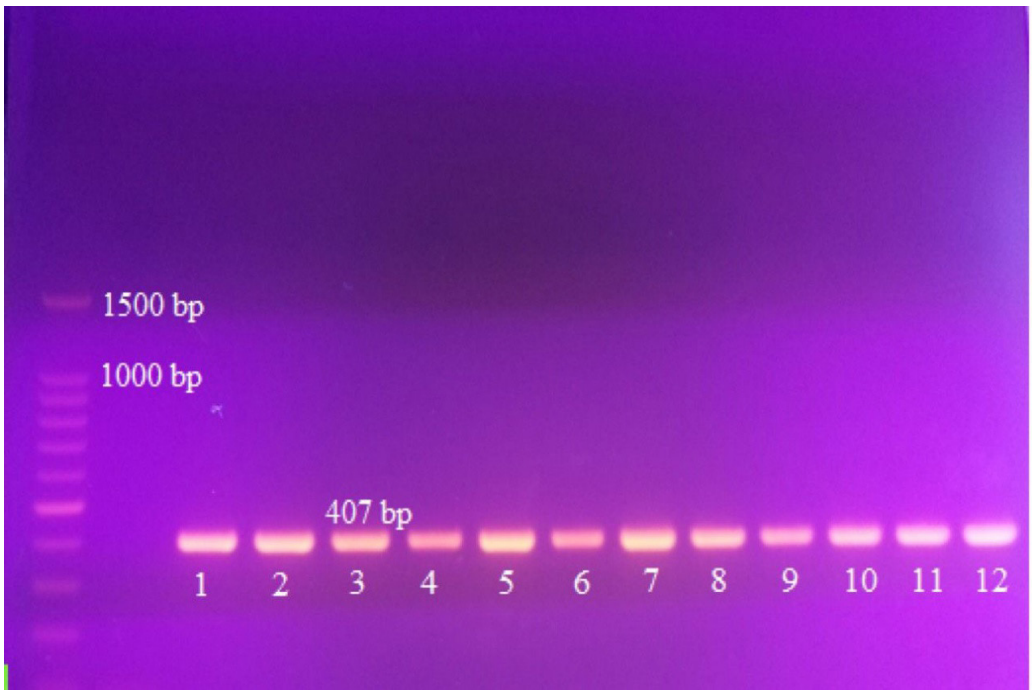

Figure (4) : Gel electrophoresis for PCR product of SPY1258 gene.

Discussion

Our study revealed that *Streptococcus pyogenes* was detected based on bacteriologicl methods (Bacitracin procedure and vitek compact 2) and identified by PCR technique. In our study observed that the SPY1258 gene was generated from whole 13 *Streptococcus pyogenes* strains only,but not from all 43 ß – hemolytic streptococci isolated, this results conferm that SPY1258gene was

definit gene only for *Streptococcus pyogenes* and can be used as amarker for its detection [9]. These results were in a greement with studies conducted by[10] this study revealed that SPY1258 gene was particular for GAS only. But not from another species of the genus *Streptococcus*.

And also a greement with results of [11] showed that the SPY1258 gene was present in all *Streptococcus pyogenes* isolates our study finding were disagreement with [9]who reported that out of 24 *Streptococcus pyogenes* isolate only 21 isolates had this gene.

This study shows that the prevalence of (3.3%)in males was higher than that recorded in females (1%).

These results agreed with that reported by Al-Gebori [12] in Iraq that indicuted male have more prevalence of infection than females . while our study results was in disagreement with [13] who reported the females were more susceptibile and response for infection with males ti ratio (3:1)the prevalence of *streptococcus pyogenes* infection in this study was (4.3%) this is in agreement with finding of [14] who reported a prevalence of 5.3% for *streptococcus pyogenes* pharyngitis . This results were approach to the ration 2.96% obtained by the researcher [15] who reported the prevalence of *streptococcus pyogenes* infection was (2.5%). The results in this study was higher than the results of (1.96%) reported by [16].

The result is lower than a percentage (15.3%) reported by [17].

The lower prevalence of GAS reported in this study, me be due to the fact that major of the pateints examined were found to be on antibiotic as most pharyngitis is viral in origin, streptococcus pyogenes pharyngitis is responsible for around 15% over all cases and 80-90% of cases are caused by viruses.

Our study finding that the percentage of resistance for the Erythromycin and Azithromycin were 69% and 38% respectively . This result agreement with findings of [18] who reported high macrolid resistance (40-70%) in Taiwan. This result is dramatically different from that has been repoeted in Saudia Arabia showed that the resistant was only 6.3% [19].

The main reason for this high rate of resistance that found in the current study, could be attributed to high misuse of Macrolid antibiotic in Iraq.

The present study revealed excellent activity of trans-cinnamic acid and 4-amino-2-hydroxybenzoic acid against *Streptococcus pyogenes* .

This result is in agreement with a work carried out by [20] Who reported that the cinnamic acid is considered and interesting for the evolution of novel antimicrobials.

This finding is in agreement with a research done by [21] who revealed that the paraaminobanzoic acid is one of a series of propargyl-linked antifolates , which is very active against GAS and dihydrofolate reductase enzyme from bacterial.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Design in line LMA-10 Photonic Crystal Fiber MZI for Detection and investigation of Beta Thalassemia

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Abstract

Consequent to the scarcity of removable rapid diagnostic examines for detecting the thalassemia, specially beta thalassemia trait or Cooly's Anemia, present detection methods include the collection of the blood, which then required being more tests before reports can be made by the treating physicians.Traditional methods like hemoglobinopathy tests and blood picture are time expending, needing specialized technical personnel, extra huge and costly laboratory instrument. Here, we simulated and demonstrated micro- hole collapsing, type of Mazh-Zehnder interferometer (MZI) that formed using LMA-!0 fiber, with a laser beam for detection the most common type of anemia, beta thalassemia trait or Cooly's Anemia .this interferometer made-up of two conventional optical fibers splicing to apart of photonic crystal fiber (PCF).this method makes a high sensitive regions for the optical characteristics in the blood sample. Biological changes in the blood sample consequent to the presence the beta thalassemia trait lead to changes optical characteristics (refractive index and absorption) of the blood sample the demonstrated laser biosensor has the ability for use as a removable, rapid diagnosis examines and the high sensitive which rises to 24.367 ABS/RIU.

Keywords: *Beta Thalassemia; Crystal Fiber MZI; LMA-10 Photonic*

Introduction

DUE TO the special characteristics of optical fibers, they are used in a wide variety of applications specially as a biosensor for high precision measurement [(1)]. These have a large role in different biological applications after production a new design of these fibers, fiber Bragg grating and photonic crystal fiber,[(2,3)]. Designing Laser biosensor needs a new modification of fiber core either using a new material to produce a fiber Bragg grating (FBG) or by using a new fiber cladding that has many holes arranged in a honey comb with either hollow or silica core [(4-7)]. Different techniques must be used for producing in line fiber sensor that are scraping ,cleaving and fusion splicing either solid or hollow cores photonic crystal fiber may be with 19 or 7 cells [(8,9,10)]. Laser biosensors have a many roles in different fields, such as immunoassays and drug detection due to their high precision and sensitivity [(11,12)]. In general, optical sensors can be distributed into four classes based on changing in light parameters such as polarization modulation intensity modulation, wavelength modulation, and phase modulation.

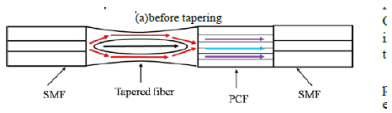
Intensity modulation was used in early optical sensor development due to its low cost, simplicity, and reliability [(13,14)]. This intensity modulated signal comes from Fiber Bragg grating as a sensing head that may be used as optical band pass or notch filters [(15,17)] .The traditional tests of beta thalassemia trait is by hemoglobinopathy analysis and complete genotype which identified the beta thalassemia trait .Beta thalassemia trait also is an important manner for decreasing the expression of HbA2 with level as low as 3.6% on high performance liquid chromatography (HPLC).This type of anemia needs regular blood transfusions and special medical care through their life. The beta thalassemia trait is the most widespread anemia in the south chines populations. This type of anemia may be not easy to do correct diagnostic who examined on HPLC [(18,19)]. In this study, we present invasive beta thalassemia trait detection biosensor using in-line PCF MZI and laser beam.This type of sensor depends on light intensity measurement through a modified optical fiber.

LASER BIOSENSOR BASED ON MICO-HOLE COLLAPSING METHODS: DESIGN AND PRINCIPLE

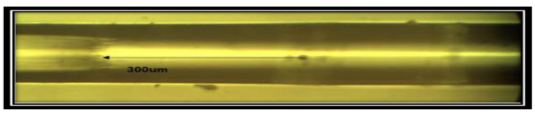
At the begging, single mode fiber (SMF-28) had been spliced with an inexpensive 1.5cm solid core photonic crystal fiber length, LMA-10, with cladding diameter: 125μm and core diameter: 7.0μm. The cross section of the PCF (LMA-10).

The splicing was done by using Fujikura (FSM-60S) splicing machine.according to trial and error. The fusion power and fusion time have been changed.

After the fusion splicing, the air holes of PCF collapsed and an elliptical air cavity was created in the tapering region. The schematic diagram and image of the microscope of the MZI are shown in Fig. 1



(a)



(b)

Fig. 1. schematic and microscope image of MZI.

As we known, the output intensity of this interferometer is given by [20]:

Where *I* represent the intensity of the interference signal, λ is the wavelength, *L* denotes the interference length, Δ*n*eff denotes the effective refractive index difference between two interference arms. The shift in the output transmission spectrum is caused due to the optical path difference Δ*n*eff *L* [14,16-19]. The MZI after tapering is based on the combination of two types of in line-fiber Mazh-Zehnder interferometer [14-16,18,19].

The interference in the air cavity. At the collapsing region, the beam of the input light is split into two beams. One of these beams transmits through the silica wall, while the other travels along the internal air cavity, and the interference obtains when two output beams recombine at the collapsing region on the other side of the air cavity. In this case, the effective refractive index difference (marked as Δ) can be given as: Δ = −, where and Refer to the effective RI of the silica wall and the air cavity, respectively.

The interference in the photonic crystal fiber. The incident beam gradually expands in the collapsing region between the air cavity and the PCF, and lots of high order modes are excited, including the cladding modes. The high order modes are recombined by the collapsing section on the second side of photonic crystal fiber and interference with the fundamental core mode. Thus, the effective refractive index difference (marked as Δ) can be expressed as: Δ = −, where and Refer to the effective RI of the core mode and the cladding mode of the photonic crystal fiber. Finally, and, are sensitive to the external refractive index while and Are not. So Δof both types of interference will change with external refractive index.

sample testing:

The Absorption Spectrum Measurement of the Blood Sample

A spectrophotometer (SP-8001) which runs in wavelength range from (190-1100) nm has been used to find the UV-Visible absorption spectrum of blood sample. Figure 6. shows the set-up of UV-Visible-IR Spectrophotometer. Sample of blood had been filled in the quartz cuvette and the absorption spectrum found .The selection of the light sources is done according to absorption spectrum have been obtained.

The Refractive Indices Measurement of the Blood Samples

The refractive indices of health and patient blood samples were measured by using Abbe refractometer.

The experimental measuring can be calculated by using the following equation:

Where is the experimental RI value measured at temperature, T.

In this study, difference blood samples lead to change the modes effective refractive index of the clad of the fiber. This change of the cladding refractive index (ncladding) due to the change in refractive index value of blood samples changes transmission intensity.

Results and Discussion

The Absorption Spectra for the Blood Sample

The absorption spectra for blood sample and

urine sample are measured by using (T60 UV-VIS) Spectrophotometer to select the suitable wavelength of laser source for the detection set-up. A figure 2.shows the absorption spectra for the blood sample.

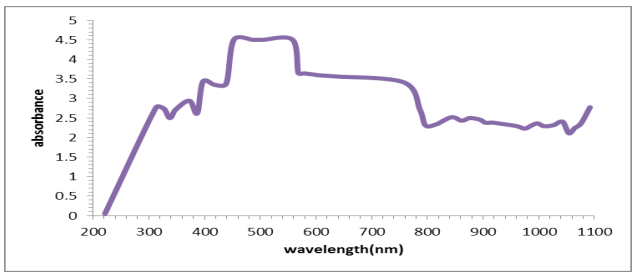


Figure 2.the maximum absorbance of the blood sample is for wavelengths in the range (470-590) nm. Thus, using green laser 532nm as laser source for laser biosensor for blood test is quite convenient.

Detection of Beta Thalassemia Trait by Using Laser Biosensor

As mention previously laser source is used (532nm wavelength, power 12.2nw) which chosen according to the blood absorption spectrum. PCF (LMA-10) with 1.5cm length is used in this experiment. The transmitted intensity of this laser without any blood sample is shown in the figure below:

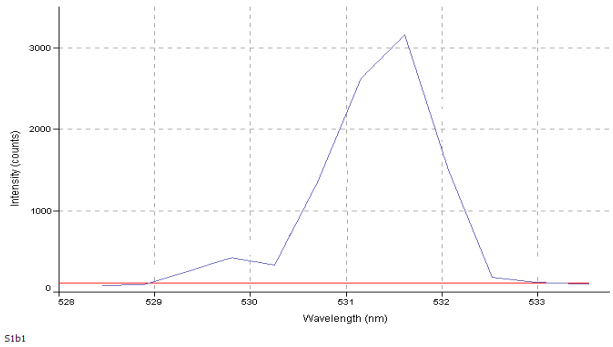


Fig. 3.The output spectrum of the green laser 532nm.

The maximum output reference intensity before immersing sensor with any blood sample was 3159. Blood samples were taken from healthy and patients with beta thalassemia trait. The patient samples were diagnostic from pathological analysis. According to the changes in the intensity modulation of the transmission laser, different blood tests are investigated. The sensor is embedded with these blood samples and the transmission spectra are shown in Fig. 4

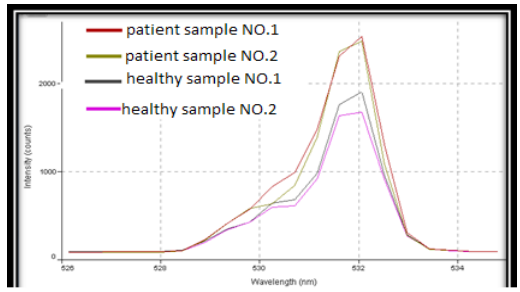


Figure (4): transmission spectra of laser biosensor for blood samples at 1.5cm LMA-10 PCF length.

The refractive indices and absorbance of each blood samples which is measured according to the reference intensity and the maximum intensity are tabulated in Table 1.

Table 1. The values of maximum intensity and absorbance for the blood samples with different Hb concentration at (1.5) cm PCF length.

Blood sample	**Refractive Index n**	**Maximum intensity I_0 (a.u)**	**Absorbance A=Log⁡〖Iref/Io〗**
Patient No.1	1.34354	2488	0.1036
Patient No.2	1.34591	2470	0.1068
Healthy person No.1	1.34893	1886	0.224
Healthy person No.2	1.35192	1619	0.2903

As a final result, the calibration curve of laser biosensor for detection beta thalassemia trait is shown in Figure below.

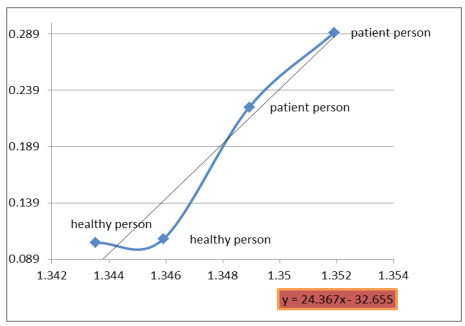


Fig. 5. The calibration curve of laser biosensor for detection beta thalassemia trait at 1.5cm LMA-10 PCF length (sensitivity = 24.367 ABS/RIU).

It's shown from Figure 5 that the presence of beta thalassemia trait leads to decreasing the refractive index and then decreasing in the absorbencies of the blood samples to (0.1036 and 0.1068) comparing with absorbencies of the blood samples of healthy people (0.224 and 0.2903).

Conclusions

According to the results found in this study, by using 1.5 cm PCF length, a fabricated laser biosensor based on MZI for detection beta thalassemia trait shows sensitivity of 24.367 ABS/RIU .the maximum absorbance of the normal blood sample is at wavelengths from (470-590) nm. The presence of beta thalassemia trait shows a decreasing in the refractive index of blood sample. Moreover, the absorbance of blood sample for healthy people showed higher values than those for the sample with anemia.

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The effect of oral magnesium supplements on lipid profile and oxidative stress in adult albino female rats

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Abstract

The present study was designed to show the effect of magnesium on lipid profile and oxidative stress. The present study used 30 adult albino female rats that distributed to following groups (each group consist 6 rats); control group received ad libidium, second group administrated magnesium (orally, 35.7mg /kg) for eight weeks, third group administrated magnesium (orally, 71.4mg /kg) for eight weeks, fourth group (pregnant rats) administrated magnesium (orally, 35.7mg /kg) for eight weeks, fifth group (pregnant rats) administrated magnesium (orally, 71.4mg /kg) for eight weeks, and then killed. The results showed high significant increased ($P < 0.05$) in levels of lipid profile (total cholesterol, total glyceride, high density lipid (HDL), low density lipid (LDL) very low density lipid (VLDL)), especially in pregnant female rats (third and fifth groups) compared with control group. On the other hand, the results showed significant changes ($P < 0.05$) in levels of malondialdehyde (MDA), *Superoxide dismutase* (*SOD*) and catalase especially in pregnant female rats (third and fifth groups) compared with control group. It was concluded that the prolong using and overdose of magnesium lead to elevated the lipid profile and oxidative stress in rats especially in pregnant female rats.

Keywords: *Magnesium; Lipid profile; Oxidative stress.*

Introduction

Magnesium (Mg2+) has several functions in the human body. It acts as a cofactor for more than 300 enzymes, regulating a number of fundamental functions such as muscle contraction, neuromuscular conduction, glycemic control, myocardial contraction, and blood pressure [1]. Intracellular Mg stores are found in high concentration in mitochondria [2], where this element plays a pivotal role in the synthesis of ATP (adenosine triphosphate) from ADP (adenosine diphosphate) and inorganic phosphate [3]. About 60% of the magnesium is present in bone, of which 30% is exchangeable and functions as a reservoir to stabilize the serum concentration. About 20% is found in skeletal muscle, 19% in other soft tissues and less than 1% in the extracellular fluid [4]. Magnesium levels in the blood are higher than 1.1 mmol in general hyperglycemia which can be clinically observable in patients with nausea, vomiting, lethargy, headaches. When magnesium reach above 3.0 mmol, it may cause acute cardiac disorders such as low blood pressure, lengthening of QRS, PR and QT intervals. Excessive hyperglycemia can lead to coma, stunting, and death by cardiac arrest [5-8]. So, the aim of study is detecting the toxicity effect of Hypermagnesemia in female adult rats.

Materials & Methods

Animal model

30 adult female rats (Rattuss norwegicus), (wt: 240-280 with age: 4-6 Mon) obtained from Veterinary college/Baghdad, and kept on standard pellet diet and water for two weeks before experiment.

Experimental design

30 adult female rats were used and distributed in five groups (six rats in each group) as following and administrated orally:

Control group received normal saline and normal diet for seven days.

Second group administrated magnesium (orally,

35.7mg /kg) for eight weeks, and then killed.

third group administrated magnesium (orally, 71.4mg /kg) for eight weeks, and then killed.

Fourth group (pregnant rats) administrated magnesium (orally, 35.7mg /kg) for eight weeks, and then killed.

Fifth group (pregnant rats) administrated magnesium (orally, 71.4mg /kg) for eight weeks, and then killed.

Prepare of blood solution

5 ml of blood collected by cardiac puncture under anesthesia and put in test tubes. Then using centrifugation 5000 cycle/min for 15 min. Sera were taken and stored by deep freezing to estimate the biochemical measurement.

Measurements

Lipid profile

Total cholesterol and triglyceride were measured by technique according to the instructions of manufacturer company kit (Randox) [9].

Oxidative Stress and Antioxidant Parameters

MDA (malonedialdehyied) was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer [10]. SOD and Catalase was measured by using the procedure of Biovision-USA kits**.**

Statistical Analysis

Data of study were analyzed by using a statistical program known as Minitab. data Means were compared using Duncan's Multiple Range test. Probability levels of more than 0.05 were regarded as statistically non-significant, whereas values less than 0.05 were considered as significant [11].

Results

Lipid profile

Lipid profile (TC, TG, HDL, LDL and VLDL) in second and fourth groups show significant changes ($P < 0.05$) compared with control group. While, Lipid profile in third and fifth groups (pregnant rats) show high significant changes ($P < 0.05$) compared with control group as shown in table (1).

Table (1): levels of lipid profile in all groups

Parameters Groups	S.Cholesterol mmol/L	S. Triglyceride mmol/L	HLDL mmol/L	LDL mmol/L	VLDL mmol/L
Control group	2.15 ± 0.34 b	1.29 ± 0.27 b	1.07 ± 0.23 b	0.82 ± 0.50 a	0.26 ± 0.05 b
Second group	2.47 ± 0.23 b	2.19 ± 0.81 b	1.20 ± 0.18 b	0.84 ± 0.18 a	0.44 ± 0.16 b
Third group	2.66 ± 0.59 b	2.09 ± 0.43 a	1.23 ± 0.19 b	1.01 ± 0.61 a	0.42 ± 0.09 a
Fourth group	3.45 ± 0.92 a	1.89 ± 0.33 a	2.19 ± 0.05 a	1.09 ± 0.77 a	0.38 ± 0.07 a
Fifth group	3.78 ± 1.01 a	2.11 ± 0.28 a	2.16 ± 0.16 a	1.40 ± 0.32 a	0.42 ± 0.06 a

Oxidative stress and antioxidants

MDA, SOD and catalase in second and fourth groups show non-significant changes ($P < 0.05$) compared with control group. Also, MDA, SOD and catalase in third and fifth groups (pregnant rats) show non-significant changes ($P < 0.05$) compared with control group as shown in table (2).

Table (2): levels of MDA, SOD and catalase in all groups

Parameters Groups	Malondialdehyde µmol/L	SOD mmol/l	S. catalase U/L
Control group	1.79 ± 0.39 a	147.40 ± 7.3 a	126.60 ± 13.28 a
Second group	2.30 ± 0.63 a	187.20 ± 55.93 a	118.00 ± 14.46 a
Third group	1.85 ± 0.35 a	194.20 ± 53.4 a	119.60 ± 14.31 a
Fourth group	2.43 ± 0.63 a	153.20 ± 29.138 a	122.60 ± 14.83 a
Fifth group	1.98 ± 0.35 a	155.80 ± 26.58 a	124.20 ± 14.70 a

Discussion

The results of present study show significant changes in lipid profiles and oxidative stress. Hypermagnesemia has often been described with the use of magnesium containing cathartics for treatment of drug overdose, in patients taking magnesium-containing cathartics and antacids for therapeutic purposes [12]. Arpaci et al referred in their study there was no correlation between magnesium level and levels of cholesterol, LDL and triglycerides [13], that is not agreement with results of the present study. On the other hand, Philips et al, referred that hypermag-nesiumia lead to necrosis and collapse of liver parenchyma, perivenular hepatocellular and canalicular cholestasis with mild macrovesicular steatosis [14], that explain the increased the levels of lipid profile in the present study. About oxidative stress in this study. The present results show non-significant changes between groups that administrated with magnesium and control group. Same findings reported by Barbagallo et al. who established a strong, direct correlation between RBC Mg levels and GSH/ GSSG concentration (circulating reduced/ oxidized glutathione) [15]. In another study, a negative correlation between Mg levels and OS stress markers (plasma superoxide anions and malondialdehyde) was observed in groups of the population chronically exposed to stress [16]. Interestingly, no correlation between Mg intake and antioxidant capacity has been found among Korean adults [17].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Evaluation of Amateur and Professional Tattoo Removal by the Q Switched Nd:YAG Laser

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Abstract

Background: A tattoo is visible and permanent pigmentation of the skin secondary to the deliberate or accidental deposition of exogenous pigment within the dermis. A variety of procedures have been used to remove tattoos, such as laser therapy, surgical excision, and dermabrasion. QS laser treatment can result in good cosmetic outcomes and complete or near-complete removal of many unwanted tattoos.

Objective: to evaluate the QS Nd:YAG laser effects for amateur and professional tattoos removal.

Materials & Methods: The study was done on 293 tattoo lesions from 176 patients (in both genders) , there were 239 amateur tattoos (136 patients) and 54 professional tattoos(40 patients). Tattoos on a wide range of body sites (limbs, feet, face, chest, shoulders and legs) were treated. Ages were between 17- 60 years. In this work, tattoos were divided to amateur and professional tattoos. A Q-switched Nd:YAG laser system was used throughout this study. All tattoos containing black and blue pigment were treated at 1064 nm. Tattoos containing red pigment were treated at 5 32nm. Treatment was scheduled at 4-6 weeks intervals and continued until maximum clearance of the tattoo was achieved, and the number of sessions varies from one to five sessions. For assessment of degree of lightening the method proposed by Lanigan was used: grade 1, complete response (> 95% lightening); grade 2, excellent response (76–95% lightening); grade 3, good response (51–75% lightening); grade 4, fair response (26–50% lightening); grade 5, poor response (0–25% lightening).

Results: Amateur tattoos were often more numerous than professional tattoos. Eighty per cent of tattoos (198 of 239) were clinically clear at the time of analysis, this included 70.7% (169 of 239) which were ≥ 95% clear. Overall 56.6% of amateur tattoos were clinically clear after two treatments, and 82% (110 of 134) were clinically clear after 2-3 treatments, while the clinical clearance rate was 40% after six treatments for professional black tattoos. It is anticipated that response rates will increase when tattoos at an early stage of treatment receive further treatments,and P value was (0.004) which mean that there is significant difference between two groups.

Conclusion: Amateur tattoos generally require fewer treatment sessions and the response is better than professional tattoos.

Keyword: *Q-switched Nd: YAG laser, Amateur tattoo, Professional tattoo.*

Introduction

Tattoo removal is an increasingly common office procedure often performed by dermatologists with special training in tattoo removal. A variety of procedures have been used to remove tattoos, such as laser therapy, surgical excision, and dermabrasion. Quality-switched (Q-switched, QS) lasers are the standard of care for tattoo removal and treatment can result in good cosmetic outcomes. A tattoo is visible and permanent pigmentation of the skin secondary to the deliberate or accidental deposition of exogenous pigment within the dermis. There are five major subtypes of tattoo [1,2]:

●**Professional tattoos** – are decorative tattoos placed by professional tattoo artists using a handheld tattoo gun that delivers uniformly deep dense dermal injections of ink. Pigment is deposited more deeply in

the dermis than most amateur tattoos, which can make professional tattoos more difficult to remove. Over time, the ink colors fade as a result of pigment migration into the deeper dermis and to regional lymph nodes via lymphatic's.

●**Amateur tattoos** – are decorative tattoos performed by nonprofessionals, and are often placed by using a handheld needles that deliver India ink or carbon injected at variable depths into the skin . Amateur tattoos are most often black and may contain ingredients such as charcoal, soot, or pen ink and are more superficially placed than professional tattoos.[2-4]

Cosmetic tattoos - are often applied freehand by cosmetologists to provide permanent makeup in areas where one would apply eyeliner, lip liner, or eyebrow pencil.

Medicinal tattoos- are small gray or blue-black markings placed by medical personnel to designate radiotherapy fields or port placement sites. Similar to amateur tattoos, they are typically composed of a sparse amount of India ink or carbon pigment.

Traumatic tattoos- result from deposits of foreign particles such as metal, glass, dirt, and carbon-containing particles into the skin following mechanical penetration, often follow blast injuries or trauma.[4,5]

Laser tattoo removal is based on the concept of selective photothermolysis. This theory, first described in the early 1980s by Anderson and Parrish, revolutionized the landscape for laser therapy by allowing for precise tissue targeting, thus ameliorating the risk of dyspigmentation and scarring associated with earlier therapies such as depigmenting agents, cryotherapy, and dermabrasion [6–10]

The longer wavelength of the Q-switched Nd:YAG laser at 1064 has proven to be more effective in removal of black tattoo, with better penetration of the dermis, less likelihood of absorption by epidermal melanin, and a reduced risk of hypopigmentation. The improved efficacy is attributed to the longer wavelength, higher fluence, and shorter pulse width, and the Nd/YAG laser, at 5 32nm, has also been used successfully on red tattoos. [4,11,12]

Patients and Method

262 consecutive tattooed patients were treated at my privet dermatology clinic, between June 2010 and December 2018. From these 262 patients, 66 were excluded because they did not come back after the first laser appointment; therefore a total of 176 patients (159 male and 17 female), and 293 tattoos was included in our study. The patients were aged 17-60 years with skin types III-IV. There were 239 amateur tattoos (136 patients) and 54 professional tattoos(40 patients). Tattoos on a wide range of body sites (limbs, feet, face, chest, shoulders and legs) were treated.

A Q-switched Nd:YAG laser system was used throughout this study. The operating characteristics of the laser were as follows: wavelength 1064nm and 532nm, with spot sizes 3mm in diameter and frequency 6 Hz. The pulse duration was ≤15 ns and the energy output 800mj and 500mj respectively. Radiation was delivered via an articulated arm and hand-piece, at a fixed energy density of l0 J/cm^2. This energy level was selected on the basis of a previous report which showed that energies of 10 or 12 J/cm^2 cleared tattoos more effectively than lower energy levels [13].

All tattoos containing black and blue pigment were treated at 1064 nm. Tattoos containing red pigment were treated at 5 32nm. Treatment was scheduled at 4-6 weeks intervals and continued until maximum clearance of the tattoo was achieved, and the number of sessions varies from one to five sessions. .

Pretreatment evaluation included a clinical description of the site, shape, color and density of each tattoo and photographs taken at each visit, progress was assessed by estimating the percentage area of the tattoo that visibly clear.

For assessment of degree of lightening the method proposed by Lanigan [14] was used: grade 1, complete response (> 95% lightening); grade 2, excellent response (76–95% lightening); grade 3, good response (51–75% lightening); grade 4, fair response (26–50% lightening); grade 5, poor response (0–25% lightening). The occurrence of adverse events, such as itch, pain, infection, hyperpigmentation and hypopigmentation, were recorded at each visit.

The Nd/YAG laser pulse, at 1064 nm produces an immediate ash white discoloration and slight elevation of the tattooed skin at the site of impact. These changes are accompanied by an audible cracking sound, a brief flash of white light at the target site, and a shock wave which is palpable in the surrounding skin. A typical

wheal and flare reaction follows and the residual erythema fades over 24 hour, and protective shielding of the operator and patient are required. Although transient hyperpigmentation is common, the normal texture of the skin and epidennal markings are retained. The 1064 nm pulse has no significant effect on normal non-tattooed skin.. The Nd/YAG laser pulse at 5 32nm produces an immediate pure white discoloration and elevation of the skin at the impact site. There is a marked reduction in the auditory component and palpable pressure wave, compared with the l064nm wavelength. Hypopigmentation occurs commonly at the treatment site, due to the absorption of the 532 nm pulse by epidermal melanocytes.

After laser treatment antibacterial cream was applied, and the patients were advised not to scrub the area, and if a scab should form, it should not be picked, scratched, or removed prematurely.

Results

The age of all patients was ranged from 17-60 years. Amateur tattoos were, in general, acquired at an older age (mean age 33.02 ± 7.9, range 17-60) than professional tattoos (mean age of 31.12 ± 7.16, range 19-45 years).

Amateur tattoos were often more numerous than professional tattoos, the patients had two or more tattoos, and one had five tattoos **Table 1**. The majority of tattoos were on the arms 127 (43.3%), forearms 91 (31%), hands 37 (12.6%), Face 17 (5.8%), chest 8 (2.7%) , shoulders 6 (2%), legs 5 (1.7%), and feet 2 (0.6), **Table 2**

Table 3 shows the current status of 239 amateur black tattoos after treatment at 1064 nm. A clinical clearance was defined as a ≥ 75% area clearance of the tattoo. Eighty per cent of tattoos (198 of 239) were clinically clear at the time of analysis. This included 70.7% (169 of 239) which were ≥ 95% clear as shown in **figure 1**. **Figure 2** shows the clearing of a typical amateur tattoo following a one session of treatment.

Table 4 shows the progressive clearing of a typical amateur tattoo following a series of treatments and the cumulative response by the number of treatments, for 136 amateur tattoo patients. The average number of treatments to achieve clinical clearance was two. The higher number of treatments associated with lower percentage clearance in this group suggests that some tattoos are relatively slow responders. Tattoos achieving less than 50% clearance had received less treatment than the other groups. Overall, 56.6% of amateur tattoos were clinically clear after two treatments, and 82% (110 of 134) were clinically clear after 2-3 treatments.

Table 4 shows the responses of 54 professional black tattoos after treatment. Seventy-four per cent (40 of 54*)* were clinically clear, including 50% (27 of 54) which were ≥ 95% clear. The progress of a professional tattoo after six treatments is shown in **table 3** and **figure 1.** The cumulative response by number of treatments for 40 professional tattoo patients is shown in **table 4**. Overall, the clinical clearance rate was 40% after six treatments for professional black tattoos**. Figure 2** shows the progressive clearing of a professional tattoo following a third session of treatment.

It is anticipated that response rates will increase when tattoos at an early stage of treatment receive further treatments and the P value was (0.004) which mean that there is significant difference between two groups.

Table (1) Details of 293 tattoos (239 amateur, 54 professional) in 167 patients.

No. of tattoos per patient	Amateur	Professional
1	64	26
2	43	11
3	19	2
4	7	-
5	1	-

No. = number

Table (2) Site involved and number of tattoos.

Site involved	Amateur	Professional	Total
Arms	101	26	127 (43.3%)
Forearms	79	12	91 (31%)
Hands	31	6	37 (12.6%)
Face	12	5	17 (5.8%)
Chest	6	2	8 (2.7%)
Shoulders	5	1	6 (2%)
Legs	3	2	5 (1.7%)
Feet	2	0	2 (0.6%)

Table (3) Current status of 239 amateur and 54 professional tattoos after laser treatment.

Area cleared	Amateur	Professional
≥ 95	168 (70.29%)	27 (50%)
75-95	29 (12.13%)	13 (24.07%)
50-75	17 (7.11%)	12 (22.22%)
25-50	12 (5.02%)	0
0-25	13 (5.43%)	2 (3.7%)

Table (4) Number of laser treatments used for different tattoo types.

No. of sessions	Amateur	Professional
2	77 (56%)	3 (7.5%)
3	33 (24.2%)	4 (10%)
4	14 (10.2%)	6 (15%)
5	2 (1.4%)	10 (25%)
≥ 6	8 (5.8%)	16 (40%)

No. = number

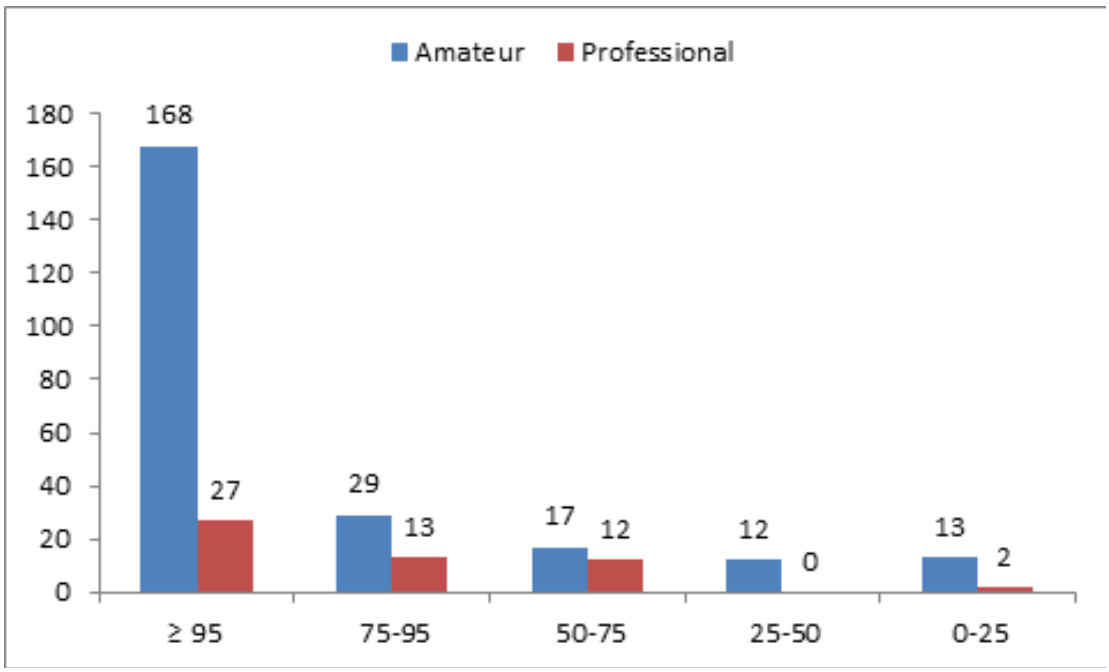

Figure (1) Area cleared of 239 amateur and 54 professional tattoos after laser treatment.

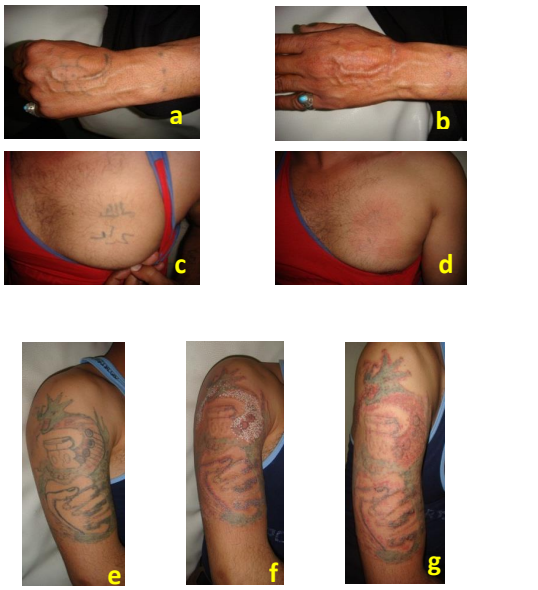

Figure 2 Amateur tattoo on the back of the hand: (a) before treatment: (b) 1 month after the first treatment. And amateur tattoo on the chest: (c) before treatment: (d) 1 month after treatment. Both Show ≥ 95% clearance of tattoo. Professional tattoo on the right arm: (e) before treatment: (f) immediately after first session: and (g) 1 month after the third treatment. Showing slight clearance of black and red tattoo and persistence of green tattoo.

Discussion

Complete laser tattoo removal requires multiple treatment sessions, typically spaced at least 4- 6 weeks apart. At each session, some but not all of the tattoo pigment particles are effectively fragmented, and the body removes the smallest fragments over the course of several weeks. The result is that the tattoo is lightened overtime. [15, 16] Immediately after laser treatment, a slightly elevated, white discoloration with or without the presence of punctuate redness is often observed. This white color change is thought to be the result of rapid, heat-formed steam or gas, causing dermal and epidermal vacuolization. Pinpoint bleeding represents vascular injury from photo acoustic waves created by the laser's interaction with tattoo pigment. [17]

We evaluated in our clinic the efficacy of a laser system providing QS lasers with the wavelengths of 1064 nm and 532 nm treat various types of tattoos. The Nd:YAG laser is an effective treatment for amateur black tattoos and has a low incidence of side effects.

We did not use topical anesthesia in our study because our patients tolerated well to procedure. The energy that selected is 600-800mj because at this range this energy is enough to cause immediate whitening without punctuate bleeding or immediate blistering to target tattoo lesions at 3 mm spot size. [4,18] And this explains no scar formation i.e. in higher energy density may cause skin damage and subsequently scaring.

Previous study regarding amateur tattoo show that the majority (60%) attain an excellent response after 1 or 2 treatments, but a minority (3.3%) require up to five treatments. Those tattoos with a < 50% response had received very few treatments. [4]

In our study also show that the majority of patients (82%) attained excellent response in 2-3 sessions, and the minority of patient (5.8%) require more than six sessions in amateur tattoo. While professional tattoo about 40% of patients need more than six sessions, the increasing response with the increasing number of treatments suggests that this group are likely to respond to further treatments.

In other previous study was showed those ten amateur tattoos and two professional tattoos. Of these, amateur tattoos showed an improvement of 69% by GAS and 58% by the PA. In contrast, professional tattoos showed a 40% lightening by GAS scoring and 35% improvement by PA. [19]

Similar finding was seen in our study, an average improvement ≥ 95% in 168(70.29%) patients with amateur tattoos, while response in professional tattoo was 50% (27 patients) .

Amateur tattoos had a quicker clearance rate than professional tattoos. Professional tattoos may require 4-6 sessions more for clearance as compared to amateur tattoos, and this goes with many previous studies. [20-22] Amateur tattoos are less dense with more superficial location of pigment, which is easier to clear. However, few amateur tattoos have deeply placed ink and may be difficult to remove.

In comparison between two group professional and amateur tattoo in response to Nd:YAG laser the P value was (0.004), which means that there is a significant difference between two groups .

Conclusion

The Q switched Nd/YAG laser is an effective treatment for both black and red tattoos with a low incidence of significant adverse effects and provides excellent cosmetic results. Amateur tattoos generally

require fewer treatment sessions and the response is better than professional tattoos.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Serum Levels of Novel Biochemical Marker (Irisin) in Relation to the Duration of Type 2 Diabetes & in Cases of Type 2 diabetes with Coronary Artery Disease in Iraqi Patients Aged (40- 60 year)

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a metabolic syndrome that affects a large proportion of the population, if not well controlled; this will lead to serious metabolic problems, including atherosclerosis, predominantly coronary artery disease (CAD).

Irisin is a peptide hormone, secreted mainly by the heart and skeletal muscle. It has a role in converting white adipose tissue to brown adipose tissue. It is one of the novel biochemical markers that link diabetes with CAD.

Objective : To explore the relationship between serum Irisin level and duration of diabetes, in cases of presence and absence of CAD, As well as the possibility of using it as a marker for the assessment of the severity of the disease.

Method: One hundred sixty-one volunteers aged [(40-60 year), body mass index (20- 25Kg/m^2)], with normal blood pressure. They divided into six groups, that distributed as [(I_a = control (negative catheterization without DM), I_b = control (apparently healthy), II_a = DM (with negative catheterization) II_b = DM (diagnosed by history and clinical examination), III_a = CAD (without DM, positive catheterization), III_b = CAD + DM (positive catheterization)]. The diabetic groups with and without CAD had been divided depending on the duration of the diabetic onset into three periods (<5, 5-10, and > 10 years). The parameters that measured were FPG, HbA1c and fasting serum (Irisin, lipid profile).

Results: The present findings showed the Means (± SD) value of Irisin levels was a significant decrease in (II_a, II_b, III_a, III_b) groups as compared with control groups (I_a, I_b). In addition, there is an inverse relationship between serum Irisin and the duration of DM in the total DM groups (II_a +II_b) and the CAD + DM group (III_b). Moreover, higher statistical decrease in mean serum level of Irisin with duration of DM was found in CAD + DM group as compared with the total DM group. Also, there was a significant decrease in mean serum level of HDL-C for (II_a, II_b, III_a, III_b) groups than in (I_a, I_b) groups. Besides, there was a significant decrease in the mean of serum HDL level in CAD groups (III_a, III_b) than in DM groups (II_a, II_b). While the means of FPG level, HbA1c, serum cholesterol level, were significantly elevated in groups (II_a, II_b, III_b) as compared with (I_a, I_b) groups. Also, there was a significant increase in the mean serum levels of triglyceride, VLDL-C and LDL-C for (II_a, II_b, III_a, III_b) groups than in the control groups.

Conclusion: Irisin was lower among patients with long-standing diabetes (with or without CAD) as compared to those with short duration of T2DM that can be included as a marker for assessment the severity of diabetes and prediction of CAD.

Keywords*: Irisin, duration of T2DM, CAD.*

Introduction

TYPE 2 Diabetes Mellitus (T2DM) is a heterogeneous syndrome, manifested by abnormalities in carbohydrate and fat metabolism. It is characterized by insulin resistance, a relative deficiency of insulin secretion and abnormal insulin action as a result; abnormal glucose homeostasis [(1)].

Abnormality of insulin secretion and insulin action at the target tissues associated with a defect in management leads to hyperglycemia. When hyperglycemia persists for prolonged periods, patients can develop various complications, including both microvascular like, nephropathy, retinopathy, and peripheral neuropathy, and macrovascular, e.g., cardiovascular disease (CVD) [(2)].

In January 2012, Bostrom and colleagues identified a new muscle tissue secreted peptide, which they named Irisin. It is a cleavage product of fibronectin type III domain-containing protein FNDC5 [(3)].

Irisin containing (112 amino acid), acts as a hormone (glycosylated protein- hormone), that is released from skeletal muscle following exercise. Irisin secreted by the response of peroxisome proliferator-activated receptor-gamma co-activator (PGC-1α) activation via training [(4)].

Irisin is mainly produced in the heart, skeletal muscle, kidney, and liver. It is essential to convert white adipose tissue to brown adipose tissue [(5)].

Irisin affect glucose homeostasis. It had also been regarded as an anti-inflammatory marker, in correlation with diabetes and insulin resistance [(6)]. Circulating Irisin is positively associated with endothelium-dependent vasodilation in diabetic patients without clinical angiopathy, indicating that low level of circulating Irisin tightly related to endothelial dysfunction and could be a marker for atherosclerosis in T2DM [(7)].

Subjects & Methods

Subjects

One hundred sixty-one voluntaries from (The specialized center for endocrinology and diabetes) and (Cardiologic clinics of Ibn- Al-Bitar hospital) were encountered in this study (September 2017 - September 2018). They were divided into six groups, table (1).

Table (1): Groups numbers and distributions

Groups	Criteria	Voluntaries NO.
I_a	Control / negative catheterization without DM	28
I_b	Control /apparently healthy	25
II_a	DM / with negative catheterization	30
II_b	DM / diagnosed by history and clinical examination	25
III_a	CAD without DM / (positive catheterization)	20
III_b	CAD + DM / (positive catheterization)	33

Method

Two methods have been used:-

Enzyme-linked immunosorbent assay (ELISA) for measuring Irisin (after three months stored in deep freezing -80^{o}C) in the central health laboratory, using the kit supplied by my biosource/USA.

Enzyme colourimetric methods for measuring FPG, HbA1c, Lipid profile, by a spectrophotometer using kits provided by Human Gesellschaft fur Biochemica and mbHMax- Planck Germany.

Besides [the electrocardiogram (ECG), ECHO, tread mill] were done for the groups (I_b, II_b) while groups (I_a, II_a, III_a, III_b) diagnosed by clinical examination of coronary computed tomography angiogram (CCTA), and they undergo catheterization (either diagnostic or therapeutic), this is according to their cardiologist decision (Regardless of search requirements).

Statistical Analysis

The statistical package for social sciences version (SPSS-23) has been used. The statistical significance of the difference in mean of a normally distributed quantitative variable was assessed by the analysis of variance (ANOVA) test, and the statistical significance of the difference in mean between all possible pair of groups was assessed by the least standard deviation (LSD) test. P-value ≤ 0.05 considered being statistically significant.

Results

There was a significant decrease in means serum levels of Irisin for overall groups when compared with that found in control groups [P-value = 0.003]. While the results showed no significant difference in mean of Irisin level [between control groups (I_a and I_b), between diabetic groups (II_a and II_b), and among groups (II_a, II_b, III_a, III_b)], table (2), figure (1).

Table (2): The difference in means of serum Irisin

Parameter Groups	Serum Irisin (ng/ml) (mean ± SD)
I_a	**A** 29.341± 16.213
I_b	A 30.149 ± 16.032
II_a	**B** 9.014 ± 2.009
II_b	**B** 9.675 ± 1.931
III_a	**B** 5.777 ± 0.450
III_b	**B** 3.728 ± 1.283
LSD	7.427
P- value	Sig. 0.003
The Letters (A, B, C) are significant at P ≤ 0.05 (comparison among groups)	

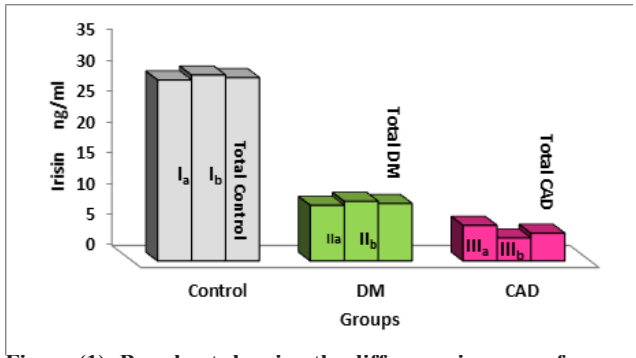

Figure (1): Bar chart showing the difference in mean of serum Irisin

There was an inverse relationship between serum Irisin level and the duration of incidence of T2DM in the [total DM groups (II_a + II_b) and CAD + DM group (III_b], table (3) figure (2).

In the total DM group, the mean serum level of Irisin was [(12.347±0.712ng/ml), (10.922±1.316 ng/ml) and (8.295±1.259ng / ml) when DM duration [(<5y), (5-10y) and (>10y), respectively], There were significantly decreased in mean of serum Irisin levels when the duration of DM was increased (LSD =0.954) [P- value (0.0018)].

In the (III_b) group, the mean serum level of Irisin was [(4.9527±0.3178 ng/ml), (4.0562±0.5580 ng / ml) and (2.4649±0.9561ng / ml)] when DM duration [(<5y), (5-10y) and (>10y), respectively], The results show significant difference in mean of serum Irisin level between duration (<5y, 5-10y) compared (>10y) (LSD >1.07). While there was no significant difference in mean of serum Irisin level between duration (<5y) and (5-10y). (LSD < 1.07), [P- value (0.0026)].

The highest statistically difference in mean serum levels of Irisin with a duration of DM was found in (III_b) group as compared with (total DM) groups, [P- value (0.00031, 0.00046, 0.00053)] for duration [(<5y), (5-10y) and (>10y), respectively], as shown in table (3), figure (2).

Table (3): The difference in mean between serum levels of Irisin and the duration of T2DM in Total DM group & in CAD + DM group

Group Duration (year)	Total DM (IIa + IIb) (mean ± SD)	CAD+DM (IIIb) (mean ± SD)	LSD	P-value
<5	A,a 12.347±0.712	A,b 4.9527±0.3178	3.281	0.00031
5-10	B,a 10.922±1.316	A,b 4.0562±0.5580	2.185	0.00046
>10	C,a 8.295±1.259	B,b 2.4649±0.9561	2.966	0.00053
LSD	0.954	1.07	0.0026	
	P-value	0.0018		

The Letters (A, B, C) are significant at P ≤ 0.05 (comparison in the same group)
The Letters (a, b) are significant at P ≤ 0.05 (comparison between Total DM group and CAD + DM group)

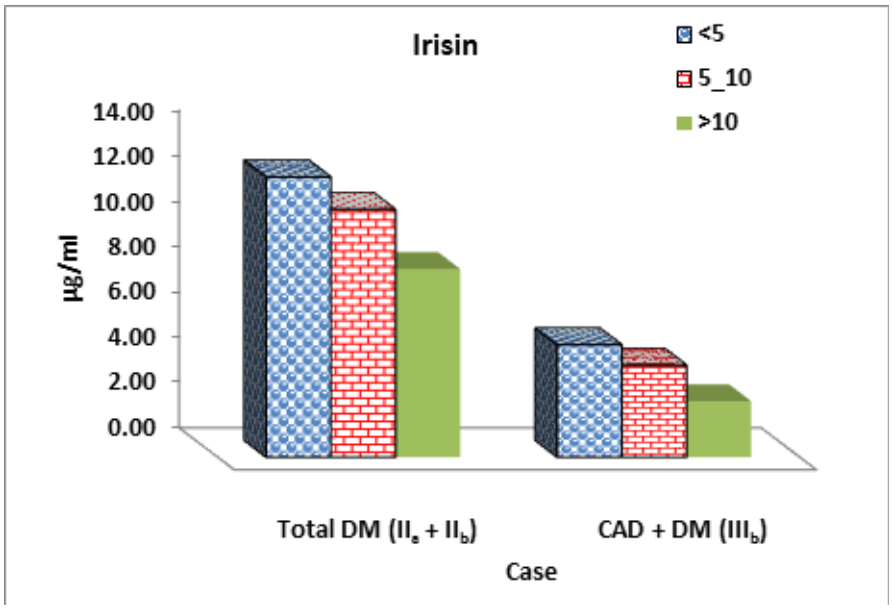

Figure (2): Bar chart showing the difference in means between serum Irisin level and duration of T2DM in total DM and in CAD + DM group

There was a significant increase in means of FPG and HbA1c for overall groups when compared with that found in control groups except in (III_a group), it was no significant difference. The results showed no significant difference in mean of FPG and HbA1c levels [between control groups (I_a and I_b), between diabetic groups (II_a and II_b), and among groups (II_a, II_b, III_b)], table (4).

Table (4): The mean serum level of fasting plasma glucose and HbA1c

Parameters Groups	FPG (mean ± SD)	HbA1c (mean ± SD)
I_a	**A** 94.68 ± 6.99	**A** 5.146 ± 0.453
I_b	**A** 95.08 ± 6.37	**A** 5.392 ± 0.526
II_a	**B** 215.13 ± 79.85	**B** 8.497 ± 2.015
II_b	**B** 214.24 ± 85.59	**B** 8.360 ± 2.324
III_a	**A** 95.10 ± 5.73	**A** 5.125 ± 0.343
III_b	**B** 229.76 ± 99.44	**B** 8.615 ± 1.528
LSD	52.649	1.170
P- value	Sig. 0.00027	Sig.0.00014
The Letters (A, B, C) are significant at P ≤ 0.05 (comparison among groups)		

There was a significant increase in means of serum cholesterol and triglyceride for overall groups when compared with that found in control groups except in (III_a group), it was no significant difference. The results showed no significant difference in mean of cholesterol and triglyceride levels [between control groups (I_a and I_b), between diabetic groups (II_a and II_b), and among groups (II_a, II_b, III_b)].

There were a significant decrease in means serum levels of HDL-C for overall groups when compared with that found in control groups. While there was a significant increase in means of serum LDL-C and VLDL-C for overall groups when compared with that found in control groups. The results showed no significant difference in mean of HDL-C, LDL-C and VLDL-C levels [between control groups (I_a and I_b), between diabetic groups (II_a and II_b), and among groups (II_a, II_b, III_b)], table (5).

Table (5): The mean serum level of lipid profile

Parameters Groups	**Cholesterol (mg/dl))mean ± SD))**	**TG (mg/dl) (mean ± SD)**	**HDL-C (mg/dl) (mean ± SD)**	**LDL-C (mg/dl) (mean ± SD)**	**VLDL-C (mg/dl) (mean ± SD)**
Ia	A 99.07 ± 23.37	A 151.93 ± 29.13	A 46.143 ± 6.559	A 85.93 ± 32.42	A 19.857 ± 4.625
Ib	A 94.16 ± 35.60	A 152.72 ± 28.98	A 46.920 ± 12.926	A 85.68 ± 26.08	A 18.880 ± 7.114
IIa	B 173.20 ± 28.54	B 210.70 ± 36.07	B 35.667 ± 9.319	B 141.10 ± 41.23	B 34.667 ± 5.809
IIb	B 174.16 ± 66.11	B 212.96 ± 48.19	B 37.680 ± 9.728	B 136.92 ± 45.42	B 34.920 ± 13.260
IIIa	A 128.95 ± 29.68	B 237.20 ± 35.96	C 28.200 ± 9.950	C 182.90 ± 39.81	C 25.750 ± 5.946

***Cont...* Table (5): The mean serum level of lipid profile**

IIIb	B 168.61 ± 66.73	B 238.64 ± 46.73	C 22.606 ± 6.113	C 179.12 ± 55.57	B 33.758 ± 13.339
LSD	36.840	30.683	7.289	33.328	7.380
P- value	Sig.0.00011	Sig.0.00011	Sig. 0.00009	Sig. 0.00017	Sig. 0.00024
The Letters (A, B, C) are significant at P ≤ 0.05 (comparison among groups)					

Discussion

There was a significant decrease in mean serum level of Irisin for (II_a, II_b, III_a, III_b) as compared with that found in (I_a, I_b) groups. Also, when compared the serum levels of Irisin between DM subgroups with and without CAD showed that Irisin decreased when CAD existed but did not reach the significant level (maybe due to small sample size) as shown in the table (2), figure (1).

A study performed by Zhang *et al*. supports the current study the authors found a significant decrease in serum Irisin in T2DM, which further confirmed the potential role of Irisin in glucose metabolism and diabetes. Additionally, when compared the serum levels of Irisin between diabetic patients with and without macrovascular disease, they had found that Irisin significantly decreased when macrovascular disease existed. So they suggested that Irisin would be a potential target for monitoring and intervention of T2DM and its associated vascular complications such as CAD [(8)]. Besides, the founding of the current study resembles those found by El-Lebedy *et al*., who reported that serum Irisin was significantly lower in diabetic patients and CVD as compared to the control group. Also, Deng reported that serum Irisin level was lower significantly in patients with CAD as compared with healthy controls [(9)].

The above results were in agreement with more recent results, by Khorasani *et al*., who found that serum Irisin level was lower in diabetic patients with cardiovascular complication compared with uncomplicated diabetic patients [(10)].

Studies that have been done to compare the levels of circulating Irisin with the healthy control group have shown a protective effect of Irisin against the development of CVD. Several potential mechanisms have been proposed for this issue. Irisin plays a vital role in the preservation of endothelial cell function and reduces endothelial damage by inhibiting inflammation, and oxidative stress, so the low levels of Irisin affect the endothelial function and increase the incidence of atherosclerosis. Besides lower circulating, Irisin levels can increase the accumulation of advanced glycation end-products (one of the causes of vascular complications in diabetic patients) [(11)].

The data and the results in this research did not show any significant difference between the two DM groups (II_a, II_b) for all measured parameters in this study. Therefore, they can be merged and deal with as a single group (total DM) as shown in table (3), figure (2).

The present study showed a significant inverse relationship between serum Irisin and the duration of incidence of T2DM in the [total DM group (II_a + II_b)] and the [CAD+DM group(III_b)], Irsin decreases significantly as the period of diabetes increases as shown in table (3), figure (2).

These results resemble the finding of Liu *et al*., who pointed out that long-term diabetes is associated with a significant reduction in levels of Irisin [(12)]. Also, the results confirmed those found by two previous studies in which there was a negative correlation between duration of diabetes and Irisin level [(13)].

Conclusion

The serum level of Irisin is affected inversely by the persistence of diabetes with or without coronary artery disease for an extended period, and the level of decline is significant decrease with an increase in the period of diabetes. Therefore it can be used as a prognostic marker for estimation of the severity of the T2DM.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and

scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Study the Effect of Temperature Variable on the Patient with Jaundice Stay Period inside the Incubator

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Abstract

This study is used for measuring the temperature of incubator during its used in the treatment of newborns who suffering from jaundice. The present system is characterized as low cost, easy to use and does not require a highly skilled operator, in which that used of DHT22 sensor based on the Arduino hardware platform. This system facilitates the continuous monitoring and the true knowledge of the temperature of the incubator for determination the period of stay of the child with jaundice in the hospital. In addition, the current system represents not only the possibility of knowing the temperature of the incubator but also a tool to assess the incubator's performance, and find out the percentage of error in the temperature and the difference between the required temperature and the real temperature.

Keywords: *Jaundice ; Patient ; physical parameters;*

Introduction

Newborns are at raised risk of jaundice, a condition during which excess bilirubin accumulates in the blood. Left untreated, jaundice will result in neurological impairment and death. Jaundice ensuing from unconjugated hyperbilirubinemia is well treated with exposure to blue light[1].

Immediately once giving birth, the child is transferred to a new and completely different surroundings for the mother's womb environment. This setting is certainly not protected and isn't appropriate initially for the child's life because of the changing factors surrounding the newborn particularly the temperature. The premature newborn is homoeothermic, however over a long amount, he cannot maintain the thermal processes. The energy he provides is employed within the following order of priority: for the functioning of important organs, for thermoregulation and growth[2]. Thus it should give a healthful hydrothermal environment to decrease the risk of body hypothermia or hyperthermia for newborn infant[3].

The process of measurement the temperature of the therapeutic environment and so understand the perfect conditions for the treatment process is one among the most necessary things that should be of high interest, because it's an excellent link between the conditions and factors related to the treatment method, and therefore facilitate the study of the impact of temperature alone or combined with different factors in effecting on diagnosing and treatment method.

The Arduino system is considered one among the simplest and most versatile electronic solutions in terms of easy use and speed of learning still as low price of producing components as well as simple to connect and programming by computers, additionally the availability of those electronic components in the local markets, permitting the research worker to work in a very wide space of ideas to dynamical developing and therefore let to get output a lot of comprehensive solutions and optimality.

The main goal of this study is to manufacture an easy system which will provides a precise perception of measurement the temperature within the incubator and therefore facilitate to calibrate and determine the appropriate degree of treatment.

Theory

Neonatal jaundice is one among the foremost

common conditions needing medical attention in newborn babies. About 60% of term and 80% of preterm babies develop jaundice within the 1st week of life, and concerning 10% of breastfed babies are still jaundiced at age one month. Neonatal jaundice is usually harmless, however high concentrations of unconjugated bilirubin could occasionally cause kernicterus (permanent brain damage)[4].

Neonatal jaundice is common, as a result of physiological jaundice or breastfeeding. In some neonates, unconjugated bilirubin concentration, in addition to different risk factors, is sufficient to permit free bilirubin to cross the blood-brain barrier and cause kernicterus. Another subgroup of infants is jaundiced as a result of elevated conjugated bilirubin; a marker for several pathological conditions. Bilirubin measuring should determine those infants in danger[5].

Bilirubin is produced from the catabolism of heme within the reticuloendothelial system as shown in Fig. (1). This unconjugated bilirubin is released into the circulation wherever it's reversible however tightly bound to albumin. When the bilirubin-albumin complicated reaches the liver cell, it's transported into the hepatocyte wherever it combines enzymatically with glucuronic acid, manufacturing bilirubin mono- and diglucuronides. The unconjugated bilirubin is reabsorbed into the bloodstream by way of the enterohepatic circulation, adding bilirubin load to the already overstressed liver. This enterohepatic circulation of bilirubin is a crucial contributor to neonatal jaundice[6].

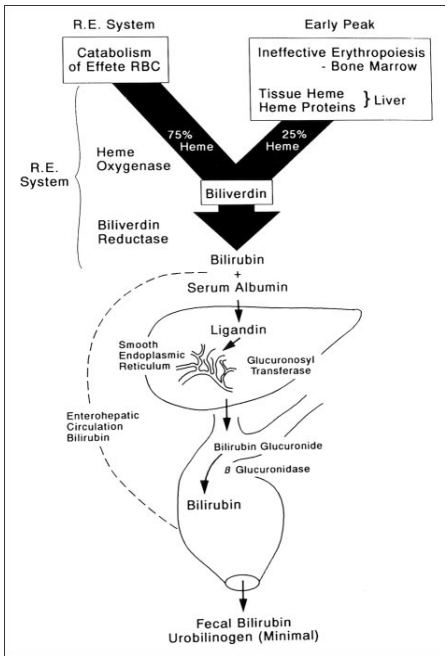


Fig. (1) Bilirubin Cycle.

Materials and Method

The hardware and software are the main components of this technique, the hardware consists of two elements that are the AM2301 thermal sensing element and also the Arduino Uno platform.

The incubator was in the hospital which has its heat supply, through this sensing circuit the temperature of incubator is measured during treated the newborn with jaundice.

DHT21

The AM2301 thermal sensing element, that sensing digital temperature which contains the compound that has been calibrated the digital signal output of the temperature sensor. Application of a dedicated digital modules collection technology and also the temperature sensing technology, to make sure that the merchandise has high reliability and wonderful long-term stability. The sensing element includes a capacitive sensing element wet components and a high-precision temperature measuring devices and connected with a high-performance 8-bit microcontroller. The merchandise has wonderful quality, quick response, sturdy anti-jamming capability, and low price. The shape of procedures, the standardization coefficients hold on within the microcontroller, the sensor among the process of the heartbeat to call these calibration coefficients. Standard single-bus interface, system integration fast and simple. Small size, low power consumption, signal transmission distance up to twenty meters, creating it the most effective selection of all types of applications and even the most demanding applications. Product for the 3-lead (single-bus interface) connection convenience. Special packages in keeping with user wants[7]. Fig. (2) Illustrates the DHT21

sensor.

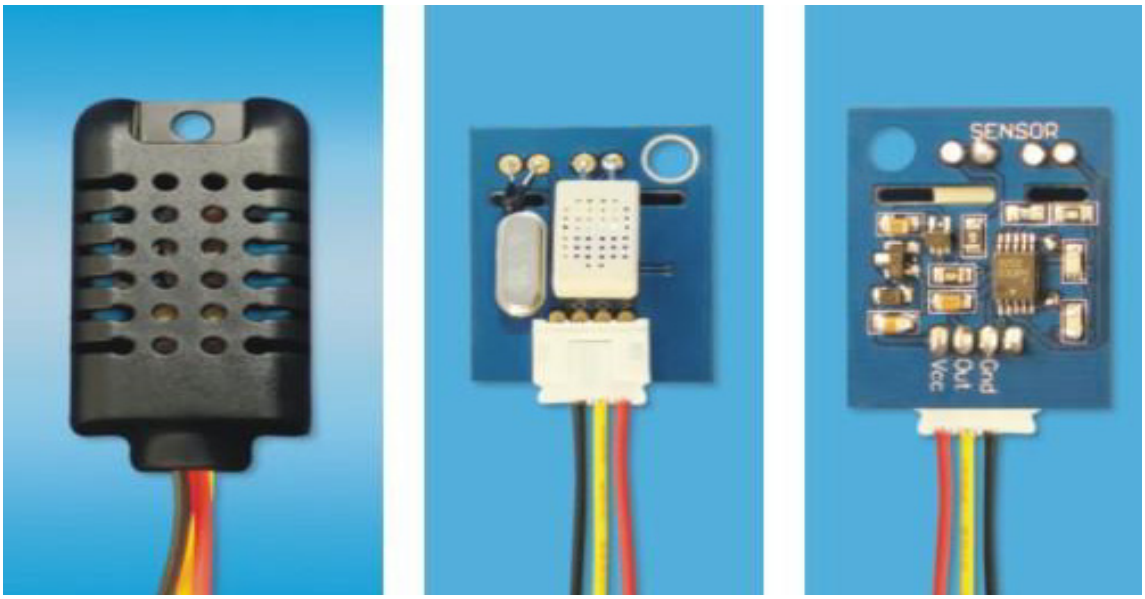


Fig. (2) The configuration of DHT21 sensor.

ARDUINO UNO

The Arduino Uno has shown in fig. (3) utilized in this study, that is a microcontroller board grounded on the ATmega328 (datasheet) was chosen as the microcontroller for this study. This was a perfect choice, as the processor is extraordinarily strong and cost-effective. It contains of 14 digital input/output pins (out of that 6 is utilized as PWM outputs), six analog inputs, a 16 MHz ceramic resonator, facilitation for USB connectivity, a power jack, an ICSP header, and a button. Its styles comprise of help that supports the microcontroller in each possible way. To induce to figure with it one has to merely connect it to a computer with a USB cable or power it with an AC-to-DC adapter or battery[8].

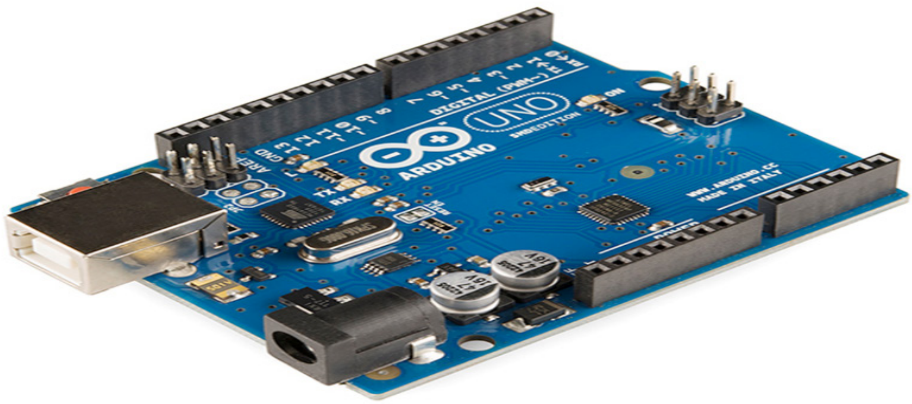


Fig. (3) The Arduino (Uno) type.

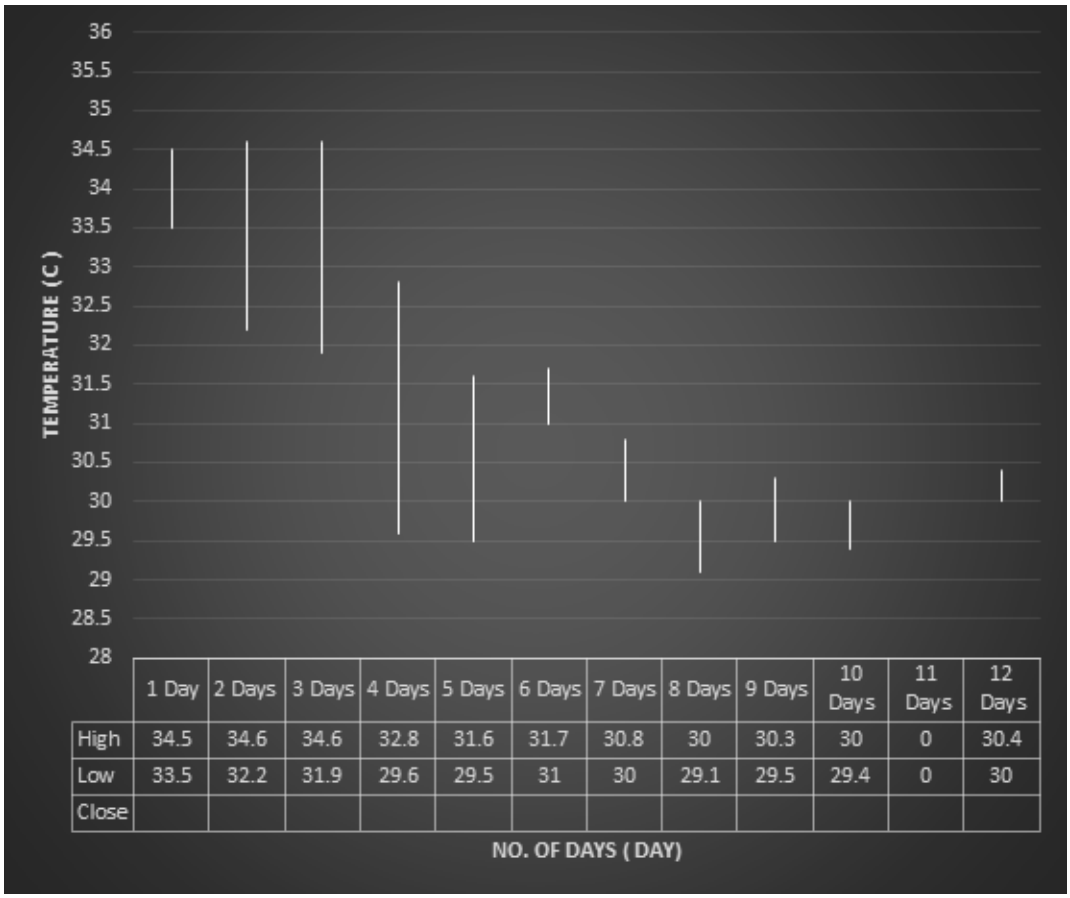
A systematic strategy was used for the design of this study, during which the obtained temperature of the incubator was to be detected for 135 patients with jaundice.

So, the information collected from the external sources that are the incubator were obtained employing a sensor. The strategy of management used is a microcontroller, that reads the results obtained from the sensing element to be then mentioned and show the impact on the speed of the healing process for a patient with jaundice.

Results

The measurement system that used in this study enables a real perception of temperature in the incubator environment during the presence of the newborn with jaundice, thus the results obtained represent exactly the conditions experienced by the newborn with jaundice during the treatment, which directly affect the speed of treatment.

To check the real readings of the proposed approach, the temperature of 135 patients with jaundice was compared to the time spent in the hospital is shown in table (1), and also see fig. (4) Which represent the relationship between the time which the child spent at the hospital and the incubator temperature range (° C) for all patients.



	1 Day	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days	8 Days	9 Days	10 Days	11 Days	12 Days
High	34.5	34.6	34.6	32.8	31.6	31.7	30.8	30	30.3	30	0	30.4
Low	33.5	32.2	31.9	29.6	29.5	31	30	29.1	29.5	29.4	0	30
Close												

Table (1) Illustrates the relationship between incubator temperature ranges with staying period in the hospital.

NO.	Period of staying in hospital	Incubator temperature range (
1	1	33.5-34.5
2	2	32.2-34.6
3	3	31.9-34.6
4	4	29.6-32.8
5	5	29.5-31.6
6	6	31-31.7
7	7	30-30.8
8	8	29.1-30
9	9	29.5-30.3
10	10	29.4-30
11	11	-
12	12	30-30.4

Fig. (4) The relationship between the period of staying in hospital and corresponding temperature ranges.

Discussion

The incubator is considered an artificial womb and

will have the most vital effect of protecting newborn. One of the most important duties of the incubator is to control the temperature of the newborn to achieve thermal neutrality, which is achieved by control temperature stability in the incubator. However, this study shows that there is a large swing in the temperature from one to another, although the fixed value is the same, and that is due to the difference in efficiency of each incubator parts because of its operational life and its ability to work such as heat source and heat radiator and efficiency of thermal insulation of the incubator walls, In addition to the external effect of room temperature at the temperature of the incubator.

This work opens the way for comprehensive study on the conditions affecting the temperature in the incubator to design an ideal incubator that provides ideal conditions that will help to actually reduce the treatment period for patients with jaundice.

Conclusions

The concluding observations of this study explain the effect of the temperature which is the most popular variable affected on the patient stay period in hospital.

It was clearly found that the performances of the fixed environment factors including temperature, humidity and other factors in the mother's womb will disappear immediately after birth. This situation places the newborn in a state of sudden environmental change which requires a necessary and quick adaptation. The child who cannot cope with the new environment and if the mother is different in the blood group and RH factor in addition to children who born before 37 weeks (pre-term) the baby will suffer from jaundice disease.

It is obvious that the changes in baby temperature from the mother's womb temperature is considered as one of the conditions causing or helping diseases and therefore difficult to cure.

It is clear that the patient stay period of the children with jaundice inside the incubator was closely related to the incubator's high temperature, for example the incubator temperature of the children who had been cured in a short period varied between 1 and 3 days were at its lowest rates of 31.9 ° C and at its highest rates of 34.6 ° C.

It was found that the speed of recovery for children with jaundice which the temperature in its incubator environment is higher than those no closer to the temperature of the mother's womb. This is due to the thermal regulation of the newborn inside the incubator is closer to the degree of the mother womb.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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The Role of Global Longitudinal Strain in Early Detection of Doxorubicin Induced Cardiotoxicity in Breast Cancer Patients

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Abstract

Objective : Cardiotoxicity is a recognized side effect of many antineoplastic drugs, particularly anthracyclines which used for breast cancer treatment. The identification of patients at high risk for developing cardiotoxicity would be a good strategy to decrease mortality. the aims was to assess the incidence of chemotherapy induce cardiotoxicity and identifying the reduction in global longitudinal strain in various treatment cycles for the early detection of cardiotoxicity, prior to ejection fraction reduction.

Method: A prospective cohort study of confirmed and newly diagnosed breast cancer patients, who attend the Babylon oncology center in Marjan medical city between the fifth of January and the first of July of 2019.

Results: The mean patient's age was 51.3(±10.5) years, There is no association difference in systolic function during the follow up period. The reduction in global longitudinal strain by 8%-15% from baseline was found in 8 patients, while reduction ≥15% from baseline was found in only 2 patients during the follow up period.

Conclusions: Global longitudinal strain drop was observed three weeks after chemotherapy onward without significant reduction in ejection fraction.

Keywords: *breast cancer; Doxorubicin; longitudinal strain*

Introduction

Breast cancer is the high frequent cancer among women, effecting 2.1 million women yearly, it also causes the highest number of cancer-related deaths among women(1). As result of that the breast cancer is presently a important public health and economical problem and many research on new therapies and monitoring their safety use, should be a priority (2). Cancer therapy has studied in a recent years and it has been proved to risen average of cure in breast cancer, as well as to decrease the recurrences.

However the cardiac toxicity from antineoplastic therapy has become a notable risk of morbidity and mortality in cancer pateints. In patients who develop heart failure from chemotherapy, the mortality rate is increase to 60% by 2 years (3) . Cardiotoxicity may appear early or late in the course of the disease, and have different clinical feature from subclinical myocardial dysfunction to irreversible heart failure or even death(4). The cardiotoxicity refers to a direct influence of the chemotherapy on the entire CVS and also to an indirect effect by a thrombogenic status or to a hemodynamic flow alteration(5). In 2016, the European Society of Cardiology has published a position paper recommending the diagnosis of cardiotoxicity occur when the ejection fraction (EF) reduction >10% for values below normality (53%)(6).

Early detection of cardiotoxicity has frequently depended upon many cardiac imaging to identify a reduction in LV function without signs or symptoms of heart failure (stage B heart failare)(7). The use of ejection fraction has important limitations.

The measurement of left ventricular ejection fraction is subject to technique-related variability, which can be higher than the thresholds used to define cardiotoxicity (8).

The decreasing in left ventricular ejection fraction is often a late phenomenon, with failure to recover systolic function in up to 58% of patients despite intervention (9, 10).

The common measurements allow only the late diagnosis of cardiac dysfunction, which might be mostly irreversible. Therefore, there is major need for other precise and reproducible parameters, able to diagnose the early, subclinical, LV dysfunction and can detect the patients at risk for rapid progression toward irreversible cardiac failure and who can benefit from early therapeutic treatment [(11)]. The using of tissue Doppler-based strain imaging and GLS has most constantly determined early myocardial changes during therapy, whereas STE in form of GLS appears to be the best measure[(12)].

Global longitudinal strain (GLS) is used in clinical practice aimed at the early detection of changes in myocardial contractile function However, neither GLS use nor its cutoff value determined to predict cardiotoxicity have been standardized [(13)] .

The American Society of Echocardiography and the European Association of Cardiovascular Imaging have pointed that deformity changes prior ventricular dysfunction. A reduction > 15% in GLS, immediately after or during anthracycline treatment, was the most informative parameter to predict cardiotoxicity, while a reduction < 8% might exclude its diagnosis.However, there is a grey zone between those two values [(14)].

Doxorubicin is an anthracycline widely used in many cytostatic treatment regimens, but has limited applicability due to cardiotoxic effect. This cardiotoxicity increases patient morbidity and mortality. The mechanisms of cardiotoxicity induced by anthracyclines include the formation of oxygen free radicals, destruction directly to the DNA, apoptosis, activation of immunological reactions in the myocardium, changes in calcium ions transport, histamine release, and activation of coagulation with the formation of thrombus in coronary arteries [(15)].Doxorubicin-induced cardiotoxicity may be augmented by increased cumulative dose, association with other cytotoxic drugs, and patients' advanced age [(16)]. In spite of this toxicity, anthracyclines stay the cornerstone of treatment in a lot type of malignancies, including lymphomas, leukemias, and sarcomas, and are still widely used in both advanced and early-stage breast cancer [(17)]. This study was palliated the early global longitudinal strain changes in breast cancer patients.

Patients and Method

This is a prospective cohort study included a convenient patients conducted in the fifth of January and the first of July of 2019 and who came to Babylon oncology center were asked to participate in this study. Sample size of 40 patients was included. The patients with Age ≥ 18 years, newly diagnosis of breast cancer with neither antineoplastic treatment nor radiotherapy, Normal baseline echocardiography study and Antineoplastic treatment planning with doxorubicin. Exclusion patients with Lacking of accurately assessing GLS because of an inappropriate acoustic window, cardiac arrhythmias and/or non-sinus rhythms, Usage of beta-blockers and/or angiotensin-converting-enzyme inhibitors and/or angiotensin receptor blockers, Moderate or severe heart valve disease or Ischemic heart disease and Prior history of treatment with cardio toxic drug and thoracic radiotherapy

The baseline information was recorded in form of patient's age, medical history of DM, HT and dyslipidemia, smoking history, oncological history (side and type of cancer), weight and height was measured by researcher.

Heart rate was measured manually and blood pressure was measured by sphygmomanometer then patient underwent Doppler echocardiogram at the baseline, before initiating the doxorubicin (60 mg/m2 IV infusion), three weeks after the first dose and three weeks after the second dose (according to the protocol of Breast cancer chemotherapy). Data was analyzed using SPSS version 23 and used ANOVA test used to find association between variables. Echocardiographic examination was performed with the patient at rest in the left lateral position, using the Vivid E9-GE device (GE, Vingmed Ultrassound Horten, Norway), LCD 17" monitor, with image acquisition with a M5Sc transducer and harmonic imaging. All tests were performed with the same device. Sector and depth were adjusted to optimize the image. The measurements and image acquisition followed the recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging

The following echocardiographic variables were assessed: EF, calculated by using of the biplane Simpson's method, considering the normal value of EF > 54% for the female sex[(18)].

According to the current recommendations ;diastolic function, evaluated by use of mitral flow with anterograde values of E wave and A wave, tissue Doppler of lateral mitral annulus, measures of S' wave (systolic velocity of the mitral ring) and ; indexed left

atrial volume (mL/m^2); tricuspid annular plane systolic excursion (TAPSE).

The GLS was acquired by use of Automated Functional Imaging (AFI) of three clips with images of the left ventricle on three apical views, so that all myocardial segments could be well visualized: 4-chamber, 2-chamber and 3-chamber views. The events of aortic valve opening and closure were marked. The images were acquired at a frame rate of 40-90 fps (> 70% of heart rate).

Results

40 patients was included. No loss to follow up, the baseline information was showed in table -1-.

Table -1- Patients baseline characteristics.

Variable	Mean(±SD)	Maximum	Minimum
Age in years	51.3(±10.5)	76	32
BMI in Kg/m2	20.5(±4.1)	33.6	11.9
BSA in m2	1.7(±0.1)	1.9	1.4
HR in b/m	78(±7.3)	95	64
SBP in mm Hg	13.4(±0.7)	140	120
DBP in mm Hg	83.1(±7.3)	95	60

History of diabetic was found in 12.5% of patients, 10%had a history of Dyslipidemia, 70% of patients had left breast cancer. Most of patients had invasive ductal type of breast cancer (82.5%), table -2-.

Table -2- Patient medical history.

Variable		NO.	Percentage
History of DM	Yes	5	12.5%
	No	35	87.5%
History of Dyslipidemia	Yes	4	10%
	No	36	90%
Laterality	Right	12	30%
	Left	28	70%
Type of cancer	Invasive ductal	33	82.5%
	Lobular	5	12.5
	Others	2	5%

The baseline ejection fraction reading was 66.2% (±5.9) and there was no significant difference in mean between the three readings (p value >0.05). Also for LAVI , TDI, TAPSE and GLS there were no significant difference in mean between the three readings (p value >0.05), table -3-.

Table -3- Comparison of echo parameters changes during follow up period.

Variable	Mean ±SD			P value
	Reading at base line	Reading after 3 week from first dose	Reading after 3 week from second dose	
EF%	66.2%(±5.9)	66.8%(±5.5)	67.5%(±5)	0.58
LAVI	26(±3.8)	26.7(±3.9)	26.4(±3.9)	0.72
TDI(s-)	8.9(±1.3)	9.1(±1.2)	9(±1.1)	0.84
TAPSE in mm	22.1(±2.6)	22.9(±2.4)	22.5(±2.5)	0.39
GLS%	-19.3(±2.4)	-19.8(±2.3)	-19.4(±2.2)	0.59

ANOVA test.

Regard GLS, the reduction in GLS after 3 week from the first dose by 8%-15% from baseline was found in 6(15%) patients, while reduction ≥15% from the baseline GLS was found in 2(5%) patients only figure -1-.

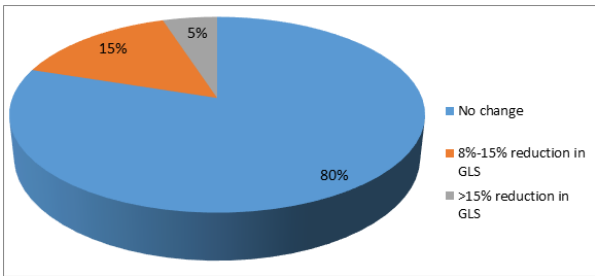

Figure -1- PIE chart for the percentage of reduction in GLS after 3 weeks from the first dose.

The reduction in GLS after 3 week from the second dose by 8%-15% from baseline was found in 2 additional patients , while reduction ≥15% from baseline GLS wasn't found in any additional patients, figure -2-.

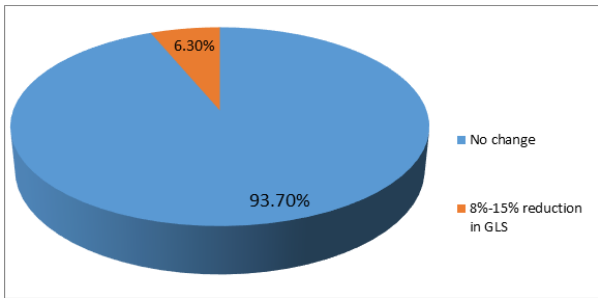

Figure -2- PIE chart for the percentage of decreasing in GLS 3 weeks after the second dose.

Discution

This study was considered as low morbidity profiles and the risk factors that could be related to cardiotoxicity was relatively low(mean age 51 years, mean BMI was 20.5 Kg/m^2 , history of DM only in 12.5%, history of dyslipidemia only in 10%, no hypertension, no smoking, no arrhythmia, no previous chemotherapy or radiation). The incidence of cardiotoxicity is fundamental, because it is not uniform in different studies. The cardiotoxicity incidence in a systematic review published in 2014 ranged from 13% to 32% [(12)]. Studies published by US at 2011 [(19)] and at 2013 [(20)] have found an incidence of 20%. Other study found a cardiotoxicity incidence of 10%, lower than that reported by those studies [(14)]. That could be explained by the different morbidity profile of population studied and different age, type of cancer, strain technique and timing of follow up. Despite this hetrogenicity, all uniformly demonstrate that changes in myocardial deformation occur earlier than change in LVEF.

In this study, reduction in the longitudinal strain level was found in 20% of patients three weeks after the first dose and in 6.3% of additional patients three weeks after the second dose and without any decrease in systolic function were noted via conventional echocardiography this similar to many other studies like Iranian study that show considerable reduction in GLS in 13% of patients [(21)], Ireland study showed, 18 (26%) of the chemotherapy group, global longitudinal strain was below the lower limit of the control group[(22)].

Early initiation of heart failure treatment appears important for the recovery of the Left ventricular function in patients who experience a significant reduction in LVEF due to anthracyclines [(3)]. Therefore, early identification of impaired systolic function is vital.

Furthermore, given that many breast cancer patients first treated with anthracyclines will then receive trastuzumab, identifying patients at greater risk of developing cardiotoxicity is of considerable advantage. Early identification of those with significant reductions in strain would enable targeted monitoring, together with the institution of supportive therapy with angiotensin-converting enzyme-inhibitors or β-blockers, should further treatment with trastuzumab be required [23].

In this study, there was no significant reduction in global longitudinal strain with preserved LVEF , this compatible to result of Australian study [23] and this contrary to other study which showed considerable reduction in GLS after chemotherapy like Iranian study which found considerable reduction in GLS before and after treatment [24] and European study which also found Global systolic strain was significantly reduced immediately after, and 6 months after anthracyclines [25]. the reason behind this may be due to short follow up period 6 week only, we studied patients after anthracycline treatment only (with no radiotherapy or adjuvant trastuzumab) and the patients received only 120 mg/m^2 cumulative dose of doxorubicin where the Cardiotoxicity related to anthracycline treatment has strong positive correlation with a cumulative dose.

Conclusion: There was a reduction in GLS between 8%-15% from the baseline in 8 patients and a reduction in GLS between >15% from the baseline in 2 patients throughout study period and there were no significant differences in the ejection fraction.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Personal Hygiene and Safety among governmental Hospitals Nurses Staff in Mosul City

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Abstract

Objective: The present study aimed to knowing weak points which is lead to negligence it could be transmission of nosocomial infection.

Material and Method: The sample of the study included 100 nurses were selected randomly from the pediatric, surgical, emergency, I.C.U and operation room departments in AL-Salam and AL-Jamhory Teaching Hospitals in the Mosul City during a period which extended from the 1/10/2018 to 20/1/2019, the data were collected through an observation of each subject of the sample during working with the patients and fulfill in the checklist sheet.

Result: The findings of this research revealed significant values between gender and personal hygiene practices to the advantage of female, also there is significant relationship between level of education and personal hygiene practices to the advantage of colligate nurses.

Conclusion: the present study demonstrated that there was negligence in some personal hygiene practices among nurses such as (hand washing, use protective devices, use protective measures, and hepatitis B vaccination).

Keywords: *hygiene, safety, nurses staff*

Introduction

we can be prevented and controlled Transmission of infections in health care system through the implementation of special precautions which can be standard and basic precautions, which must be applied to all cases from the admission to hospital until discharge, regardless types of diagnosis or infectious degree , and addition (transmission-method) .[(1)] The patient's care managements in the health facility should be the same level of a "Standard precaution" applied work practices that are essential to formulate a high level of patient's protection, patient's visitors, and health care practitioner.

Warnings points to prevent infection transmission include the following:

hand washing and antisepsis (hand hygiene),use of personal protective like gloves, masks and equipment when handling blood samples and body secretions pay attention when handling of surgical care equipment and soiled linen, protection of needle stick and knife sharp injuries, environmental management, cleaning from pollution ; and recycled of waste.[(2)] the Infection can be control for most factors which that influencing to the transmission of infection between patients or health workers by using a prophylaxes method starting from hand washing, to all body hygiene and vaccination [(3-9)] Many researchers and studies have been seeking over the world health centers about nosocomial infection which are reviled careless to nosocomial infection control and no standards precaution in multi countries. Our research aimed to study the personal hygiene of health care giver staff. In addition the objective was to identify weak points which could lead to transmission of nosocomial infections.

Methodology

Descriptive study design depended to, 100 nurses were selected randomly from the pediatric and carried out that study in, surgical, emergency, I.C.U and operation room departments in (AL-Salam and AL-Jamhory Teaching Hospitals in the Mosul City

during a period which extended from the 1/10/2018 to 20/1/2019. Data were collected by checklist, which is as a guidelines and reference to established [8, 9]. A questionnaires format consists of multiple fields and items as the purpose of this study. Checklist papers tested to pilot study in two hospitals on ten nurses to discover any problems and modified according to results. Data were collected through an observation of each subject of the sample during working with the patients and fulfill in the checklist sheet. To describe and analyze the findings of the study, SPSS program (version 17) was used to analyze the data by using mean, percentage, and ANOVA test at P.=0.05.

Results

Table (1) Demographical characteristics of the study sample

Sex	No.	%
Male female	53 47	53% 47%
Age	No.	%
Less than 25 25-29 30-34 35-39 More than 40	5 44 19 13 19	5% 44% 19% 13% 19%
Place of work	No.	%
I.C.U Pediatric Surgical Emergency Operation room	20 20 20 20 20	20% 20% 20% 20% 20%
Years of experiences	No.	%
Less than 5 5-10 More than 10	38 32 30	38% 32% 30%
Graduate of	No.	%
Nursing school preparatory nursing Institute College	4 18 38 40	4% 18% 38% 40 %

Table-1- reveals that (53%) of the sample were males, (44%) of the sample were between (25-29) years of age, (38%) of them had experiences less than (5 years), and (40%) of them had bachelor degree in nursing science

Table (2) checklist scores for personal hygiene practices among nurses

Practice	Frequency	
	Yes	No
Use uniform according to hospital policy	96	4
Uniforms are clean	78	22
Use proper shoes (not slippers)	96	4
Have short fingernails	80	20
Do not wear jewellery (rings/bracelets)	78	22
Use needle-cutter or specific container to discard used syringes, needles and other sharp items	100	0
Wash hands before contact with patients	40	60
Wash hands after each step of working	80	20
Wash hands after contact with patients	40	60
Wear gloves when needed	40	60
Use protective devices (gown, mask, gloves and goggles) when in contact with infectious patients or if there is possibility of splashing blood or other drainages	40	60
Use protective measures if drawing blood (gloves, masks and goggles)	22	78
Hepatitis B vaccination complete (3 times)	50	50

Table -2- shows that 96% of the sample wear uniform according to hospital policy, 78% from them own uniform are clean, 96% use proper shoes, 80% of keep short fingernails, 78% not wear jewellery, 100% from the sample uses specific container to discard used syringes,60% from them didn't wash their hand before contact the patients, 80% from them wash hands after contact the patients, 60% didn't wear gloves and never use protective devices when contact with infectious patients, 78% of nurses didn't use protective measures when drawing blood, Finally 50% from the sample didn't take Hepatitis B vaccine.

Table (3) Comparison of the differences between nurse's practice regarding hand hygiene rate and their gender.

Practice	sex	No	X	SD	DF	T.obs	P.value
Personal hygiene	Male	53	3.3	1.4	98	1.5	N.S
	female	47	3.5	1.4			
Hand wash	Male	53	3.4	2.2	98	0.04	S
	female	47	3.5	2.1			
Contact with patients	Male	53	4.3	1.7	98	0.03	S
	female	47	4.8	1.8			
Protective measurement	Male	53	1.6	1.0	98	0.8	N.S
	female	47	1.4	1.0			
Vaccine	Male	53	2.9	1.9	98	1.6	N.S
	female	47	3.2	1.6			

T critical=1.9

Table (3) shows that there was statically significant differences between hand hygiene rates and the gender of staff at p value= 0.05.

Table (4) one – way analysis of variance for the difference between hand hygiene practice by nurses and their age

Practice	S.O.V	SS	DF	MS	F.obs
Personal hygiene	Between Groups	1.630	4	6.157	1.011 N.S
	Within Groups	16.530	95	1.753	
	Total	17.160	96		
Hand wash	Between Groups	1.712	4	18.893	1.053 N.S
	Within Groups	22.288	95	5.269	
	Total	24.000	96		
Contact with patients	Between Groups	1.712	4	18.893	1.053 N.S
	Within Groups	22.288	95	5.269	
	Total	24.000	96		
Protective measurement	Between Groups	1.359	4	14.165	0.843 N.S
	Within Groups	15.801	95	3.432	
	Total	17.160	96		
vaccine	Between Groups	1.936	4	18.893	2.585 N.S
	Within Groups	24.064	95	5.269	
	Total	26.000	96		

F critical=3.32

Table -4-demonstrate that there were no statically differences with regards nurse's hand hygiene rates and their age at p value= 0.05.

Table -5-one – way analysis of variance for the difference between nurse's practice regarding hand hygiene rates and their level of education.

Practice	**S.O.V**	**SS**	**DF**	**MS**	**F. obs**
Personal hygiene	Between Groups	3.712	3	8.720	5.011 S
	Within Groups	22.288	96	1.003	
	Total	26.000	99		
Hand wash	Between Groups	9.132	3	3.044	19.653 S
	Within Groups	14.868	96	0.155	
	Total	24.000	99		

Cont... **Table -5-one – way analysis of variance for the difference between nurse's practice regarding hand hygiene rates and their level of education.**

Contact with patients	Between Groups	9.132	3	3.044	19.653 S
	Within Groups	14.868	96	0.155	
	Total	24.000	99		
Protective measurement	Between Groups	1.392	3	1.464	13.875 s
	Within Groups	15.768	96	0.164	
	Total	17.160	99		
vaccine	Between Groups	8.26	3	2.675	15.132 s
	Within Groups	16.974	96	0.177	
	Total	25.000	99		

F critical=3.78

Table -5- revile that a significant differences for nurse's hand hygiene rates and their level of education at (p value= 0.05. the majority of staff had bachelor degree in nursing science.

Table (6) one – way analysis of variance for the difference between nurse's hand hygiene rates and their years of experience.

Practice	S.O.V	SS	DF	MS	F. obs
Personal hygiene	Between Groups	3.330	2	6.975	6.311 S
	Within Groups	16.830	97	2.987	
	Total	20.160	99		
Hand wash	Between Groups	0.163	2	113.907	10.653 S
	Within Groups	23.837	97	10.454	
	Total	24.000	99		
Contact with patients	Between Groups	9.132	2	3.044	19.653 S
	Within Groups	14.868	97	0.155	
	Total	24.000	99		
Protective measurement	Between Groups	0.181	2	8.500	5.166 S
	Within Groups	16.979	97	1.645	
	Total	17.160	99		
vaccine	Between Groups	0.219	2	29.565	5.536 S
	Within Groups	24.781	97	5.341	
	Total	25.000	99		

F critical=4.6

Table -6- shows that there were a significant differences between nurse's hand hygiene rates and the years of experience at p value= 0.05.

Discussion

Since the era of Islamic message, Islam religion encouraged people to maintain personal hygiene especially hand washing before and after eating, before and after use of toilet to prevent transmission of disease from one person to others. [(2)], Florence Nightingale in 1854 was stated the relationship between nursing performance and infection control that was first identified during the Crimean war, when she worked at a military hospital in Scutari/ Italy, the services in that hospital was too bad . Nightingale's notes and her believes in this health facility led her to believe the enhancing hygienic conditions would decrease the number of deaths. "championed the cause of improved hygiene, food, and living conditions for the hospitalized soldiers she attacked the hospital conditions and called for basic public health, infection control measures, cleanliness, hygiene and education about the importance of the issue" . The confirmation was come from Jean Lawrence, which is chairman of the Infection Control Nurses Association (ICNA) he also said that Florence Nightingale was maybe the first nurse mentioned to infection control without recognized it [(8)]. Now the nursing staff are the key which are players the important role during heath care to ensure the survival of infection control. [(7)]. Nosocomial infection control is conceder important for three main cusses : the first one to avoiding transmission of infections from patients to health care provider staff and from them to patients ,second one to development the bacterial support and resistance, finally to limitation of financial consuming resources [9,10]. in 3 ways could be Transmission of nosocomial infections in hospitals, from patient to patient, patient to health staff and health caregivers to patients [11]. A huge reason for transporting of microorganisms is because forgetting hand washing, protective devices such as gloves and masks and a weak of personal hygiene in HCWs, in particularly improper disposal of sharp tools, [12]. The major findings of this study were as follows: hand washing compliance was significantly higher among female nurses (p=0.04) than male nurse (see Table-3).This finding is supported by the study of (Askarian, 2014) which concluded" that the Female nursing staff in CCU unit pay attention to hand washing significantly more than males after patient care contact.[(4)] [Malekzadeh, 2015], said that inter gender behaviours differences with regard hand washing may be as a result of basic differences in the education of parents on hand hygiene for girls and boys. However females were more look after. Which also add more support to this result. Similar findings have been stated, that led us to think females were more likely to wash their hands than male in HCWs [5]. The study also revealed slightly significant relationship between the level of education and hand washing (p=0.04), majority of the staff (%40) were staff nurses which had bachelor degree (see Table-4). This results is a similar with the study did in Italy [6] which stated that "nurses how are graduated from a higher level of education and with a higher knowledge of risk a transmitting and infectious disease as well as during working were more focusing to doing appropriate antisepsis of the surgical wound with hand washing pre and post medication care .[13]. [Health centre, 2016] suggested that to be careful a high level of hand cleanses adherence, HCWs need education, clear guide notes, and some understanding to infection complications. This study reveals also that there were statistically significant difference between years of experience and hand washing practice (see Table-6). While the exact reasons for these observed patterns are not clear, several possibilities exist. The first, are that of all health care settings put a lot of effort to make sure that all staff should compliance with WHO Quid lines of hand washing whenever contact with the patients., the second is that the dramatically increase in the number of high educated nursing staff.

Conclusion

Hand washing is important. This study has shown that there was negligence in some personal hygiene practices among nurses such as (hand washing, use protective devices, use protective measures, and hepatitis B vaccination).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Molecular Detection for Nosocomial *Pseudomonas aeruginosa* and its Relationship with multidrug Resistance, Isolated from Hospitals Environment.

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Abstract

Pseudomonas aeruginosa is an important pathogen in hospitalized patient's causative to their morbidity and mortality due to its multiple resistance mechanisms. Therefore, as a therapeutic option becomes restricted, the search for a new agent is a priority. So *Pseudomonas aeruginosa* is an extremely versatile Gram-negative bacterium capable of thriving in a broad spectrum of environments, and this performs main problems to workers in the field of doctors and nurses. One hundred and fifty samples were collected from different sources from Al-Kut hospitals, divided into two main groups: clinical (80)samples and (70) samples as a Nosocomial, collected from October to the December of the year 2018. All of these samples were cultured by specific and differential media, Forty (40) isolates of *P.aeruginosa* bacteria were identified by using microscopic examination, biochemical tests. The identification of (40) isolates of *P.aeruginosa* confirmed VITEK-2 system. The antibiotic sensitivity test recognized for all bacterial isolates and the results showed high sensitivity to amikacin, gentamicin, ciprofloxacin and Chloramphenicol, and high resistant to oxacillin and Cefoxitin. A molecular diagnosis is recognized by conventional PCR technique to detect the specified gene amplification products of the *bla*OXA-1 gene for *Pseudomonas aeruginosa*.

Keywords: *Hospitals ; Pseudomonas aeruginosa ; multidrug Resistance.*

Introduction

Pseudomonas aeruginosa is a Gram-negative bacillus, straight or slightly curved rods, non-spore forming, capsulate; it is usually motile by one polar flagellum (monotrichous), measuring about 1-5 µm long and 0.5-1.0 µm wide. It occurs as single bacteria, in pairs and occasionally in short chains possess mucous layer due to material alginate slime layer as well as, multi-layers of extracellular polysaccharide(1). The bacterium is ubiquitous in soil, a variety of aqueous solutions, including disinfectants, soaps, eye drops, as well as sinks and respiratory equipment, some strains hemolysis blood completely (β-hemolysis) by producing hemolysis toxin. *P. aeruginosa* forms smooth round colonies and irregular edge with a fluorescent greenish to yellow pigment pyoverdine. It also produces the non-fluorescent bluish pigment pyocyanin, which diffuses into the agar. Many types of media may be used for selective isolation of *P. aeruginosa* like cetrimide agar which promotes pyocyanin, pyoverdine, and pyoyrubin red colour and pyomelanin black colour production (2).

Pseudomonas aeruginosa is one of the most important secondaries of the hospital-acquired infection in burnt patients (3), and it is leading to the nosocomial pathogen, causing infections that usually accrue late during hospital stay (4) .*P. aeruginosa* groups have a tendency to form biofilms, which are compound bacterial groups that stick to a variety of surfaces together with plastics, medical transplant materials, and tissue. They are very difficult to destroy (5, 6) *P. aeruginosa* produces the resource of virulence factors, as well as pili, flagella exopolysaccharides, proteases, elastase, lipases, iron chelators and a variety of various toxins, including pyocyanin, chemical compound, exotoxin A, and the Type III Secretion System (T3SS) toxins ExoS, ExoT, ExoU, and ExoY (7).

Materials and Method

Specimens' Collection:

Through the period prolonged from October to December of the year 2018, One hundred and fifty specimens were collected from some hospitals including

80 clinical samples from hospitalized patients and 70 samples from Nosocomial sources. The clinical samples included four main clinical sources: Urine samples, exudate samples from wounds of the burn units patients, stool samples, Sputum samples from Cystic Fibrosis(CF) patients and Ear Swabs. The nosocomial samples included many hospital nosocomial sources: ICU, Operations Hall, Birth Hall, burning gowns, Devices and medical equipment, hand wash and hospital bed rooms.

Bacterial Isolation

Both of the clinical and Nosocomial samples were cultured on MacConkey agar, Nutrient agar, Blood agar, and Cetrimide agar, incubated for 24 hours at 37ºC. this isolates may belong to *Pseudomonas aeruginosa* growth on Cetrimide agar for characterization of *Pseudomonas aeruginosa* such as blue-greenish colour, mucoid colony, smooth in shape, and have a fruity odour. colonies were picked and re-cultured on another MacConkey agar plates in order to be appeared as pale greenish and lactose non-fermenter colonies, while on Blood agar they appeared as large, opaque irregular colonies with butyrous consistency(shiny) give fruity odour and *B*-hemolytic colonies. and on Nutrient agar appeared as yellowish green, smooth in shape with flat edges and elevated center. Further identification tests included the morphological characteristics and biochemical tests were carried out depending on Forbes *et al*. [8].

Bacterial Identification:

Colonial morphology on MacConkey agar, Nutrient agar, blood agar, and Cetrimide agar was depended initially to identify bacterial isolates, colony shape, texture, colour and edges were examined. In addition to macroscopic characteristics; microscopic examination of a gram-stained slide was examined under a light microscope with special regard towards cell shape and arrangement. Biochemical tests and VITEK 2 system were depended to complete the identification of *P. aeruginosa* isolates.

PCR amplification:

DNA template of all bacterial isolates was prepared by boiling method (30 min in 100°C). The DNA of isolates was targeted only for the *bla*oxa-1 gene using the primer (Z. Tavajjohi, *et al* Iran [9] listed in Table 1. A reaction mixture (25 µl) contained 2 µl of DNA, 1 µl of each primer, 12.5 µl of Master Mix 2X (Z.Tavajjohi, *et al* ., 2011), and 8.5 µl of nuclease free water. The experiment was sustained according to the following program: initial denaturation at 95°C for 5 minutes, followed by 30 cycles at 95°C for 30 Sec. , 55°C for 30 Sec. , 72°C for 30 Sec. and a final extension at 72°C for 7 minutes. The PCR products were analyzed using gel electrophoresis (1% agarose) and stained with safe dye and visualized by Gel Doc apparatus (BioRad, USA) (Table 2).

Table (1): Primers used in this study.

Gene	Primer Sequence		Product size (bp)	References
bla OXA-1	F	5`-AGCCGTTAAAATTAAGCCC-3`	908	Z.Tavajjohi, et al ., 2011
	R	5`-CTTGATTGAAGGGTTGGGCG-3`		

Table (2): Condition of PCR Reaction for the gene *bla* OXA-1 of *Pseudomonas aeruginosa*.

Steps	Temperature	Time	Number of Cycle
Initial Denaturation	95°C	5 min.	1
Denaturation	95°C	30 Sec.	30
Annealing	55°C	30Sec.	
Extension	72 °C	30Sec.	
Final extension	72 °C	7 min.	1
Hold	10 °C	10 min.	1

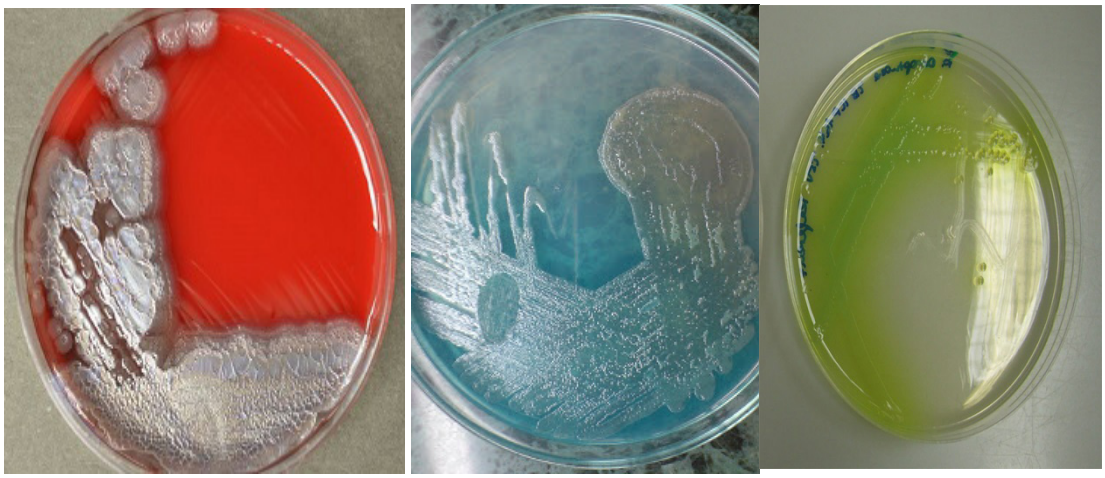
Results and Discussion

Isolation and Identification of *Pseudomonas aeruginosa:*

Out of One hundred and fifty samples, clinical and Nosocomial specimens were analyzed for the presence of *Pseudomonas aeruginosa*, the results of bacterial isolation and identification revealed the detection of forty (40) isolates of *Pseudomonas aeruginosa*.

Biochemical Tests:

Some biochemical tests were performed for more validation. showed 40 isolates of *P.aeruginosa* provided by some biochemical tests, results showed positive results for oxidase test, catalase test, motility test, , and production of *B*-hemolysis while (40) isolations negative results to citrate utilization tests, indole production and urease production tests negative to Gram's stain and capable of growing on cetrimide agar as blue greenish colonies (at 37˚C for 24 hrs.). (figure 1, table 3).



A B C

Figure (1): *Pseudomonas aeruginosa* colonies on (A) Blood agar r and (B) Cetrimide agar and (C)Nutrient agar, after 24 hours of incubation at 37ºC.

Table (3): The biochemical tests of *Pseudomonas aeruginosa*

Biochemical test	Result
Gram stain	G- rods
Kligler's iron agar	K/K
Growth at 42ºC	+
Motility	+
Oxidase production	+
Catalase production	+
Hemolysis(ß-hemolysis)	+
Indole production	-
Urease production	-
Methyl-red	-
Voges-Proskauer	-
Lactose fermentation	-
Simmon's citrate	+

(+) Positive result, (-) Negative result

P. aeruginosa has a wide growth temperature range, optimum growth at 37°C. Slower growth rates are seen at 4˚C. *P. aeruginosa* is distinguishable from other clinically *Pseudomonas* spp. by its capability for growth at 42˚C. They also grow well at pH range 6.6-7.0. It was tolerant of a wide variety of physical conditions, including temperature and pH. Also, it was resistant to high concentrations of salts and dyes. It is typically given a positive result to the oxidase test and catalase. It does not ferment carbohydrates, but many strains oxidize glucose [10]. The identification was performed with the automated VITEK-2 system using the GN-ID cards which contains 64 biochemical tests, from (40) isolate of *P. aeruginosa* (40) positive result of the *P. aeruginosa* demonstrated.

Distribution of *P. aeruginosa* according to Type of Specimens :

A percentage of about 50% (40) isolates belonged to *P.aeruginosa* from total 80 isolates, 28(70%) isolates were positive to clinical *P. aeruginosa* and 12(30%) isolates were positive for Nosocomial sources. The percentage of the positive results from burns, ears and wounds were 5 %, 27.5% and 12.5% respectively (table 4).

Table (4): Distribution of *Pseudomonas aeruginosa* isolates in Nosocomial samples

Site of samples	Numbers of sample and Percentage
ICU	2 (5%)
Operations Hall	4 (10%)
Birth Hall	2 (5%)
Burn Hall	2 (5%)
Devices and medical equipment	2 (5%)
Total	12 (30%)

The low percentage was found in burn specimens which accomplished 5%. Results obtained reported that the highest percentage of *Pseudomonas aeruginosa* from ear swab (27.5%). In comparison with Nosocomial isolates of *Pseudomonas aeruginosa* the highest percentage isolation of Operations Hall (4%) where are the isolates reported low present in burn hall, birth hall and Devices and medical equipment (2%) in comparison with clinical findings.

P. aeruginosa is well-adapted to the respiratory tract environment, especially in patients with the chronic obstructive bronchopulmonary disease, who are hospitalized in intensive care units or immunocompromised, [11]. *Pseudomonas aeruginosa* is very hard to kill because of its ability to build resistance to many cleaners and medicines. *Pseudomonas aeruginosa* is accountable for 16% of healthcare facility respiratory disease cases, 12% of hospital-acquired UTI, 8% of surgical wound contaminations, and 10% of blood stream infections. [12]. Vandeale *et al*. (2005) [13] studied the epidemiology of *P. aeruginosa* in cystic fibrosis rehabilitation centre, sixty-seven isolates of *P. aeruginosa* were isolated from patient's sputum, the infection by patient-to-patient-transmission could have happened in the past, the risk of patient-to-patient transmission during the study period (63day) was relatively (10%) and the risk of persisting colonization with a newly acquired strain during the study period was (4%).

Antibiotics Resistance in *P. aeruginosa*

In the present study the in vitro antibiotic sensitivity test of isolated

P. aeruginosa strains examined revelled Amikacin and Gentamicin to be the most effective antibiotic followed by Ceftriaxone, Ciprofloxacin & Cefotaxime-clavulanic acid in decreasing order as reported in the table(5).

Table (5): Antibiotic resistance and sensitivity of *Pseudomonas aeruginosa* to antibiotics

Antimicrobial Agent	Pseudomonas aeruginosa (40 isolates)			
	S		R	
	No.	%	No.	%
Amikacin(AK)	40	100%	0	0%
Gentamicin(GN)	40	100%	0	0%
Cefotaxime-clavulanic acid (CEC)	30	75%	10	25%
Aztreonam(AT)	20	50%	20	50%
Cefoxitin(CX)	3	7.5%	37	92.5%
Ceftriaxone(CTR)	35	87.5%	5	12.5%
Oxacillin(OX)	0	0%	40	100%
Ciprofloxacin(CIP)	35	87.5%	5	12.5%

Antibiotic sensitivity testing is an essential tool for treatment. *P. aeruginosa* is considered as one of UTI infection ,burn, CF , otitis ear and wound infection is

resistant to a range of antibiotics due to the permeability barrier provided by its outer membrane for hydrophilic substances , although using sometimes surfactant as a permeabilizing agent to enhance the interaction of hydrophilic material with the hydrophobic cell wall did not enhance the antibacterial activity of some agent against *P. aeruginosa* [14]. Malla *et al* [15] reported that *P. aeruginosa* were susceptible 100% to Amikacin and Gentamycin which are similar to our study.

Another study by V. Saegeman1a *et al,*. (2010)[16] stated that *P. aeruginosa* had shown high sensitivity to a β-lactamase antibiotic with 100% for Amikacin(AK), 95 % to Gentamicin(GN) and 87.5% to Ciprofloxacin(CIP). Osazuwa *et al*., (2011)[17] in Nigeria recorded 100% resistance for both oxacillin and Cefoxitin(CX). These results agree with the percentages in this study.

Resistance to some antibiotics such as cefotaxime, neomycin and kanamycin showed an increase in comparison with previous studies, this might be due to the variation in the usage of antibiotics[18].

Genomic DNA Extraction:

Using a genomic DNA purification kit (Promega), Genomic DNA was extracted from *Pseudomonas aeruginosa* isolates. Extraction genomic DNA from 40 isolates that were confirmed as bands by gel electrophoresis. The DNA concentration and purity were measured by Nanodrop spectrophotometer, all the isolates had DNA concentration between (50-100 ng/µl) and purity of the DNA.

Molecular Detection of *Pseudomonas aeruginosa* by $bla_{oxa1\ like}$ gene:

The result of PCR analysis concerning of the found the *bla*oxa 1, showed that studies *P. aeruginosa* possess the gene $bla_{\ oxa\ 1\ like}$ gene from 40 isolates, 10(25 %) isolates positive $bla_{oxa1\ like}$ gene showed 4 (40%) from clinical isolates and 6 (60 %) from nosocomial (figures not shown).

From 10 positive isolates, 4 strains of clinical isolates; urine and ear isolates were multi-drug resistant. The prevalence of *bla*oxa-1 positive isolates of ESBL *P. aeruginosa* occurred in 40% of isolates and MDR phenotype was common in *P. aeruginosa*. The most prevalent gene among ESBL producers were *bla*OXA-1 which compromises the efficacy of MDR bacteria especially in clinical(urine, ear, and stool samples) and nosocomial samples (operation hall, burning gown, and ICU) according to the results in this study and tend to be geographically limited.

The *bla*OXA-1 ESBLs provide *P. aeruginosa* with an additional powerful resistance mechanism with potentially serious clinical implications, including limitation of the therapeutic options. ESBLs manufacturing organisms create distinctive challenges to clinical microbiologists, clinicians, infection control professionals and scientists engaged in finding new antibacterial agents [19]. In this study, the prevalence of *bla*oxa-1 of *P. aeruginosa* isolates was 25% that less than values reported in different studies like in Iran; Malaysia; and Pakistan

This study reported 10 isolates (25%) of *bla*OXA-1 ESBL of *P. aeruginosa* in clinical and nosocomial isolates from 40 isolates. The studies conducted by others depicted low rates, 7.7% (J Jacoby GA)[20],3.7% (Neil Woodford *et al*)[21], 4.2% (Lim *et al*., [22] respectively, of ESBL production of *P. aeruginosa*.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Knowledge Assessment of Working Women Regarding Breast Cancer / Control and Prevention in Kirkuk Technical Institute

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Abstract

Breast cancer is the most common cancer in women both in the developed and less developed world In Iraq, it is the commonest type of female malignancy, accounting for approximately one-third of the registered female cancers according to the latest Iraqi Cancer Registry.

A descriptive cross-sectional study was conducted on working women in Kirkuk Technical Institute during the period from 1st April till 30th June / 2016 to assess their knowledge regarding breast cancer prevention and control methods.

The total number of included studied women was 71from different scientific departments after distributing a special questionnaire form and face to face interview was done after receiving the written agreements to participating in the study.

Majority of working women are technical (52.2%), married (74.7%) aging above 40 years (47.8%) with employment years above 15 years (50.0%).

Both electrical and survey dept. go with the establishing of breast cancer prevention at adulthood after fully maturated (44%, 45%) respectively in comparison to 57.7% of study women from electronic dept. go with the starting of cancer prevention at the birth of a first child .

The main recommendation of the study is that further researches should be done because there is a need for increased efforts towards developing and widely available cancer educational program that focuses on the main preventive measures and encouraging the women with breast disease, not only providing them with information but also for the ability to make her decision to control the disease

Key words : *Knowledge, breast cancer, assessment, control and prevention.*

Introduction

Women play a critical role in maintaining the health and well being of their communities because families and communities health is tied to the health of women. Woman illness or death has serious and far-reaching consequences for their children, families, and communities [(1)] .

Among women, breast cancer is the most common cause of cancer mortality accounting for 16% of cancer deaths in adult women [(2)]. Breast cancer is a serious life-threatening public health problem of great concern. Increases in the incidence of breast cancer are being observed in both industrialized and developing world [(3).]

WHO promotes breast cancer control methods within the national cancer control program which is directed for both non- communicable diseases and other related health consequences his program includes (prevention, ***early detection of the disease, diagnosis, treatment, rehabilitation with the assistance of palliative care***) [(3, 4).]

The key strategies for population-based breast cancer control are :

1- Increasing the awareness of the general public about this health problem.

2- Appropriate policies and health programs [5].

Nowadays countries with low and middle income facing a double burden of breast cancer which is regarded as a top killer in women aging over 30 years old. These countries need further specific strategic methods that concentrated for effective ways of disease prevention [5].

A study was done by Nada *etal* during 2014 mentioned that breast cancer constitutes about one fourth of the reported cancer cases among Iraqi population which is regarded the main cause of death and each year many women are exposed to the vicious ramifications of breast cancer which include death if left untreated or the negative outcomes that would experience, cosmetically and psychologically, after exposure to surgical intervention [6].

Another study was done in Iraq about the breast cancer incidence trends from 2000- 2009 which reported that the disease incidence of all female breast cancer in Iraq (all ages) has risen and there is a rapid increase in the age-specific incidence rate among the age group 60-69. However, this disease among Iraqi women still affects younger age groups than their counterparts in developed countries [7].

Reduction in the incidence of breast cancer needs control of both modifiable risk factors with the effective integrated preventive measures that promote (intake of the healthy diet, daily physical activity, alcohol, and weight control) which eventually have a direct impact for the future outcomes. [8,9].

In more recent years, many studies have indicated the effect of diet and other behaviors on the occurrence of breast cancer. These additional risk factors are environmental factors such as tobacco use, radiation, endocrine disturbance and shift work [10] .

The main three important screening tests usually considered for controlling and preventing the disease which is 1- clinical breast examination (CBE) 2-X-ray mammography 3- breast self-examination (BSE). In industrial countries, breast cancer mortality is declining where screening mammography is the standard for care. BSE is appealing as a patient-centered, non-invasive screening procedure which allows women to become more comfortable with their own bodies [11, 12].

The aim of the study: to assess the knowledge of working women regarding breast cancer control and prevention methods

Methodology-

1- Ethical issues

Official agreements were taken from Kirkuk Technical Institute before establishing the study

2- Study design and setting:-

A descriptive cross-sectional study was carried out in Kirkuk Technical Institute on three scientific departments (Electronic, Electrical, and Survey).

3- Study sample and sampling method:-

A randomly selected sample from 71 working women (25 from the electronic department and 26 from the Electrical department and 20 from the Survey Department).

A specific special questionnaire form was distributed to them after receiving their written consent and the data was collected by interviewing with the study women after complete explanation of the main objectives of the study.

4- Study period :

The study was conducted during the period from 1st April till 30th June / 2016.

5- Data collection tool:-

Certain designed prepared questionnaire form has been conducted by the investigator utilizing a recent updated related literature to the study subject included three main parts:-

Part-1- Demographic characteristics like (age, employment year, type of work, and martial status).

Part-2- Information about their knowledge regarding disease control and preventive measures with the main risk factors). Part -3- Their knowledge about the specific time for starting the prevention of breast cancer.

6- Reliability of the questionnaire form:-

The data was presented to (4) experts in different fields, they were (2) Clinical physicians, (1) Community physicians and (1) statistical experts.

Inclusion criteria: Any married woman

Exclusion criteria: History with chronic diseases such as hypertension, Diabetes's mellitus, or renal disease.

7- Statistical analysis of data:-

The information regarding each participant was transferred into a code sheet and the data entry was done using a computer Pentium IV and the statistical analysis was calculated by mine tab program /2016 [13].

All the statements with Yes and No answer, number and percent will be calculated.

Chi-square test was used to detect the relation between the studied variables and the level of significance is taken at level 5% ($P < 0.05$).

Results

The total number of working women are 89 distributed among three scientific departments.

The number of women responded to study was 71 and the response rate was (79.7%).

Table (1): Socio- demographic characteristics of the study women

Socio- demographic parameter		**Number(N=71)**	**Percent**
Age group in year	<20 20-25 25-30 35-40 >40	8 10 19 24 10	11.2 14.3 26.7 **33.8** 14.0
Employment years	5- 10 years 11- 15 years > 15 years	9 23 39	12.6 32.4 **55.0**
Type of work	Official Technical Teacher	12 37 22	16.9 **52.2** 30.9
Marital status	Single Married	18 53	25.3 **74.7**

Table 1 shows that most of the study women are technical (52.2%) aging between 35-40 years (33.8 %) with employment years above 15 years (55.0%), married (74.7%).

Table (2): Frequency distribution of study women according to family history and type of relationship

No.	**Items**	**Yes**		No		Total	
	1-Family history of breast cancer 7	No.	%	No.	%	No.	%
		9.8	64	90.2	71	100.0	
2- Relation of breast cancer with the relatives 4	Mother	Sister		**Aunt**		**Grand mother**	
	3	0		0			

Table 2 indicated that 90.2 % of study women had a negative family history of breast cancer and only 9.8 % of them had a positive history of breast cancer with a first degree (mother and sister).

Table (3): Study women distribution according to their knowledge about the risk factors, preventive and control measures for breast cancer.

Knowledge parameter	Number of working women (=71)				*P value
	yes	%	No.	%	
Risk factors of breast cancer a- Aging factor b- Contraceptives pills c- Life style d- Family history	 12 21 10 3	 26.1 45.6 21.8 6.5	 11 7 5 2	 44.0 28.0 20.0 8.0	0.000
Total	46	100.0	25	100.0	
Control measures : a- Advanced educational program b- Raised general public awareness c- Control risk factors like contraception d- Regular cheek up	 19 10 8 2	 48.7 25.6 20.6 5.1	 12 8 5 7	 37.5 25.0 15.6 21.8	0.240
Total	39	100.0	32	100.0	
Preventive guidelines : a- Screening by mammography b- Modifying healthy life style c- Regular breast self exam d- Breast clinical exam	 18 20 13 3	 29.4 39.2 25.5 5.8	 6 3 4 7	 30.0 15.0 20.0 35.0	0.000
Total	51	100.0	20	100.0	

Table 3 shows that there was a statistically significant agreement among study women knowledge regarding risk factors was for contraceptive pills (45.6%), (48.7%) for the advanced educational program as a control measures and (39.2%) for modifying healthy life style as a preventive measure for breast cancer prevention with a P value = 0.000 .

Table 4: Frequency distribution of study women according to their knowledge about the specific time for starting the prevention of breast cancer

Specific time for starting the prevention of breast cancer	Study women N=71						Total	P* value
	Electronic dept. N= 26		Electric dept. N= 25		Survey dept . N=20			
	N0.	%	No.	%	No.	%		
1- Child hood before and during breast development	2	7.7%	7	28%	4	20%	13 (18.3%)	0.166

Cont... **Table 4: Frequency distribution of study women according to their knowledge about the specific time for starting the prevention of breast cancer**

2- Young adult hood before fully maturation of the breast	5	19.3%	1	4%	5	25%	11 (15.5%)	0.124
3- Adult hood after the breast fully mature	4	15.3%	11	44%	9	45%	24 (33.9%)	0.045
4- The birth of a women first child	15	57.7%	6	24%	2	10%	23 (32.3%)	0.015

***χ^2 – test was used**

Table 4 shows that both electrical and survey dept. go with the establishing of breast cancer prevention at adulthood after fully maturated (44%, 45%) respectively in comparison to 57.7% of study women from electronic dept. go with the starting of cancer prevention at the birth of a first child with a P value = 0.015

Discussion

Regarding the family history of breast cancer, the study shows that most of the working women had a negative family history of breast cancer. This result goes with the American cancer society (ACS)report about the disease / 2007[14] The report stated that any women who had a history of breast cancer in her family is more prone to be a risk factor for it and this will lead gradually to increase the level of education about the disease occurrence

Shambhavi *etal* conducted a study in Mangalore, Karnataka / India to assess the teacher's knowledge regarding breast cancer and to find the association between the level of knowledge and some selected demographic variables. They found that the highest number of school teachers (60%) were in the age group between 25 -35 years and least were about > 55 years of age (6%) [15].

Concerning the general knowledge of the breast cancer , the current study show the about half of study women knowledge regarding risk factors was for contraceptive pills and for the advanced educational program as a control measures. This result may be explained because of better information sources from the scientific references and the declaration of the Ministry of health on the television about the danger and risk factors of the disease.

Samir /2011 [16] conducted a study in the college of nursing / University of Sulaimani who found that the general informative background of study students regarding breast cancer, clinical manifestation, risk factors and control measures were ranged between highly significant and moderate. He explained that because of the student scientific study and general information in their college.

Another study was prepared by Alkhasawneh /2007 [17] at Pamukkale University Denizli hospital / Jordan among nurses. H e found that 74 (57.6%) of them were correctly knew at least four risk factors of the disease and the increasing age 72%, familial history 94.4%, childlessness 85.6%, absence of breastfeeding 82.4%, and taking birth control pill or hormone replacement therapy 50.4% were well-known risk factors. However, a small percentage of them believed that early menarche 23.2% and late menopause 28.8% were the risk factors of breast cancer. He discussed these results because of breast cancer is being considered as a disease of the developed countries and much efforts with strong emphasis had been given to this issue in addition to that further preventive and control measures was conducted against breast cancer.

A similar study was conducted by Nada *et al*. / 2012 [18] in Kirkuk University about knowledge, attitude and practice towards breast cancer and breast self-examination as the most effective preventive measure towards breast cancer.

They found that the general knowledge about breast cancer and the practice of breast -self-examination was relatively well in Kirkuk yet the positive attitude towards learning the screening techniques and the intention to teach others were fairly encouraging hey recommended

for promoting public health awareness campaigns through various media.

Dr. Golditiz *et al* / 2015[19] published a study about the prevention of breast cancer mentioned that control and prevention should start early in life because nearly 25% of breast cancer patient is diagnosed in women younger than age 50 years in developed countries and 22% of it is diagnosed in premenstrual women and is often aggressive than cancer diagnosed in postmenopausal women . They recommended for adaptation of a specific strategy to decrease cancer risk by calculated how much the differences would occur in a sample of women .

A study was done by Coleman / 2008 [20] to assess the survival rate of breast cancer. He found that the disease range was from 80% or more in North America, Sweden, and Japan to reach up to 60% in Middle income- countries and very low in low income-countries which reach to 40% and they explained these differences because of lack of preventive measures like early detection health programs which later on lead to increase the proportion of women complaining of late and advanced stage of the disease with the lack of adequate specific diagnosis of the disease .

Conclusion

Informative background for studied women was good but still, there is a defect in disease prevention and time for early detection.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Colorectal Cancer in Nineveh: Five Years Survival Rate

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Abstract

Overall**,** colorectal cancer ranks third in terms of incidence but second in terms of mortality, according to the latest GLOBOCAN worldwide estimation in 2018**.** The aim of this study is to show the survival rates of colorectal cancer patients in Nineveh province for the period 2010 – 2014, a retrospective cohort study, conducted among patients at the Mosul Cancer Registry center. Survival analysis was carried out using the actuarial method to construct the needed life tables. Chi-square test, was used to study the relationship between cancer occurrence and sex, age and stage. The result of this study indicates that the 5 year survival rate (50.1%) with 95% confidence intervals (0.4985-0.5035) in Mosul was between developed and developing countries. The survival of colorectal cancer in men (50.0%) was significantly better than women (43.0%), older patients ≥ 70 years, had a poorer survival rate (25.9%) compared to younger patients <50 years (70.5%) and the survival rate by tumor stage was better in the localized stage (75.4%) than regional and distant stages of (63.6% and 11.7%) respectively.

Keywords: *Colorectal cancer, 5-year survival rate, Mosul Cancer Registry center*

Introduction

Over 1.8 million new colorectal cancer cases and 881,000 deaths are estimated to occur in 2018[1]. Colorectal cancer (CRC) is the third most common cancer in men and the second in women, according to the latest GLOBOCAN worldwide estimation in 2012[1,2]. About 55%of the cases are reported in the more developed countries. The highest rates were estimated to be in Australia/New Zealand: 44.8 and 32.2 per 100,000 in men and women, respectively, and the lowest in Western Africa (4.5 and 3.8 per 100,000)[2]. Colorectal cancer is a major cause of morbidity and mortality throughout the world[3]. It accounts for over 9% of all cancer incidence [4,5]. It is the third most common cancer worldwide and the second most common cause of death[1,4]. It affects men and women almost equally[3–6]. Colorectal cancer is one of the ten leading cancers in Iraq, accounting for 4.8% of estimated cancer cases in males and 3.8% of estimated cancer cases in females[7].

Aim of The Study

The aim of this study is to show the survival rates of CRC patients in Nineveh province for the period 2010 – 2014.

Objectives

To describe the sociodemographic characteristics of the CRC patients.

To calculate the 5-year survival rate (5YSR), according to the age, sex and stage of the cancer.

Materials and Method

Study Settings:

This was a retrospective cohort study, conducted among patients at the Mosul Cancer Registry center (MCRC). This study protocol was approved by the local ethics committee of Medical Collage, University of Nineveh and also obtained from the directorate of health in Nineveh

Study sample:

The sample of the present study included all colon and rectum cancer patients registered at the Mosul cancer registry center in Nineveh in 2010 to 2014 of all ages and both sexes. A total of 460 cases of CRC, recorded were retrieved from the medical records of MCRC.

The cancer cases are registered with respect to patient number, age, sex, clinical stage and cancer coded according to the International Classification of Diseases

(ICD10)[8,9]. All the data are fitted on Can Reg 3 format and fed into the computerized database of the MCRC.

Staging is done according to the summary stage system[10,11]. The cancer has become invasive and is categorized as local, regional, or distant based on the extent of spread[12]. Stage "unknown" has been recorded to include tumour morphologies for which the summary stage system is not strictly applicable and patients with cancer of an unknown stage or carcinoma in situ were excluded from the present study.

The criteria of the age groups chosen were those used for the international standard cancer patient population[13]. With age been categorized into three groups (<50, 50–69 and 70+ years).

The sex was examined as a potential confounding variable for CRC for presentation of some descriptive results.

Survival Analysis

Survival analysis was carried out using the actuarial method to construct the needed life tables [14]. ***Observed survival rate*** **"OSR"** is the probability of surviving from all causes of death in a group of cancer patients under study. Observed survival was measured from the date of diagnosis to the date of death or censoring, whichever occurred first[14,15,16]. Cancer patient survival was estimated as the cumulative probability (range 0 to 1) of survival up to a stated time after diagnosis [17]. This method has been used in this study to estimate the absolute survival probability [18]. In the present study, period analysis was used to derive 5-year survival estimates for 2010-2014 [19, 20].

Statistical Analysis

95% confidence intervals and calculation of the standard error (s.e.) of the 5YSR obtained by the actuarial method uses the Greenwood's formula[14, 21]. Chi-square test was used to study the relationship between variables and some results[22].

Micro Soft Excel 2010 was used for statistical analysis.

Results

The total number of the CRC cases, according to age, sex and stage of extension are shown in Table 1. The result is significant at $p \leq 0.05$, except for sex, the result is not significant and the chi-square statistic is 2.513.

Table 1; The frequency distribution of the colorectal cancer cases, according to age, sex and stage of disease.

Colorectal Cancer Cases (460) 2010		**Date of Diagnosis**					**Total**	**%**	**p-value**
		2011	**2012**	**2013**	**2014**				
Age group	<50	22	41	30	29	32	154	33.5	< 0.00001
	50-69	38	34	49	42	61	224	48.7	
	≥70	11	8	20	21	22	82	17.8	
Sex	Female	28	42	50	48	45	213	46.3	0.11291
	Male	43	41	49	44	70	247	53.7	
Stage	Local	14	8	26	17	27	92	20.0	< 0.00001
	Regional	30	42	34	25	51	182	39.6	
	Distant	26	21	27	27	28	129	28.0	
	Unknown	1	12	12	23	9	57	12.4	

To CRC death, about half of people (50.3%) die, is of 55-69 year of age, as shown in Table 2,

Table 2; The frequency distribution of the colorectal cancer deaths, according to age, sex and stage of disease.

Colorectal Cancer Deaths (163) 2010		Date of Diagnosis					Total	%
		2011	2012	2013	2014			
Age group	<50	0	3	11	9	13	36	22.1
	50-69	3	7	17	21	34	82	50.3
	≥70	6	2	6	12	19	45	27.6
Sex	Female	2	6	15	17	36	76	46.6
	Male	7	6	19	25	30	87	53.4
Stage	Local	0	0	3	9	6	18	11.0
	Regional	2	5	11	16	17	51	31.3
	Distant	7	7	16	15	35	80	49.1
	Unknown	0	0	4	2	8	14	8.6

Table 3, shows the CRC survival rate declined from 70.5% in the ≤50 age group to 25.9% among those aged ≥70 years. There was an greater difference in survival rates between female and male, which were 43.0% and 50.0%, respectively and between stages.

Table 3: The 5- year observed survival rate of the colorectal cancer cases, according to overall, age group, sex and stages for the period from (2010-2014)

Colorectal Cancer		OSR	95% CI
	Overall	0.501	0.4985-0.5035
Age Group	**<50**	0.705	0.7003- 0.7100
	50-69	0.518	0.5127- 0.5240
	≥70	0.259	0.2152- 0.3032
Sex	**Female**	0.430	0.4251- 0.4343
	Male	0.500	0.4954 - 0.5045
Stage	**Local**	0.754	0.7469- 0.7603
	Regional	0.636	0.6305- 0.6411
	Distant	0.117	0.0975- 0.1363

Discussion

Previous studies in other countries have reported variable CRC survival rates.

In Asia, the highest survival rates were found in China (68.0%) [23], the lowest rate was in India (33.6%) and Malaysia (34.3%) [24,25]. This study showed that the overall 5YSR for patients with CRC was 50.1%. Various studies from Iran have reported 5YSR of CRC of 47%, 41% and 61% respectively[26-28]. In Saudi Arabia, the overall 5YSR of the CRC was 44.6% [29]. The disparities in CRC survival between Japanese, Mediterranean countries could also be attributed to many factors, together with variations in socioeconomic standing, stage at identification, treatment, medical practitioner characteristics, and hospital factors. In Japan in 2005, the 5YSR was reported at 61.4% [30]. Also, studies in Germany, USA, England, France, and Italy were reported the 5YSR 65%, 65%, 52.7%, 60.3% and 59.3% respectively[31-34]. The 5YSR of patients with CRC in our city is lower than the developed regions. Therefore, we conclude that the survival rate of patients with CRC in Mosul is between developed and developing countries, like other patients in the world depend on various factors such as demographics, pathologic and tumor characteristics, geographical distribution and gene mutation or have a family history of [35,36].

In this study, there is no significant difference in the occurrence of CRC between females and males (p-value= 0.11291), but differences in survival rates were reported, 43.0% and50.0%, for females and males, respectively. Yet, other studies had reported a lower 5YSR in females[2,37]. The absence of gender differences in survival rates was reported in some of the previous studies[23,25]. In a study in Germany and Cuba, the 5YSR in women was better than men that were not similar to the result of our study[38,39]. The reason for better survival in men than women in this study may be due to a higher participation rate in men compared with women for screening programs like fecal occult blood test and colonoscopy[40].

This study showed that the hazard of death increased significantly with increased age being the highest in the age of ≥70 years. This result was reported in other studies [27] that showed that older patients had a poorer survival rate compared to younger patients. However, other studies[26,41] reported no difference in survival according to age. The contradictory results of previous studies on age may be due to the inclusion of patients from single referral centers and poor adjustment for the effect of possible confounds.

One of the most important factors that influence the survival of patients with CRC was the stage of diagnosis. Which were 75.4%, 63.6% and 11.7%, for local, regional and distant stages respectively. This finding is consistent with findings of other studies; in the USA, 5YSR is 90%; survival in local stage, declines to 71% and 14% with regional and distant stages, respectively[34], also in European countries, localized CRC reached levels close to 90% and 15% in the case of distant stage[38], while in Saudi Arabia, 63.3% for localized disease, 50.2% for those with regional disease, and 14.7% for patients with distant stage[29]. But the most important factor for our CRC patient is that the disease diagnosed at an advanced stage[42]. So this significant difference in 5YSR may be due to, poorly established screening program like the fecal occult blood test, sigmoidoscopy and colonoscopy and limited accessibility in developing region[43].

Conclusion

In conclusion, the result of this study indicates that the 5YSR of patients with CRC in Mosul was between developed and developing countries. The survival of CRC in men was significantly better than women, older patients had a poorer survival rate compared to younger patients and the survival rate by tumor stage was better in the localized stage than other stages of CRC.

Conflict of Interest: no conflict of interest.

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Ethical Clearance: From Ethical Committee, Medical College, University of Nineveh

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The Effect of Esomeprazole on Cell Line Human Cervical Cancer

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Abstract

Esomeprazole is an inhibitor of the proton pump that reduces gastric acidity and is used in indigestion treatment; Esomeprazole is a promising treatment for tumors, such as melanoma and B cell leukemia, through several mechanisms, including rapid cellular acidification and activation of many types of caspase enzymes, which in turn lead to the activation of cellular apoptosis

This work was designed to assess the toxicity of the (Esomeprazole) on (HeLa cell line) in vitro at concentrations starting from one - ten thousand µg/ milliliter and compared with cytotoxicity of vincristine and methotrexate at concentrations between one - ten thousand µg/ milliliter In 3 incubation periods

The results showed that the cellular toxicity of one µg / milliliter was a lot of more than the cellular toxicity of ten and a hundred µg / milliliter. The results additionally showed that toxicity of esomeprazole was considerably more than vincristine and methotrexate on HLA cells at seventy two hours.

Key word: *esomeprazole, vincristine, Hela cancer cell line.*

Introduction

Cervical malignant tumor is a common categories of cancer between female, lead to women's sexual ability and fertility [(1)]. Chemotherapy is that the backbone strategy for cancer treatment especially in the early stage in case of cervical cancer, is treated by surgery or irradiation with chemotherapy [(2-5)]. Chemotherapy kills both cancer cells and normal cells with a high split rate [(6-14)], Common side effects of chemotherapy are nephrotoxicity, which usually associated with vincristine and methotrexate therapy

Several approaches to solve the toxicity of conventional cancer treatments have been developed, like using of natural agents as plant extract that may show a promising anticancer activity [(15-23)] .

Investigation about the efficacy of some drugs that already used to treat diseases other than cancer. it show a hopeful role in the handling of malignancy, such as aspirin, which acts as a protective agent for various types of Cancer (colorectal cancer), through a mechanism like suppress platelets aggregation [(19)], also acetaminophen at the therapeutic dose was capable of killing liver cancer cells by turn activate apoptosis [(24)]. On the other hand, Omeprazole has the ability to rise the sympathy of the immune B-cell leukemia cell line to the vinblastine by several mechanisms, including an increase in cellular acidity [(4)].

Omeprazole have been shown to have potential to reduce resistance of tumor cell to chemotherapy by altering the pathway of the transfer of lysosomes contains and autophagy that activate programmed cell death mechanism [(3)]. A mixture of esomeprazole and amygdalin reveled ability to suppress the growth of cervical cancer cells in vitro [(5; 23)].

Martial and Method

Proton pump inhibitor: Esomeprazole (nexium) ® 40 mg (AstraZeneca) was used in different concentrations ranging from one to 10000 µg / mL. These concentrations were set by thinning of the medication with bovine serum unrestricted medium.

Chemotherapeutics agent; a)Vincristine sulphate 1 mg/ml vial (hospira UK.) was used at different concentration ranged between (1-10000) µg/ml, after

dilution with bovine serum free medium

Methotrexate vial (50 mg/5cc - kocak / turkey) was used at different concentration ranged between (1-10000) µg/ml, after diluted with bovine serum free medium

$$\text{Inhibitor rate \%} = \frac{\text{The optical density of control} _ \text{The optical density of test.}}{\text{The optical density of control}} \times 100$$

Cell line culture:

The human cervical cell line cancer gotten from the unit of tissue culture in the Iraqi Center for Cancer and Medical Genetics Research . It were planting in tissue culture flasks with size 75 cm 2 and with controlled conditions involving 5% of humidified, carbon dioxide on 37 ° C in RPMI-1640 media (Kigma Chemical, England) with fetal bovine serum at 10% (FBS), with add some anti biotic include pen.-strep. With concentration 1% ([10]) .

Cytotoxicity Assay:

Human cervical cancer cells grown in microtiter plate (96wells) have been handled with a range of concentrations of each (esomeprazole, vincristine and methotrexate), cell density increases throughout the development period and also the cytotoxic effect of analytical agents strongminded once many incubation periods, [10] each milliliter contains 1X105 cells / ml, serum calf medium (10 percent) Used for seeding, plates were then incubated at 37CO for 24 hours to achieve cell attachment, followed by various serial dilution (1-10000 µg / ml) for esomeprazole, vincristine and methotrexate by victimization maintenance medium.

After twenty-four hours of incubation, cells were exposed (for each concentration tested, six replicates at 200µl), two hundred µl of maintenance medium in addition to each control group well, the days of exposure were twenty-four, forty-eight, and seventy-two hours. The cultures were wrapped with self-adhesive paper, then transferred to incubators and then colored cells with MTT stain. Each well's optical density was scanned via a micro-ELISA at a wavelength of 550 nm (17 ; 10) ; the measurement of the substance frequency by using the subsequent equation[11]

Statistical Analysis

The Statistical Analysis System (SAS) [18] used it to determine the outcome of varying parameter factors in the study.

Results and Discussion

Esomeprazole cytotoxic effect:

The findings of esomeprazole productivity inhibition of Hela cancer cells show an increase in productivity inhibition with an increase in the concentration of esomeprazole ranging from (10-10000) µg / ml, for every 3 incubation periods, except (1 µg / ml) wherever growth inhibition was significantly higher than 10 µg / ml to all incubation cycles, whereas there was no significant variation between the development reserve of (1 µg/ml) with (1000 µg/ml) for 24hr. and (1 µg/ml) with (100 µg/ml) for 48hr. and 72hr. incubation periods table (1).

Table (1) Effect of concentration and time in growth inhibition rate for Esomeprazole on Hela cancer cell line

Concentration (µg/ml)	Time (hr.)			LSD value
	24	48	72	
1	B 44.50 ± 7.03 a	A 32.46 ± 9.08 a	B 25.76 ± 6.87 a	26.754 NS
10	C 9.66 ± 2.28 a	B 6.86 ± 3.43 a	C 3.93 ± 1.45 a	16.012 NS

Cont... **Table (1) Effect of concentration and time in growth inhibition rate for Esomeprazole on Hela cancer cell line**

100	C 16.20 ± 1.10 a	A 14.90 ± 8.02 a	B 29.40 ± 3.14 a	17.356 NS
1000	B 35.30 ± 6.36 b	A 23.83 ± 2.45 b	A 89.66 ± 0.66 a	13.70 **
10000	A 83.63 ± 0.24 b	A 32.56 ± 2.31 c	A 88.76 ± 0.97 a	5.037 **
LSD value	13.845 *	20.198*	11.828 *	----
* (P<0.05).				

*Capital letter to compare among column means.

**Small letter to compare among row means,

Hormosis will explain the fluctuated growth inhibition of esomeprazole; hormetic result may be a common biological development in pharmacological medicine characterized by a reverse action of low concentrations compared with high concentration [16]. The carbon-based biochemical pathway by hormesis action aren't well clear, it's imagined that low amounts of poisonous ingredient might start the healing process in the body [6]. This compound might increase the activity of anticancer agent whereas others may forestall the carcinogenic effect of some genotoxic compound [9]

Several recommended mechanisms will explain the toxicity of esomeprazole on Hela cancer cells, because the ability of esomeprazole to induce a lysosomes membrane permeabilization, inflicting increase outflow of lysosomes contains to the cytoplasm, inflicting lysis of cellular elements then death, additionally to hydrolytic activity, the lysosomal enzymes also can offer acidic atmosphere that encompasses a role in killing tumor cells [8 ; 13 ; 15] . Another mechanism in vitro via a fast intracellular acidification and activation of many caspase enzyme [5], esomeprazole has ability to produce ROS in B cells neoplasm by many ways [4] .

Comparison among the cytotoxic effect of Esomeprazole, Vincristine and Methotrexate:

As shown in table (2) the manner of vincristine growth inhibition is dose and time dependent reflecting the fact of increase in vincristine cytotoxicity occurred with increase in time of its persistence inside cancer cells which occurred by several mechanisms including

mitosis blocking in metaphase, and blocks the ability of tubulin to polymerize to form microtubules binding by binding of microtubule protein, (tubulin) with esomeprazole then. Consist of Tubulin dimers that resulting from paracrystalline aggregates, leading to Spindle apparatus dysfunction, frozen in metaphase, impaired chromosomal segregation and cellular proliferation.

Permeabilization of lysosomal membrane is one of the vincristine anticancer mechanisms which Causing increase of lysosomes contains leakage to the cytoplasm and occurrence of cell death by the Degenerative activity of lysosomes contains and acidic conditions which equip by lysosomes acidic ambience.

When comparing between the growth inhibition of vincristine with esomeprazole as shown in table (4,5,6), the results establish the development reserve of esomeprazole was more than vincristine gro7wth inhibition on Hela cancer cells, especially at 24 hr. and 72hr. incubation periods . reflecting the ability of esomeprazole to enhance permeabilization of lysosome membrane was more than the vincristine ability , that may related to the development of resistance toward vincristine action by efflux of vincristine through the special transport system P-glycoprotein [20] leading to less in persistence time of vincristine inside cancer cells , producing less in antineoplastic activity of vincristine which including inhibition in microtubules formation , with less ability to induce lysosomes membrane permeabilization as compared with the inducing ability of esomeprazole , where esomeprazole has a superiorities on vincristine to induce lysosomes membrane permeabilization which mainly related to the less resistance development toward esomeprazole activity.

While the result of methotrexate. Growth inhibition as shown in table (3) the growth inhibition at 72hr. was less than the growth inhibition at 24 hr. especially at 10,100 and 1000 µg/ml, this result reflecting the developments of resistance toward methotrexate Cytotoxicity ,where there is several mechanism by

which cancer cells follow to resistance the cytotoxicity of MTX included (weakening in methotrexate moving into cells , making of altered form of DHFR that have a fewer affinity for the dysfunction , intracellular height of intracellular DHFR , by quality enhancement or modified quality guideline and diminished capacity to combine methotrexate polyglutamate) [7].

when comparing between the cytotoxicity of esomeprazole and methotrexate as shown in table (4,5,6) and, the result of comparison demonstrated the growth inhibition of esomeprazole was more than the growth inhibition of methotrexate , that may relate to less resistance development toward esomeprazole .

Table (2) Effect of concentration and time in growth inhibition rate for Vincristine on Hela cancer cell line

Concentration (µg/ml)	Growth inhibition			LSD value
	24 hr.	48 hr.	72 hr.	
1	C 12.00 ± 2.54 a	B 18.00 ± 2.54 a	B 20.00 ± 2.54 a	8.791 NS
10	BC 17.00 ± 1.73 b	B 19.00 ± 1.73 b	A 36.00 ± 3.46 a	8.476 *
100	BC 18.00 ± 4.04 b	B 23.00 ± 4.04 b	A 40.00 ± 4.04 a	13.985 *
1000	B 24.00 ± 4.73 b	B 25.00 ± 4.73 b	A 45.00 ± 5.19 a	16.932 *
10000	A 36.00 ± 3.17 b	A 45.00 ± 3.17 ab	A 47.00 ± 1.73 a	9.616 *
LSD value	10.758 *	10.758 *	11.343 *	----
(P<0.05), NS: Non-significant.				

Table (3) Effect of concentration and time in growth inhibition rate for methotrexate on Hela cancer cell line

Concentration (µg/ml)	Inhibition effect of Methotrexate on cell viability %			LSD value
	24 hr.	48 hr.	72 hr.	
1	C 0 ± 0.00 a	C 0 ± 0.00 a	C 0 ± 0.00 a	0.00 NS
10	B 17 ± 0.62 a	C 4 ± 0.06 b	C 0 ± 0.00 b	7.95 *
100	B 19 ± 0.59 a	BC 10 ± 0.37 b	C 0 ± 0.00 c	9.53 *
1000	A 30 ± 1.24 a	AB 14 ± 0.51 b	B 17 ± 0.55 b	7.69 *
10000	A 37 ± 2.49 a	A 19 ± 0.62 b	A 31 ± 1.07 a	7.85 *
LSD value	8.531 *	8.336 *	4.602 *	-
(P<0.05), NS: Non-significant.				

Table (4) Effect of Esomeprazole, methotrexate and Vincristine on Hela cancer cell line at 24hr .

Concentration (µg/ml)	Treatment			LSD value
	Esomeprazole	Vincristine	MTX.	
1	B 44.50 ± 7.03 a	C 12.00 ± 2.54 b	C 0.00 ± 0.00 b	14.942 *
10	C 9.66 ± 2.28 b	BC 17.00 ± 1.73 a	B 17.00 ± 1.73 a	6.688 *
100	C 16.20 ± 1.10 a	BC 18.00 ± 4.04 a	B 19.00 ± 3.46 a	10.86 NS
1000	B 35.30 ± 6.36 a	B 24.00 ± 4.73 a	A 30.00 ± 4.04 a	17.79 NS
10000	A 83.63 ± 0.24 a	A 36.00 ± 3.17 b	A 37.00 ± 2.30 b	7.85 *
LSD value	13.845 *	10.758 *	8.531 *	----
(P<0.05).				

Table (5) Effect of concentration and time in growth inhibition rate for Esomeprazole, methotrexate and Vincristine on Hela cancer cell line at 48hr .

Concentration (µg/ml)	Treatment			LSD value
	Esomeprazole	Vincristine	MTX.	
1	A 32.46 ± 9.08 a	B 18.00 ± 2.54 a	C 0.00 ± 0.00 b	18.850 *
10	B 6.86 ± 3.43 ab	B 19.00 ± 1.73 a	C 4.00 ± 1.15 b	14.335 *
100	A 14.90 ± 8.02 a	B 23.00 ± 4.04 a	BC 10.00 ± 2.30 a	18.52 NS
1000	A 23.83 ± 2.45 a	B 25.00 ± 4.73 a	AB 14.00 ± 4.04 a	13.36 NS
10000	A 32.56 ± 2.31 b	A 45.00 ± 3.17 a	A 19.00 ± 3.46 c	10.46 *
LSD value	20.198 *	10.758 *	8.336 *	----
(P<0.05).				

Table (6) Effect of concentration and time in growth inhibition rate for Esomeprazole, methotrexate and Vincristine on Hela cancer cell line at 72hr .

Concentration (µg/ml)	Treatment			LSD value
	Esomeprazole	Vincristine	MTX.	
1	B 25.76 ± 6.87 a	B 20.00 ± 2.54 a	C 0.00 ± 0.00 b	14.64 *
10	C 3.93 ± 1.22 b	A 36.00 ± 3.46 a	C 0.00 ± 0.00 b	9.761 *
100	B 29.40 ± 3.14 b	A 40.00 ± 4.04 a	C 0.00 ± 0.00 c	10.23 *
1000	A 89.66 ± 0.66 a	A 45.00 ± 5.19 b	B 17.00 ± 2.31 c	11.43 *
10000	A 88.76 ± 0.97 a	A 47.00 ± 1.73 b	A 31.00 ± 2.31 c	6.086 *
LSD value	11.828 *	11.343 *	4.602 *	----
(P<0.05).				

*Capital letter to compare among column means.

**Small letter to compare among row means,

Conclusion

This study prove that Esomeprazole have a promising anticancer activity against human cervical cancer cells with superiority above vincristine and methotrexate cytotoxicity.

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Evaluation of Salusin β in Patients with Prediabetes and Type 2 Diabetes Mellitus

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Abstract

Background: Type 2 Diabetes mellitus is a group of metabolic disease characterized by increase blood glucose level (hyperglycemia) resulting from either defect in insulin production by pancreas or increase insulin resistance that lead to decrease insulin effect in the body. prediabetes It is stage before diabetes mellitus including impaired fasting glucose (IFG)where the fasting glucose level (6.1-7.O mmol/L) and impaired glucose tolerance (IGT after glucose load) glucose level (7.8-11 mmol/L).**(salusin-β)** It is a newly glass of peptide it is synthesized in many body tissues, like (skeletal muscle, human vascular smooth muscle cells, and endothelial muscle) it have important role in Diabetes pathogenesis it has been reported to involve in vascular inflammation and the relation between salusin β and diabetes through endothelial injury, salusin β increase inflammation in diabetes. It have essential role in pathological endothelial dysfunction. **Method:** by using ELISA kit, we measured serum level of salusin β in 30 patients with prediabetes and 30 patients with type 2 DM compare with 30 healthy volunteers enlisted as normal controls. **Result:** serum salusin β level in patient with type 2 DM were significantly higher than prediabetes group and normal subjects. Salusin β in prediabetes group no significant higher than in healthy control subjects. **Conclusion:** Serum Salusin β level in patients with type 2 DM are expressed at significantly high levels were no difference in Salusin β level of prediabetes group and normal control subjects.

Keywords: *Salusin β, diabetes mellitus, prediabetes, ELISA.*

Introduction

Diabetes mellitus is a group of metabolic disease characterized by increase blood glucose level (hyperglycemia) resulting from either defect in insulin production by pancreas or increase insulin resistance that lead to decrease insulin effect in the body. Feature of hyperglycemia include polyuria, weight loss, polyphagia, polydipsia, unclear **.**

Prdiabetes It is stage before diabetes mellitus including impaired fasting glucose (IFG)where the fasting glucose level (6.1-7.O mmol/L) and impaired glucose tolerance (IGT after glucose load) glucose level (7.8-11 mmol/L), to forms of prediabetes in which increase glucose level more than normal range but below the renal threshold of diabetes level, the HbA1c range in the two. state . The presence of prediabetes is associated with the presence of β cell dysfunction and insulin resistance and about 5-10% of population are development to**.** Salusin β It is a newly glass of **.** salusin-β have special physicochemical properties, It is present in biological fluids such as human plasma , urine and vascular .the bioactive peptide (salusin-β) is synthesized in many body tissues, stomach, bone marrow, small intestine, thymus, lymph, salivary glands, adrenal medulla, adrenal cortex, spleen, liver, brain, skeletal muscle, testes, human vascular smooth muscle cells, and endothelial muscle. salusin β it have important role in the diabetes**.** it has been reported to involve in vascular inflammation and the relation between salusin β and diabetes through endothelial injury, salusin β increase oxidative stress, cardiac dysfunction and inflammation in diabetes. It have essential role in pathological endothelial**.**

The aim of the present study was to evaluate serum level of Salusin β in patients with type 2 DM , Prediabetes, and compare this group with control healthy group as well as to investigate whether it can be used as

a biomarkers for detection of diabetes.

Materials and Method

Study design case control study

The present study include 90 Iraqi participants (30 with type 2 DM, 30 with prediabetes, 30 normal healthy control group) the Age range (30-65)years, the age and gender matched to the patients and control group. Blood sample collected between September 2018 and april 2019. The following biochemical parameters have been studied. Salusin β, hs- CRP by ELISA method, fasting blood sugar (FBS), HbA1c, blood urea, serum creatinine, Lipid profile by colorimetric method by (cobas C111). Measure body mass index (BMI), and measure (WC). Fasting serum Samples were collected from patients and control by taking eight (8) ml of venous blood will be collected in plane tube (without anticoagulant). Blood are stand for 30 minutes then centrifuged in 1800 RPM for 15 minutes. Serums are transferred to new tube and keep in -20 C until assay.

Salusin ELISA

A total 100 µl serum was analyzed using a commercially available ELISA kit Salusin β (melsin) according to manufactures recommendations. Prepare all re a g e n t s before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate. Add standard: Set Standard wells, testing sample wells. Add standards 50µl to standard wells. Add 10µl of testing sample of sample wall .Then add 40µl of sample diluent to testing sample well, doesn't add anything to the Blank well. Add HRP-conjugate reagent 100µl to each well, covering with an adhesive strip , incubate for 60 minutes at 37°C. Aspirate each well and wash, repeating the wash 4 times for a total of 5 washes. Wash by filling each well with Wash Solution (400µl) using a squirt bottle, manifold dispenser or auto washer. Removal of liquid completely at each step is essential to good results. After the last wash, remove any remaining Wash Solution by decanting or aspirating. Invert the plate and blot it on a clean paper towels. Add 50µl of chromogen solution (A) and added 50µl of chromogen solution B to each well. mix Gently and incubate for fifty minutes (15) minutes at 37°C. and Protect from light. Add 50µl from Stop Solution to each well. The color should change from blue to yellow of the walls. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing. Read the Optical Density (O.D.) at wave lengths 450 nm and using a microtiter plate reader within 15 minutes.

Statistical Analysis

Statistical analysis was carried out by using SPSS version 20 and Microsoft excel 2013.the numerical data expressed as mean ± SD. Comparison between mean serum Salusin β concentration and (IFG, DM) groups were performed. Receiver Operating Characteristics (ROC) Curve was calculated to estimate the sensitivity and specificity of the used Salusin β.

Result

There was highly significant difference in mean Salusin-β among study groups ($P < 0.001$); the level was highest in diabetic patients with highly significant difference ($P < 0.001$) in comparison with that of both prediabetics and control subjects; however, there was insignificant difference between control and prediabetic groups ($P = 0.736$),

Table 1. Salusin-β in patients and control groups

Characteristic	Control		Prediabetic		Diabetic		P	P1	P2	P3
	Mean	SD	Mean	SD	Mean	SD				
Salusin-β (pg/ml)	5.12	1.15	5.27	0.83	7.80	2.47	<0.001 † HS	0.736 ¥ NS	<0.001 ¥ HS	<0.001 ¥ HS

n: number of cases; data were presented as either mean and standard deviation; †: One way ANOVA; ¥: post hoc LSD test; HS: highly significant at $P \leq 0.01$; NS: not significant at $P \leq 0.05$; S: significant at $P \leq 0.05$; *P1*: Control vs prediabetic; *P2*: Control vs DM; *P3*: Prediabetic vs DM

Result

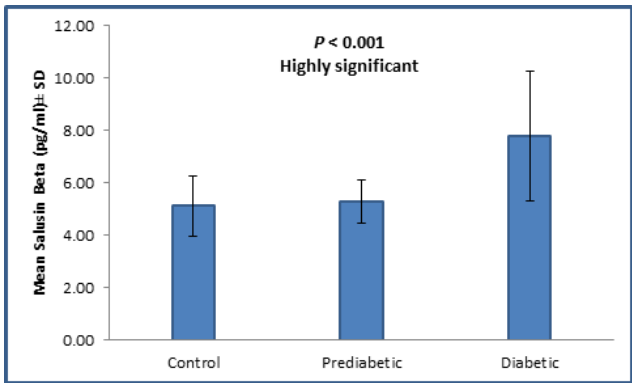

Figure 1. Bar chart showing the mean serum salusin beta in diabetics, prediabetics and control subjects

Because of the highly significant difference in the level of salusin-β in diabetic patients when compared to prediabetics and control groups, the authors of the current study suggested the presence of a cutoff value that can predict a diagnosis of diabetes with certain level of accuracy. For that reason, receiver operator characteristic (ROC) curve analysis was carried out and the results are shown in figure 3.1 and table 3.6. The cute off value was > 6.5 with an accuracy level of 88.2 %, a sensitivity level of 80 % and specificity level of 93.3 %, table 3.6.

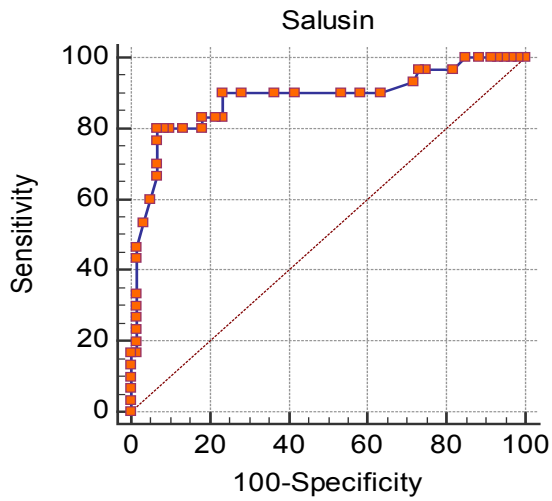

Figure 2. Receiver operator characteristic (ROC) curve to predict the Salusin β cutoff value with a diagnosis of diabetes mellitus

Table 2. Cutoff value , sensitivity and specificity of Salusin- β.

Characteristic	Salusin-β
Cutoff value	> 6.5
AUC (95 % CI)	0.882 (0.796 - 0.940)
Accuracy	88.2 %
P	<0.001
Sensitivity	80.00
Specificity	93.33

Discussion

. It is a peptide that present in endothelium tissue and in cell membrane it is play important role in endothelial function and pathogenesis of diabetes the increase level of salusin β in patients with type -2 diabetes mellitus related with the complication of diabetes due to endothelial injury **(7)**. In the present study found increase in Salusin β concentration in diabetes group and high significant deference between diabetes group and another two groups at p value (<o.oo1) and normal level of prediabetes and control group . And this agreement with study done by **(7)** salusin β was higher in level with high glucose (diabetes group). Salusin beta was positivelycorrelated to female gender There was no significant correlation among markers in all three study groups, as shown in table (3.3) Because of the highly significant difference in the level of salusin-β in diabetic patients when compared to prediabetics and control groups, the authors of the current study suggested the presence of a cutoff value that can predict a diagnosis of diabetes with certain level of accuracy. For that reason, receiver operator characteristic (ROC) curve analysis was carried out and The cute off value was > 6.5 with an accuracy level of 88.2 %, sensitivity level of 80 % and specificity level of 93.3 %, result show in table (3.6). Salusin beta was negatively correlated to age, positively correlated to FBS, positively correlated to HBA1c, negatively correlated to cholesterol and negatively correlated to LDL, in the present study the result show there was positive correlation between salusin-β and FBS. And no correlation between salusin-β and adiponectin, no correlation with lipid bound sialic acid and no correlation with lipid profile.

Table 3. Correlations of biochemical markers to demographic, disease characteristic lipid profile and renal function in prediabetic group

Characteristic	salusin (pg/ml)	
	r	***P***
Age	0.312	0.094
Gender	0.449	0.013*
BMI	0.171	0.365
Waste (cm)	-0.134	0.479
FBS	0.103	0.587
HbA1c	0.025	0.896
cholesterol (mg/dl)	-0.194	0.305
TG (mg/dl)	-0.206	0.276
HDL (mg/dl)	0.048	0.801
LDL (mg/dl)	-0.312	0.093
VLDL (mg/dl)	-0.205	0.278
UREA (mg/dl)	-0.122	0.52
creatinine (mg/dl)	0.223	0.237

* significant at $P \leq 0.05$; ** highly significant at $P \leq 0.01$

Conclusion

Salusin β was significant increase level in patients with type -2 diabetes mellitus as a compared with prediabetes and control healthy group with high sensitive and Specific of cutoff value for diagnosis type 2 DM, there was significant correlation between salusin β and FBS and HbA1c. no significant deference of salusin-β level in prediabetes and control groups**.**

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Vitamin D and its Association with the Severity and / or the Control of Asthma among Adult Iraqi Asthmatics

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Abstract

Background: asthma is one of the common chronic diseases with variable presentations and pathogenetic mechanisms. Vitamin D is thought to influence the expression as well as the severity, control and treatment responses in some asthmatic patients. Aim of study: to examine the possible association between asthma severity and level of control with vitamin D level in a sample of Iraqi adult asthmatics. **Method:** randomly selected 50 patients with asthma diagnosis were evaluated for medical factors of asthma harshness and control with measurement of 25-hydroxyvitamin D serum level (mean age ± SD: 48.5 ± 14.05 years, 30% males, FEV1:1.44 ± 0.63 L, 60 % severe, 60% poorly controlled). **Results:** most of asthmatic patients (96%) were found to have vitamin D level of less than 30 ng/ml (17.66 ± 7.06 ng/ml) and this was related to severity of airway obstruction (mild: 28.71± 5.52 ng/ml, moderate: 19.9 ± 4.56 ng/ml, severe: 13.81 ± 4.22 ng/ml, $p < 0.001$) as well as control of asthma (controlled: 27.13±6.12 ng/ml, partly controlled: 20.62±4.21 ng/ml, uncontrolled: 17.66±7.06 ng/ml, $p < 0.001$). In addition, the 25(OH)D level was significantly associated with history of ER visits and hospitalizations. No significant association between the gender, age, BMI, the presence of allergic rhinitis and the status of vitamin D level. **Conclusions:** low levels of 25(OH)D were common in this sample of Iraqi adult asthmatics and was most prevalent and severe in those with severe and/ or uncontrolled asthma. This finding supports the hypothesis of possible vitamin D role in asthma.

Key words: *vitamin D, severity and / or the control, asthma, Iraqi asthmatics.*

Introduction

asthma is a common chronic illness with growing prevalence in many parts of the world for many years. More than 300 million persons from all ages, and different ethnics, grieve from asthma and its burden on patients, the families, and economy of the health authorities and is increasing internationally. It is defined by "a history of symptoms including wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation". (1)

It has been suggested that there is heterogeneity in asthma expression and phenotypes and these respond differently to treatment options which suggests different pathogenic mechanisms. In most asthmatics, i.e. the classical type, the symptoms are well controlled by beta 2 agonists and inhaled corticosteroids. However, some phenotypes of asthma do not exhibit similar response to corticosteroids and those patients are labelled as steroid – refractory and are challenging to manage clinically. (2-6)

Vitamin D affects the pathophysiology of asthma by effect on several mechanisms. It may help in maintaining the immune homeostasis especially the innate immunity by the direct stimulation of the expression of the gene responsible for the production of the antimicrobial peptide cathelicidin, which is known to be active against viruses, bacteria and fungi, and its deficiency may predispose to infections and consequent asthma exacerbations.(7)

In addition, vitamin D can reduce inflammation and smooth muscle proliferation by inhibiting the synthesis and release of cytokines related to the Th1 immune response and it may decrease the expression of TNF-α which lead to enhanced inflammatory response in asthmatics. In addition, it has been suggested that the level of 25(OH)D may facilitate steroid response in asthmatics. (8). The purpose of study is to find any link between vitamin D level and the severity and / or the control of asthma in a sample of adult Iraqi asthmatics.

Method

This is a cross sectional study of randomly selected 50 patients (35 females and 15 males) with asthma who regularly attend the respiratory medicine outpatient clinic at Al-Imamein Kadhimein Medical City. Patient interviewing and testing period was from the first of December 2014 till 31st of December 2015. **Inclusion criteria:** confirmed asthma diagnosis, age 18 years and above

Exclusion criteria: presence of smoking history, use of supplemental vitamin D, presence of co-morbid chronic condition like chronic renal failure, diabetes mellitus, heart failure and use of prolonged daily systemic steroids in the last year.

Asthma severity and control: the level of control and asthma severity were assessed via the simple questions suggested by Global Initiative for Asthma (GINA)[1] by asking about the frequency of daytime symptoms, night symptoms, use of rescue inhalers and limitation of activity. Patients were grouped as well controlled, partly controlled and poorly controlled. Patients were grouped according to the forced expiratory volume in one second (FEV_1) percentage of predicted into mild intermittent/persistent (≥ 80% predicted), moderate persistent (≥60% but <80% predicted) and severe (<60% predicted). [13]

Spirometry: according to the American Thoracic Society guidelines [14], by a practiced operator using Masterscreen system (Jaeger Co., Höchberg, Germany) at the pulmonary function test laboratory in Al-Imamein Kadhimein Medical City. FEV_1 and forced vital capacity (FVC) were recorded. For those with FEV_1 of less than 70% predicted, reversibility testing using nebulized salbutamol 2.5 mg for 5 minutes and repeating the spirometry testing after 5 minutes was done. Reversibility was documented with both 200 ml increase in FEV1 and 12% increase in FEV1.

Vit D level assessment: vitamin D level was determined using Vidas® 25 OH Vitamin D Total test (bioMérieux SA, USA) according to the manufacturer recommendation. Levels of < 20 ng/ml considered deficiency, 20 – 29.9 ng/ml considered insufficiency and ≥ 30 ng/ml were considered as sufficient according to the previous recommendations. [14]

Other measurements: height and weight were recorded by a trained personnel during spirometry and the body mass index was calculated using the standard equation weight (kg)/height $(m)^2$.

Statistical Analysis : done by SPSS 22 , data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). Chi square test was used for categorical variables and Pearson's Correlation two tailed test was used for parametric variables. P value was equal or less than 0.05 mean significant results.

Results

50 patients involved in the study, mean age was 48.5 ± 14.05 years (range 19 – 72). There were 35 (70%) females and 15 males (35%). Body mass index (BMI) was 30.72 ± 7.86 (range 15.79 - 53.69). The mean level of vitamin D was (17.66 ± 7.06) ng/ml and range from 8.1 – 39.80 ng/ml. The FEV1 mean was 1.44 ± 0.63 L with a range from 0.53 to 3.7 L. as in table (1).

Table 1: Age, BMI, FEV1, FVC and 25 (OH) D level in relation to gender (mean ±SD)

Variable	Female	Male
Age (mean ± SD) years	48.17 ± 14.56	49.27 ± 14.83
BMI (mean ± SD)	32.76 ± 7.90	25.94 ± 5.4
FEV1 (mean ± SD) L	1.33 ± 0.46	1.69 ± 0.88
FVC (mean ± SD) L	2.07 ± 1.72	2.34 ± 0.98
25(OH) level(mean ± SD) ng/ml	17.01 ± 6.75	19.16 ± 7.77

In this study, only 8 patients (16%) had mild persistent asthma, and only 9 patients (18%) had well controlled asthma. Figures 2 and 3 display the control and severity of asthma among this study sample respectively. Regarding treatments used for patients, 34 patients (68%) were using beta agonist inhalers, 19 (38%) were on inhaled corticosteroids (ICS) and 17(34%) on combination ICS and long acting beta agonist (LABA). There was no significant association between the treatment use and the level of 25(OH)D. There is a significant positive correlation between the level of 25(OH)D and FEV1, $r(50) = 0.772$, $p < 0.001$. There was negative significant correlation between the level of vit D and the number of emergency visits, $r(50) = -0.749$, $p < 0.001$. Other correlations are shown in table 2.

Table 2 - Correlations of parametric variables with 25(OH)D level

25(OH)D correlations		
Variables	**Pearson's Correlation (r)**	**Significance (p)**
Age	-0.109	0.452
BMI	0.195	0.174
FEV1	0.772	<0.001
FVC	0.224	0.118
FEV1/FVC	0.557	<0.001
Emergency Visits	-0.749	<0.001
Hospitalizations	-0.465	0.001

The level of asthma control was found to be significantly related to the severity of asthma ($p < 0.001$) with 60 % (n=30) of patients having poorly controlled asthma, of whom 86.7% (n=26) having severe persistent asthma (Figure 1). In addition, the severity of asthma was significantly related to the status of vitamin D ($p < 0.001$) with 81.8% (n=27) of patients with severe asthma having deficiency of 25(OH) (level < 20 ng/ml) (Figure 2). Moreover, the level of control was also significantly related to the status of vitamin D ($p < 0.001$) with 96.7% (n= 29) of poorly controlled asthmatics having deficiency of vitamin D (Figure 3).

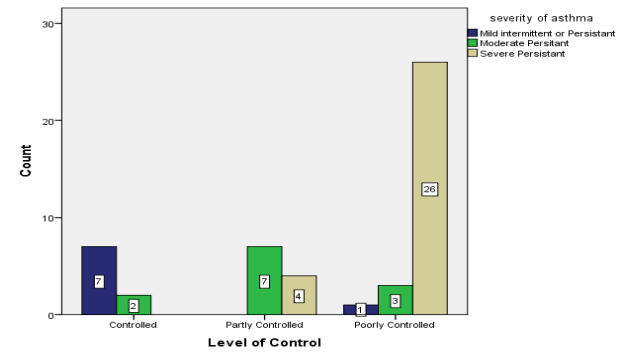

Figure 1: Level of asthma control and its severity

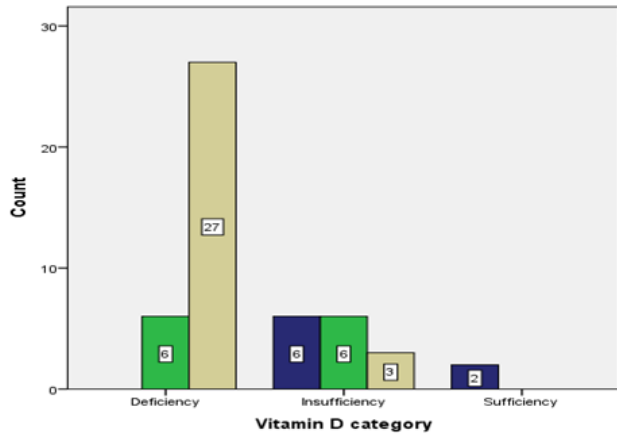

Figure 2: Level of asthma severity and Vitamin D status

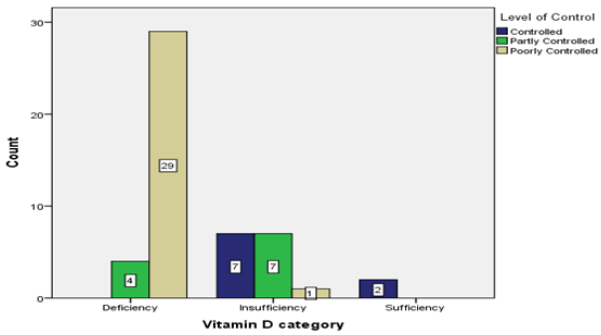

Figure 3: Level of asthma control and Vitamin D status

Mean and SD of levels of 25(OH)D, FEV1, FVC, FEV1/FVC according to the level of asthma severity and control are presented in tables 3 and 4.

Table 3 - means, standard deviations of 25 (OH) D level, FEV1, FVC, FEV1/FVC and patient numbers in relation to the level of asthma control.

Asthma Control Level	25 (OH) level (ng/ml)	FEV1(L)	FVC (L)	FEV1/FVC
Controlled (n=9)	Mean ± SD			
	27.13±6.12	2.1±0.68	2.51±1.01	85.57±10.63
Partly controlled (n=11)	Mean ± SD			
	20.62±4.21	1.67±0.55	2.12±0.59	74.69±8.72
Poorly Controlled (n=30)	Mean ± SD			
	17.66±7.06	1.15±0.45	2.02±1.87	67.00±13.63

Table 4 - means, standard deviations of 25 (OH) D level, FEV1, FVC, FEV1/FVC and patient numbers in relation to asthma severity

Asthma Severity	25(OH)D(ng/ml)	FEV1 (L)	FVC (L)	FEV1/FVC
Intermittent / Mild Persistent	28.71± 5.52	2.2 ± 0.68	2.5 ± 1.06	89.72 ± 9.77
Moderate Persistent	19.9 ± 4.56	1.7 ± 0.54	2.4 ± 0.66	72.97 ± 7.87
Severe Persistent	13.81 ± 4.22	1.1 ± 0.36	1.9 ± 1.85	66.94 ± 13.0

There was no significant association between the age, gender, BMI, the presence of allergic rhinitis and the status of vitamin D level.

Discussion

In this study, high prevalence of vitamin D deficiency and inadequacy among this sample of Iraqi adult asthmatics (96%, n=48). This high ratio is similar to that stated in Chinese (90%) and Costa Rican (90%) adult asthmatics but is higher than that recorded in German adult asthmatics (64%). [12-14] However, this percentage is much higher than that seen in childhood asthma reported in previous studies; 20% in Iranian, 28% in Costa Rican, 38% in Egyptian, and 44% in Puerto Rican childhood asthmatics. [15-18]. These discrepancies between adults and children asthmatics may be related to behavioral variables seen in areas with plenty of sunny days as by avoiding sunlight, dress habits and racial difference in skin color. Importantly, in Iraq the cultural and religious background may play a role in limited sun exposure. Other variables for low vitamin D level like being overweight or female gender can also play a role but there was no significant association. In this study, the level of vitamin D was not associated with markers of allergy; namely the presence of allergic rhinitis used in our study. This lack of association of vitamin D level and markers of allergy has been described in some studies. [9,12,13] However, such associations have been reported in other studies [10,14,15,18] These contradicting results may be related to the different populations studied, the difference in allergen exposure between regions and the variability in outdoor exposure to sun in different parts of the world. These findings need cautious interpretation since the sample size is small and the other parameters of allergic response (like IgE level, sputum eosinophilia, allergen specific IgE antibodies, IL-4, and IL-5) have not been studied in this study. The major result of this study was the presence of significant correlation between the levels of vitamin D and the FEV1, FEV1 percent of predicted (as a marker of asthma severity), and FEV1/FVC ratio (as a marker of airway obstruction), $p < 0.001$ for all parameters. This finding has been recorded in most of the studies in both adults and children. [11-19]. Moreover, vitamin D level was significantly associated with the level of asthma control, patients with higher level of vitamin D tend to have better asthma control (27.13±6.12 in controlled, 20.62±4.21 in partly controlled and 17.66±7.06 in uncontrolled, $p < 0.05$). This finding was also observed in a cross-sectional studies in Italian children and German adults. [11,14]

In this study, the risk of emergency department visits and hospitalizations were found to be significantly associated with vitamin D level, $p < 0.001$ for both. These findings are consistent with the results obtained from other studies, both in adults and children. [9,10, 12-14, 16-18]

However, a causal relationship between asthma and vitamin D deficiency cannot be established due to the sample size and cross-sectional project. In specific, the possibility of reverse causation cannot be eliminated as the patients with severe, poorly controlled asthma are more likely to be home bound with less exposure to sun and may be exposed to frequent steroid rescue courses that might have induced the deficiency state in vitamin D. Additional issue is the selection bias as there was unbalanced male to female ratio (30% males vs. 70% females). However, comparable gender inequality in asthma have been recorded in previous studies conducted on Iraqi adult asthmatics. [19, 20] Also This study was conducted in a tertiary center and in a specialized respiratory unit, which may not reflect the overall population of asthmatics in Iraq. Additional limitation of this study is the absence of control group of well persons to regulate that the level of vitamin D has a solid association with asthma or this observation of low vitamin D level is just a coincidence.

Conclusion

this study demonstrates that a low level of vit D is related with clinical parameters of poor asthma control plus severe asthma as predicted FEV1.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Determination *Enterococcus faecalis* in Asymptomatic Urinary Tract Infection Associated with Diabetes Type 2 Patients in Suwayrah General Hospital-Iraq

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Abstract

Background: Asymptomatic Urinary Tract Infection is common in diabetes type-2 patient and predisposes these patients to more severe urinary tract infections (UTIs). Enterococci have been documented to cause infection of the urinary tract and other sites. Although several species of Enterococcus have been recognized, *Enterococcus faecalis* has been considered one of the major agents of urinary tract infections.

Objectives: To determine the percentage *Enterococcus faecalis* of ASB , antimicrobial resistance in diabetics and non-diabetics in urine sample and detection of enterococcal surface protein (*esp*) gene .

Method: The study involved a total of 611 participants including 197 diabetes mellitus patients and 414 non-diabetics in Suwayrah-Wasit-Iraq. Mid-stream urine was collected from consented subjects and dipstick , general urine examination and bacterial culture were conducted for each sample. Isolates were identified using standard biochemical tests and VITEK-2 system .

Results: All collected samples are asymptomatic bacteriuria. There was a significant difference in the percentage of asymptomatic bacteriuria (ASB) between diabetics and non-diabetics ($P = 0.010$). There was no significant difference in resistance between diabetics and non-diabetics ($P > 0.05$). *Enterococcus faecalis* isolates showed (50%) resistance to Erythromycin and Levofloxacin. The *esp* gene was observed in all *Enterococcus faecalis* isolates .

Conclusion: The females of age more than 60 years old have the higher percentage of asymptomatic UTI. Erythromycin and Levofloxacin were more resistance in Enterococci isolated .

Keywords: *Diabetes mellitus, percentage, asymptomatic bacteriuria, antimicrobial resistance.*

Introduction

Diabetes type-2 has been recently associated with urinary tract infections. The mechanism of pathogenesis for this association is not fully elucidated , however, it is suggested that high glucose concentration in urine may favor the growth of pathogenic microorganisms and stimulate urinary tract infection[(1)] . Asymptomatic bacteriuria is a form of UTI characterized by the presence of significant amount ($>10^5$ cfu/ml) of bacteria in urine [(2)].

Several studies have documented the association of asymptomatic UTIs with diabetes; however, reports on the prevalence of ASB appear contradictory [(3)].

Most studies reported the percentage to be higher in people with diabetes than people without diabetes (4). On the contrary, another study reported no significant difference in the percentage of ASB between diabetic and non-diabetic ($P > 0.05$) [(5)], others report that *Enterococcus spp*. are more often associated with UTI among diabetics and cause 13% of asymptomatic bacteriuria in diabetics compared to 4.9% in non-diabetics [(6)].

The incidence of urinary tract infections due to *Enterococcus faecalis* has risen steadily over the years and *Enterococcus faecalis* urinary tract infection now outnumbers *Enterococcus faecium* urinary tract infection 5:1 [(7)].

Recent results indicate that *Enterococcus faecalis* can not only adhere to epithelial cells in the urinary tract, but also invade cells, leading to formation of intracellular bacterial communities in the bladder. The combination of these multiple virulence mechanisms, the ability to survive in harsh conditions, and both intrinsic and acquired resistance to many antibiotics explains the high frequency of infection by *Enterococcus faecalis* [8].

The enterococcal surface protein (Esp) is a high-molecular-weight surface protein of unknown function whose frequency is significantly increased among infection-derived Enterococcus faecalis isolates. Esp is another surface protein and has been shown to promote colonization and persistence in bladder (but not kidneys) Esp also influences, at least in some strains, biofilm formation in vitro [9].

Method

This work has applied on 611 asymptomatic UTI patients (197 of them had diabetes mellitus and 414 were non-diabetic), 208 males and 403 females, their ages ranged from 20 – 70 years old attended to emergency and consulting unite in the Suwayrah general hospital in Wasit province during the period from November 2018 to October 2019. Out of 197 asymptomatic UTI diabetic patients, seven showed *Enterococcus faecalis* culture results. And five out of 414 asymptomatic non-diabetic patients showed *Enterococcus faecalis* culture results which considered as a control group.

The study participants were educated on how to collect a "clean-catch" midstream urine specimen and the importance to avoid contamination. They were advised on washing of hands prior to collection and labia separation, especially in females. Participants who had difficulties collecting their specimens were assisted by trained personnel. Urine samples were collected into sterile containers, placed in a cool box (0 ^{o}C) and transported to the laboratory within 2 hours. The samples were then stored in a refrigerator and analysed within 8 hours of collection. Each sample was separated into two parts under sterile conditions; one part for urinalysis and the other for culture. The dip stick test was performed using urine test strips as described by the manufacturer (Condor-Teco, Beijing, China). Wet preparation of centrifuged urine was observed using 40 times objective lens to detect blood, pus and other cells. A calibrated 10µl wire loop was used to inoculate uncentrifuged urine into MacConkey, blood and plates incubated aerobically at 37^{o}C for 24 hours. Significant ASB was defined as urine culture of > 10^5 cfu/ml without symptoms of cystitis. Isolates were identified using standard biochemical techniques and VITEK-2 System .

Antimicrobial susceptibility testing was performed with the automated VITEK-2 compact system by using AST-P580 cards. These included; Levofloxacin ,Erythromycin , Linezolid ,Teicoplanin ,Vancomycin ,Tetracycline ,Tigecycline and Nitrofurantoin (BioMérieux , France).

The PCR technique was also used detection virulence gene of *Enterococcus faecalis* after DNA Extraction from all bacterial isolates.

Results

Of the 611 participants enrolled into the study 208 were males while 403 were females. Majority of participants were in the age group more than 60 years , while the least age range was less than 40 years as showed in Table (1). The overall percentage of ASB in this study was (1.96 %). There was a significant difference in the percentage of ASB between diabetics and non-diabetics (P = 0.010) .

Table 1 shows the distribution of ASB with respect to age and sex. There was no significant difference in the distribution of ASB with age (P = 0.357) among the study participants. However, women demonstrated a higher percentage than men in both diabetic and non-diabetic groups.

Table 1: Distribution of *Enterococcus faecalis* asymptomatic UTIS with respect to age and sex .

Age / Sex	No.	Diabetics with ASB No. (%)	Non-diabetics with ASB No. (%)	Total with ASB No. (%)
20-30 year	102	0(0 %)	0(0 %)	0(0 %)
30-40 year	130	1(0.77 %)	1(0.77 %)	2(1.54 %)
40-50 year	121	1(0.82 %)	1(0.82 %)	2(1.64 %)
50-60 year	120	2(1.66 %)	1(0.84 %)	3(2.5 %)
60 +	138	4(2.89 %)	1(0.72 %)	5(3.62 %)
X2,P value		4.681 , 0.322 (NS)	0.823, 0.935 (NS)	4.379, 0.357 (NS)
Male	208	2(0.96 %)	1(0.48 %)	3(1.44 %)
Female	403	6(1.48 %)	3(0.74 %)	9(2.23 %)
X2,P value		0.295, 0.587 (NS)	0.147, 0.702 (NS)	0.446, 0.504 (NS)
Overall Percentage	611	8\197 (4.06 %)	4\414 (0.96 %)	12\611 (1.96 %)

X^2 chi-square value , NS : Non-significant difference (P >0.05) , ASB : Asymptomatic Bacteriuria .

All *Enterococcus faecalis* isolates were (100%) sensitive to Linezolid ,Teicoplanin ,Vancomycin ,Tetracycline ,Tigecycline and Nitrofurantoin antibiotics while they were (50%) resistant to Levofloxacin and Erythromycin in both diabetes and non-diabetes patients as showed in Table (2).

Table 2: Antibiotic resistance of urinary *Enterococcus faecalis* in diabetics and non-diabetics

Antimicrobial agent	Diabetics	Non-Diabetics
Levofloxacin	4(50%)	2(50%)
Erythromycin	4(50%)	2(50%)
Linezolid	0(0 %)	0(0 %)
Teicoplanin	0(0 %)	0(0 %)
Vancomycin	0(0 %)	0(0 %)
Tetracycline	0(0 %)	0(0 %)
Tigecycline	0(0 %)	0(0 %)
Nitrofurantoin	0(0 %)	0(0 %)

enterococcal surface protein (*esp*) virulence gene of *Enterococcus faecalis* was detected in all 12 *Enterococcus faecalis* isolates showed in Figure (1).

Figure (1) : Agarose gel electrophoresis (1.5%) showing PCR product of a partial fragment of *esp* gene under U.V. light after staining with ethidium bromide. M: 100 bp marker; lane 1-12: *Enterococcus faecalis* isolates. The size of product is 887 bp.

Discussion

The association of diabetes mellitus and urinary tract infections is increasingly being reported. Especially, infection by Enterococcus bacteria. Asymptomatic bacteriuria is common among diabetic patients and may lead to serious complications if not properly managed [(10)]. ASB has been identified as a risk factor for acquiring symptomatic UTIs especially in diabetic women [(11)]. UTIs are more severe in diabetic patients involving life-threatening complications such as emphysematous pyelonephritis and renal papillary necrosis. The present study revealed an overall percentage of ASB of 1.96%; 4.06% in diabetics and 0.96% in non-diabetics (table 1).

This result is concurrent with that of earlier reports which recorded prevalence of high percentage in diabetics and low percentage in non-diabetics [(13)]. In other earlier reports which recorded percentage low percentage in diabetic and high non-diabetic [(14)]. Consequently, the issue of prevalence of ASB remains debatable. This inconsistency has been attributed to variations in sample size, geographical location, culture or screening method. In the present study, ASB was significantly higher in diabetics than non-diabetics (P = 0.010). This is in line with majority of previous reports [(12)]. however, it is suggested that high glucose concentration in urine may favor the growth of pathogenic microorganisms and stimulate urinary tract infection [(15)]. The females are more susceptible to UTI than males, because their urethra is shorter and closer to anus more than in males, so that the intestinal flora which may ascend to urinary tract and cause UTI [(17)]. The most affected age group in females were these of more than 60 years , it may be due to Estrogen deficiency , diabetes mellitus , gynaecological diseases (cystocele) and operations, urological diseases (incontinence, residual urine, cystopathy) , operations , immunological changes and diabetic cystopathy [(18)].

Results of the antibiotic susceptibility test revealed no significant difference (P>0.05) in the resistant

pattern between diabetics and non-diabetics (Table 2). Resistance to Levofloxacin and Erythromycin may be due its frequent use in our study area to treat UTIs and other infectious diseases [16].

In figure (1) , shows all isolated have *esp* gene , the ability of enterococci to adhere the epithelial cells of urinary tract is a key initial step in UTI pathogenesis due to encoded by the *esp* gene facilities colonization and persistence of *Enterococcus faecalis* in urinary tract infections [19].

Conclusion

The study revealed diabetic patient have a higher percentage of asymptomatic UTI than non-diabetic patients. Females of age more than 60 years old have the higher percentage of asymptomatic UTI. Erythromycin and Levofloxacin were more resistance in Enterococcus isolated . Therefore the need to speed up sensitization against antibiotic abuse in Suwayra -Wasit – Iraq.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Oral Desmopressin as an Add-on Therapy for Men with Benign Prostate Hyperplasia they Suffering from Persistent Nocturia

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Abstract

Background: men with benign prostate hyperplasia suffer from urinary tract infection especially in lower part of the tract and common manifestation of this infection is nacturia. Treatment of nocturia depends mainly on α-blockers; however, these drugs have limited success in relieving this symptom. **Aims:** This study aims to assess the efficacy of desmopressin (0.2mg) as an add-on therapy with α-blockers in relieving refractory nocturia for men with BPH. **Patients and Methods**: Inclusion criteria for this prospective, non-randomized, open-label trial included men ≥60 years old with LUTS due to BPH; a total IPSS of ≥14 points; and persistent nocturia with ≥2 voids/night despite using α-blockers for at least 10 weeks prior to inclusion. Patients with diabetes mellitus, known neurogenic bladder dysfunction, uncontrolled hypertension, congestive heart failure, and those using diuretics were excluded from the study. Fifty one patients were included and categorized into two groups; those who continued on α-blocker treatment (n=22), and those for whom oral desmopressin (0.2 mg) was used as an add-on therapy (n=29). After four weeks of treatment, the International Prostate Symptom Score (IPSS), storage and voiding subs-scores, nocturia episodes, maximal flow rate ($Q_{max)}$ and bother score were measured and compared with their respective baseline values. **Results:** there were no difference significantly between the two groups in all measured demographic and clinical parameters (all *p*-values >0.05). After four weeks of treatment, the total IPSS, storage sub-score, nocturia episodes, and bother score were all significantly better in the group were desmopressin add-on (P-values ≤0.05). No significant differences were observed in the voiding sub-score and Q_{max} value between the two groups (both *p*-value >0.05). **Conclusion:** adding desmopressin to α-blockers is active therapy for men with BPH and suffering nocturia and no initial response to α-blockers.

Keywords: *nocturia, desmopressin, benign prostate hyperplasia, lower urinary tract infection*

Introduction

Benign prostatic hyperplasia is one of the most common causes of lower urinary tract symptoms (LUTS) in ageing men. BPH can cause nocturia via different mechanisms including detrusor overactivity and a high post void residual (PVR), both of which can result in decreased voiding volume and frequent voiding [(1)]. However, LUTS is not the sole cause of nocturia. Rather, nocturia is considered as a multifactorial condition and five main causes have been identified including nighttime polyuria, worldwide polyuria, decrease bladder volume, sleep problems and circadian clock illnesses [(2)]. It is very common to find a patient with more than one of these factors involved [(3)]. Nocturia is a critical condition that necessitates clinical attention. Persistent nocturia can result in chronically disturbed sleep, with a negative effect on patient's class of life (QoL) and general health. In elderly, frequent voiding during night can particularly expose the patients to slipping and fractures [(4)]. Moreover, the impact of nocturia extends beyond being a medical issue. For example, the annual direct cost of managing nocturia in Germany, including the costs of medical consultations, investigations, medicines and treatments of falls and fractures, is about 2.32 billion euros and the annual indirect costs, by loss of labor due to diminished efficiency or absence at the workplace, is about 20.76 billion euros [(5)]. The treatment approaches for nocturia usually depends on pharmacological therapy

that is assigned principally for BPH, namely α-blockers, 5α-reductase inhibitors, and anticholinergics. However, in most cases with BPH, using these drugs is not associated with satisfactory results in reducing voiding frequency [6]. Thus, this study aims to assess the efficacy of desmopressin (0.2mg) as an add-on therapy with α-blockers in relieving refractory nocturia for men with BPH.

Patients and Method

A prospective study for four weeks single center, open-label, non-blinded comparative study. Inclusion criteria were men ≥60 years old with LUTS due to BPH; a total IPSS of ≥14 points; and persistent nocturia with ≥2 voids/night despite using α-blockers for at least 10 weeks prior to inclusion. Patients with diabetes mellitus, known uncontrolled hypertension, neurogenic bladder dysfunction, congestive heart failure and those using diuretics were excluded from the study. From February 2018 to February 2019, patients who attended the Urosurgery department at Al-Imamain Al-Kadhumain Medical City in Baghdad were assessed for inclusion. After excluding ineligible cases, a total of 51 patients were included and assigned into two groups, those who remained on an α-blocker (n=22), and those who received oral desmopressin 0.2 mg add-on therapy with an α-blocker (n=29). All included patients were assessed at baseline and after four weeks for IPSS, including storage and voiding sub-scores, as well as bother scores and Qmax levels. A consent form explaining the aims of the study was obtained from all patients. The study was approved by the ethical committee of Al-Imamain Al-Kadhumain Medical City. **Statistical Analysis:** Statistical Package for Social Sciences (SPSS) was used for data analysis. Paired student t-test was used to compare means of total IPSS, voiding and storage sub-scores, and Q_{max} at baseline and 4 weeks after the onset of treatment within the same group, while independent t-test was used to compare the means between the two groups. A p-value of ≤ 0.05 was considered as significant.

Results

Baseline Characteristics of the Patients: continuous data were subjected for normality test (Shapiro Wilk test) and were found to be normally distributed. The mean age and stander deviation of the patients on α-blockers alone is (63.95 ±10.15) years compared to (62.43±10.72) years for those patients using desmopressin add-on with no significant difference (P-value = 0.607). Likewise, no significant differences between the two groups in IPSS total score, storage and voiding sub-scores, nocturia episodes, bother score, PSA level, prostate size and Q_{max} (all P-values >0.05) (Table 1).

Table 1: Baseline characteristics of patients

Variables	α-blocker alone (n=22)	Desmopressin add-on (n=29)	p-value*
Age (years)	63.95±10.15	62.43±10.72	0.607
IPSS total score	24.18±3.69	25.75±4.94	0.221
IPSS voiding sub-score	11.8±1.8	12.1±2.4	0.306
IPSS storage sub-score	13.1±1.9	13.65±2.6	0.903
Nocturia episodes	3.95±0.78	4.0±0.75	0.835
Bother score	4.64±1.0	4.52±1.05	0.685
PSA (ng/ml)	1.7±1.0	1.66±0.98	0.874
Prostate size (cm3)	52.5±13.86	54.11±12.97	0.675
Qmax (ml/s)	17.68±5.54	15.59±4.32	0.135

Data presented as mean ± SD; IPSS: international prostatic symptom score;
PSA: prostate specific antigen; Q_{max}: maximum urine flow rate
*Using independent sample t-test.

Effect of desmopressin add-on on IPSS scores, bother score, and Q_{max}:

As seen in table (2), when each treatment group was assessed separately before and after treatment, the total IPSS, storage sub-score, voiding sub-score, bother score, and nocturia episodes were significantly lower following treatment in both groups (all p-values ≤0.05). However, Q_{max} values were significantly higher only in the desmopressin add-on group (p-value=0.025). In a head-to-head comparison for the same parameters following four weeks of treatment (table 2), the mean IPSS score for the desmopressin add-on group (14.07±3.66) was significantly lower than that of α-blocker alone group (17.09±4.5), with P-value of (0.012). Similarly, desmopressin add on-treated group had a better IPSS storage sub-score compared to α-blocker alone group (6.92±1.71 vs. 7.98±2.87, P-value 0.018), with a significantly lower bother score (2.83±0.96 vs. 3.91±1.19, P-value 0.001) and a significantly less nocturia episodes (1.59±0.82 vs. 3.32±0.99, p-value 0.0001). On the other hand, there was no significant difference between the two groups in the voiding sub-score (P-value 0.366) and Q_{max} (P-value 0.074).

Table (2): Effects of desmopressin add-on on IPSS scores, sub-scores, and Q_{max}

Variables	**Status**	**α-blocker alone**	**Desmopressin add-on**	**$p^{(a)}$-value**
IPSS total score After treatment	Baseline	24.18±3.69	25.75±4.94	0.012
	17.09±4.5	14.07±3.66		
$P^{(b)}$-value		< 0.001	< 0.001	
IPSS voiding sub-score After treatment	Baseline	11.8± 1.8	12.1±2.4	0.336
	9.12±2.7	7.15±2.2		
$P^{(b)}$-value		<0.001	<0.001	
IPSS storage sub-score After treatment	Baseline	13.1± 1.9	13.65±2.6	0.018
	7.98±2.87	6.92±1.71		
$P^{(b)}$-value		<0.001	<0.001	
Nocturia episodes	Baseline	3.95±0.78	4.0±0.75	0.0001
	After treatment	3.32±0.99	1.59±0.82	
$P^{(b)}$-value		0.001	0.0001	
Bother score	Baseline	4.64±1.0	4.52±1.05	0.001
	After treatment	3.91±1.19	2.83±0.96	
$P^{(b)}$-value		0.014	0.0001	
Q_{max} (ml/s) After treatment	Baseline	17.68±5.54	15.59±4.32	0.074
	16.91±3.2	18.35±5.58		
$P^{(b)}$-value		0.289	0.025	

IPSS: International Prostate Symptom Score; Q_{max}: maximum flow rate

$p^{(a)}$: Designed using independent t-test; $p^{(b)}$: Designed using paired t-test

Discussion

For greatest knowledge, this study is the first study that show usage of desmopressin in management of patients with benign prostate hyperplasia and suffering from nocturia. The current results confirms the efficiency of desmopressin as an add-on therapy in the alleviation of nocturia through reducing the total IPSS, storage sub-score, nocturia episodes, and bother score after four weeks of treatment compared to those treated with an α-blocker alone. Several previous studies have reported similar findings.

Shin et al. (2014) enrolled 405 men with persistent nocturia due to BPH in a large prospective randomized trial to investigate the role of desmopressin (0.2 mg) as add-on therapy in reducing LUTS in those patients. It was found that the number of episodes of nocturia, nocturnal urine volume, and nocturnal index were significantly decreased using an α-blocker plus an antidiuretic agent [7]. In a cross sectional study including 136 patients, Chen et al. (2016) reported that long-term treatment with low dose desmopressin, 0.05 mg increased to 0.2 mg as required, was effective in the treatment of nocturia in Chinese patients with LUTS/BPH with or without nocturnal polyuria [6]. In another study, Kim et al. (2017) divided a total of 86 men with persistent nocturia in spite of the usage of α-blocker therapy for 8 weeks into two collections, one of this collection of patients treated with desmopressin (0.2 mg) and another collection of patients take placebo. The desmopressin added group was significantly improved when compared with placebo group in the no. of nocturnal occurrences, nighttime urine volume, entire IPSS, nocturnal polyuria index, and International Consultation on Incontinence Questionnaire score. Furthermore, the authors found that the adverse events in desmopressin add-on group were not different from that in the placebo group. Regarding QoL, no significantly differences between 2 groups in the variation of IPSS QoL score, so the International Consultation on Incontinence Questionnaire-Nocturia Module (ICIQ-N) agreed with this results that there was significant improved when used desmopressin add-on compared to the group not used it [8]. More recently, Taha et al. (2018) published a systematic review that included 3072 patients with BPH/LUTS and persistent nocturia. The study demonstrated that a combination of α-blockers and desmopressin add-on resulted in a 64.3% redused in the occurrence of nocturnal voids compared to 44.6% of patients used α-blockers a lone [9]. On the other hand, a Turkish study by Koca et al (2012), which is similar to our study, found that there was no significant difference in terms of IPSS, Q_{max}, and QoL between alfuzosin-alone and desmopressin add-on treatment after a treatment duration of three months, although both groups had a significant improvement in these parameters when compared to their baseline measurements [10]. The effect of desmopressin can be explained by the fact that in most cases nocturia is considered as an associated condition within LUTS, and accordingly treated with drugs allocated for BPH or overactive bladder. Usually, these drugs include α-blockers and (or) 5α-reductase inhibitors. However, the response to such treatment was not satisfactory in most cases which could be due to the multifactorial nature of nocturia [6]. Furthermore, nocturia can be improved by some behavioral performs such as decreasing fluid intake at bedtime, thus several non-pharmacological factors can interfere with the drug activity (Km et al., 2017). Desmopressin acetate (Minirin®) is an artificial equivalent of arginine vasopressin, alike antidiuretic action but have no vasopressor actions. Have active effects in the management of conditions with polyuria: such as primary nighttime enuresis and essential diabetes insipidus. Therefore, it can be expected to possess a dual activity when it is used as an add-on therapy beside α-blockers in reducing nocturia as well as other LUTS [11]. Collectively, these data support the use of oral desmopressin (0.2 mg) as an add-on treatment to α-blockers for the management of men with BPH/LUTS they suffering from refractory nocturia. The present study has limitations including: absence of blinding and randomization, small sample size, the lack of input from a bladder diary or from a nocturia-specific questionnaire and not addressing the side effects of desmopressin. Further studies that include women with LUTS are required to popularize the use of desmopressin as essential element for treatment of this disorder.

Conclusions

Adding desmopressin to α-blockers is active therapy for men with BPH and suffering nocturia and no initial response to α-blockers.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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The Practicing and Attitude of Some Medical Students at Al-Nahrain College of Medicine Towards Cosmetic Rhinoplasty

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Abstract

Background: Rhinoplasty in universal is connected with cosmetic surgery carried out to produce alterations in the interior and exterior components on the nose for merely cosmetics good reasons to get better look. It was common cosmetic procedures around the world because the nasal deformities effect on the psychology of the person as a negative impact on their self-confidence and social interaction with others.

Objectives: The study is an attempt to display the practicing and attitude of some medical students towards cosmetic rhinoplasty and its relation to some studied variables.

Methods: A cross-sectional study was conducted at Al-Nahrain medical college, during March 2016. A total of 200 undergraduate students were given a semi constructed questionnaire .The sample selected randomly by systematic random sampling.

Results: The practicing of rhinoplasty among this sample was 10.5%. Most of them happy with their nose appearance. 56.5% of the sample see this surgery socially accepted in Iraq. There was significant relation between person undergoing rhinoplasty and presence of family history of it.

Conclusion: More than half of the medical student found cosmetic rhinoplasty is socially accepted in Iraq. Despite the majority of them had no family history of undergoing rhinoplasty but there was significant difference between doing such surgery and the presence of family history. More than two third of the students included in this study like more awareness program on cosmetic surgery.

Recommendation: Encouragement for more studies to be done for this subject, and developing an educational program to increase awareness about rhinoplasty.

Keywords: *rhinoplasty, attitude, cosmetic.*

Introduction

Among males and females the nose aesthetic is become occupied, the nose due to it's central prominent part of the face, so when the nose is taller than other facial part of face or not complement the face, it can affect self-perception and confidence.(1)

Rhinoplasty in universal is connected with cosmetic surgery carried out to produce alterations in the interior and exterior components on the nose for merely cosmetics good reasons to get better look.(2)

Currently there is always a developed picture attention among a variety of racial and ethnic communities around the world. Possibly because individuals living longer, a larger feeling of self-stem driven by way a person's looks are suffering from, (3) and the nasal deformities effect on the psychology of the person as a bad effect on their self-confidence and public communication with others (4). Rhinoplasty presently increasing everywhere in the world, particularly in the Gulf countries, according to the statistics done worldwide; it is become the fifth most usually done plastic technique international.(5) While

in Saudi Arabia it is the second most common plastic surgery.[6]

Plastic rhinoplasty customized to repair, reconstruct or replace any physical defect due to: (Birth disorders, Trauma, Burns, Developmental abnormalities, Infections, Tumors, Preference and self-choice).[7]

Complications of Rhinoplasty include: (Complications of anesthesia. Epistaxis, Septal perforation and hematoma, Unsatisfactory appearance and deformity, Infection, Worsening rather than improvement in shape or in the nasal functions).[8]

In Iraq despite their lifestyle was largely dedicated by costumes, traditions and some religious interpretation, there has been growing interest in the past few years in cosmetics procedures, especially in rhinoplasty. And their plastic surgeons have gained huge experiences and skills in reconstructive and cosmetic surgery to help rehabilitate many thousands of people wounded by bombs or bullets. [9]

Subjects and Method

A cross-sectional study was adopted at Al-Nahrain College of medicine. The sample selected randomly by systematic random sampling and choose 200 students, during March and April 2016, were given a semi constructed questionnaire especially prepared for this study and collected by the fourth year students, which include **Demographic information** e.g. age in years, gender, stage order, father and mother education in years and **attitude questions like:**

(How do you feel about your nose?, In the past , Have you had cosmetic Rhinoplasty ?, Do you think cosmetic Rhinoplasty are necessary ?, Family history of having cosmetic procedures?....)

Data was translated into a computerized data base structure .Statistical analysis was done by using SPSS Version (20) computer software and Microsoft Excel 2010 used to draw figures present in this study .

Verbal consent was taken from all participants before they answered the questionnaire.

Result

Among 200 students there were 86 (43%) males and 114 (57%) females. The percent of students whom practicing rhinoplasty among this sample was 10.5%. See figure1

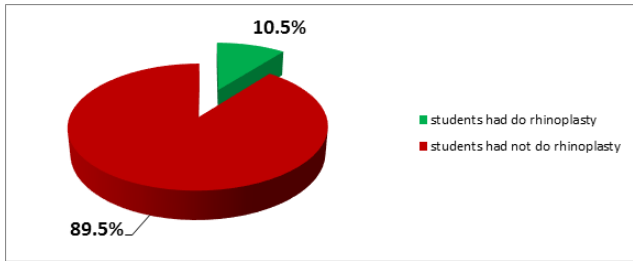

Figure-1- The percent of students whom practicing rhinoplasty among the sample from Al-Nahrain medical students,2016.

-All college stages were included in this study according to their total numbers, first stage= 33(16.5%), second stage 37(18.5%), third stage 39(19.5%), fourth stage 34(17%), fifth stage 30(15%) and sixth stage 27(13.5%). See figure -2-

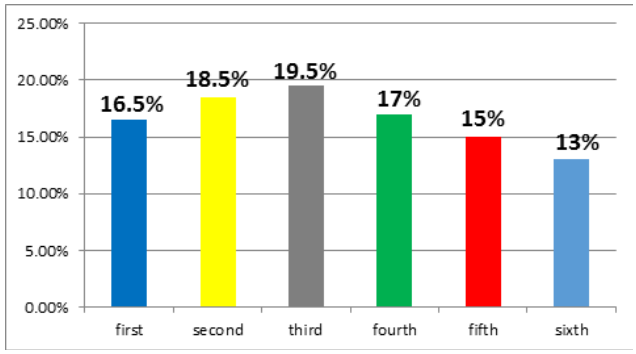

Figure-2- Distribution of the sample according to the student's stages in Al-Nahrain medical college, 2016.

-The age of the study group ranged (17 – 25) years with mean ± SD (21.2 ± 1.76)years.

-Concerning the questions about the attitudes of AL-Nahrain Medical Students towards cosmetic rhinoplasty. See table1

Table(1):- Attitudes of the sample of Al-Nahrain medical students towards cosmetic rhinoplasty .

Questions	Attitude	Numbers (%)
1- feeling about Your nose	Happy Not happy Don't care	96 (48%) 37 (18.5%) 67 (33.5%)

Cont... **Table(1):- Attitudes of the sample of Al-Nahrain medical students towards cosmetic rhinoplasty .**

2- any previous cosmetic Rhinoplasty?	Yes No	21 (10.5%) 179 (89.5%)
3-are rhinoplasty necessary?	Yes No Not sure	57 (28.5%) 76 (38%) 67 (33.5)
4-any family history of cosmetic intervention?	Yes No	30 (15%) 170 (85%)
5-your friend has previous cosmetic surgery?	Yes No	102 (51%) 98 (49%)
6- you embarrassed from cosmetic procedures if anybody identified about it?	Yes No	59 (29.5%) 141 (70.5%)
7- You support your friends experiencing cosmetic procedures if they told you?	Yes No Uncertain	96 (48%) 51 (25.5%) 53 (26.5%)
8-If cosmetic surgeries were done free, would you go for any cosmetic operation ?	Yes No Not sure	55 (27.5%) 94 (47%) 51 (25.5%)
9-If you were cognizant that somebody did cosmetic surgery, this effect on relationship with him?	Yes No Not sure	16 (8%) 141 (70.5%) 43 (21.5%)
10-it is socially accepted in Iraq?	Yes No Not sure	113 (56.5%) 29 (14.5%) 58 (29%)
11-If (10) yes, to how much it is acceptable?	Widely Averagely Not acceptable	29 (14.5%) 135 (67.5%) 36 (18%)
12-any awareness program on cosmetic operation ?	Yes No Not sure	142 (71%) 32 (16%) 26 (13%)

Comparing students, who had rhinoplasty and those had not:-

There was no significant association regarding the gender of the students and the history of doing rhinoplasty (P=0.36). See table (2).

-Table (2) Rhinoplasty according to the gender of Al-Nahrain medical students.

Gender	**Do rhinoplasty**	**Don't do rhinolasty**	**Total**	**Significance**
Male	11 (12.8%)	75 (87.2%)	86(100%)	$\mathbf{X^2}$= 0.842 P=0.36
Female	10 (8.8%)	104 (91.2%)	114(100%)	

-While there was significant difference between gender of students and the attitude of their relation with a person who undergo rhinoplasty (P=0.002). See table (3).

Table (3) Negatively effect of operation on the student relation according the gender.

Gender	Yes	No	Not sure	Total	Significance
Male	13 (15.1%)	60 (69.8%)	13 (15.1%)	86(100%)	X^2= 12.422 P= 0.002 2
Female	3 (3.6%)	81 (71.1%)	30 (26.3%)	114(100%)	

-Finally there was significant relation between undergoing rhinoplasty and the presence of family history of doing such surgery at (P= 0.002). See table (4).

Table (4) Family history association of undergoing rhinoplasty and doing such surgery.

	Family history	No family history	Total	Significance
Had rhinoplasty	8 (38.1%)	13 (61.9%)	21 (100%)	X2= 9.816 P= 0.002 Df= 1
Had not rhinoplasty	22 (12.3%)	157 (87.7%)	179 (100%)	

Discussion

The concerning about the appearance is progressively increase both in the developed and the developing countries, so this lead to increase in cosmetic surgeries done yearly [(10)]. The mean age of student involved in our study was similar to study done by Ahmed M…. et al in Pakistan [(11)] and by Tanthry D…et al in India [(12)] , this is due to the sample take in our study and samples of them is taken from the college whom their age closely in the same variety.

The prevalence of rhinoplasty was 10.5% in this study and this more than the global percentage 8.2% ,[(5)] and this may be due to selection of the sample from medical students whom more aware about this surgery, also the age of them which had tendency to do such procedure.

Regarding their satisfaction about their nose appearance, it was found that less than half of the sample were happy about their nose appearance like what was found in Iran Aliasghar M….et al. [(8)]

Most of the students included in this study see rhinoplasty surgery as not necessary like what Rozin…. et al found, as they preferred the natural things over unnatural things. [(13)]

The majority of this sample 70.5% were not embarrassed about undergoing cosmetic procedures and this like what was found in London by Swami V …et al. [(14)].

Regarding socially acceptance of rhinoplasty more than half found it socially accepted in Iraq and this like what found by Swami V. ….et al. [(14)]This may represent the opinion of medical students and not all Iraqi's people. Concerning the students relationship with a person undergoing rhinoplasty, about two third of them their relation were not affected negatively ,and this like what found by Swami V….et al in London, [(14)] while Delinsky in United States found there was a negatively affect on relationship with such person. [(15)]

Less than third of the included students in this study like to do cosmetic surgery if it was done free in Iraq, and this nearly like what found by AL Mohanna Sh. … et al in Saudia Arabia. [(16)]

2/3 of the defendants suggested the need for more consciousness of beautifying operation in the culture like what was originate in Nigeria by Opeyemi A…. et al in 2014. [(17)]

Regarding the gender there was no significant difference in doing rhinoplasty and this like what found in Nigeria, [(17)] while in other study done in Saudia found significant relation between both gender [(16)] and this may be due to take the sample whom had nearly same socio-demographic and educational levels.

Also there was significant relation between male and female according to their relationship with a person who undergo rhinoplasty, this like what Swami V. … et al. [(14)]

Finally this study found a significant relation between doing rhinoplasty and the presence of the family history of rhinoplasty and this like what was found in Iran [8] and in Saudia. [16] And this means friends and relatives influence a major motive for undergoing cosmetic rhinoplasty.

Conclusion

As the number of rhinoplasty operations are increase, so further research concerning the reason of this increase. It also recommend doing more movements to increase consciousness toward rhinoplasty problems.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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The Level of Serum Progesterone on the day of HCG Administration in Assisted Reproduction Cycles

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Abstract

Introduction: During stimulated IVF- ET cycle spontaneous LH surge occur in about 20 – 50 % of cycle stimulated with gonadotropin without the pituitary suppression of GnRH agonist. This has been related to maturation and recovery of oocyte together decrease fertilization and implantation rate, this spontaneous LH surge is associated with increase in serum progesterone , so without pituitary suppression with GnRH , the rise of serum progesterone in the per ovulatory period may indicate the occurrence of spontaneous of LH surge, the aim of study is to indicate cutoff point of progesterone in the day of HCG administration. **Method:** This was a retrospective, non-interventional cohort study of patients undergoing ICSI at Tiba infertility center in Babylon, from period 2015 - 2019, all patient age from 20 – 45 years old under long protocol, had at least one grade I embryo transferred, total amount of patients was 1457. At second day of menstrual cycle we stimulate by used recombinant FSH, measured on second day of menstruation serum FSH, LH and estrogen levels, transvaginal ultrasound used to monitored the follicles. when follicle became 14 mm in size we used antagonist. 3 or more follicles gotten 18 mm in diameter. Activation gave either hCG alone or mixture of hCG and agonist. After 14 days from embryo transfer we checked HCG and after that after 10 days transvaginal ultrasound was done. **Results:** In our study showed the significant association between positive pregnancy outcome and progesterone level at day of HCG trigger were 79.2 % of female with positive pregnancy at progesterone level < 1.5, while 9.3 % of female with positive pregnancy at progesterone level > 2.5, There is a significant differences of LH at day of HCG activate rendering to progesterone level day of HCG activate, progesterone level (< 1.5) had more chief level at day of HCG activate with mean and SD (5.38 ± 2.52), and reduction when the level progesterone rise. **Conclusion:** pregnancy cannot depend on progesterone level at day when HCG administration for assisted reproduction cycles treated with GnRH agonists and gonadotrophins. So cutoff point of progesterone can reach to above 1.5 ng/ ml and expected of pregnancy at this level.

Key word: *serum progesterone , HCG , IVF, estrogen, HCG trigger.*

Introduction:

During stimulated IVF- ET cycle spontaneous LH surge occur in about 20 – 50 % of cycle stimulated with goDDuring stimulated IVF- ET cycle spontaneous LH surge occur in about 20 – 50 % of cycle stimulated with gonadotropin without the pituitary suppression of GnRH agonist (1,2). This has been related to maturation and recovery of oocyte together decrease fertilization and implantation rate (3–5), this spontaneous LH surge is associated with increase in serum progesterone , so without pituitary suppression with GnRH , the rise of serum progesterone in the per ovulatory period may indicate the occurrence of spontaneous of LH surge (6), with the use of GnRH agonist to suppress pituitary gonadotropin increase in serum progesterone in the day of HCG administration has been reported sporadic cases (7), in such cases the usage of plasma progesterone as a conception predictor is controversial with some studies reported that peri- ovulatory rise in progesterone has negative outcome, some studies reported that peri-ovulatory rise in progesterone has negative outcome in pregnancy rate during assisted reproductive cycle (8,9), while other studies disagree and consider the rise in progesterone in the daytime of HCG management as unconnected to the proportion of pregnancies in assistant reproductive cycles(7), premature progesterone elevation during IVF cycles and its effect of endometrium respectively (10–12), the exact mechanism of peri-ovulatory plasma progesterone increase during stimulation IVF cycle is not fully clear as the rise proceeds the HCG administration for the final maturation of

oocyte and is not associated with LH surge during IVF cycles relatively high dose of exogenous FSH require to achieve multiple follicular maturation (13), some study contribute the premature progesterone elevation FSH stimulation (14,15). Recently publish study on sample of humen ovary cortices shows that FSH stimulate the enzymatic activity of 3B- HSD which are salting increase conversion of progenenolone to progesterone (16). Different cutoff points have been used to difine the progesterone elevation during stimulation IVF cycle that is range from 0.8 – 2 ng / ml (10,17), significant decrease in pregnancy rate with 1.5 ng/ ml or above progesterone level at the day of HCG administration (18,19). The aim of study is to indicate cutoff point of progesterone in the day of HCG administration.

Materials and Method

This was a retrospective, non-interventional cohort study of patients undergoing ICSI at Tiba infertility center in Babylon, from period 2015 - 2019, all patient age from 20 – 45 years old under long protocol, had at least one grade I embryo transferred, total amount of patients was 1457. At second day of menstrual cycle we stimulate by used recombinant FSH, measured on second day of menstruation serum FSH, LH and estrogen levels, transvaginal ultrasound used to monitored the follicles. when follicle became 14 mm in size we used antagonist. 3 or more follicles gotten 18 mm in diameter. Activation gave either hCG alone or mixture of hCG and agonist. After 14 days from embryo transfer we checked HCG and after that after 10 days transvaginal ultrasound was done, pregnancy occur when at least one gestational sac and detectable cardiac pulse. Progesterone measurement on the day of hCG administration. Samples were tested with electrochemiluminescence immunoassay. Statistical analysis: Progesterone 1.5 ng/ ml; this cut-off on the day of hCG administration. Comparisons were made by Student's t test and Chi square analysis where applicable. P <0.05 was considered statistically significant

Results:

In our study showed the significant association between positive pregnancy outcome and progesterone level at day of HCG trigger were 79.2 % of female with positive pregnancy at progesterone level < 1.5, while 9.3 % of female with positive pregnancy at progesterone level > 2.5 as show in table 1.

Table 1: Association between progesterone level at day of HCG trigger and pregnancy outcome (age 20-45)

Study variables	Pregnancy		Total	X2	P-value
	Positive	Negative			
Progesterone level at day of HCG trigger					
Less than 1.5	570 (79.2)	537 (72.9)	1107 (76.0)		
1.5- 2	52 (7.2)	75 (10.1)	127 (8.7)		
> 2- 2.5	31 (4.3)	39 (5.3)	70 (4.8)	8.226	0.042 *
More than 2.5	67 (9.3)	86 (11.7)	153 (10.5)		
Total	720 (100.0)	737 (100.0)	1457 (100.0)		

*p value ≤ 0.05 was significant.

In our study the distribution of patients with infertility according to pregnancy outcome. Those patients those get pregnant represent (49.4%) of study sample. (age 20-45).

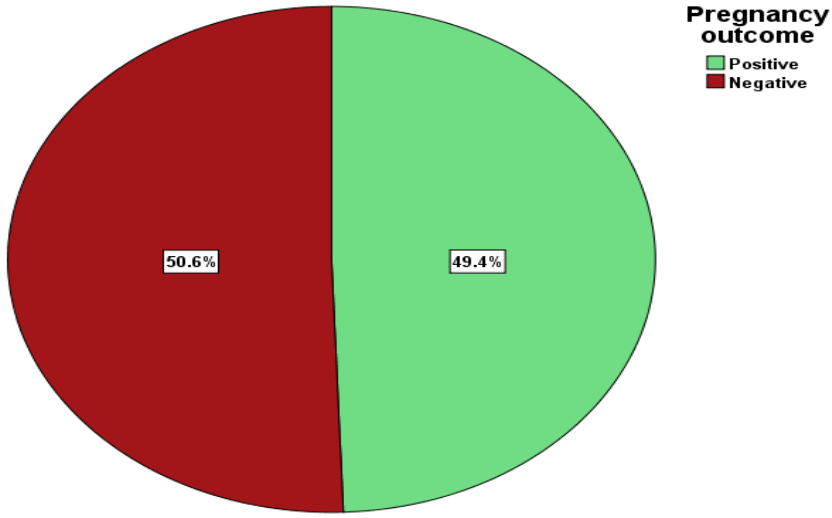

Figure 1: Show distribution of patients with infertility according to pregnancy outcome

The mean differences of age (years) according to pregnancy outcome including (positive and negative).There were significant differences between means of age between these two groups (N= 1457, t= -7.316, and P=<0.001*). Were 30.9% get pregnant at age 20-40 years as show in fig (2)

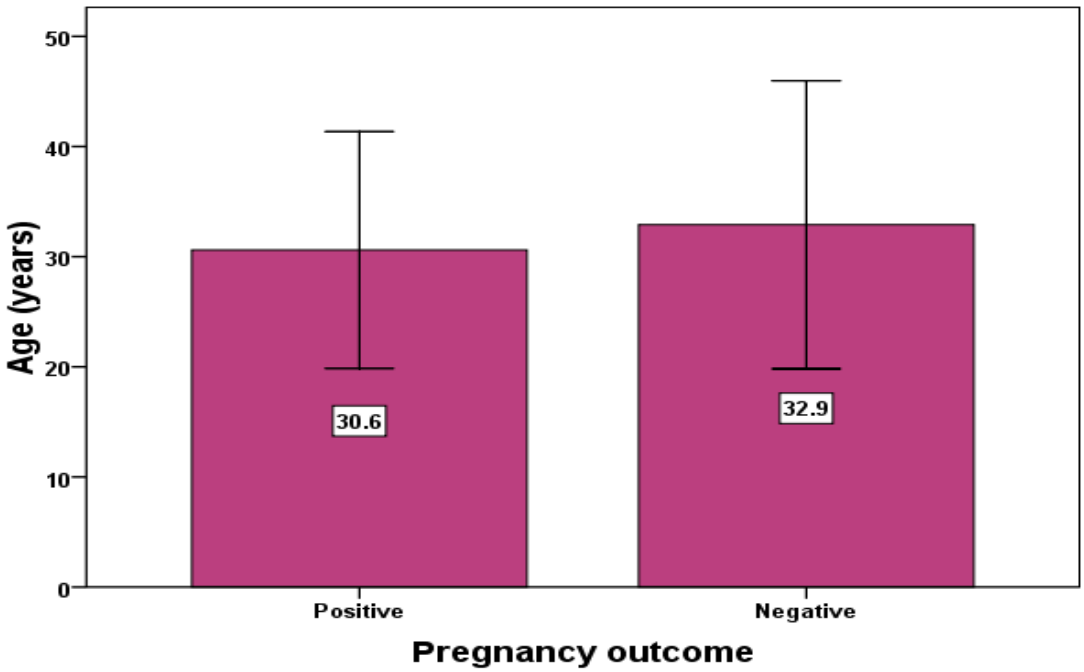

Figure 2: The mean differences of age according to pregnancy outcome.

The mean significant differences of estrogen at day of HCG activate rendering to progesterone level day of HCG activate, progesterone level (>2 - 2.5) had more predominant level at day of HCG trigger with mean and SD (2625.73 ± 65.62), as show in table 2.

Table 2: The mean differences of estrogen at day of HCG trigger according to progesterone level day of HCG trigger.

Progesterone level at day of HCG trigger	N	Mean	SE	P-value
Less than 1.5	1107	1870.16	23.20	<0.001*
1.5 - 2	127	2231.16	72.35	
>2 - 2.5	70	2625.73	65.62	
> 2.5	153	1849.71	72.50	
Total	1457	1935.78	21.00	

There is a significant differences of LH at day of HCG activate rendering to progesterone level day of HCG activate, progesterone level (< 1.5) had more main level at day of HCG activate with mean and SD (5.38 ± 2.52), and decrease when the level progesterone increase as show in table 3. while in table 4; There is no significant differences of LH at day of HCG activate rendering to progesterone level day of HCG activate, progesterone level (< 0.9) .

Table 3: The mean differences of LH level at day of HCG trigger according to progesterone level day of HCG trigger.

Progesterone level at day of HCG trigger	N	Mean	SE	P-value
Less than 1.5	1107	5.38	2.52	<0.001*
1.5 - 2	127	3.60	0.32	
>2 - 2.5	70	3.43	0.50	
> 2.5	153	2.47	0.23	
Total	1457	4.83	1.91	

Table 4: Association between progesterone level at day of HCG trigger and pregnancy outcome (age 20-45)

Study variables	Pregnancy		Total	X2	P-value
	Positive	Negative			
Progesterone level at day of HCG trigger					
Less than 0.9	358 (49.7)	353 (47.9)	711 (48.8)		
0.9-below 1.5	210 (29.2)	184 (25.0)	394 (27.0)		
1.5- 2	56 (7.8)	75 (10.1)	131 (9.0)	8.144	0.086
> 2- 2.5	31 (4.3)	39 (5.3)	70 (4.8)		
More than 2.5	65 (9.0)	86 (11.7)	151 (10.4)		
Total	720 (100.0)	737 (100.0)	1457 (100.0)		

*p value ≤ 0.05 was significant.

Discussion

During IVF/ICSI and embryo transfer, the rise of progesterone in late follicular parts of cycles has controversial affect (7,8,10,12), in our study significant association between positive pregnancy outcome and progesterone level at day of HCG trigger were 79.2 % of female with positive pregnancy at progesterone level < 1.5, while 9.3 % of female with positive pregnancy at progesterone level > 2.5, similar to study done by Jawa Ashmita and similar the study done by Ze Wu in China (20,21). In our study the distribution of patients with infertility according to pregnancy outcome. Those patients those get pregnant represent (49.4%) of study sample. (age 20-45), The pregnancy rate in 45% (65/143) in study done by Kinnari Vilaschandra Amin (22). The mean differences of age (years) according to pregnancy outcome including (positive and negative).There were significant differences between means of age between these two groups (N= 1457, t= -7.316, and P=<0.001*). Were 30.9% get pregnant at age 20-40 years, similar to study done by Jawa and study done by Ze Wu in China (20,21). In our study mean significant differences of estrogen at day of HCG trigger according to progesterone level day of HCG trigger, progesterone level (>2 - 2.5) had more predominant level at day of HCG trigger with mean and SD (2625.73 ± 65.62) , in difference to study done by Francisca Martínez; no significant differences in progesterone /estrogen ratio between conception and no conception cycles (23). Un obvious mechanism responsible for increasing plasma progesterone, exposure to FSH lead to increase sensitivity of granulosa cells (GCs) to LH this occur after hMG treatment this lead to premature Latinization even low plasma LH (24). IVF-ET have been lesser during the existence of pre-hCG rise in plasma P, lead to decrease embryo and oocyte quality, when increase in plasma progesterone the oocyte got from follicular have this rising lead to pregnancy rate decrease (24,25). So in our study 1107 women get pregnancy when level of progesterone < 1.5 ng/mL, 127 get pregnant in level of 1.5- 2 ng/mL, 70 get pregnant in level of >2 - 2.5 ng/mL, and the no. increase in level > 2.5 ng/mL to 153 women get pregnant. a premature elevation of progesterone 0.9 ng/mL was observed in 62% of 166 IVF cycles pretreated with GnRH-a. We do not see an obvious explanation for the discrepancy between Loughlin's results and hose reported by other investigators (24).

Conclusion

Pregnancy cannot depend on progesterone level at day when HCG administration for assisted reproduction cycles treated with GnRH agonists and gonadotrophins. So cutoff point of progesterone can reach to above 1.5 ng/ ml and expected of pregnancy at this level.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Protective Role of Quercetin plus Vitamin C on Infection of Rats with *Klebsiella pneumonia*e and *Pseudomonas aeruginosa*

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Abstract

This study was performed on fifty adult male laboratory rats (*Rattus norvegicus*) of 175 – 200 grams weights. The animals were randomly allocated into five equal groups of ten rats each. The groups of rats were: First, control group where the animals were fed on a standard diet along the experiment period; the Second and Fourth groups, where also maintained on a standard diet while the Third and Fifth groups were also maintained on a standard diet and they were orally dosed with Quercetin (20 mg) and vitamin C (500 mg) daily. Suspension in phosphate buffer solution (PBS) was made for the bacteria and colony forming unit (CFU) count was done after making 10-folds serial dilutions. The rats of the second and third groups were intramuscularly injected with 16×10^6 (CFU) of *Klebsiella pneumoniae* while those of fourth and fifth groups were intramuscularly injected with 20×10^5 (CFU) of *Pseudomonas aeruginosa*. After 48 hours of challenge with bacteria, all the rats of the second group and 4 rats of the third group died. On the other hand, all the rats of fourth group and 3 rats of the fifth group died. The organs of died animals of all groups like the stomach, spleen, liver, and thigh muscle were immediately picked up and weighed after death and well homogenized with (PBS) to obtain counting of (CFU) in the organs mentioned. Passing 3 days later, the survived 6 rats of third group and the survived 5 rats of fifth group were euthanized and their organs were treated as the same as the process mentioned before to gain the organs (CFU) count. The results in this study clarify that the use of Quercetin plus vitamin C has led to increase the survival of rats challenged with bacterial infection and the organs bacterial count of them was significantly less than those rats maintained without supplementation with Quercetin and vitamin C at ($P\leq0.05$).

Keywords; *Quercetin, Vitamin C, Pseudomonas aeruginosa, Klebsiella pneumoniae.*

Introduction

Opportunistic microbial pathogens are serious challenging obstacle in fields of human or veterinary medicines and *Pseudomonas aeruginosa* comes in the queue of these pathogens [(1)]. The other paramount pathogen is *Klebsiella pneumoniae* which is considered as a major problem in patients who suffer from malignancies and who are dwelling in hospitals [(2)]. Vitamin C is well proved for its fantastic features in defensing against diseases, protecting and promoting all bodily functions. Of these effects relating to vitamin C are its role as an antioxidant agent, collagen and bone formation assistant, and assistant in enzymes and coenzymes models [(3)]. In addition, vitamin C was approved to be protective in different ways and in different systems of the body and it can be gained naturally by consuming different vegetables and fruits especially citrus fruits, berries, tomatoes and others [(4)]. Furthermore, vitamin C was clearly reported to cause healing, repairing and protection against heavy metals intoxication in the vital organs like liver and kidney of laboratory animals [(5)].

Polyphenolic compounds are a vast family spreading in the world of plant kingdom and comprise flavonoids as a member of them [(6)]. Quercetin belongs to the family of flavonoids and it is of highly antioxidant capacity [(7)] and it is fluently found in apples, beans, broccoli and onions in particular [(8)]. The antioxidant potency of quercetin was found to be as four times as that of tocopherol [(9)]. The effects of quercetin were linked to its antioxidant potency and its modulating effects on antioxidant enzymes beside its genomic stability effect

[10]. Vast range of beneficial features of quercetin were reported like anticancer, anti-inflammatory, antiulcer, antibacterial, antiviral, cataract preventing role, cardiovascular system protector and other [11]. Quercetin also has a protective role against lipid peroxidation and prevent cellular injury [12]. Besides, quercetin was reported to be protective against poisonous compounds and has ameliorating and protective role for the hematological and reproductive aspects of laboratory animals [13, 14].

Materials and Method

- Animals and Diets

Fifty adult laboratory male rats (Rattus norvegicus) of weights 175 – 200 grams were purchased from a local licensed laboratory medical centre and they were housed in a suitable cages in a very typical laboratory conditions and allocated randomly into five groups of ten rats each. The rats were fed and treated as:

1- Control group, fed and maintained on a standard diet (AIN-93) referenced by [15].

2- Second group, fed and maintained on a standard diet.

3- Third group, fed and maintained on a standard diet and they were orally dosed with Quercetin (20 mg) and vitamin C (500 mg) daily. The doses of quercetin and vitamin C were chosen depending upon LD50 (161mg/kg for quercetin, and 11,900 mg/kg for vitamin C) as referenced by [16, 17] respectively.

4- Fourth group, fed and maintained on a standard diet.

5- Fifth group, fed and maintained on a standard diet and they were orally dosed with Quercetin (20 mg) and vitamin C (500 mg) daily.

- Experimental Challenges

The protocol of the experiment was continued for one month during which the rats were maintained on standard diets and the treatment with quercetin and vitamin C was done as mentioned before. After that, the rats of the second and third groups where intramuscularly injected with 16×10^6 (CFU) of *Klebsiella pneumoniae*. Organs of these animals including spleen, liver, kidney, lung and thigh muscle were homogenized in sterile PBS after being removed and weighed. Viable bacteria number in the organs was obtained by plating on specific agar; BHI agar and the expression of results was the number of CFU/g – tissue. The rats of the fourth and fifth groups were intramuscularly injected with 20×10^5 (CFU) of *Pseudomonas aeruginosa*. Organs of these animals were processed as before mentioned and viable bacteria number in these organs determined by plating on specific agar plates; cetrimide agar and the expression of results was the number of CFU/g – tissue. All plates where incubated for 24 hours at 37 C° and the scoring of CFU was done. The bacteria isolates used in this experiment where obtained from Basrah general hospital. Cetrimide agar was prepared according to the manner described by [18].

- Statistical analysis

At ($P\leq0.05$), (t) test was used to find out the significant differences among groups by using SPSS program version 20.

Results and Discussion

It is obvious from the results shown in tables below that the use of quercetin and vitamin C was very effective against the experimental infection of rats with *Klebsiella pneumonia* and *Pseudomonas aeruginosa* bacteria where it causes a significant increase in the survival numbers and ratios of rats (table 1) and a significant decrease in the numbers of bacteria isolated from different rats organs (tables 2 and 3).

Quercetin has been reported and documented by vast numbers of researches to be a very potent against viral, fungal, and bacterial infections beside its beneficial effects on all bodily systems and immunity. One of these studies [19] who mentioned that quercetin has a wide range of antiviral, antibacterial, antioxidant properties. As anti-inflammatory agent in animals, quercetin was found to be very potent against inflammation and has a modulatory effect on the immune system beside boosting immunity as a whole [20]. It was found to decrease TNF-α of adipose tissue and nitric oxide generation in obese rats [21]. The mechanism of quercetin effects against bacterial infection might be related to its anti-inflammatory role where it has a very strong antioxidant potency and cause increase the secretion of cytokines [22, 23].

Vitamin C on the other hand, is well known for its drastic effects against infections such as protozoal, viral and bacterial infections [24]. Infections with microbial agents in humans or animal lead to oxidative stress and

then activation of phagocytes which in turn will cause elevation in reactive oxygen species ROS production [25, 26]. These ROS are known to cause damages to the cells and it was found in many cases of infection that vitamin C contents in macrophages or leukocytes declines severely in cases of infection [27, 28]. One can exclude that vitamin C is required as antioxidant in case of infection and it can exert its action throughout its antioxidant effects against ROS beside its boosting role to other immune system compartments [29, 30, 31, 32].

Table (1). Role of quercetin and vitamin C on rats' survival after being challenged with *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

Groups	Total rats number	Number of died rats	Number of survived rats	Ratio of survived rats %
Control	**10**	**0**	**10**	**100**
Second *Klebsiella*	**10**	**10**	**0**	**0**
Third *Klebsiella + Vit C and Quercetin*	**10**	**4**	**6**	**60**
Fourth *Pseudomonas*	10	10	0	**0**
Fifth *Pseudomonas + Vit C and Quercetin*	**10**	**3**	**7**	**70**

Table (2). The role of Quercetin +vitamin C on the cultured *Klebsiella pneumonia* bacterial numbers isolated from different rats organs

Groups	Organs					
	Muscle	**Lung**	**Kidney**	**Liver**	**Spleen**	**Stomach**
Control	C 0	C 0	C 0	C 0	C 0	**C** 0
Second ***Klebsiella***	**A** **62×10^7**	A 27×10^7	A 40×10^7	A 9×10^7	A 16×10^7	A 12×10^7
Third ***Klebsiella + Vit C and Quercetin***	**B** **15×10^6**	B 7×10^6	B 10×10^6	B 27×10^6	B 24×10^6	**B** 16×10^6

Table (3). The role of Quercetin +vitamin C on the cultured *Pseudomonas aeruginosa* bacterial numbers isolated from different rats organs

Groups	Organs					
	Muscle	**Lung**	**kidney**	**Liver**	**Spleen**	**Stomach**
Control	C 0	C 0	C 0	C 0	C 0	**C** 0
Fourth ***Pseudomonas***	**A** **37×10^7**	**A** **25×10^7**	A 31×10^7	A 8×10^7	A 13×10^7	A 11×10^7
Fifth ***Pseudomonas + Vit C and Quercetin***	**B** **13×10^6**	B 6×10^6	B 8×10^6	**B** 21×10^6	**B** **21×10^6**	**B** **14×10^6**

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Study The Role of Selenium in The P-Selectin and E-Cadherin Serum Levels in Women with Preeclampsia

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Abstract

Preeclampsia (PE) is characterized by endothelial dysfunction with vasoconstriction due to cell adhesion molecules or mediators released by defective placentation. Platelet selectin (P-selectin) and epithelial cadherin (E-cadherin) are the cell adhesion molecules, is elevated in many inflammatory conditions including PE.

Objective: To investigate if P-selectin and E-cadherin levels can be used as a marker for adverse outcomes in pregnancy complicated with PE.

Patients and Method: This study involved 90 pregnant women in their third trimester of pregnancy divided into2 group:

Group A: involves 45 pregnant women with PE.

Group B: involve 45 healthy pregnant women and normal fetal growth (Control group).

Result: The results show there were a high significant increase in the mean values of P-selectin with high increase in the mean values of E-cadherin in PE group when compared to control group.

ROC curve shows sensitivity and specificity and false positive rate for predication of PE by P-selectin and E-cadherin in control and patients groups, and the maximum accuracy for the P-selectin and E-cadherin in discrimination between control and PE patients groups.

Conclusions: There is an inverse relationship between cell adhesion molecules and selenium in PE compared with normal pregnancy.

High level of serum P-selectin and E-cadherin are associated with the presence PE, which provide additional information for predicting of PE and preventing its complications and for better understanding pathogenesis of PE.

Key words*: platelet-selectin and epithelial-cadherin, with preeclampsia & with normal pregnancy.*

Introduction

Preeclampsia (PE) is a disorder of pregnancy, is a multisystem syndrome that is primarily, characterized by the onset of high blood pressure (HBP) (hypertension) and often a significant amount of protein in the urine (proteinuria). When it arises, the condition can appear any time from the twenty weeks of pregnancy (1). The exact causes of PE and eclampsia - a result of a placenta that doesn't function properly, insufficient blood flow to the uterus could be associated. Genetics plays a role as well, and have many risk factor for PE is most often seen in first-time pregnancies, in pregnant teens, and in women over 40 years old While it is defined as occurring in women have never had high blood pressure before, other risk factors include:

A history of high blood pressure prior to pregnancy.

A history of preeclampsia.

Having a mother or sister who had preeclampsia.

A history of obesity.

History of diabetes, kidney disease , lupus , or rheumatoid arthritis.

P-selectin functions as a cell adhesion molecule (CAM) on the surfaces of activated endothelial cells, which line the inner surface of blood vessels, and activated platelets. In inactivated endothelial cells, it is stored in granules called Weibel-Palade bodies. In inactivated platelets P-selectin is stored in α-granules of platelets, have been shown to correlate with the level of oxidative stress in pregnancy [(2)].

Other names for P-selectin include CD62P, Granule Membrane Protein 140 (GMP-140) and Platelet Activation-Dependent Granule to External Membrane Protein (PADGEM) [(3)].

Cadherin (named for "calcium-dependent adhesion") are a type of cell adhesion molecule (CAM) that is important in the formation of adherence junctions to bind cells with each other [(4)]. E- Cadherin is a class of type-1 transmembrane proteins. It is dependent on calcium (Ca^{2+}) ions to function.

Cell-to-cell adhesion mediated by E- Cadherin plays an important role in tissue differentiation, holding the tissues together as they form during embryonic development.

Role of Adhesion in Preeclampsia:

Increase expression of adhesion molecule interfere with homeostasis. For example adhesion molecules can activate platelets through their contact with exposed epithelial wall [(5;6)]. Abnormal active platelet are involved in the pathogenesis of several pathologies including PE.

Patients and Methods

A case control study was conducted on 90 pregnant women (aged 18-45 years old) from the obstetrics and gynecology department of Al- Immamain Al-Kadhmain Hospital and Baghdad Teaching Hospital, after taking an approval from institutional review board (I.R.B.) of the Medical Collage / Al-Nahrain university and taking a written informed consent from all women in the study.

The subject in the study were 45 pregnant women with PE patient and 45 health pregnant women with gestational age matching to the cases at sampling, which remain normotensive and deliver a healthy baby. Blood pressure for each pregnant women was measured twice in four-hour intervals while resting, at third trimester as well as protenuria detected in urine sample by a simple dip-stick test.

The blood sample were taken from each women that participate in the study and used to measure P-selectin and E-cadherin by using enzyme linked immunosorbent assay (ELISA) method.

Result

Serum Levels of Adhesive Molecules

Mean serum level of P-selectin in PE patients was 261.69± 101.64 ng/mL which was higher than that of controls (204.13± 85.68 ng/mL) with a significant difference (Figure 1).

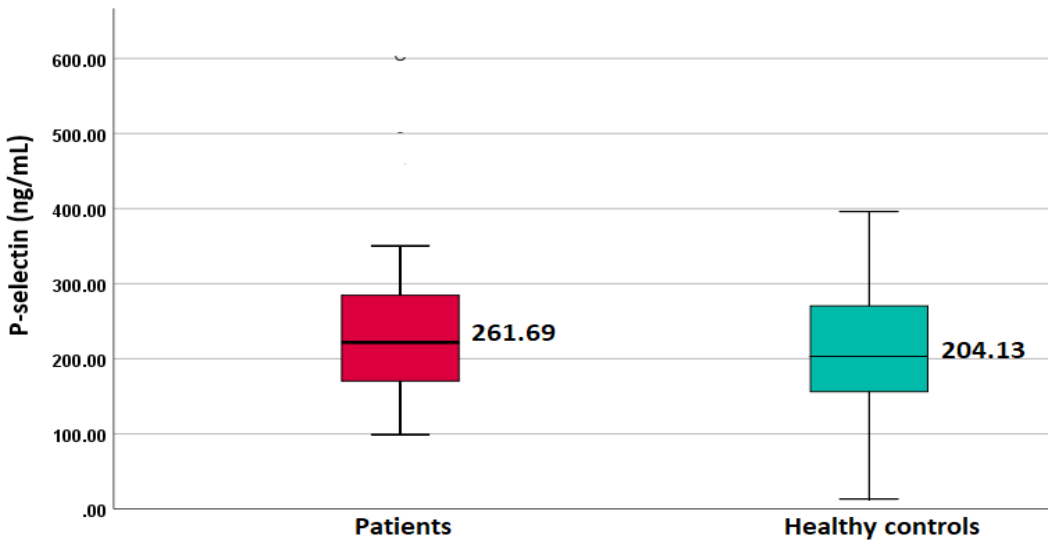

Figure 1: Mean serum level of P-selecting in PE patients and healthy controls

Similarly, mean serum level of E-cadherin in patients (69.45±15.26 ng/mL) was higher than that of controls (58.6 ± 21.69 ng/mL) with a significant difference (Figure 2).

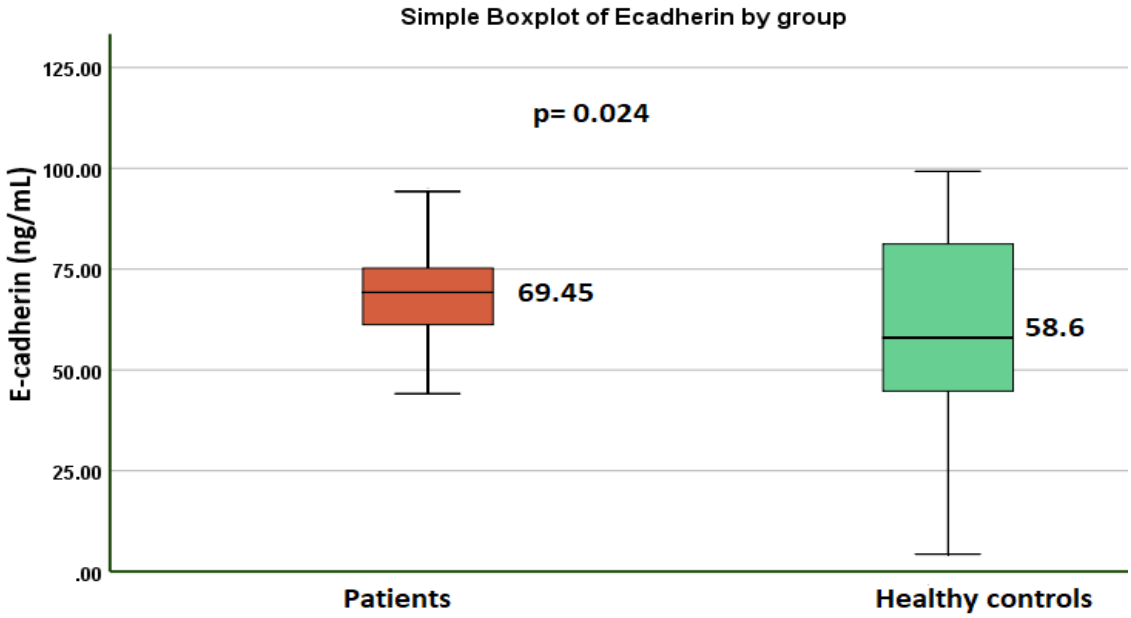

Figure 2: Mean serum level of E-cadherin in PE patients and healthy controls

Diagnostic Value of Adhesive Molecules

Receiver operating characteristic curve was used to explore the diagnostic value of adhesive molecule in the context of discrimination between PE patients and healthy controls (Figure 3).

For P-selectin, the AUC was 0.586, 95% CI= 0.469-0.704, p= 0.159. The sensitivity and specificity of the test atcut off values of P-selectin= 211.0 ng/mL were 0.60 and 0.556 respectively.

For E-cadherin, the AUC was 0.617, 95% CI= 0.495-0.739, p= 0.056. The sensitivity and specificity of the test atcut off values of E-cadherin= 64.87 ng/mL were 0.622 for both.

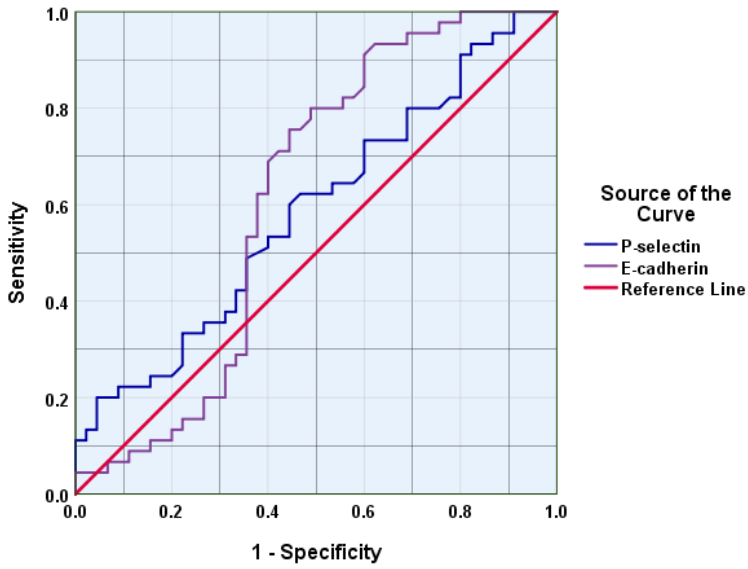

Figure 3: Receiver operating curve for P-selectin and E-cadherin in the context of discrimination between PE patients and healthy controls

The combination of the two markers did not improve the discriminative value (Figure 4). The AUC was 0.613, 95% CI= 0.496-0.729, p= 0.056. The sensitivity and specificity of the test at the corresponding cut off values were 0.60 and 0.51 respectively.

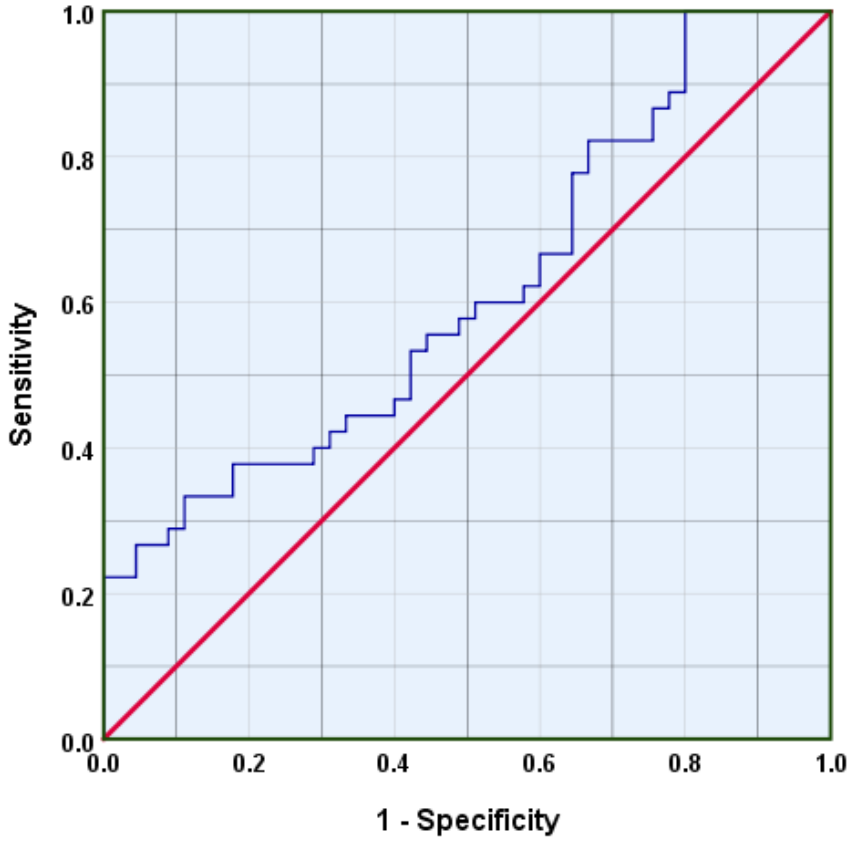

Figure 4: Receiver operating curve for a combination of P-selectin and E-cadherin in the context of discrimination between PE patients and healthy controls

Discussion

In the present study, markers were chosen to reflect inflammation, endothelial dysfunction and poor placentation which are the hallmark of PE. Previous studies which have done on the these markers in other similar clinical situations such as CVD, had shown great association of the markers with the disease processes.

Nevertheless, these clinical condition are known to share more than one physiological and vascular factors. So that this study aimed to investigate the role of current markers with PE development.

Serum Levels of Adhesive Molecules

In the current work, the mean level of P-selectin was found to be significantly higher in women with PE compared with the control group. Presented a significantly higher expression of P-selectin in PE than in apparently healthy pregnant women.

Same results of increased levels of sP-selectin in preeclamptic pregnancies were obtained by [7]. The authors proposed that their findings support an inflammatory model for PE, while the endothelial cell activation might be a secondary to a primary inflammatory response. However, [8] observed significantly increased levels of both P-selectin and sP- selectin form in women with PE in comparison with normal pregnant women and came to the result that normal pregnancy is connected with increased platelet activation followed by further alteration in these parameters in pregnancies complicated by hypertension.

Also, the present findings are in accordance with the results reported by [9], who suggested that the elevated level of the P-selectin is associated with PE (Figure 1), sP-selectin acts as a marker for platelet and endothelial activation and as an initiator of the pro-coagulant state as well as playing an important role in atherosclerosis. The current results of elevated sP-selectin in PE seem

to confirm that these processes occur in preeclamptic pregnant women and the excess circulating sP-selectin aid to the pathogenesis of this pregnancy-specific disorder.

There are two sources of sP-selectin. A minor proportion of this marker is produced through alternative splicing of mRNA which remover the exon that coding the transmembraneous domain.

However, the main proportion of sP-selectin is primarily derived from proteolytic cleavage of the ectoderm part of membranous P-selectin.

Interestingly, shedding of sP-selsectin requires leukocyte adhesion to activate platelets or endothelial cells through interaction of PSGL-1 on leukocyte [10].

PE involves an endothelial cell activation to coagulation hyperactivity and inflammation, which implies the availability of PSGL-1 for activation of platelets and endothelial cells with eventual increase in sP-selectin shedding.

The circulating platelet count is reduced reflecting a decreased platelet lifespan [11]. Features of platelet activation in PE include an increased expression of platelet surface P-selectin (CD62P) [6]. As can be observed from receiver operating characteristic curve (ROC) (Figure 3). Analyses of P-selectin demonstrated the low descrimitive value of this marker to diagnose PE both in terms of sensitivity and specificity. The sensitivity was 0.60 and specificity 0.556 respectively. And the AUC was 0.586, 95% CI=0.469-0.704, P=0.159. This is because sP-selectin can elevated in many pathologies. For example in a case-control study, including women with polycystic ovary syndrome (PCOs) and healthy control women, found that sP-selectin levels were significantly higher in PCOs women than controls.

The current study revealed a significant elevation in serum level of E-cadherin in PE compared with control. This result in accordance with during human placental development, cytotrphoblast(CTB) differentiate and fuse with the syncytio-trophoblast cell layer (STB), allowing for growth of the syncytium and for re-epithelization of aged or damaged regions of villi. This process of trophoblast turnover is regulated to ensure normal fetal development. Localization and expression of cadherins are highly relevant to the morphology of trophoblasts at different stages of placental development. E-cadherin, a classical cell-cell adhesion molecule, is expressed in villous CTB, but is lost when they differentiate and syncytialise, resulting in the absence of E-cadherin expression in the STB of both healthy 1 and 2 trimester placentae [12]. The reduction of E-cadherin expression from 1 trimester to 3 trimester is reported in normal term placenta [13]. A number of studies have shown that the expression of E-cadherin was increased in PE resulting in CTB proliferation [14], although more recent studies have shown the reduced expression of E-cadherin in PE, or no changes in E-cadherin expression in TNF-α induced reduction of trophoblast invasion [15]. Elevated expression of E-cadherin raise the proliferation of CTB and inhibits their differentiation into STB in PE, which suggests the alterations of E-cadherin levels are potentially important to the pathogenesis of PE [16]. Consistent with other studies, this data showed that E-cadherin was expressed in a higher level in the preeclamptic patient women compared with normotensive pregnant women. Studies have shown the villous trophoblast undergoes increased apoptosis in preeclampsia and the increased apoptosis was observed in STB [17].

As can be observed from receiver operating characteristic curve (ROC) (Figure 3), analyses of E-cadherin demonstrated poor diagnostic value of this marker to diagnose PE. The sensitivity and specificity was 0.622 for both.

The combination of the two markers did not improve the discriminative value (Figure 4). The AUC was 0.613, 95% CI= 0.496-0.729, p= 0.056. The sensitivity and specificity of the test at the corresponding cut off values were 0.60 and 0.51 respectively.

Conclusions

There is an inverse relationship between cell adhesion molecules and selenium in PE compared with normal pregnancy.

High level of serum P-selectin and E-cadherin are associated with the presence PE, which provide additional information for predicting of PE and preventing its complications and for better understanding pathogenesis of PE.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they

have no conflict of interest.

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Histological Sections of Pancreas and Serum Biochemical Changes in Rats after *Dexamethasone* and Zinc Oxide Nanoparticles Injection

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Abstract

Zinc Oxide Nanoparticles have been prepared using a simple green synthesis method by green tea extract (*Camellia sinensis*) as a reducing and capping agent, XRD measurement of Nano powder stated that Zinc Oxide had a hexagonal wurtzite structure, UV-Vis. The maximum absorption peak was approximately 318 nm and the energy gap was nearly 3.8eV calculated using Planck's equation, blood serum sugar levels, liver function after ZnO NPs injection for rats was recorded. Forty-eight adult male rats were used and separated randomly into six groups of eight (n=8) rats in each group.Results stated that blood glucose levels, liver enzymes were increased in groups that injected by dexamethasone while considerably modified in rats administered by the first dose (100 mg/kg) of ZnO Nanoparticles while the other dose (300 mg/kg) hadn't any significant effects.

Keywords: *Dexamethasone, XRD, Zinc Oxide NPs, AST, HDL-C*

Introduction

recently, a strategic development has been frequently implemented in the context of a novel green synthesis technique that forms biocompatible nanoparticles because of the possibility of developing bio-nanomaterials with particular biological functionalities [(1)]. Today, non-toxic, environmentally friendly techniques have gained more importance because of their ability to generate a wide variety of nanomaterials to cover better suite biomedical applications [(2)] most of these apps are due to the fact that the Nanometer scale has considerably unique characteristics compared to the bulk. Nanoparticles have good surface bonding, The large surface-volume ratio in nanoparticles is the primary reason for a structural deformation that can influence the physical characteristics of nanoparticles [(3)] In particular, the synthesis of zinc oxide nanoparticles has gained considerable interest due to the fact that it has excellent characteristics for biomedical apps, zinc [(4)] plays a significant part in the preservation of blood sugar and activate more than three hundred of body enzymes. [(5)], it is also known to maintain the structure of insulin and has an important roles in insulin biosynthesis, storage and secretion [(6)].There are several zinc transporters in pancreatic β- cells [(7)]. This report intended to investigate the ability of prepared Zinc Oxide by green synthesis to recover blood glucose, liver enzymes and lipids in rat's body after dexamethasone administration.

Materials and Method

Zinc Acetate Dehydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) (0.2 M) is mixed with 70ml of Deionized Water using a magnetic stirrer, stirring 1500 rpm a few minutes. Then 20 ml of the previously prepared green tea leaf extract was obtained from the stock solution (stored in the refrigerator) and gradually added. Now the blend was stirred continuously for 2 hours at 40ºC. The solution was then cooled at room temperature and centrifuged twice at 4000 rpm for 15 min after a thorough washing and then dried at 60°C for 3 hours, lastly the produced ZnO nano-powder pale white colored using for further studies.

Experimental Protocols and Grouping

(Forty-eight) Male adult Wister albino rats weighing (175-225 g) and (10-14) weeks old housed in cages

made of polypropylene under controlled conditions (25±5) ° C and 12/12 hrs light / dark cycles, drinking water and diet food were provided ad libitum, animals were grown and housed at the University of Baghdad / animal house in the college of science. The experimental animals were randomly grouped into six groups, each of which consisted of eight rats (n=8) treated for 30 days and designed as follows: G1 the first group was served as negative control, G2 the second group was injected intraperitoneally with (0.5 mg/kg) dexamethasone sodium phosphate, G3 and G4 were injected intraperitoneally with (100 and 300) mg/kg ZnO NPs [8] and (0.5 mg/kg) of dexamethasone respectively, G5 was injected with 100 mg/kg ZnO NPs and G6 injected with 300 mg/kg ZnO NPs. The Laboratory animals were fasted 24 hrs before dexamethasone injection; they were weighed and anesthetized by inhalation in a glass dome.

Biochemical assessment & Statistical Analysis

When the experiment period end; blood samples collected from animals by heart puncture, the blood centrifuged at 4000 rpm for 10 min, serum was separated for test of several biochemical parameters such as blood glucose level (BGL) measured by commercially kit (Biosystem, Germany), cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Triglyceride (TG) and Low Density Lipoprotein Cholesterol (LDL-C) estimated using an available kit (Linear kit, Spain), serum levels Alanine Amino transferase (ALT), Aspartate Amina transferase (AST), Alkaline Phosphate (ALP) and Total Bilirubin (TB) were assessed using commercially available kit (Reflotron kit, Germany). Results were stated as means ± standard Errors. The Statistical Analysis System applied one way (ANOVA) analysis of variance to verify (LSD) using statistical package of Prism software 8.1.2 (Version 8.1.2, GraphPad, San Diego, CA, USA). The Probability (P) value ≤0.05 has been considered to be of statistical significance. When pancreatic samples collected from animals after organs separation and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70%), cleared in xylene, and embedded in paraffin. 5 micron thick paraffin sections were prepared and then regularly stained with Hematoxylin and Eosin (H&E) [9] and examined microscopically.

Results and Discussion

Structural characterization of Nano-powder was recorded by Pro Penalty CAL with Cu-Kα radiation (1.5406 Å). X-ray diffraction was indicating the structure and crystalline nature of as synthesized ZnO Nanoparticles. Results show all diffraction peaks are in good agreement with the international crystallographic data table; JCPDS card No. 01-075-0576. No impurities peaks can associate to the XRD spectrum of the gotten powder, showing high purity of ZnO products and the peaks intense of the samples proof to good crystallinity. The broadening of the diffraction peaks clearly indicates the presence of Nano-size, large broadening in X-ray diffraction lines occur when particle size is less than 100 nm, The peak width at half height using the Debye–Scherer formula [10] gives indication for average crystallite size of green synthesized ZnO NPs, The calculated average crystallite size was 9.27 nm, result of X ray pattern agreed with [11][12].

Table (1): The structural parameters of as synthesized ZnO Nanoparticles

2θ (Deg.)	G.S (nm)	FWHM (Deg.)	dhkl Exp.(Å)	dhkl standard (Å)	(hkl)
30.81	14.7	0.539	2.877	2.902	(100)
34.32	8.4	0.941	2.593	2.613	(002)
35.3	9	0.883	2.523	2.537	(101)
46.7	5.33	1.497	1.943	1.941	(102)
57.97	5.54	1.470	1.592	1.595	(111)
62.15	5.25	1.516	1.492	1.493	(013)
66.93	10.3	0.773	1.396	1.398	(201)
76.5	15.7	0.510	1.243	1.268	(202)

The optical behavior recorded by VARIAN spectrophotometer (Cary 5000 Scan), to explain the optical properties and energy gap value of ZnO NPs; UV–Vis absorption spectrum has been studied. Fig. (1) Shows the exciton maximum absorption is about 318 nm. Actually, energy absorbed in UV or visible region causes a change in the electronic excitation of the molecule, and hence results in corresponding change in its ability to absorb light in the UV-visible region of the electromagnetic radiation. The optical energy band gap for Zno NPs was 3.8 eV has been calculated using Planck's relationship [13].

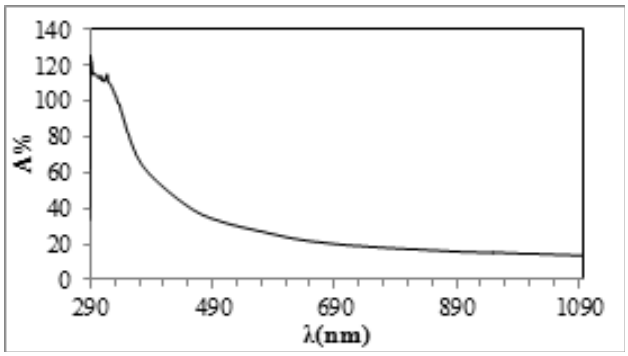

Figure (1): Absorption Spectrum of ZnO nanoparticles

Table (2) shows blood glucose of the study groups, mean blood glucose level (BGL) of **G2** after 30 day administration of dexamethasone showed a significant increasing compared with control group, the reasons behind increasing glucose is generated oxidative stress by sugar causes the generation of free radicals that attack and damage beta cells, thus inhibiting the formation and secretion of insulin, and then stops glucose metabolism and glycogen decomposition, Oxidative stress is involved in creation of ROS that damage cellular membranes, proteins and nucleic acids, the auto oxidation of glucose that produces hydrogen peroxide (H_2O_2), a strong ROS [14], these results similar to previous study [15] that reported the dexamethasone cause tissue damage in pancreas.

Treatment with 100 mg/kg of ZnO NPs were responsible of significant reduction to BGL ($P \leq 0.05$) may be due to that Zinc has been elucidated to be a potent metal that Improves glucose utilization and metabolism through its potent influence on enhancement of hepatic glycogenesis through actions on the insulin signalling pathway [16], interestingly, on the basis of Umrani and Paknikar [17]; ZnO did not possess the risk of hypoglycemia in living organisms so it can act as an insulin secretion, more researches have the same results revealed that ZnO NPs could increase serum insulin level in diabetic animals [18].

Table (2): Changes of Serum biochemical parameters in treated rats with CeO_2 Nanoparticles after 30 days, Each value represents the mean± standard error of (n=8), The values were found to be statistically significant at P≤ 0.05, (*P<0.01, **P<0.001, ***P<0.0008, ****P<0.0001)

	Means± SE					
Groups Parameters	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**
BGL (mg/dL)	113.2±6.4	395.2±29.7	130.2±18.9**	236.7±5.1*	110.25±0.8	121.25±6.4
ALT(IU/L)	35.7±1.6	66.2±4.7	33.7±1.6*	46.5±0.6	41.2±0.6	43.7±1.6*
AST(IU/L)	27.2±4.4	48.2±3.5	34.5±2.3*	58±3	31±1.6	38.2±1.2
ALP(IU/L)	151±17.8	383.2±30.7	124±7.5**	317.5±8.5	159.5±12.7	238.2±20.5*
TB(mg/dL)	0.33±0.01	1.42±0.08	0.54±0.02**	1.27±0.04	0.45±0.06	0.62±0.1
TC(mg/dL)	178.5±12.5	255±5.4	139.7±13.8**	232.7±5.9*	164±4.2	173.2±5.5
TG(mg/dL)	152.2±10	231.7±14.8	170.5±2.2*	203.7±3.8	157±1.9	165.7±2.9
HDL-C(mg/dL)	45±1.6	26±3	46.2±2.3*	29±1.4	40.2±2.2	35±1.7
LDL-C(mg/dL)	91.2±8.1	172±13.1	115.2±3.9*	163.5±5.1	101.7±2.4	113.7±2.4

ALT and AST are very sensitive enzymes and have been closely connected with hepatic damage these levels enzymes will rise when liver is in dysfunction [19] [20], Maiti et al. [21] have suggested that increases in the actions of ALT, AST, and ALP in the serum of diabetic rats as a consequence of hepatic damage, ALT and AST levels increased is associated with insulin deficiency has been interrelated to increased gluconeogenesis during hyperglycemia [22].

The results showed there was a significant increase in liver functions parameters such as ALT, AST and ALP in experimental animals compared with negative control group (**G1**) table (2), increases of liver functions parameters levels possibly due to hepatic damage and increase synthesis of these enzymes in the liver [23], and a significant decrease (P≤0.05) with liver enzymes when animals treated with (100 mg/kg) of ZnO NPs while there was no statistically significant effect of 300 mg/kg dose for infected rats respect to **G2**. Increases of Bilirubin levels may be attributed to the changes in cell membrane permeability with change of effective stress of the membrane under the oxidative stress [24], ZnO nanoparticles have reduced Bilirubin levels in **G3**; **G5** and **G6** were nearly the normal ranges.

G2 had a significant increase in cholesterol; this increasing attributed to high exposure of free radicals which stimulate the rate limiting enzyme hydroxyl-methyl glutaryl which is responsible for liver synthesis [25]. When these animals treated with 100 mg/kg of ZnO NPs, a significant recovery in cholesterol levels occurred. Higher levels of Triglycerides (TG), LDL-C and reduction in HDL-C levels in **G2** is possibly due to a direct consequences of conversion white adipocytes to brown adipocytes [26], or may be attributed to decrease the activity of lipoprotein lipase which is responsible for TG removal. However, triglyceride and LDL-C levels ($P \leq 0.05$) decreased was evident. With the exception of raises of serum HDL level.

Histological section in (Fig. 2a) shows pancreatic lobules containing acini (*), islets of Langerhans (**) and unstained connective tissue septa (***) for control group. Fig. (2b and c) showed damage and necrosis of endocrine cells of islet (*), Langerhans with shrinkage of islet (**), degenerated acini cells and islet of Langerhans (***) and presented different alterations of rat's pancreas and many histological changes were detected in pancreas section of (**G2**) that administrated by dexamethasone, such as necrosis and damage of endocrine cells of islet Langerhans with shrinkage of islets, Dexamethasone induced necrosis is mediated by reactive oxygen species mediated lipid peroxidation which causes bursting of plasma membrane of the cell and disturbance of osmotic balance this osmotic alteration ultimately leads the cell towards necrosis. The histological sections showed several modified in rats pancreas of rats in groups that treated with 50 mg/kg of ZnO Nanoparticles (**G3**), whereas group that treated with (100 mg/kg) (**G4**) had no clear changes after dexamethasone and nanoparticles injection, in fig. (3a) the section shows animal from **G3** had a normal histological structure of islets of Langerhans of pancreas which consist of α and β cells, in between capillaries blood vessels, while fig. (3b) section shows no effects of Nanoparticles on pancreatic cells, abundant necrosis, degenerated acini and damage of endocrine cells, **G5** and **G6** in fig. (3c and d) appears look like normal pancreatic structure.

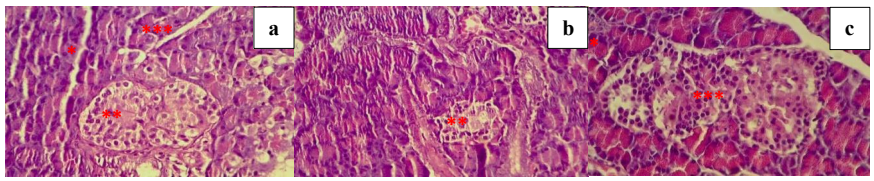

Figure (2): Cross sections of pancreas (a) Control group G1, (b and c) G2 X400 (H&E)

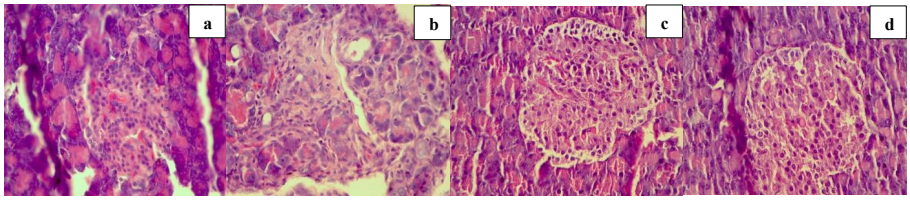

Figure (3): Cross section of pancreas (a) G3 (b) G4 (a) G5 (b) G6 X400 (H&E).

Conclusions

Biocompatible nanoparticle synthesis was produced by using green tea extract (Camellia Sinensis) method for the preparation of Zinc Oxide nanoparticles. Successful formation of nanoparticles by examining its structural and optical properties was investigated. Results indicated that blood glucose concentrations, liver enzymes and lipids were enhanced in dexamethasone-injected groups, while significantly altered in rats administered 100 mg / kg dose of ZnO NPs and the other dose (300 mg / kg) did not significantly improve BGL, liver enzymes and lipids.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Study the Role of Trichomoniasis in In Vitro Fertilization (IVF) Failure

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Abstract

Trichomoniasis is a sexual transmitted disease that affects human fertility. In women, trichomoniasis has been related to infertility due to pelvic inflammatory disease that compromises tubal patency, while in men, trichomoniasis may contributed to infertility by deficit of sperm cell quality and function due to physical damage. In this article, asymptomatic fifty couples with unexplained infertility, undergoing In Vitro Fertilization (IVF) were examined for Trichomonas vaginalis (T.V.) infection. In 70% of couples, embryo implantation failed and pregnancy was not achieved, probably as a consequence of trichomoniasis. The result indicates that T. vaginalis pathogenicity, adverse reproductive health outcomes, in time diagnosis, and treatment may improve implantation rate in patients with unexplained infertility undergoing Assisted reproductive technology (ART).

Key word : *Trichomonas vaginalis* , (IVF) Failure**,** infertility; sexual transmitted disease

Introduction

Trichomonas vaginalis is a globally occurring anaerobic/ microaerophilic protist parasite which colonizes the epithelium of the human urogenital tract [(1)]. Aside from human papillomavirus, trichomoniasis is the most common sexually transmitted infection worldwide, based on estimates of the World Health Organization (WHO) from 2016 [(2)], *T. vaginalis* infection , affect approximately 156 million people every year. Medical opinion has traditionally held that it plays little role in health complications in women, and it is rarely seen in men, the Centers for Disease Control and Prevention (CDC), reports that only 30 percent of people with trichomoniasis report any symptoms at all [(3)]. However, evidence has recently accumulated implicating *T. vaginalis* as a contributor to a variety of adverse outcomes among both sexes. Among both women and men, the association of *T vaginalis* with human immunodeficiency acquisition and transmission has been shown in multiple studies [(4, 5)]. Among women, trichomoniasis may play a role in development of cervical neoplasia, postoperative infections, and adverse pregnancy outcomes and as a factor in atypical pelvic inflammatory disease and infertility [(6, 7)]. Among men, trichomoniasis has emerged as a cause of nongonococcal urethritis and contributes to male factor infertility [(8)]. Published reports clearly show that the rate of trichomonas infections among infertile couples is higher than that among fertile ones [(9)].

The interaction of vaginal pathogens with epithelium and mucosa of the reproductive tract affects the immunological harmony needed for the success of embryo implantation. Nowadays, *T. vaginalis* is a latent pathogen in the reproductive tract, in this way is responsible of the adverse reproductive health outcomes in humans. Trichomoniasis proves the way for several bacterial intruders of the inflammation processes, thereby increasing the risk of failure in reproductive capacity and increasing the risk by 1.5-3 times of HIV and VPH acquisition [(10,11)]. *T. vaginalis* evades host immunity by the presence of adhesion proteins, cysteine proteases, and lipophosphoglycan molecules, all of which increase the pathogenicity of this intruder. The parasite adheres to the vaginal and cervical epithelial cells and triggers an immunosuppressive response from monocytes, macrophages, and dendritic cells. Also, *T. vaginalis* carries viruses and other parasites, such as mycoplasma

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and gardenella, causing chronic mucosal damage and an inflammatory reaction which gives rise to severe consequences in reproductive outcomes [12-15]. Goodman *et al*. [15] reported the presence of a Totiviridae viral family, which is a virus with a doubled stranded RNA that concurrently, is infecting the *T. vaginalis* parasite [15-17]. Infection of *T. vaginalis* by such a virus increases severely the immunological genesis of trichomona virulent factors by changes in its genome organization, protein coding, and replication signals. Trichomona virus increases the recurrence of the parasite infection and resistance to the metronidazole treatment [15,18,19-21].

Materials and Method

Ethics Statement: All human participants were enrolled in conformity with informed consent, privacy and confidentiality of patients who were sampled and analyzed anonymously during study (No. 53.2017).

Sampling:

From August 2017 through to August 2019, a prospective and case-control study at Dr. Khawer center for infertility and In vitro fertilization (IVF) in Erbil province of Kurdistan region of Iraq, were conducted. For research purpose only, fifty women under the age of 35 years, had normal body index, and nonsmokers and their spouses (normal seminal fluid analysis according to the world health organization parameters (WHO) at 2010), [22] with unexplained infertility, were enrolled in this study. High vaginal swab and semen sample had been collected from the participate couples.

DNA extraction and PCR:

The genomic DNA was extracted by the extraction kit gSYNC^{TM} (Geneaid England) directly from high vaginal swab, following the manufacturer's instructions.

PCR steps

The primer set targeting beta tubulin gene 9/2 which is a conserved region found in all of *T. vaginalis* (gene bank accession No. L05470) were used to amplify a 112 bp piece of the gene, the sequence of the primers.

Forward primer sequences (5'–3'): CAT TGA TAA CGA AGC TCT TTA CGA T

Reverse primer sequences (5'–3'): GCA TGT TGT GCC GGA CAT AAC CAT

PCR protocol:

The PCR master mixture prepared at final volume of 20 µl in 0.2 mL PCR tubes. Component and their volume are explained in Table (1).

Table (1): the PCR construct master mixture for amplification of (BTUB) gene:

Reagents	Volume for 1X
Master mix(Prime taq Primemix)	10.0 µl
Forward primer	1.0 µl
Reverse primer	1.0 µl
Nuclease free water	4.0 µl
DNA	4.0 µl
Total	20 µl

The PCR amplifications were performed on a program according to the cycle

The construct reaction conditions for (BTUB) as shown in table (2):

Table (2): the construct reaction conditions for (BTUB):

Stage	Temperature	Time	Cycles
Initial Denaturation	95	5 minutes	1 cycle
Subsequent denaturation	95	30 second	10 cycles
Annealing	(64-55)	30 second	
Extension	72	1.0 minute	
Subsequent denaturation	95	30 second	40 cycles
Annealing	55	30 second	
Extension	72	1.0 minute	
Final Extension	72	10 minutes	1 cycle
	4	To time end	

A 3 µl volume of each reaction was subjected to electrophoresis on 3.0 % agarose gel in Tris-acetate-EDTA buffer pH 8.5 (45 min at 10 V/cm) and staining

with safe dye. The DNA bands were visualized under UV light (transillumenator).The size of the amplified products was assessed by comparison with a commercial weight marker, Smart Ladder (50 bp) GeneDiex|Korea and photographed by digital camera.

PCR products sequencing:

To reconfirm the PCR results, 10 amplified PCR products were directly sequenced by targeting Beta tubulin gene in both directions using, the Big Dye Terminator method. Sequencing was performed bi-directionally using the same primers used in PCR amplification of the beta tubulin genes (Macrogen /South Korea). The obtained sequences were aligned (by using Bio edit software) with normal sequence from GenBank.

Results and Discussion

Unfortunately, all the examined women for trichomoniasis were positive by detection of BTUB gene and 40% of their spouses also. Only 30% of these women successfully achieve pregnancy, as shown in table 3.

Table (3): the percentage of trichomoniasis in males and females and the successful percentage of IVF.

Gender	T.V. infection percentage	IVF percentage	IVF success percentage	IVF failure percentage
Female	100%	100%	30%	70%
Male	40%			

Although, couples that enrolled in this study were chosen very carefully, to overcome the IVF failure causes and to insure getting good eggs quality (eggs without chromosomal changes) and quantity, not defective embryo (that can implanted successfully in the uterus), yet it has long been regarded as a sexually transmitted infection of minor importance, trichomoniasis is not routinely screened in asymptomatic patients and the infection can persist for 3-12 months in the genital tract. [23-26]. Patients with trichomomiasis are asymptomatic in 70-100% of male cases *versus* 35-85% of female cases [23], however, the asymptomatic women may also experience, at least part of pathological signs, could be due to the genetic diversity of the parasite strains to express virulence factors, which increase cytoadherence, cytolysis and cell detachment [27].

Additionally, previous studies have reported that trichomoniasis has high rates of reinfection due to resistance of the parasite to metronidazole treatment [18, 26] making this microorganism a serious reproductive tract pathogen.

The *T. vaginalis* protozoan attaches to vaginal epithelial cells through its barbed tail, membrane expression of surface protein p270, secretion of proteases and a cell-detaching factor, leading to an intense host inflammatory response, inducing local cytotoxic effects, genital tract damage, and reproductive effects [28,29]. Because, trichomonas has been been isolated from fallopian tubes, peritoneal fluid, and the pouch Douglas, scientists suggesting that motile trichomonas may be able to invade the whole genital tract [30,31]. There are several studies that conclude that *T. vaginalis* causes urogenital damage to different types of cells and tissues, such as connective and muscular tissues, due to an excessive cytotoxic local effect suggesting high risk of reproductive failure [31-37]. Trichomonas bind to the cells inducing membrane retraction, cell blebbing, and apoptosis. These changes of cell architecture can be evidenced under microscopy and characterized by condensed chromatin and intense cytoplasm vacuolization [37]. In this study, the female partners did not report any infectious symptom and, therefore, they were not tested for *T. vaginalis*, but they might have been affected with latent trichomoniasis. They are an examples of asymptomatic patient who obtained a negative outcome of implantation failure after ART. More than 20% of women with trichomoniasis have a chronic inflammatory process in the reproductive tract and that may explain the embryo implantation failure after ART procedure. Consequently, the clinical implication of embryo implantation failure depends on both the quality of the embryo and the receptivity of endometrium mainly marked by the correct and exact immune environment needed for a successful pregnancy.

On the other hand, men infected with *T. vaginalis* display abnormal motility of the spermatozoa and high semen agglutination. A relevant tropism from *T. vaginalis* microorganism to the head or flagella of the human spermatozoa has been reported. The adhesion of *T vaginalis* and sperm affects sperm motility followed by phagocytosis, lysis, and digestion of sperm cell [38, 39]. In 2008, Benchimol *et al*.

[40] report that, after one hour of interaction between *T. vaginalis* and sperm cells in an *in-vitro* environment, 75% of the sperm cells were immotile or dead. Additionally, cyto-adhesion and phagocytic activity of trichomonas to ingest and digest spermatozoa in an *in-vitro* environment suggest a similar behavior in an *in-vivo* environment as a cause for decreasing numbers of motile sperm directly affecting reproductive success. Latent trichomoniasis infection could be the cause of the unexplained infertility in men, since sperm damage evidenced by severe asthenozoospermia.

This search showed that, Couples with unexplained infertility must be widely screened and tested for sexually transmitted pathogens to ensure adequate conditions of the female reproductive tract needed to achieve pregnancy. Further studies are required to accurately fill the gap of knowledge between trichomoniasis, unexplained infertility, and implantation failure.

In conclusion: The result indicates that T. vaginalis pathogenicity, adverse reproductive health outcomes, in time diagnosis, and treatment may improve implantation rate in patients with unexplained infertility undergoing Assisted reproductive technology (ART).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Unexpected Adverse Medical & Surgical Health Conditions in Neonates after Elective Cesarean Sections within the Context of the Influence of Local Socidemographic Factors in Babylon

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Abstract

Background: Caesarean section was first introduced to reduce the risks of normal delivery, and is recommended only when the lives of the mother and fetus are compromised, as it is associated with certain risks. However, some women's perceptions may be influenced by local culture. According to international statistics , the rate of C-section is increasing globally, and in Iraq it is 2-3 times higher than other nations. More than 1/3 of women in Babylon choose C-section voluntarily, believing it is safer, healthier, and less painful.

Aim of the Study: Identify the unexpected medical and surgical conditions of newborns by elective cesarean delivery in Al-Hillah city, and assess the association between the actual gestational age at birth and decision by which elective caesarean sections were done as influenced by local sociodemographic factors and perceptions; that resulted in those conditions.

Methodology: This study is a cross sectional study conducted in Al-Hilla General Teaching Hospital from the beginning of March 2018 until the end on August 2018, and included a total of 150 neonates born via elective cesarean section who had unexpected medical and surgical conditions.

Results: Mean maternal age was (26.27±9.56) year ranging from (16-43). Seventy percent of them had timing of < 39 weeks of gestation, and 36.7% had actual gestational age at birth of < 37 weeks. Almost half the them chose C-section for being safe. Respiratory compromise was the commonest medical complication among mothers. There was significant relationship between decision basis and actual gestational age at birth, as well as between maternal age and medical and surgical complications (P-value<0.05).

Conclusion: Neonates born after elective cesarean delivery in Al-Hillah city have significantly higher rates of respiratory morbidity and longer length of hospital stay, with increased risk of certain medical and surgical complications. The unexpected medical complications are inversely related to gestational age at which elective cesarean section is timed.

Keywords: *cesarean section; medical complications; surgical complications.*

Introduction

Child delivery in Babylon is associated with a multitude of physical, emotional, social, physiological, ethnocultural , and psychological dimensions. Cesarean section rates are increasing in Babylon Province. Given the steady rise of C-sections carried out, the unexpected adverse health outcomes in neonates born via this mode of delivery it seems necessary to investigate and forge out sufficient information about this area of obstetric practice and the factors that attract women's decision of choosing this mode of delivery given that few scholarly articled in Iraq are available currently[1].

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In an ethnographic based study carried out by Latifnejad-Roudsari et al. in 2014, non medical factors including dread of mesmerizing normal vaginal delivery related pain,local Iraqi social attitudes and values, and social network were blamed as the reasons affecting the choice of opting for elective cesarean delivery and act partly for a proportion of the observed increase [2].

Caesarean sections are either planned electively or emergent. Planned sections are done before delivery commences, as compared to emergency sections, which are undertaken before or after labour has commenced[3]. The reasonable gestational age for scheduled sections has become a matter of heated debate among professionals dealing with maternal and child health care [4].

Over nearly a half century, maternal and child health care professionals have defined "term pregnancy" based on the assumption that fetal maturity is completed at the end of 37th week[5]. However, it recently became clear that neonatal respiratory complications are reduced when increasing the gestational age to 39 weeks. There is evidence that babies born by planned C-section at 37 weeks have more occurrences of certain compilations that those at 39 weeks[6]. Therefore, experts recommend that elective C-section be conducted from 39 weeks onwards to achieve fetal maturity[7].

Although pregnancy is a physiological process, its end is a painful experience for mothers and associated with fear, dread and anxiety[8]. Local culture in Babylon province has a major impact on women's beliefs and attitudes towards labor pain, coping mechanisms, and observed behaviors. This attitude can influence women's decisions about her mode of delivery[9].

Safe delivery is the target of every medical team dealing with childbirth. C-section was introduced primarily to reduce the risks maternal child complications. Unfortunately there is a false assumption nowadays that scheduled C-section provides a safe and healthier means of escaping vaginal delivery pain . That is why it is estimated that more than half of women voluntarily choose C-section as the preferred mode of delivering their babies[10].

C-section rate is an important health insurance index. According to WHO, C-section rate was 15% in 1985, significantly increasing in 2009 worldwide[11]. In North America notably the United States of America, C-section accounted for 26.1% of all deliveries in 2002. In Europe, this rate was one quarter of births in some EU states. In addition, in Latin America this rate is also quite high reaching up to 40%[12].

According to regional health records and statistics, the prevalence of C-section in Iraq is up to three times higher than the international rate[13]. Although scheduled operations should be decided when the health of the mother or baby are compromised; this mode of delivery has become a way of avoiding distress of labor pain, due to a common notion that its less painful, safer, and healthier than vaginal delivery[14].

Aim of the Study

Identify the unexpected medical and surgical conditions of newborns by elective cesarean delivery in Al-Hillah city, and assess the association between the actual gestational age at birth and decision by which elective caesarean sections were done as influenced by local sociodemographic factors and perceptions; that resulted in those conditions.

Patients and Methods

This is a cross-sectional study conducted in Al-Hilla General Teaching Hospital from the beginning of March 2018 until the end on August 2018, and included a total of 150 neonates born via elective cesarean section who had unexpected medical and surgical conditions following the procedure, who were born at various gestational ages. Excluded cases were those who did not develop unexpected outcomes, as well as those born via emergency C-section or normal vaginal delivery.

Results

Timing of caesarean section was less than 39 weeks in 70.0%, while it was equal or more than 39 weeks in the remaining 30.0% (Figure 1).

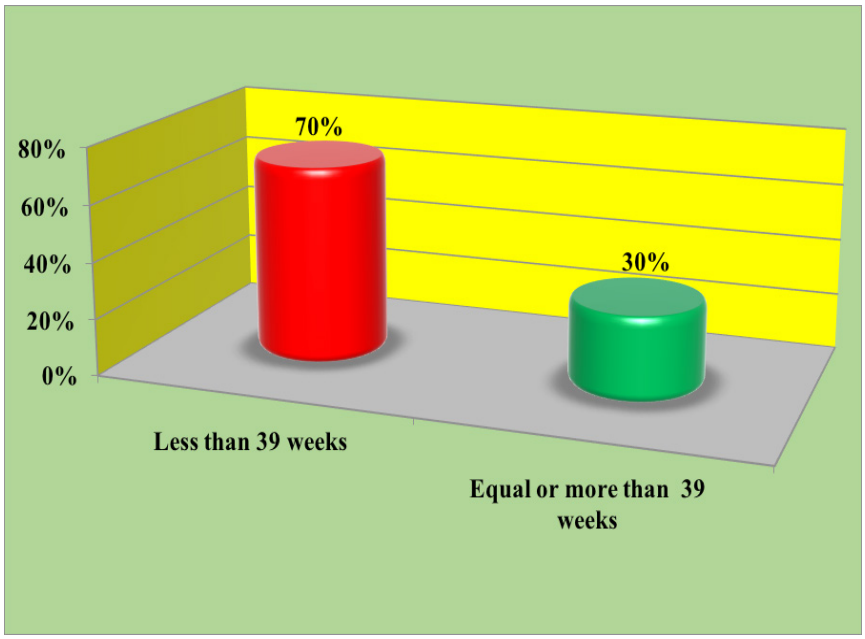

Figure 1: Distribution of the timing of elective caesarean section

Figure 2 shows the primary basis for decision to time elective caesarean section, which is clinical in 26.75% and by ultrasound in 73.3%.

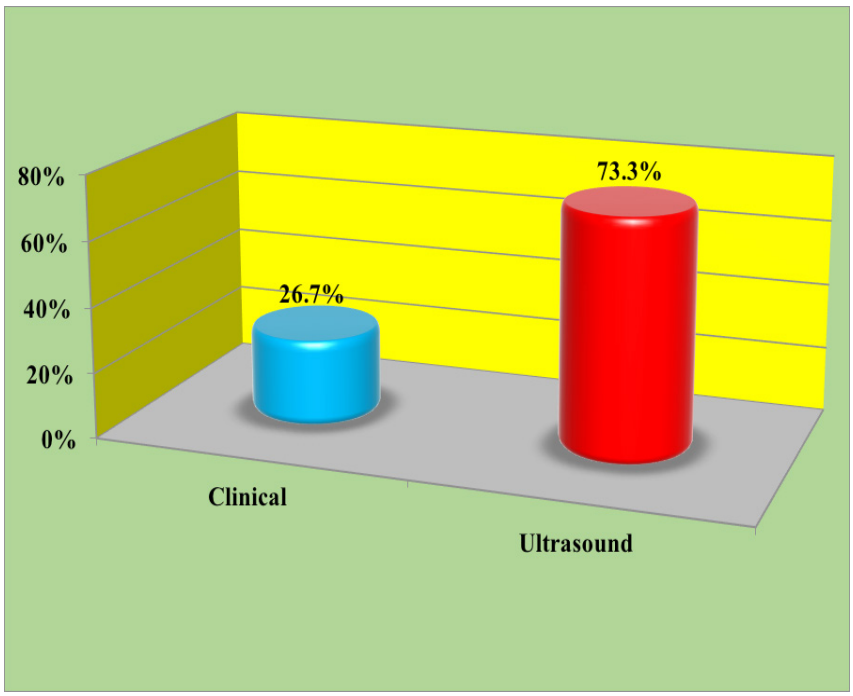

Figure 2: The primary basis for decision to time elective caesarean section

Figure 3 represents the reasons for opting for elective C section cited by women.

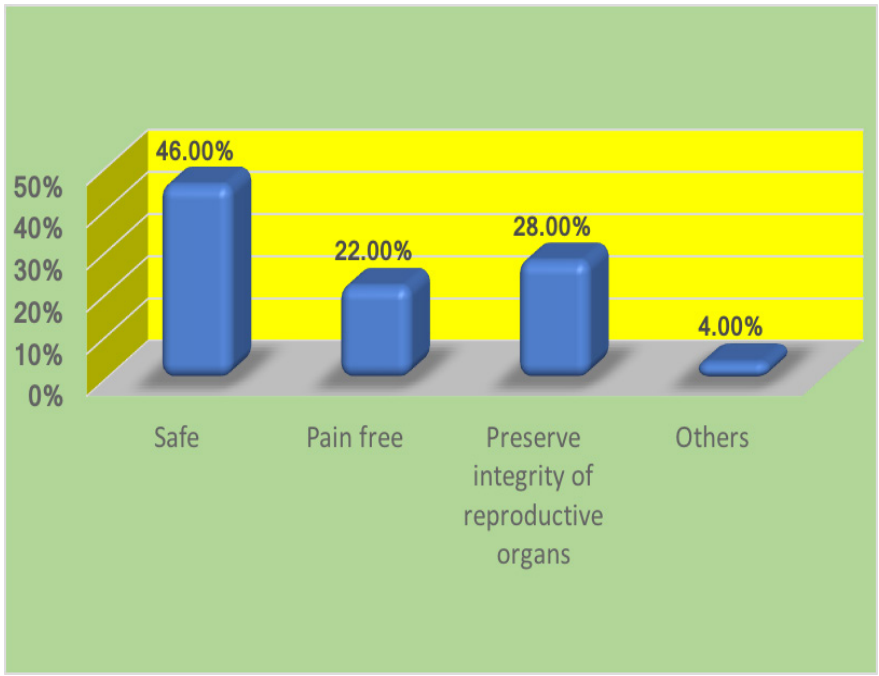

Figure 3: Reasons for opting for elective C-section cited by women

Table 1 shows that the higher percentage of unexpected medical complication of pregnant women who underwent elective caesarean section was respiratory compromised which represented 23.3%, followed by nosocomial infection and neonatal sepsis, prematurity, congenital heart disease which represented 16.7%, 14.7%, and 12.7% respectively.

Table 1: Distribution of unexpected medical complication of pregnant women who underwent elective caesarean section.

Medical complication	Number	Percentage(%)
Respiratory compromised	35	23.3%
Prematurity	22	14.7%
Postmaturity	5	3.3%
Birth asphyxia	10	6.7%
Nosocomial infection &neonatal sepsis	25	16.7%
Reluctant to feed,artificial feeding & GERD	9	6.0%
Seizure and electrolyte disturbance	15	10.0%
Hypothermia and hypoglycemia	5	3.3%
Congenital heart disease & other anomalies	19	12.7%
Stillbirth	5	3.3%
Total	150	100.0%

Table 2 shows that the higher percentage of surgical complication was injury followed by intestinal obstruction, pneumothorax and trachea esophageal fistula in a percentage of 40.0%, 30.0% ,20.0% and 10.0% respectively.

Table 2: Distribution of unexpected surgical complication of pregnant women who underwent elective caesarean section.

Surgical complication	Number	Percentage(%)
Pneumothorax	10	20.0%%
Intestinal obstruction	15	30.0%
Tracheo eosophageal fistula	5	10.0%
Others (injury)	20	40.0%
Total	50	100.0%

Table 3 shows that chi-square test was conducted to find an association between variables (decision by which caesarean section was conducted and actual gestational age at birth) and timing of caesarean section (less than 39 weeks and equal or more than 39 weeks).

Table 3: Association between variables and timing of caesarean section

Variables	Timing of caesarean section		Total	X2	P-value
	<39 weeks	≥39 weeks			
Decision					
Clinically	20(19.0%)	20(44.4%)	40(26.7%)	10.39	0.001*
Ultrasound	85(81.0%)	25(55.6%)	110(73.3%)		
Total	105(100.0%)	45(100.0%)	150(100.0%)		
Actual GA at birth(week)					
< 37	55(52.4%)	0(0.0%)	55(36.7%)	44.99	<0.001*
37 – 42	40(38.1%)	25(55.6%)	65(43.3%)		
≥ 42	10(9.5%)	20(44.4%)	30(20.0%)		
Total	105(100.0%)	45(100.0%)	150(100.0%)		

*P-value≤0.05 was significant

Discussion

Depending on the results of this cross sectional study, vaginal birth should be highly encouraged to avoid unexpected negative maternal child health outcomes . The results are highlighted by the findings of Knoblauch H.[15]. In similar international studies, participants assumed that vaginal delivery was necessary for baby's lungs functional maturation, propping up of mother-child emotional attachment, reduction of unnecessary drug usage and other interventions. Overall, they assumed that vaginal delivery was less risky than C–section as it eliminates maternal child adverse outcomes[16].

The highest percentage of unexpected medical complications of pregnant women who underwent elective C-section was respiratory compromise in 23.3%, followed by nosocomial infection and neonatal sepsis in 16.7%, prematurity in 14.7%, and congenital heart disease in 12.7%. This study is in line with international studies that indicate lung complications are the commonest cause of neonatal morbidity following elective C-sections[17].

These are in part attributed to the absence of hormonal and physiological cascade of changes associated with birth which are vital for lung function

in newborns, which do partially occur in neonates delivered as preterm[18]. Term neonatal respiratory distress syndrome is also associated with higher need for oxygen and assisted pulmonary ventilation, lengthy hospital stays and higher mortality than other causes of respiratory morbidity in term counterparts[19].

Infants delivered by planned C-sections in Babylon have an increased risk of adverse respiratory morbidity. The relative risk increased with decreasing gestational age. Nearly one quarter (23.3%) of unexpected neonatal medical conditions following elective C-sections were neonatal respiratory compromise that required SCBU or RCU admission, including (respiratory distress syndrome, transient tachypnea of newborn and assisted ventilation in respiratory care units). These complications are similar to international studies and surpassed other medical conditions.

As shown by this current research paper elective C-section can elicit some unexpected negative outcomes in neonates. The findings are in agreement with those of Fenwick and associates, who concluded maternal and fetal health, mother-child bonding and attachment, and transition to motherhood as the main positive benefits of vaginal delivery[20]. An outstanding issue pointed out by those with scheduled cesarean experience was mothers' "paralysis" literally and helplessness to care for the child with unexpected negative outcomes and meet the maternal role; the results were in harmony with those of Cranley and colleagues.[21].

Thus results of the present study demonstrate that vaginal delivery remains and should remain a symbol of well being and health as it is a defining point in life. Since childbirth pain is usually ends with positive outcomes ,this distinguishes it from other types of painful experiences. The study of Manthata also explicitly emphasized this fact[22]. Selecting C-section electively in Babylon according to this paper was mainly out of fear of labor pain. The study of Poikkeus likewise concluded that women's preference for elective C-section is due to their unfound anxiety of pain during vaginal delivery and unrealistic thoughts about their inability to withstand vaginal birth[23].

Almost all participants had positive attitude towards scheduled C-section despite its adverse outcomes. They also attributed many advantages to it. The most important conceptions were that it is harmless, painless and maintain beauty of reproductive organs, with no negative impact on intimacy and sex life[24]. Gungor and associates, in a study in 2008, also documentd similar social beliefs. They also found that the frequency and mode of delivery are greatly influenced by cultural beliefs[25].

Conclusions

Neonates born after elective cesarean delivery in Al-Hillah city have significantly higher rates of respiratory morbidity and longer length of hospital stay, with increased risk of certain medical and surgical complications. The unexpected medical complications are inversely related to gestational age at which elective cesarean section is timed. The recommended gestational age at which elective cesarean section should be timed is at 39 weeks.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Isolation of MRSA from Drinking water Supplies in Al-Anbar Province, Iraq

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Abstract

This study was aimed to isolate MRSA from water supplies from different places in alanbar province, fifty water samples were cultured on nutrient, macConkey and blood agars then subcultured on MSA and Staph 110, then gram stain and biochemical test done to confirm this bacteria. Then antibiotic susceptibility test were done on S. aureus by using different antibiotics including Methicillin, erythromycin, Doxycycline, Sulfa-trimethoprim, penicillin, chloramphenicol, ciprofloxacin.

The results showed that 12 (24%) samples were diagnose as S. aureus, these were showed complete resistance to methicillin, erythromycin, Doxycycline and showed different percent for resistance to other antimicrobials that used.

In conclusion, the MRSA were isolated from drinking water in significant percentage and this strain of bacteria were showed higher resistance to antibiotics.

Key words: *MRSA, drinking water, Antibiotic.*

Introduction

The WHO reported that MRSA was an important pathogenic bacteria causing high infections that was initially detected in 1961 & became a main threats to the public health (1).

In a study of Chen et al. (2), they reported that above two million of MRSA infection cases around the different countries in the world producing human death. As described in the UK, above five thousands deaths each year were showed to be linked with MRSA, although in Netherlands, MRSA has been found to be causes for in excess of twenty percent of all infections for MRSA (3). In Europe, it has been reported that MRSA was responsible for above 170,000 infections each year, consistent to forty four percent from all infections linked to the health care (4).

Staphylococcus aureus is an environmentally widespread but it is found chiefly on the skin as well as mucous membranes of human and animals. Staphylococci are sometimes identified in the GIT and has been discovered in a sewage. S. aureus can become free by human direct contact with the water environments for example the pools for swimming as well as fun waters. It has been also identified in a drinking water supplies (5), there is an indication of spread through the drinking of such water. While Staph. Spp. are somewhat resist to residuals chlorine than the E. coli, their occurrence in water is restricted by conventional therapy as well as disinfection procedures (6).

This study aimed to isolate MRSA from human water supplies and detection of resistance to different antimicrobials by using antimicrobials sensitivity test.

Materials and Method

A Fifty drinking water specimens were taken from a different places found in Al-anbar province (Fallujah, Heet, Ramadi, Ana, Rawa, Al-qaim, Saqlawya, Garma), these were cultured on primary media (nutrient, macConkey, blood agars), then were subcultured on other media such as mannitol salt agar and Staph 110, then gram stain as well as biochemical tests were done including catalase, oxidase as well as coagulase (7).

The antibiotics sensitivity test were done according to Bauer et al. (8) by using different antibiotics including Methicillin, erythromycin, Doxycycline, Sulfa-trimethoprim, penicillin, chloramphenicol, ciprofloxacin.

Results and Discussion

The current results were showed detection of 12 isolates of MRSA from water specimens (Table 1, Fig. 1&2).

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Table 1- Number and percentages of MRSA from different location

Location	Number of MRSA	Percentage (%)
Fallujah	1	8.33
Ramadi	2	16.66
Heet	1	8.33
Ana	1	8.33
Rawa	0	0
Al-qaim	1	8.33
Saqlawya	4	33.33
Garma	2	16.66
Total	12	24%

Figure -1- S. aureus on MSA (left)

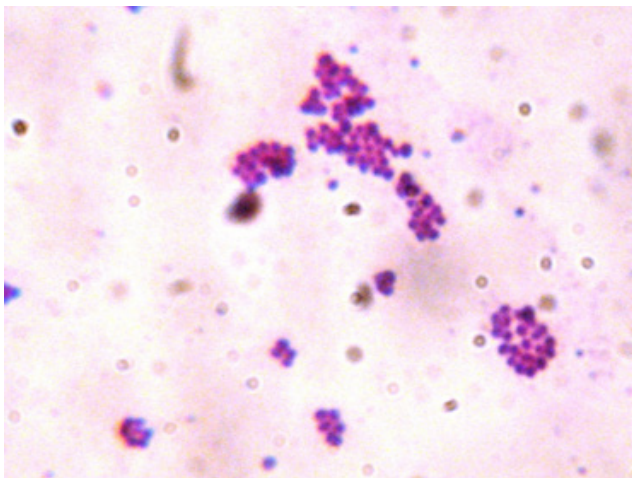

Figure -2- Gram positive cocci

The WHO reported that above 80% of all illness as well as the diseases on the world is made by insufficient hygiene, contaminated water or unreachability of water & at least five million deaths occur in each year which attributed to the water-borne illnesses (9). These results were in agreement with results of (10) who isolated S. aureus from the water provisions. Also, these similar to results of another study in gana that showed S. aureus was detected from about 64% of the specimens, with about 34.38% from well water supplies, 32.81% from hole supply & 31.25% from the tap water (11).

A study of Salim et al. (12) in Iraq found that Staphylocossus aureus were isolated from bottled drinking water during storage for one year at 18c. Also, in Nigeria (13) isolated *S. aureus* from a drinking water. Moreover the bacteria that resist antibiotic were upper in the tap water than in treated water (14). Additional studies have also recognized *S. aureus* that are MDR in seaside societies in their tap water as well as fresh water (15).

The results were disagree with a results of Hillo (16) who reported that *S. aureus* not detected in drinking water in Albasrah city.

The present results of antimicrobial sensitivity test for presumptive *S. aureus* (12 isolates) showed complete resistance to methicillin, erythromycin, Doxycycline and showed different percent for resistance to other antimicrobials that used (Table 2, Figure 3).

Table 2 Antimicrobial susceptibility test for *S. aureus*

Antimicrobials	Resistance (%)	Sensitive (%)
Methicillin	12 (100)	0
erythromycin	12 (100)	0
Doxycycline	12 (100)	0
Sulfa-trimethoprim	5 (41.7)	7 (58.3)
penicillin	10 (83.3)	2 (16.7)
chloroamphenicol	3 (25)	9 (75)
ciprofloxacin	4 (33.3)	8 (66.7)

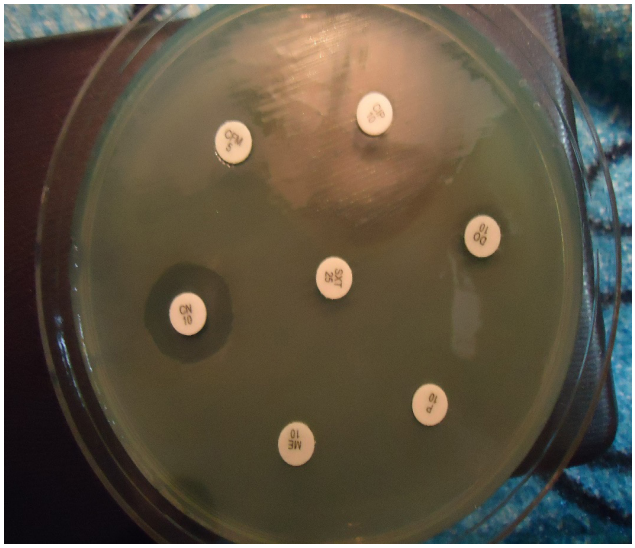

Figure 3- showing MRSA on Muller hinton agar

The current results were in agreement with results of many studies, Younis et al. (17) reported that 96.6% of *S. aureus* isolates were resistant to penicillin. Shi et al. (18) reported that 87.6% of isolates were resist to penicillin. Abera et al. (19) recorded that 94.4% of isolates were resist to the penicillin. Vanderhaeghen et al. (20) reported that all isolates (118 *S. aureus* isolates) in their study were resist to tetracycline. Tiao (21) found that none of the 20 *S. aureus* isolates tested in her study carried the mecA gene and one showed methicillin resistance phenotypically. Turutoglu et al. (22) stated that out of 18 *S. aureus* isolates, 15 were typically resist to the methicillin but didn't carry the mecA gene.

Also, this strains presented greater amounts of resistance to the family of macrolides especially erythromycin as reported by (23).

The incidence of genes that responsible to antibiotic resistant in a drinking water is of special concern because of the amount of persons as well as animals who possibly affected by consuming contaminated water that comprising the bacteria which were antibiotic resistant that can transmit antibiotic resistance to the usual bacterial flora in the bowels of human as well as animals.

The presence of resistance for most antibiotics that used may be due to random usage of antibiotics and usage of drugs by incomplete course, these may lead to what is called multi drug resistance bacteria.

It has been concluded that MRSA isolated from water supplier in a significant number and this has been resist to multiple antibiotics.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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The Predictive Value of Red Cell Distribution (RDW) in Patients with Type 1 and Type 2 Diabetes Mellitus

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Abstract

Background: Epidemiologically speaking the diabetes mellitus is one of the common leading causes of mortality and morbidity worldwide. Prognosis of the disease is variable and depends on the development of macrovascular and microvascular complications. Researchers are nowadays spending plenty of time trying to identify prognostic factors in order to make treatment approach be tailored according to the predictive value of such prognostic factors. One of these newly introduced factors is red cell distribution width (RDW).

Aim of the study: The current study was aiming at shedding light of the possible prognostic role of RDW in patients with type 1 and 2 diabetes mellitus.

Patients and methods: The present case control study was carried out at diabetes center in Al-Diwaniayh Teaching Hospital, Al-Diwaniyah Province, Iraq. The study started on January 2019 and ended on August 2019. The study included 30 patients with established diagnosis of type 2 diabetes mellitus, 30 patients with type 1 diabetes mellitus and 30 apparently healthy control subjects. Diabetic patients were selected randomly from the pool of patients already registered in that center.

Results: We grouped diabetic patients into two groups according to HbA1c level, ≤7% and > 7% and contrasted hematological levels between those new groups. The results showed no significant difference in mean hematological values between the two groups in diabetic type 1 and type 2 patients ($P > 0.05$).

Conclusion: There was no significant role for RDW in predicting poor glycemic control of patients with type 1 or type 2 diabetes mellitus.

Key words: *RDW, diabetes mellitus type 1 and 2*

Introduction

Diabetes mellitus is represented by a heterogenous group of metabolic disorders sharing in common the criterion of chronically elevated blood sugar level [1, 2]. The disease is heterogenous because of variation in the etiology associated with raised blood sugar. The most common form is type 2 diabetes mellitus in which the etiology is shared by resistant to insulin action and some degree of defective insulin secretion; even though, early in the disease there may transient period of hyperinsulinemia [3]. Type 2 diabetes was previously called non insulin dependent diabetes mellitus and adult onset disease; however, and because of insulin requirement to control the blood sugar level in significant proportion of patents late in the disease, in addition to the identification of children with type 2 diabetes mellitus, the preferred name nowadays becomes the "type 2 diabetes mellitus" [3].

Type 1 diabetes mellitus, on the other hand, is often recognized in patients younger than 40 and is principally caused by profound insulin deficiency [4]. From etiologic perspective, the disease is autoimmune in approximately 70-90 % of cases and the rest of cases are

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labeled idiopathic as no cause can identified [5]. Other forms of diabetes mellitus included gestational diabetes, endocrine abnormality, drug induced and exocrine disease of the pancreas such as pancreatic tumors and resection [6].

Epidemiologically speaking the disease is one of the common leading causes of mortality and morbidity worldwide [7, 8]. Prognosis of the disease is variable and depends on the development of macrovascular and microvascular complications [9]. Researchers are nowadays spending plenty of time trying to identify prognostic factors in order to make treatment approach be tailored according to the predictive value of such prognostic factors [10]. One of these newly introduced factors is red cell distribution width (RDW) [11]. Red cell distribution width (RDW) is a quantitative hematological parameter which can indicate the degree of variation in size of RBC (anisocytosis); initially was considered in the differentiation of cause of anemia [12].

The data obtained from published articles dealing with the predictive role of RDW in diabetes mellitus are conflicting and no clear consensus can be inferred from these data. Besides, data from Iraqi literatures dealing with this subject are extremely rate; therefore, we were encouraged to plan and conduct the current study aiming at shedding light of the possible prognostic role of RDW in patients with type 1 and 2 diabetes mellitus.

Patients and Methods

The present case control study was carried out at diabetes center in Al-Diwaniayh Teaching Hospital, Al-Diwaniyah Province, Iraq. The study started on January 2019 and ended on August 2019. The study included 30 patients with established diagnosis of type 2 diabetes mellitus, 30 patients with type 1 diabetes mellitus and 30 apparently healthy control subjects. Diabetic patients were selected randomly from the pool of patients already registered in that center.

Variables included in the questionnaire form included: duration of illness, age, body mass index, systolic and diastolic blood pressures, a number of hematological parameters, namely (MCV, RDW, WBC and platelet count), serum lipid profile (triglyceride, HDL and LDL) and glycated hemoglobin (HbA1c %). Patients were considered type 2 diabetes bases one positive protein c results.

The study was approved by institutional ethical approval committee and verbal consent was obtained from all participants.

Data were then transformed into an SPSS (version 23) spread sheet for statistical analysis. Quantitative data were expressed as mean and standard deviation. Independent samples t-test was used to compare mean values between two groups, whereas, one way ANOVA was used to compare mean values among more than two groups. The level of significance was considered at $P \leq 0.05$.

Results

The current study included 30 patients with diabetes mellitus type 1, 30 patients with type 2 diabetes mellitus and 30 control subjects. The characteristics of subjects enrolled in the current study are shown in table 1. There was no significant difference in mean duration of diabetes mellitus between type 1 and type 2 DM patients ($P > 0.05$); however, patients with type 1 DM were significantly younger than type 2 DM patients ($P < 0.05$). There was also, no significant difference in mean BMI among study groups ($P > 0.05$).

In addition, there was no significant difference in mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) among groups ($P > 0.05$). WBC count was significantly higher in diabetic patients in comparison with control group ($P < 0.05$); however, the count was comparable in both diabetic groups ($P > 0.05$). Besides, there was no significant difference in other hematological parameters and serum lipid profile among study groups ($P > 0.05$), as shown in table 1. We grouped diabetic patients into two groups according to HbA1c level, ≤7% and > 7% and contrasted hematological levels between those new groups. The results showed no significant difference in mean hematological values between the two groups in diabetic type 1 and type 2 patients, as shown in table 2 and 3.

Table 1: Characteristics of patients with diabetes and control subjects

Characteristic	Control n = 30	DM type 1 Protein C –ve n = 30	Type Protein C +ve n = 30	P value
Duration of illness (years)	-----	7.23±691	7.1±682	> 0.05 ¥
Age (years)	55.42 ±11.7	35.64±10.81	57.84±10.7	<0.01 †**
BMI (kg/m2)	31.53±3.54	31.50±4.73	32.50±4.13±	> 0.05 †
SBP (mmHg)	127.60±6.1	129.55±16.7	130.41±15.6	> 0.05 †
DBP (mmHg)	79.1 ±6.51	73.75±7.11	74.55±5.15	> 0.05 †
MCV (fl)	83.51±7.5	81.80±7.00	81.12±7.6	> 0.05 †
RDW	12.98±1.96	13.61±1.2	13.57±1.5	> 0.05 †
WBC X106/L	5.98±2.1	6.8±1.99	7.1±2.20	<0.05 *
Platelet count X106/L	257.76±55.54	254.81±77	255.72±74	> 0.05 †
TG (mg/dl)	1.72±0.81	1.70±0.1	1.68±0.7	> 0.05 †
HDL (mg/dl)	1.2±0.37	1.1±0.08	1.09±0.2	> 0.05 †
LDL (mg/dl)	2.92±0.85	2.71±0.6	2.60±0.80	> 0.05 †

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MCV: mean corpuscular volume; RDW: red cell distribution width; WBC: white blood cell count; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; ¥: independent samples t-test; †: one way ANOVA; *: significant at P ≤ 0.05; **: highly significant at P ≤ 0.01

Table 2: Hematological characteristics of patients with type 1 diabetes mellitus according to HbA1c level

Hematological characteristic	Hb A1C ≤7%	Hb A1C >7%	P value
MCV (fl)	81.94±7.1	80.23±7.2	> 0.05 ¥
RDW	13.94±1.1	14.22±1.09	> 0.05 ¥
WBC X106/L	255.83±61.1	259.56±65.1	> 0.05 ¥
Platelet count $X10^6$/L	6.55±1.9	7.17±2.5	> 0.05 ¥

MCV: mean corpuscular volume; RDW: red cell distribution width; WBC: white blood cell count; ¥: independent samples t-test

Table 3: Hematological characteristics of patients with type 2 diabetes mellitus according to HbA1c level

Hematological characteristic	Hb A1C ≤7%	Hb A1C >7%	P value
MCV (fl)	82.94±7.1	80.93±7.2	> 0.05 ¥
RDW	13.84±1.08	14.42±1.2	> 0.05 ¥
WBC X106/L	253.83±62.1	257.56±65.1	> 0.05 ¥
Platelet count X106/L	6.67±1.9	7.19±2.2	> 0.05 ¥

MCV: mean corpuscular volume; RDW: red cell distribution width; WBC: white blood cell count; ¥: independent samples t-test

Discussion

Diabetes mellitus is a heterogenous disorder with wide spectrum of outcomes and complications that vary from to patient. These outcomes are determined by macrovascular and microvascular complications. Microvascular complications include nephropathy, retinopathy and neuropathy; whereas macrovascular complications included ischemic heart disease and cerebrovascular accidents. The development of these complications can be delayed and their effects can be minimized if patients were able to control their blood sugar strictly. For that reason, the quality of life of patients with type 1 and type 2 diabetes mellitus can be improved if treatment is directed toward strict glycemic control. Indeed, glycemic control can be determined by measuring HbA1c every 90 days in average. Recent studies have shown that the red cell distribution width (RDW) can be used as a prognostic factor since it has a correlation to HbA1c and diabetic complications and can be used as a predictive marker for diabetic control [11, 13]; however, some authors have denied such role for RDW in diabetes mellitus [14, 15].

The evidence connecting red cell distribution width (RDW) with an increasing risk of mortality has been grown since the early report of its predictive value in patients with heart failure. It has also been described to independently anticipate cardiovascular and overall mortality in various high-risk populations and in the general population [16, 17]. Besides, it has been shown to be a significant predictor of mortality in number situations such as malignancies, obesity and renal disorders [18]. For that reason, some authors suggested that linking RDW to diabetes prognosis is justified [11].

In the current study it was shown that RDW is not sufficiently affected by type of diabetes or even by degree of glycemic control indicated by HbA1c. These results are in agreement with a number of authors [14, 15] and disagree with other authors [13, 11], thus we believe that it is very early to judge the role of RDW in diabetic prognosis and that a lot of research work is needed in order to reach clear consensus about such role.

In one study, it was found that Red cell distribution width (RDW) was significantly higher in diabetic patients than in control subjects (P=0.008). It was also higher in patients with uncontrolled glycemia (HbA_{1c} >7%) than those with good control (HbA_{1c} ≤7%; P=0.035) [11].

Actually, these findings are not consistent with the present study findings; however, the author of the later study agreed with us that other hematological characteristics were not affected by diabetes or its degree of glycemic control.

It is worth to mention that WBC was significantly higher in both diabetic groups when compared to non diabetic group. This finding is similar to the findings of several other authors [19-21]; however, it contradicts other authors [11]. Several explanations have been suggested to explain the high WBC in association with diabetes such as increased insulin resistance, development of complications and high rate of infections [19-21].

In conclusion, there was no significant role for RDW in predicting poor glycemic control of patients with type 1 or type 2 diabetes mellitus.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Histopathological Changes Associated with Cutaneous Leishmaniasis in Patients with Baghdad Boil/South of Iraq

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Abstract

Cutaneous leishmaniasis is an endemic disease in Iraq and it is become epidemic in Nassirriyah city recently/ South of Iraq.

The following histopathological study were achieved in microbiology dept. at college of Medicine in University of Thiqar, targeting Nassiriyah city in south of Iraq where Baghdad boil is highly distributed during a period of time at end of April 2016. several of tissue (skin) biopsies were obtained by dermatologist under sterilizing condition and at Al-Hussain teaching hospital where the patients have entered for treatment . the result explain the following changing :

- marked epidermal hyperplasia with hyperkeratosis .
- two small granulomas of macrophages some with vaculation in the dermis .
- sever dermal hyperplasia and foliculitis also hyperkeratosis and scab formation.
- marked hyperplasia of epidermis , hyperkeratosis and scab formation .

***Key words** : coetaneous leishmaniasis , Baghdad boil , histopathology*

Introduction

The parasitic disease of Leishmaniasis caused by haemoflagellate *Leishmania tropica* . It is widespread and may cause serious health problems in communities throughout the Mediterranean regions and the Middle East, including Iraq [1-3]. There are an estimated 12 million cases worldwide, and there are about 1.5 million new cases of cutaneous leishmaniasis each year, of which over 90% occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru [4]. Old World disease primarily is caused by *Leishmania tropica minor* in urban areas and *Leishmania tropica major* in dry desert areas [3].

In Iraq, two species are present: *L tropica*, the agent of anthroponotic cutaneous leishmaniasis (ACL), and *L. major*, the agent of zoonotic cutaneous leishmaniasis (ZCL). Both ACL and ZCL were reported as causative agents of leishmaniasis in Iraq, but ACL is found mainly in suburban areas [5]. The disease is epidemiologically unstable, with large and unpredictable fluctuations in the number of cases. The total incidence rate of cutaneous leishmaniasis in Iraq varies from 2.3 / 100000 to 45.5 / 100000 [5]. Cutaneous leishmaniasis is characterized by one or more cutaneous lesions on areas where sandflies have fed. Persons who have cutaneous leishmaniasis have one or more sores on their skin. The sores can change in size and appearance over time. They often end up looking somewhat like a volcano, with a raised edge and central crater. A scab covers some sores. The sores can be painless or painful. Some people have swollen glands near the sores (for example, in the armpit if the sores are on the arm or hand).[6]

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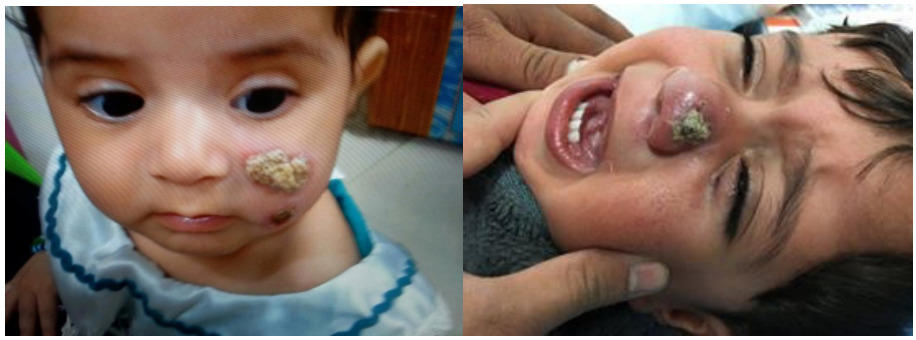
Materials and Method

- **Skin biopsy :** Biopsies were taken from patients by the dermatologist and placed in 10% of formalin to prepare it for tissue processing .(9)

- **Histology :** Tissues biopsy were processed according to [9], briefly the tissue from the skin collected and placed in 10% formalin for histopathological studies and dehydrated by several dilutions of ethanol alcohol dealcoholization with xylol, then embedded with paraffin wax blocked (3-5) mm thickness sections were obtained by microtome. The sections were put on glass slides deparaffinised with xylol, rehydrated by alcohol and stained by hematoxyline and eosin.



Fig(1) : explain the presence of Baghdad boil or cutaneous leishmaniasis

Results

The following histopathological changing has associated with Baghdad boil , the clinical infection with cutaneous leishmaniasis caused by the protozoan *Leishmania tropica* , as illustrated in the following figures;

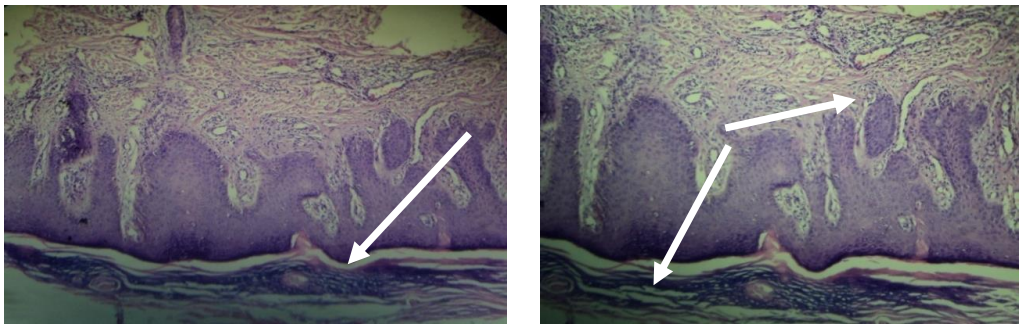
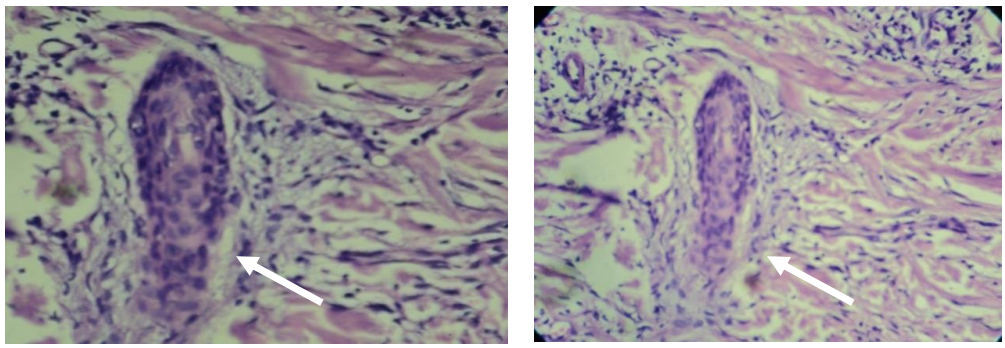


Fig (2) : marked epidermal hyperplasia with hyperkeratosis (10X)



Fig(3): small granuloma in the dermis consist of macrophages and fibroblast at the periphery (40X)

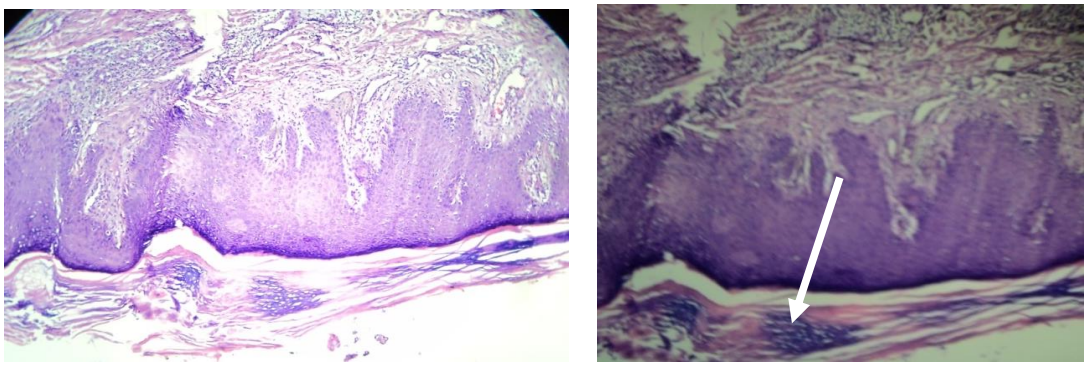


Fig (4) marked hyperplasia of epidermis , hyperkeratosis and scab formation (10X)

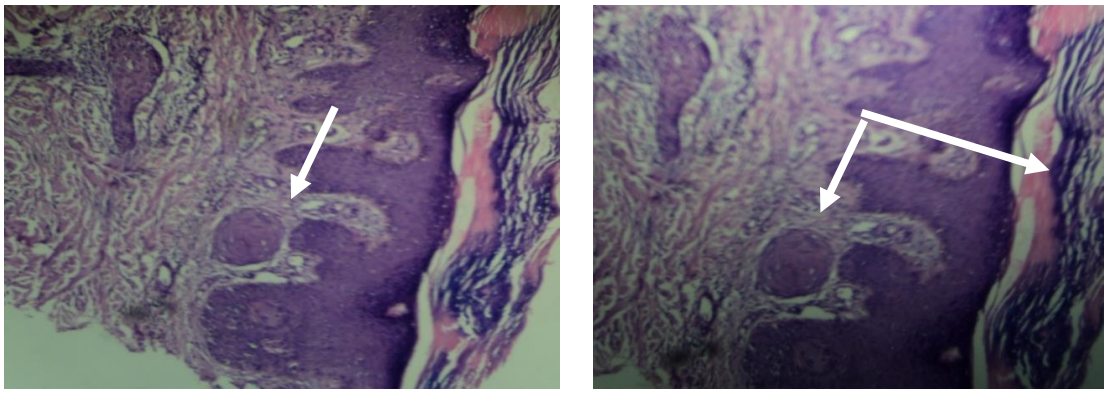


Fig (5) : marked hyperplasia of epidermis , hyperkeratosis and scab formation , keratin nest in the dermis (10X)

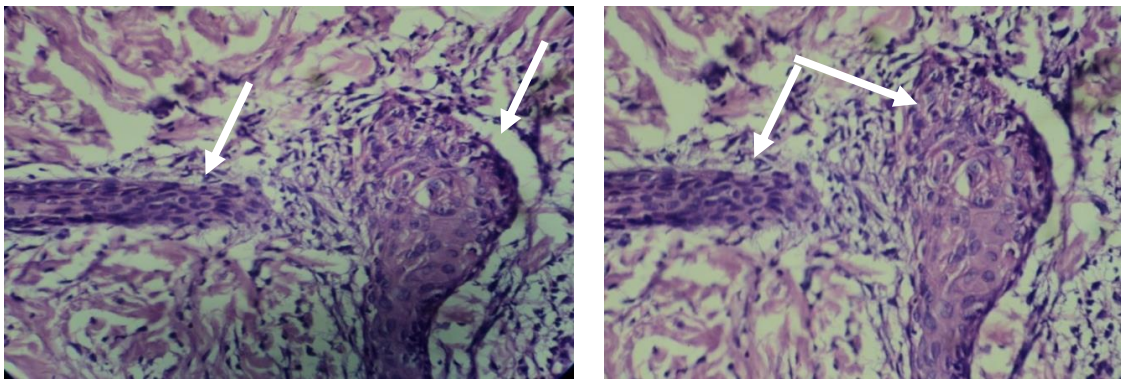


Fig (6): two small granulomas of macrophages some with vaculation in the dermis (40X)

Discussion

The skin covers the human body and is constantly exposed to the environment. As a barrier against infection, the skin not only forms a physical hindrance but also serves as a very important immunological organ.

cutaneous leishmaniasis appears in variable patterns clinico-pathologically, and sometimes a simple erythemic lesion, shows (LD) bodies and in few cases has been diagnosed clinically malignant and remained untreated for years. The CL clinically presents according

to the stage of infection and the clinical type of disease basically an ulcer appearing at the site which heals slowly, takes months to years depends upon the host immunity.

Cutaneous leishmaniasis, a wide clinical and histological spectrum. The histological picture in CL differs according to the stage of infection and the clinical type and host immunity. There is evolution of the lesion as it progresses from a papule and nodule into a soft boggy, crusted plaque or nodule [12]. The lesion breaks up after 3-4 months into a well circumscribed ulcer, which heals slowly over a period of 3 to 12 months. The histological spectrum in CL has wide range with a great variation in morphology. The appearances may range from predominance of leishmanial granuloma with macrophages showing epithelioid. Granuloma formation results from macrophages activation into epithelioid cells, considering clinicopathological relationship [11]. The histological picture in CL differs according to the stage of infection and the clinical type. A consistent finding is a moderate to heavy dermal infiltrate of lymphocytes, plasma cells, and macrophages. In approximately 80% of cases, epithelioid cell granulomas with giant cells and a rim of lymphocytes are present. It is important to search for amastigotes, which are diagnostic [13,14]. Histopathological changing of skin during cutanous leishmaniasis explain the granulomatous reaction in the site of infection together with presence of amastigote stage or LD bodies in the lesion based on the current study. The essential feature of CL pathology is the colonization by amastigotes of cells of the mononuclear phagocytic system and the resulting granulomatous inflammatory response. A granuloma is defined as a compact collection of mature mononuclear phagocytes, not necessarily accompanied by accessory features such as necrosis. Conceptually granuloma evolves in three stages: 1) the development of an infiltrate of young mononuclear phagocytes; 2) the maturation and aggregation of these cells into an unorganized granuloma; and 3) the potential maturation of these cells into an epithelioid or organized granuloma [7-10]

Cutaneous Leishmaniasis presents a spectrum of manifestations both clinically and histologically. Lesions can present as nodule, plaque or ulcer mostly present on exposed sites [8,9]. Histopathological findings in acute CL include dermal infiltrate predominantly consisting of macrophages containing large number of leishmania organism called LD bodies[5,6] In addition plasma cells and dense mixed inflammatory cell infiltrate are also present in dermis. The morphology of LD bodies in histopathological sections were rounded with a nucleus and kinetoplast, in some sections spindle shape form similar to smear morphology were detected [7, 8].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Serum Anti-Mullerian Hormone Concentration and Function of The Pituitary-Gonadal Axis in Iraqi Patients with Chronic Kidney Disease

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Abstract

Infertility among patients with chronic kidney disease (CKD) has been extensively investigated. However, reproductive function in these patients is less well-characterized. The present study was aimed to examine the associations among chronic kidney disease (CKD) and fertility status by evaluation levels of: Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH). Estrogen (E2) and Progesterone (P4) for females and Testosterone for males along with Anti-Mullerian Hormone (AMH) in CKD patients. The study has been registered at Nephrology and Transplant Center in Medical City of Baghdad- Iraq from April 2018 to July 2018. The study included 50 patients who are diagnosed to have CKD stage-5, their ages ranged between 20-50 years (25 males and 25 females) and 20 matched apparently healthy as control, their ages ranged between 20-48 years (10 males and 10 females). This study showed a highly significant ($P<0.01$) increase in LH, FSH levels in CKD patients compared to the control group. Highly significant ($P<0.01$) decrease in E2 level in CKD females patients compared to the control group, while there was non-significant ($P>0.05$) decrease in P4 level in CKD females patients compared to the control group. In parallel, there is significant ($P<0.05$) decrease in Testosterone level in CKD males patients compared to the control group. On the other hand, the study showed non-significant ($p>0.05$) increase in AMH level in CKD patients compared to the control group.

Keywords: *CKD, AMH, LH, FSH, E2, P4, Testosterone.*

Introduction

The genesis of sexual dysfunction in patients with chronic kidney disease (CKD) is multifactorial [1]. Hormonal dysfunction in CKD is clinically accompanied by sexual dysfunction that influences the life quality of these patients, in advanced stages of CKD, these sexual dysfunctions can be more evident, several changes in hormone levels have been demonstrated, these changes can be because of decreased renal excretion and disturbance of the endocrine system because of uremic effects [2]. Disturbances in the hypothalamic-pituitary-gonadal axis, resulting in alterations in signal-feedback mechanisms and hormone production, are seen already in patients with moderate reduction in the glomerular filtration rate and often become more obvious as kidney failure progresses [2,3]. Earlier studies have shown elevated levels gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [4]. Decreased levels of free and total testosterone have also been reported in patients with CKD [5,6]. In those patients, level LH and FSH rise up [7]. In uremic patients, GnRH releasing from the hypothalamus and GnRH- LH signal are impaired. In these patients, bioactivity of LH changes and then a series of function inhibitors of LH are made [8].

Anti-Müllerian hormone (AMH) is a glycoprotein with a fundamental role in male sex differentiation [9]. As such, AMH is now recognized as the best available biomarker of both the functional and true ovarian reserve [10]. In male serum AMH is correlated with spermatogenesis, the Sertoli cells secrete AMH, and it is a specific marker of Sertoli cell function [11]. CKD and its consequences affect the production of AMH, resulting in change in AMH levels, this may indicate impaired function of Sertoli cells [12]. In women with CKD the aforementioned fertility disturbances may be

caused by the damage of the ovaries by uremia, this may reflect an intrinsic dysregulation of the granulosa cells leading to higher AMH production or alternatively AMH accumulation in CKD patients requiring dialysis [13,14].

Material and methods

The study has been registered at Nephrology and Transplant Center in Medical City of Baghdad- Iraq from April 2018 to July 2018. This study included two groups, patients and control group. The study included 50 patients are diagnosed to have CKD stage 5, their ages ranged between 20-50 years (25 males and 25 females) and 20 control their ages ranged between 20-48 years (10 males and 10 females). Blood samples were collected from all groups for estimation of (LH, FSH, E2, P4, Testosterone), All those biomarkers were estimated in serum of all subjects by using an automated quantitative COBAS e 411 test (from Roche, Germany). Serum AMH was measured by ELISA using a kit supplied by Beckman Coulter- Germany.

Results

In this study the level of LH (7.10 ± 0. 57) was highly significant increased (P<0.01) in comparison with control (3.62 ± 0.45). Also, the level of FSH showed high significant increase (P<0.01) in patients group (8.01 ± 0.50) when compared with control group (4.79 ± 0.55), Table (1).

Table 1: Comparison between patients and control in level of LH, and FSH.

Group	Mean ± SE	
	LH mIU/ml	FSH mIU/ml
Patients	7.10 ± 0. 57	8.01 ± 0.50
Control	3.62 ± 0.45	4.79 ± 0.55
T-Test	1.897 **	1.732 **
P-value	0.0005	0.0004
** (P<0.01): Highly Significant		

On the other hand the mean of serum E2 and P4 levels in the females of study groups are summarized in Table (2). E2 level (7.89 ± 1.17) in patients group observed highly significant decrease (P<0.01) in comparison with control group (20.80 ± 5.04), while P4 levels showed non-significant decrease (0.407 ± 0.06) in patients compered to control group (0.408 ± 0.10) .

Table 2: Compare between patients and control (Female) in E2 and P4.

Group	Mean ± SE	
	E2 Pg/ml	P4 ng/ml
Patients	7.89 ± 1.17	0.407 ± 0.06
Control	20.80 ± 5.04	0.408 ± 0.10
T-Test	7.318 **	0.249 NS
P-value	0.0008	0.997
** (P<0.01): Highly Significant ; NS: Non-Significant.		

The present study displayed, significant decrease (P<0.05) in Testosterone level in male patients with CKD (1.57 ± 0.25) in comparison with healthy control group (2.73 ± 0.58).as shown in Table (3).

Table 3: Compare between patients and control (Male) in level of Testosterone.

Group	Mean ± SE of Testosterone ng/ml
Patients	1.57 ± 0.25
Control	2.73 ± 0.58
T-Test	1.097 *
P-value	0.0387
* (P<0.05): Significant	

On the other hand, in this study as shown in Table (4), level of serum AMH found to be slightly elevated but not significant in patients with CKD in comparison to control group. AMH levels in both groups (9.03 ± 1.58), (7.76 ± 1.75) respectively showed non-significant difference (p>0.05).

Table 4: Compare between patients and control in level of AMH.

Group	Mean ± SE of AMH ng/ml
Patients	9.03 ± 1.58
Control	7.76 ± 1.75
T-Test	5.490 NS
P-value	0.645
NS: Non-Significant.	

Statistically, the finding in Table (5) indicate a correlation coefficient between AMH hormone and other parameter in this study. As showed there is a highly significant (P<0.01) positive correlation between AMH with Testosterone, non-significant (p>0.05) positive correlation between AMH and FSH, E2. On the other hands there is non-significant (p>0.05) negative correlation between AMH with LH and P4.

Table 5: Correlation coefficient between AMH and other parameters.

Parameters	Correlation coefficient (r) and Level of significant
	AMH
LH	-0.15 NS
FSH	0.09 NS
E2	0.001 NS
P4 -0.16 NS	
Testosterone	0.22**
* (P<0.05), ** (P<0.01), NS: Non-significant.	

Discussion

End stage renal disease (ESRD), has a strong influence on the hypothalamic-pituitary-gonadal axis resulting in hormonal disturbances and deterioration in gonads function. [2,15]. The results of this study on elevated levels of LH and FSH in CKD patients are consistent with previous study reported by Anatharamaa and Schmidt [16], who found that changes in pulsatile release of GnRH and LH reduce feedback inhibition of LH production (because of low levels of testosterone) contribute to high levels of LH, FSH secretion in CKD patients. With CKD there was a significant increase in LH and FSH and development of pattern of hyper gonadotropic hypogonadism, which indicates that uremic metabolite tend to increase CKD effect on testes and ovaries more than hypothalamic or pituitary function [17].

The results of this study agree with those that have been found with previous studies [7,8,15], who showed that low concentration of E2 has been seen in uremic patients, nevertheless it has insufficient concentration in the puberty, while in the second half of menstrual cycle serum P4 concentrations are decreased due to the defective follicle luteinization. In this study P4 levels are slightly decreased with no significance compared to the control group, previous reports go in agreement with the results of this study which reported that mean P4 levels in patients were not significantly different from those of control subjects [18,19].

In this study, the results of testosterone in male patients indicated significant decrease (P<0.05) in CKD patients, and this result was also approved by several previous studies as testosterone which is normally reduced in CKD patients and when LH level is arise in response to low levels of testosterone, so that the hypothalamic pituitary axis in CKD is reset in such a way that it is more sensitive to the negative feedback inhibition of testosterone [16,17,20,21]. Rathi and Ramachandren. [22], showed that low testosterone is due to decreased production, increased metabolic and dialytic clearance, alteration in testosterone binding capacity.

The data of this study suggest that patients with CKD stage-5 have elevated but not significant AMH compared with control and this results go in agreement with those that have been found with Stoumpos *el al*. [14], who showed that CKD patients were found to have non-significant increase in AMH concentrations compared with control. Also, the results of this study was compatible with data reported by Sikora-Grabkaa *et al*. [13], who found that serum AMH concentration was higher in haemodialysed women with CKD and had menstrual cycle abnormalities in comparison to those with regular menstrual cycles, and suggested that serum AMH clearance is reduced in CKD patients and AMH similar to other protein hormones probably accumulates in those patients [14]. On the other hand, AMH is of similar size to other molecules perceived to be uremic 'toxins' [1].

This study disagrees with those that have been found with Eckersten *et al*. [1], who they reported that male patients with CKD have lower serum AMH levels versus controls and this was an unexpected finding, the author explained that this finding of low AMH levels indicate a dysfunction of both Sertoli cells and Leydig cells in men with CKD. The results of this study also in disagreement with Eckersten *et al*. [23], who showed that plasma AMH levels were lower in CKD stages 1–4 by 30% and by 70% in CKD stage 5 compared with controls, the author said that this reduction in AMH is unclear, but can be linked to altered Sertoli cell function. The cause of decreased plasma AMH levels is unknown, but it speculate on effects of inflammation, uremic toxicity, or other causes. The results of this study support that the molecular weight of AMH (140 kDa) indicates that it is not eliminated by glomerular filtration or dialysis, if this is the case, an increase in plasma AMH levels would be expected with advancing CKD stages [1,14,24].

Results of this study showed that LH has negative correlation with serum AMH in men and women with CKD, this finding disagree with another finding [25], who found no relationship between AMH and LH. Another study also showed no significant correlation between AMH and LH [26]. Results of current study showed positive no significant correlation between AMH and FSH and this result was in agreement with other study which reported that AMH positive correlated with FSH [27]. Fiza *et al*. [28], showed a negative relationship between the levels of AMH and FSH and this results disagreement with the results of this study.

Thus, the higher levels of AMH are associated with lower level of the FSH and support this result by another study which reported that the excess of AMH is involved in diminishing FSH induced aromatize activity and this results disagreement with this study [9,29,30]. High AMH levels are probably related to the follicular arrest during the selection process of the dominant follicle, through a negative interaction between AMH and FSH [31].

In this study E2 showed non-significant positive correlation with AMH and this result was in agreement with other study which reported that AMH was negative correlated to LH and positive correlated with FSH and E2 [27].

Conclusions

This study confirms the noxious role of CKD in hormonal disruption and infertility among patients with CKD.

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Infection Control Measures for Nurses Staff Concerning with Hepatitis B and C at Hemodialysis Unit in Hilla Hospitals

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Abstract

Hepatitis an infection in the liver is very contagious and is caused by the hepatitis virus. Objectives: The study aims to evaluate nurses performance concerning infection control practices, and to determine the relationship between nurses performance and their demographic characteristics. As well as, to determine the relationship between nurses performance and patients clinical data. A quantitative descriptive design was conducted in Hilla City Hospital for the period of from September 2018 to March 2019. A checklist was used as a means of data collection from those who works at Hemodialysis unit. Non probability sampling (purposive) has been performed on (30) nurses and (30) patients in dialysis unit. direct observation as mean of data collection. Nurses were observed three times while they are working in the dialysis unit. The data were analyzed through the application of descriptive and inferential data analysis. The study results reveals that (80%) sometime applied hand washing, (76.7%) were sometime apply uniform, and (66.7%) were always apply gloves, (53.3%) never wearing gown, and (86.7%) never wearing mask. A majority of (63.3%) of nurses were partially performed infection control measures. There is insignificant association with nurses demographic characteristics at *p-value >0.05*.

Keywords: *Infection, Control, Nurses, Hepatitis.*

Introduction

One of the most challenges is health care-associated infections, or infections acquired in health-care settings are the most frequent adverse event in health-care delivery worldwide. Hundreds of millions of patients are affected by health care-associated infections worldwide each year, leading to significant mortality and financial losses for health systems [1]. The World Health Organization (WHO) estimates an average of 9 million individuals are affected by nosocomial infections and approximately 1million patients die each year because of these diseases [2]. Patient safety is one of the most important aspects in evaluating the quality of healthcare. However, healthcare associated infection is a major cause of morbidity and mortality in the critical care units. It is imperative for health care administrators to ensure implementation of the infection control program in healthcare facilities [3]. Control measures is a set of specific practices and procedures performed under carefully controlled conditions with the goal of minimizing contamination by pathogens [4]. It is well known that patients undergoing dialysis treatment, and in particular hemodialysis (HD), are at increased risk for contracting viral infections. This is due to their underlying impaired cellular immunity, which increases their susceptibility to infection. In addition, the process of HD requires blood exposure to infectious materials through the extracorporeal circulation for a prolonged period. Moreover, HD patients may require blood

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transfusion, frequent hospitalizations and surgery, which increase opportunities for nosocomial infection exposure [5].After the identification of HCV in 1989 and HEV in 1990, there were still unexplained cases of post transfusion and "community-acquired" hepatitis, implying that cryptogenic hepatitis and cirrhosis may be related to viruses other than hepatitis A, B, C, D or E. By the mid-1990s, between 3% and 4% of anti-HCV-negative patients on chronic HD had elevated serum aminotransferase levels with no apparent etiology [6,7].

Methodology

Study Design:

A quantitative descriptive design was conducted to evaluate nurses performance concerning infection control practices, and to determine the relationship between nurses performance and their demographic characteristics. As well as, to determine the relationship between nurses performance and patients clinical data in has been carried out in Hilla City Hospital.

Instrument Construction:

The checklist (observation tool) include three part. They are: Nurses demographic data, patients clinical data, and observation tool. The last part consists from 55 items divided (8) section; includes: hand washing, uniform, gloves, gown, mask, instruments and equipment, intra venous iv fluids and medication, medical waste management and sharp disposal.

Participants Sample:

Non probability sampling (purposive) has been performed on (30) nurses who work in dialysis unit and (30) patients.

Data Collection:

The researcher has gathered the objective's responses through an application of direct observation as mean of data collection. Nurses were observed while they are working in the dialysis unit . The researcher observed each nurse three observations and among each observation (10) days' time period. Three correct practices out of 3 episodes were rated as always , 2-1 correct practice out of 3 episodes were rated as sometimes, and no correct practice out of 3 episodes was rated as never.

Data Analysis: Through the used descriptive statistical (SPSS) version XX analysis approach that includes, frequencies, percentages, mean of scores, standard divagation and graphical presentation of data; and inferential statistical data analysis approach that include Chi-squared test.

Results and Discusion

In light of frequencies and percent, results depicts that the nurses were female within age groups (19-29) years old and single state residences in urban areas. Concerning education, (53.3.%) were bachelor in nursing graduated, works for (1-3) years in nursing field and in Hemodialysis unit without training in-outside of country. This results come because most of them are new appointments at the same time that unity needs effort. These results agree with study deals with case control educational program on nurses knowledge find that the majority of nurses at 20-29 years [8]. Also, study conducted in Baghdad City has been evaluate the nurses' practice concerning infection control. Finds that the high percent of nurses have 1-5 years of experiences in nursing [9]. Finding reveals that 96.7% of patients with hepatitis C is for duration (1-2) years diagnosed after dialysis for a two dialysis times per week . In light of these results, a study has been assessed the "Hepatitis B and C infection in hemodialysis patients in Libya, the results indicated that the patients on maintenance HD in Libya have a high incidence and prevalence of HCV infection and lower rates of HBV infection[10].Also, in another study (Yousifetal., 2018), the result indicated that the prevalence of HBV, HCV, and coinfection in the studied subjects was 1.8%, 9.2%, and 0.2%, respectively [11]. The results reveals that the majority of nurses measures regarding hand washing and uniform were sometime apply. In contrast a study conducted in Turkey , they found that the majority of nurses have a partially level of practices concerning quality of hand washing [12]. Also in another study in Al-Najaf AL-Ashraff City Hospitals, their findings indicate that 72.5% of study sample are not applying the practices of hand washing [13]. While another study in Palestine, show that the high percentage of nurses are wearing the special uniform [14].

Concerning with personal protective equipment, the results reveals that the majority of nurses were always apply gloves, and never apply gown, and mask. In another study done by (Cason and others, 2007), which reported that 75% of nurses are wearing gloves. The researcher believes that the present result related to not available of sterile gloves, but they can be used clean

gloves during the nursing intervention [15].These results supported by (Chan et al., 2008), show that the nurses have negative attitudes and practices toward standard precaution. These results may be to not the availability of sterile gown in the hospital [16]. In another study done by (Nichol et al., 2013), their study showed that the 56% nurses reported adherence to recommended use facial mask [17].

Concerning with nurses measures deals with instruments and equipment the results depicts that the majority of nurses measures regarding instruments and equipment were sometime apply. In another study done by (Kandeel et al., 2012), which revealed that high percent of nurses were used a sterile technique in preparing the instrument in critical care units [18].

Concerning with nurses measures deals with dialysis Catheters and port catheter systems, intravenous fluids and medication and medical waste management and sharp disposal, the results show that the majority of nurses were always applied. The present results disagree with (Alkubati et al., 2015), they founded that the majority of nurses had poor compliance with the standard strategies for prevention PVC infection [19].In addition the results agree with (Dolan etal., 2010), they confirmed in their study that an infusion, safe injection, and medication vial practices in health care needed to adhere sterilization technique during the administration of intravenous fluids and medication for the patient [20]. The present result also disagrees with (Ferreira et al., 2010), they founded in their study that a healthcare worker was inadequate practice and knowledge related to the correct separation of wastes and sharps disposal [21]. Findings of present study demonstrated that there demographic characteristics of nurses have been not affected their control measures at *p-value >0.05*. The present finding agree with study done by (Bakey, 2012), which indicated that there was no significant relationship at p-value (0.464) level between nurses' practice and their age, level of education, years of experience in hemodialysis unit [22] . Also the present finding disagree with study done in Korea, they found that there were significant relationship between nursing practice concerning infection control and age and significant between the type of units and nurses practices [23]. Results demonstrated that there were non-significant association between nurses control measures and patients their clinical data at *p-value >0.05*. The result disagree with study of (Alashek, 2012), which indicated that their is a significant relationship between duration of hemodialysis and prevalence of HCV, and longer dialysis times increase the probability of hepatitis C in Libya [10]. Also in another study done by (Sarhan et al., 2015), the result show that the prevalence of HCV antibody (HCV Ab) at the start of dialysis was 45.2%, and the prevalence of HCV Ab by the time of data collection was 51%; the incidence rate of seroconversion was 10.7% after a mean duration of dialysis of 3.35±2.048 years [24].

Table (1): Distribution of Patients by their Clinical Data about Hepatitis

Demographic data	Rating	No	Percent
Type of hepatitis disease	C	29	96.7
	B	1	3.3
Duration of disease	<1 years	1	3.3
	1-2 years	26	86.7
	>3years	3	10.0
When the disease was diagnosed	Before dialysis	3	10.0
	After dialysis	27	90.0
Number of dialysis times per week	One	4	13.3
	Two	17	56.7
	Three	9	30.0

Table (2): Infection Control Measurement Concerning Hepatitis B and C in Hemodialysis Unit

	Rating	**No**	**%**	**M.S.**	**S.d.**	**Evaluation**
Hand Washing Domain	**Never**	**6**	**20.0**	**1.80**	**0.40**	**Sometime**
	Sometime	**24**	**80.0**			
	Always	**0**	**0.0**			
Uniform Domain	Never	4	13.3	1.96	0.49	Sometime
	Sometime	23	76.7			
	Always	3	10.0			
Gloves Domain	Never	0	0.0	2.67	0.47	Always
	Sometime	10	33.3			
	Always	20	66.7			
Gown Domain	Never	16	53.3	1.56	0.67	Never
	Sometime	11	36.7			
	Always	3	10.0			
Mask Domain	Never	26	86.7	1.17	0.39	Never
	Sometime	3	10.0			
	Always	1	3.3			
Instruments and Equipment Domain	Never	1	3.3	2.03	0.32	Sometime
	Sometime	27	90.0			
	Always	2	6.7			
Catheters and port catheter systems	Never	0	0.0	2.73	0.45	Always
	Sometime	8	26.7			
	Always	22	73.3			
IV fluids and medication Domain	Never	7	23.3	2.40	0.85	Always
	Sometime	4	13.3			
	Always	19	63.3			
Medical waste management and sharp disposal	Never	2	6.7	2.70	0.59	Always
	Sometime	5	16.7			
	Always	23	76.7			

Table (3): Overall Infection Control Measurement Concerning Hepatitis B and C in Hemodialysis Unit at Hilla Hospitals

	Rating	**No**	**%**	**M.S.**	**S.d.**	**Evaluation**
Overall Measures	Never	1	3.3	2.30	0.53	Sometime
	Sometime	19	63.3			
	Always	10	33.3			
	Total	30	100			

Table (4): Statistical Relationship between Nurses Infection Control Measurement and Patients Clinical data about hepatitis.

Demographic data	**Rating**	**Nurses Measures**			**Total**	**d. f**	
		Never	**Sometime**	**Always**			
Type of hepatitis disease	B	1	19	9	29	2	obs.=2.069 crit.=5.991 P-value= 0.355→NS
	C	0	0	1	1		
	Total	1	19	10	30		
Duration of disease	<1 years	0	1	0	1	4	obs.=2.672 crit.=9.488 P-value= 0.614 →NS
	1-2 years	1	15	10	26		
	>3years	0	3	0	3		
	Total	1	19	10	30		
When the disease was diagnosed	Before dialysis	0	1	2	3	2	obs.=1.696 crit.=5.991 P-value=0.428 →NS
	After dialysis	1	18	8	27		
	Total	1	19	10	30		
Number of dialysis times per week	One	0	3	1	4	4	obs.=1.024 crit.=9.488 P-value=0.906→NS
	Two	1	10	6	17		
	Three	0	6	3	9		
	Total	1	19	10	30		

Conclusions

Most of nurses new appointments young adults nurses partially performed infection control measures in the dialysis unit not affected by their demographic data, and not influence the results of patients. An intensive training courses and provide possibilities for personal protection with activate principle of reward and punishment.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing, Babylon University, Babylon Province, Iraq, and all experiments were carried out in accordance with approved guidelines.

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Gingival and Salivary Changes in Correlation with Multiple Sclerosis

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Abstract

Multiple sclerosis: chronic degenerative autoimmune disease of the central nervous system marked by patchy destruction of the myelin sheath that surrounds the nerve fibers. The gingival or oral changes that associated with multiple sclerosis might be related to disease itself or as sequel of medications that used for treatment. Aims;-to determine the gingival index and assess the SIgA, SFR and PH in MS patients in compare to control group as well as analyses theses parameters according to age group, gender and type of MS. 36 patients with proven diagnosis of MS were selected randomly between 1st Nov.2016-1st nov.2017 from MS center. Medical city .Baghdad. Iraq. Oral examination was done to measure the gingival index as well as saliva was taken to assess the salivary IgA level, salivary flow rate and PH of saliva, in addition 20 subjects who are free of MS or any others autoimmune disease were selected as control group. The GI of patients group was significantly higher than control group(3.4 ,1.4) respectively while the SIgA ,SFR and PH of saliva was significantly lower with patients group in compare to control group510,0.56,6.6 for patients group) 941.8,1.7,7.1 for control group

Key word: *Multiple sclerosis, salivary immunoglobulin A, gingival index, saliva flow rate*

Introduction

Multiple sclerosis (MS) is a progressive, neurodegenerative disorder of the myelin sheath, in which plaques created on the central nervous system (CNS) alter nerve function. (1) Although the exact cause of MS is unknown, a combination of genetic susceptibility, environmental factors, immune system response and systemic inflammation is suspected. Progression of MS and new plaque formations cause CNS changes in motor, sensory and cognitive functions, leading each individual to experience symptoms that vary in degree and severity.(2) The prevalence of MS varies considerably, from high levels in North America and Europe (>100/100,000 inhabitants) to low rates in Eastern Asia and sub-Saharan Africa (2/100,000 population). Concerning mortality, in a large French cohort of 27,603 patients, there was no difference between MS patients and controls in the first 20 years of the disease, although life expectancy was reduced by 6–7 years in MS patients ·MS starts attacks people from age 20–50 years old and the records investigated that females' attacks double than males' attacks (3) There are 4 types of MS. They're named according to the way the disease acts on the body over time. Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS), usually characterized by a relapsing-remitting (RR) course, or by disability accrual over an extreme variability of time. At diagnosis, about 75% of persons with MS suffer from RRMS, while about 25% of such persons will convert to secondary progressive MS (SPMS) within two to three decades from the onset. About 10% of patients with MS manifest primary progressive MS (PPMS) at diagnosis, and they show gradual worsening of neurological disability from symptom onset (4) . With the term progressive MS (PMS), we usually refer to the combined population of secondary progressive MS (SPMS) and primary progressive MS (PPMS) that still remains a distinct phenotype in the 2013 revision of clinical course in MS (5)

THE 4 TYPES OF MS

Relapsing-Remitting MS (RRMS). This is the most common form of multiple sclerosis. About 85% of people with MS are initially diagnosed with RRMS. People with RRMS have temporary periods called relapses, flare-ups or exacerbations, when new symptoms appear (6)

 Secondary-Progressive MS (SPMS). In SPMS, symptoms worsen more steadily over time, with or without the occurrence of relapses and remissions. Most people who are diagnosed with RRMS will transition to SPMS at some point [7]

 Primary-Progressive MS (PPMS). This type of MS is not very common, occurring in about 10% of people with MS. PPMS is characterized by slowly worsening symptoms from the beginning, with no relapses or remissions2

 Progressive-Relapsing MS (PRMS). A rare form of MS (5%), PRMS is characterized by a steadily worsening disease state from the beginning, with acute relapses but no remissions, with or without recovery2 [6]

The hypothesis states that the pathophysiology of MS is related to myelin antigen-specific CD4+ T cells which become activated. The exact trigger for the activation of the T cells is still unknown. One theory suggests that T cells might become activated by cross-reacting with a particular antigen, such as a microbial agent, known as "molecular mimicry". This theory explains why some viruses have been studied as a possible trigger for the disease. These cells then cross the blood-brain barrier, recognize myelin basic protein, attack the neuronal myelin sheaths, and trigger the onset of muscular symptoms and can lead to cognitive decline. These areas of axonal damage are known as lesions. The actual mediator of myelin and axonal destruction has not been established, but it may reflect a combination of macrophages, antibodies, cytokines, and reactive oxygen intermediates.[8] To our knowledge this is the first study in our country studying the changes in salivary IgA, SFR, PH of saliva in MS patients in compare to control health group as well as assessment the changes according to type of MS.

Subjects and methods

Between 1st Nov.2016-1st Nov.2017 and after approval was obtained from concerned authouritis,36 patients with proven diagnosis of MS who are attending the MS center of medical city. Baghdad.Iraq were selected randomly and oral examination was doing to measure the Gingival Index as well as saliva was taken to assess the salivary IgA level, Salivary Flow Rate and PH of saliva, in addition 20 subjects who are free of MS or any others autoimmune disease were selected as control group.

Saliva sample:

Un stimulated whole saliva (2 ml) were collected from patient group and control by expectoration into sterilized vials then saliva sample were centrifuged at 12000 rpm for 10 minutes and the supernatants were stored at -20˚C until use .

Estimation of SIgA by ELISA procedure of the test: -

1- Standard was constituted to 1000 pg/ ml with standard dilution buffer. a serial diluents of the standard were prepared from original standard.

2- One hundred ml of standard or sample was added per well, then 100 ml of diluent were added for each well. The plate was incubated for 4 hours.

3- The contents of well were discarded and washed by washing solution.

4- Two hundred ml of conjugate reconstituted was added into each well and incubated at 25 C° and then washed three times.

5- Two hundred ml of substrate was added into each well, followed by incubation for 15 minutes at 25 C° with continuous shaking in the dark.

6- Fifty ml of stopping solution H_2SO_4 was added into each well and mixed gently.

7- Absorbance was measured by spectrophotometer at 450 nm within two hours.

The flow rate of saliva (SFR) ml/min was estimated by dividing the total collected saliva volume (ml)by collecting time (min) that was measured by sample collection:

SFR ml/min = saliva sample volume (ml)/ collection time (min). Salivary pH was measured with a digital pH-meter (Hanna Instruments, USA) 30 to 60 minutes after saliva samples were collected, and pH was considered as a quantative variable. The BMI for a person is defined as their body mass divided by the square of their height with the value universally being given in units of Kg/m^2.

Statistical Design

SPSS version 23 was used for data entry and analysis, mean and standard deviation was used to represent the continuous data. ANOVA and student t test were used

to confirm significance. P-value≤0.05 was considered significant.

Results and Discusion

The findings indicated that the mean age of patients group was 37.2±4.3, 47.2% belong to age group of 20-40 years,75% was females and 72.2% of RRD type. With regards to control group; the results revealed that the mean age was 38.7±6.8 years, 45.5% was fall to age group of 20-40 years and 63.6% was females as seen in table.1.

Table.1. descriptive characteristics of studied groups

		Groups			
	Patients(n=36) Mean age=37.2±4.3years		Control(n=22) Mean age=38.7±6.8 years		
	No.	%	No.	%	
Age groups	<20	8	22.2%	6	27.3%
	20-40	17	47.2%	10	45.5%
	>40	11	30.6%	6	27.3%
Gender	Female	27	75.0%	14	63.6%
	Male	9	25.0%	8	36.4%
Type	RRD	26	72.2	-	-
	SPD	3	8.3	-	-
	PPD	4	11.1	-	-
	PRD	3	8.3	-	-

The results demonstrated that the mean value of GI, SIgA, SFR and PH of patients group were significantly higher than that of control group (p≤0.05 for all) as displaced in table.2.

Table.2. Mean of GI, SIgA, SFR and PH of patients and control group.

	Groups				
	Patients group		**Control group**		
	Mean	**Std. Deviation**	**Mean**	**Std. Deviation**	**p-value**
GI	3.4	0.6	1.4	0.5	0.01
SIgA	510.8	59.7	941.8	63.8	0.01
SFR	0.56	0.1	1.7	0.2	0.01
PH	6.6	0.2	7.1	0.1	0.01

Our data showed that the mean value of GI was significantly increased with aging while the mean value of SIgA, SFR and PH were decreased as the patient get elderly but the significant difference was reported with SIgA and SFR only as illustrated in table.3.

Table.3. Mean of GI, SIgA, SFR and PH of patients according to age groups

		N	Mean	Std. Deviation	p-value
GI	<20	8	2.8	0.3	0.01
	20-40	17	3.1	0.2	
	>40	11	3.4	0.12	
SIgA	<20	8	561.0	58.8	0.02
	20-40	17	512.0	62.3	
	>40	11	415.3	66.1	
SFR	<20	8	0.6	0.05	0.03
	20-40	17	0.4	0.1	
	>40	11	0.3	0.08	
PH	<20	8	6.5	0.05	0.09
	20-40	17	6.4	0.23	
	>40	11	6.1	0.18	

The results showed there was no significant difference ($p \geq 0.05$ for all) regarding the mean value of GI, SIgA, SFR and PH when compared according to the type of multiple sclerosis, however the patients who had remission relapsing type showed higher value for all these studied parameters but the difference did not reach the significant level as displaced in table 4.

Table.4. mean of GI, SIgA, SFR and PH of patients according to MS types

		N	Mean	Std. Deviation	p-value
GI	RRD	26	3.6	0.5	0.06
	SPD	3	3.4	0.3	
	PPD	4	3.5	0.7	
	PRD	3	3.4	0.4	
SIgA	RRD	26	517.4	67.3	0.06
	SPD	3	482.0	69.2	
	PPD	4	502.8	66.4	
	PRD	3	506.7	61.8	
SFR	RRD	26	0.6	0.08	0.07
	SPD	3	0.4	0.05	
	PPD	4	0.5	0.08	
	PRD	3	0.5	0.15	
PH	RRD	26	6.7	0.18	0.09
	SPD	3	6.4	0.25	
	PPD	4	6.4	0.08	
	PRD	3	6.6	0.2	

Cont... **Table (4): Association between (IgG) and (IgM) anti -*T.gondii* seropositivity and risk factors**

The result of current study demonstrated that the mean value of GI, SIgA, SFR and PH of MS patients were higher with females in compare to male patients but the difference not significant statistically (p≥0.05for all) as showed in table 5.

Table.5. mean of GI, SIgA, SFR and PH of patients according to their gender.

	gender				**p-value**
	Female(N=27)		**Male(N.=9)**		
	Mean	**Std. Deviation**	**Mean**	**Std. Deviation**	
GI	3.6	0.4	3.4	0.3	0.06
SIgA	562.4	78.8	554.3	81.03	0.07
SFR	0.6	0.1	0.5	0.09	0.7
PH	6.5	0.2	6.6	0.15	0.09

The results showed there was significant direct correlation between duration of disease in years and gingival index while negative correlation was reported between each of SIgA, SFR and PH and duration of disease as displaced in table.6.

Table.6. correlation of age,

		GI	**SIgA**	**SFR**	**PH**
Duration	Pearson Correlation	0.8**	-0.9**	-0.9**	-0.3
	p-value	0.001	0.001	0.001	0.1

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder of the central nervous system affecting white and possibly grey matter. MS characterized by multiple focal demyelinating lesions affecting the white matter, which is not infrequently associated with cortical demyelination. The finding of this study indicated that the mean age of patient group was 37.4±6.3 SD (ranged from 20-40) year while the control group was 36.3±4.8 SD years old. The studies showed that the age at MS onset follows a similar pattern across different regions, with incidence low in childhood, rapidly increasing after adolescence, reaching a peak between 25 and 35 years, and then slowly declining [11], this opinion support our work in the term of spread of the MS in this age group. With regard to gender distribution, our data indicated that female was 75% of patient group and 63.6% of control group, this result in consistent with finding of other studies that revealed the women are affected more commonly than men, with the female-to-male ratio varying between 1.5:1 and 2.5:1, with a trend toward higher values in the most recent studies [17,18]. Recent research though anyone can be affected, but MS most frequently occurs in white women ages 20 to 45. [1,2] According to some authors the lifetime risk of developing MS in high-risk populations is approximately 1 in 200 for women which coincide our study, others supposed MS 3 times more common in females than in males. Although controversy surrounding sex factor. several investigators indicated that females had a relatively favorable course [19]. For salivary IgA changes that associated with MS, a studies reported that chronic stress mostly reported with MS patients result in an increased levels of salivary cortisol and decreased level of salivary IgA. [20]. This study revealed effect of

MS on saliva immunoglobulin A comparing with saliva of healthy individuals (control) group which show decreased salivary IgA levels as well as the (SFR) with duration. Increased autoimmunity with age may result from increased numbers of auto aggressive T-cells or reduced immune regulation. Autoimmune diseases with interleukin-17 as an important messenger cytokine may also be relevant in periodontitis [27], T cells protect from autoimmune inflammation despite reduced STAT3 activation and decreased constraint of IL-17 producing T cells. This may be an appropriate explanation for this outcome in this study. However, many other studies correspond our result it is observed Individuals with MS are at increased risk for dental caries, gingivitis and periodontitis due to the physical effects of MS, as well as these patients' reduced immune response [2,10]

Conclusion

The gingival index increased but the S.IgA, SFR, and s.PH in MS patients but we could not prove these changes due to the disease itself where they might be induced by used medications that used for MS treatment

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry/Al-Mustansiriyah University and all experiments were carried out in accordance with approved guidelines.

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Impact of Caring Child with Diabetes Mellitus Type1 upon Mothers at Specialized Center for Endocrinology and Diabetes in Baghdad City

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Abstract

The purposes of the study to assess the impact of caring children with Diabetes Mellitus Type 1 upon mothers. A descriptive study was conducted at Specialized Center for Endocrinology and Diabetes in Baghdad City. To achieve the objectives of the study, a (Convenience) samples of (50) mothers who have children with Diabetes Mellitus Type 1. Data were collected by an application of direct interview as a means of data collection. The results indicated that mothers of diabetic children are experiencing a moderate level of burden regarding care of their children (86.5%), in which they are experiencing a moderate psychological and social burden (61.5% and 51.9%), while they show moderate to high financial burden of care for their children (40%). The study concluded that child with Diabetes Mellitus Type I are experiencing a moderate impact on their mothers .

Key Words: *Burden, Caring, Diabetes Mellitus Type1, Mothers*

Introduction

Type 1 diabetes mellitus one of the most common chronic diseases of childhood, can be diagnosed at any age; peaks in onset occur between 5 years and 7 years of age and at or near puberty.T1DM accounts for ~10% of all diabetes cases, and the incidence rate of childhood T1DM has risen worldwide by ~2.8%–4.0% each year in all age groups in the last several decades.T1DM is most common in Europe (129,000) and North America (108,700) and far less common in People's Republic of China, India, and Africa. [1,2] Iraq is one of the 19 countries and territories of the IDF MENA region. 415 million people have diabetes in the world and more than 35.4 million people in the MENA Region; by 2040 this will rise to 72.1 million. There were 1.2 million cases of diabetes in Iraq in 2015 [1] Type 1 diabetes mellitus is a challenge for children, adolescents and their parents as they need special support to keep it under control. After the diagnosis of T1D, the lifestyle of all family members' changes and this interferes in their quality of life [3]. Mothers were considers the primary caregivers in most families, and many had to rearrange work schedules or leave work in order to care for their child full-time. Parenting roles also changed dramatically [4,5]. Mothers are ultimately responsible for daily management of T1DM, which has a significant impact on family life. Daily management of T1DM is time-consuming and requires a change to many well-established family routines. Although technological advances have greatly enhanced the ability of parents to monitor and manage T1DM in youth, the responsibility of T1DM management can contribute to parental stress, distress, and diminished quality of life. Understanding the experience of parenting a child with T1DM can provide insight into the challenges and needs of parents [6,7]. Type 1 diabetes mellitus is early age of onset, and a major burden of care on to the family members, especially parents. Parents with psychological morbidity had more dysfunction in social, personal and cognitive domains and also had significantly poorer quality of life in the physical health, psychological health and general well being domains [8]. The diagnosis and treatment of a chronic illness or disability, such as type 1 diabetes, in a child leads to family upheaval and reorganization [9]. The effects of this stress on parents not only may adversely affect parents' mental health but also could affect the health of the child with diabetes. According to family

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systems theory, what happens to a target member of the family affects other family members and their response in turn affects the target family member (10). Moore et al; (2013) and Simsek et al., (2013) reported that 20% to 30% of parents report clinically significant distress, which has been defined as stress (life stress or parenting stress) and symptoms of anxiety, depression, and/or posttraumatic stress.6–8 Parental psychological distress at the time of diagnosis has been found to predict later parental distress(3,11). Parents and particularly mothers of children with T1DM are at increased risk for depression, which was associated with negative effects on parenting, the child's quality of life, and the child's psychosocial adjustment (12).

Methodology

A descriptive study was conducted at Specialized Center for Endocrinology and Diabetes in Baghdad City. To achieve the objectives of the study, a (Convenience) samples of (50) mothers who have children with Diabetes Mellitus Type 1.In order to assess burden of caring children with Diabetes Mellitus Type 1 upon mothers, a constructed questionnaire was design and means of an interview technique with the mothers was constructed to measure the variables underlying study. Questionnaire based on comprehensive review of relevant literature and previous studies was also used. These instruments consist of two parts Part one concerned with personal information includes, the demographic data of the mothers, child and child's illness. Part two contained of (25) items concerning burden (psychological, social and economical) of caring children with Diabetes Mellitus Type 1 upon mothers. The questionnaire was answered by one of these answers (always, sometime and never). Constant validity determined for questionnaire through the use of panel experts who are faculty members from College of Nursing. The experts were asked to review the questionnaire for content with clarity. Such changes were employed according to their suggestions and valuable comments. The data collected with constructed questionnaire through an application of direct interview as mean of data collection. Appropriate statistical approach was used

Results and Discusion

Table 1: Demographic Characteristics for Mothers

No.	Characteristics		F	%
1	Age Group:	20 – 29 years	13	25
		30 – 39 years	26	50
		40 – 49 years	12	23.1
		50 ≤ years	1	1.9
		Total	52	100
2	Educational Level:	Doesn't read & write	1	1.9
		Read & write	0	0
		Primary school	19	36.5
		Intermediate school	19	36.5
		Secondary school	5	9.6
		Institute/College	8	15.4

Cont.... **Table 1: Demographic Characteristics for Mothers**

		Total	52	100
3	Marital status:	Married	49	94.2
		Divorced	1	1.9
		Widowed	2	3.8
		Total	52	100
4	family members:	1 – 5	34	65.4
		6 – 10	17	32.7
		11 ≤	1	1.9
		Total	52	100
5	Monthly income:	> 300,000 IqD	19	36.5
		300,000 – 600,000 IqD	21	40.4
		600,000 – 900,000 IqD	9	17.3
		900,000 IqD <	3	5.8
		Total	52	100
6	Occupation:	Housewife	48	92.3
		Governmental employed	3	5.8
		Self-employed	1	1.9
		Total	52	100

The analysis of table (1) shows that half of the mothers are with age group ranged 30 -39 years old (50%), who were graduated from primary and intermediate school (36.5%). The marital status is normal for most of mothers in which 94.2% of them are married. The number of family members reveals that more than half mothers are living in family composed of 1-5 members (65.4%). Regarding monthly income, 40.4% of mothers are having monthly income of 300,000-600,000 Iraqi dinars per month. The occupational status shows that most of mothers are housewife (92.3%).

Table 2: Demographic Characteristics of Children with Diabetes Mellitus

No.	Characteristics		F	%
1	Age:	> 2 years	1	1.9
		2 – 5 years	14	26.9
		6 – 12 years	26	50
		13 – 18 years	11	21.2
		Total	52	100
2	Gender:	Male	30	57.7
		Female	22	42.3
		Total	52	100
3	Birth order:	First	14	26.9
		Second	9	17.3
		Third	14	26.9
		Fourth/+	15	28.8
		Total	52	100
4	Age at diagnosis:	> 2 years	5	9.6
		2 – 5 years	22	42.3
		6 – 12 years	24	46.2
		13 – 18 years	1	1.9
		Total	52	100

This table presents the characteristics of children with diabetes mellitus; the table shows that 26.9% of them are male toddler, preschool children with age group 2-5 years old (57.7%). 28.8% of them are fourth born in the family. More than third of children are diagnosed at school age of 6-12 years (46.2%) and 42.3% of them are diagnosed at preschool age.

Table 3: Total impact of Care of Children with Diabetes Mellitus on Mothers (N=52)

Burden	**Levels**	**F**	**%**	**M.S**	**SD**
	Low	23	1.9		
Psychological	Moderate	32	61.5	2.35	0.520
	High	19	36.5		
	Low	23	44.2		
Social	Moderate	27	51.9	1.60	0.569
	High	2	3.8		
	Low	10	19.2		
Financial	Moderate	21	40	2.21	0.750
	High	21	40		
	Low	2	3.8		
Total	Moderate	45	86.5	2.06	0.366
	High	5	9.6		

The findings in this table indicate that mothers of diabetic children are experiencing a moderate level of burden regarding care of their children (86.5%), in which they are experiencing a moderate psychological and social burden (61.5% and 51.9%), while they show moderate to high financial burden of care for their children (40%).

Table 4: The Relationship between Mothers' Burdens of Care with Their Children' Characteristics (N= 52)

	Burden	**Chi-square**	**d.f**	**P-Value**	**Significance**
Characteristics				≤ 0.05	
Age		4.691	6	0.584	N.S
Gender		0.788	2	0.674	N.S
Birth order		6.810	6	0.339	N.S
Age at diagnosis:		10.668	6	0.099	N.S

d.f: degree of freedom, P: Probability, N.S: Not significant

This table reveals that there is no significant relationship between mothers' burden of care and characteristics of their children with diabetes mellitus at p-value ≤ 0.05.

Table 5: The Relationship between Mothers' Burden of Care with Their Demographic Characteristics (N= 52)

	Burden	**Chi-square**	**d.f**	**P-Value**	**Significance**
Characteristics				**≤ 0.05**	
Age group		4.874	6	0.560	N.S
Educational level		13.608	8	0.093	N.S
Marital status		0.495	4	0.974	N.S
Family members		12.796	4	0.012	S
Monthly income		4.287	6	0.638	N.S
Occupation		0.674	4	0.954	N.S

d.f: degree of freedom, P: Probability, N.S: Not significant, S: Significant

This table indicates that there is no significant relationship between mothers' burden of care with their demographic characteristics, except number of family members that shows significant at p-value ≤ 0.05.

The finding of this study found that half of the mothers are with age grouping ranged 30 -39 years old (50%), who were graduated from primary and intermediate school (36.5%). The marital status is normal for most of mothers in which 94.2% of them are married. The number of family members reveals that more than half mothers are living in family composed of 1-5 members (65.4%). Regarding monthly income, 40.4% of mothers are having monthly income of 300,000-600,000 Iraqi dinars per month. The occupational status shows that most of mothers are housewife (92.3%).characteristics of children with diabetes mellitus described in table that 26.9% of them are male toddler, preschool children with age group 2-5 years old (57.7%). 28.8% of them are fourth born in the family.More than third of children are diagnosed at school age of 6-12 years (46.2%) and 42.3% of them are diagnosed at preschool age. The study found that table (2) demonstrates the findings indicate that mothers of diabetic children are experiencing a moderate level of burden regarding care of their children (86.5%), in which they are experiencing a moderate psychological and social burden (61.5% and 51.9%), while they show moderate to high financial burden of care for their children (40%). Helgeson (2012) agree with this study said that found that caring for a child with diabetes had implications for both parent mental health and the health of the child with diabetes, he found that both were associated with increased parent depressive symptoms and decreased parent life satisfaction [13]. Whittemore (2012) reported that prevalence of parental psychological impact ranged from 10% to 74%, with an average of 33.5% of parents reporting distress at diagnosis and 19% of parents reporting distress 1 to 4 years after diagnosis [6]. The result of this study contrast with study done by Bhadada (2011) indicates that nearly two-third of parents (64%) had psychological morbidity. Parents with psychological morbidity had more dysfunction in social, personal and cognitive domains and also had significantly poorer quality of life in the physical health [8]. Another study focused on Economic burden done by (Shorer et al., 2011; Zysberg and Lang ,2015), concluded that families with lowest incomes bear the highest relative financial burden of diabetes [14,15]. Study done by Abolhassani, (2013) indicated that findings of this study provide an insight about effects of diabetes on the parents' life. Participants' reactions for their children were denial, shock, presentiment and concern, anger, anxiety, and impatience, and crying [16]. Hanif and Felimban (1998) concluded in their study that Psychological symptoms shown by mothers such as nervousness, irritability, insomnia, fatigue, poor concentration, fear and indecision, were significantly higher in mothers of diabetic children [17].

Conclusion

Mothers of children with Type 1 Diabetes Mellitus experience considerable many burden related to the caring of diabetes. Although many mothers find ways to effectively manage this burden, such as developing routines and finding sources of support, others experience clinically significant levels of psychological distress, including symptoms of depression, anxiety, and posttraumatic stress. These symptoms have been shown to have negative effects on parenting, child quality of life. A better understanding of the experience of parenting a child with T1DM may help providers to identify parents at highest risk.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing /University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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Measures Self-Esteem among Undergraduate Nursing Students

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Abstract

A large body of research has highlighted self-esteem and self-concept for their importance and relatedness to various aspects of students' academic and personal life. Self-esteem is significantly associated with the positive psychological outcomes of both individual and society, including, but not limited to psychological adjustment, positive emotion, and academic performance. A descriptive-correlational study was done through the period of November 10th, 2017 to February, 11th, 2019. The study also aims to assessing levels of self-esteem in the study sample, and to determine the relationship between self-esteem and the following variables: age, gender, academic year, residency place, father job, mother job, family's monthly income, and interest of attending the nursing college. A non-probability, convenience, sampling of (600) undergraduate nursing students were selected from four nursing colleges located in Middle Ephorates region. A two parts questionnaire was used to fulfil the study objectives. The first part includes student's sociodemographic information. The second part is the Rosenberg self-esteem scale to measure levels of self-esteem. The finding indicates that in majority of nursing students had a self-esteem levels range between average (52.2%) to high level (44.8%).

Keywords: *self-esteem, nursing students*

Introduction

Nursing is a humanitarian profession that cares about caring for people, families and communities, so that they can be fully healthy, preserved or restored to good health in the best possible way [1]. As for nurses, they can be distinguished from each other through their theoretical and scientific training [2]. Nursing also includes independent care and cooperative care for individuals of all ages, families, groups and communities, whether healthy, sick or ill, and in all circumstances. Nursing includes health promotion, disease prevention, patient and disability care, and pre-death care. Promoting a safe environment, scientific research, participation in shaping health policies, management of disease and patient systems, and health and nursing education are key nursing roles [3]. The concept self-esteem is multi-dimensional and is present in varying degrees among people, and is considered important within the concept of self, which reflects the sense of human value and efficiency. When the attitudes of people are positive towards themselves, they are highly appreciated, and conversely, when people have negative attitudes towards themselves, with a low self-esteem, the contrary happens [4]. Self-esteem is thus the overall assessment of the value of the individual as seen by himself/herself [5]. Rosenberg (1965) defined self-esteem as a set of positive or negative attitudes towards the self; self-esteem is a prominent psychological trait that concerns a person's image of self-esteem and self-confidence in the overall aspects of human activity in general. Many studies and research in different countries have linked the level of self-esteem with psychological well-being. This influence can be explained by the extent to which one has control over the management of things, the resources available to him/her and how effectively life's problems are dealt with and solved. Moreover, there seems to be a significant effect of the strength of personality and high self-esteem on the rate of stress and regardless of the conditions experienced by the individual. High self-esteem is an essential aspect of human well-being, happiness and adaptation [6]. Individuals with low self-esteem perceive

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life in a dark and negative way. These negative views felt by a person can lead him/her to believe that life is worthless (Crocker, 2006). This feeling may lead one to despair, inability and lack of motivation to exert more, because one sees one's experiences as meaningless. Low self-esteem leads to the feeling that others are better than us, which is a feeling of lack of will, firmness and trust that leads us to make room for others to benefit us.

Materials and Method

descriptive-correlational analytic study. This design was carried out in order to attain the purposes of the study by using an assessment technique on undergraduate nursing students. The chosen period for measuring self-esteem among undergraduate nursing students was from 10th June, 2018 to 11th February, 2019. A study was conducted at Middle-Euphrates Region in Iraq, which is a group of four Iraqi provinces Babylon, Al-Kufa, Kerbala, and Al-Qadisiah Province; each province has one nursing college. The subjects of current study were selected from all the nursing colleges of the Middle-Euphrates Region. Target population: A convenient sample of 600 consenting nursing students was chosen out of a total of (1600) nursing students in the four nursing colleges in the Middle Euphrates Region. The selected study sample was used to reflect the general characteristics of target population and to improve a generalizability of study results.

Results and Discusion

Table (1) Chi-square analysis was run to determine the association between the nursing collegians self-esteem and their demographic data. The study results indicate that there is a significant association between the nursing collegians self-esteem and their academic year at p-value less than 0.05. While there is a non-significant association with the other demographic data at p-value more than 0.05. Table (2) As it is seen in Table 2, there were the students (44.8% of the sample) with high self-esteem level, while there were students (52.2 % of the sample) with Average self-esteem level, and the students (3.0% of the sample) was with poor self-esteem. T6able (3) The analysis of variance was used to find group mean difference between academic years in response to students' self-esteem. Previous table shows that there is a significant difference in nursing students' self-esteem according to their academic year. Based on the statistical mean, the study results indicate that the students in fourth academic year perceive a more self-esteem compared with those in other academic years with a mean score equal to 2.5.

Table (1) Relationship between the Nursing Students' Self-Esteem and their Demographic Data `

Demographic Data	Rating and Intervals	Self-Esteem Levels			Sig.
		Poor	Average	High	
Gender	Male	6	77	54	X2 (2.84) D.F. (2) P-Value (0.24) NS
	Female	12	236	215	
Total		18	313	269	
Age / Years	18-20	10	134	143	X2 (6.881) D.F. (4) P-Value (0.14) NS
	21-23	7	164	114	
	24-26	1	15	12	
Total		18	313	269	
Academic Year	First Year	5	57	56	X2 (13.921) D.F. (6) P-Value (0.031) S
	Second Year	8	136	115	
	Third Year	3	82	44	
	Four Year	2	38	54	
Total		18	313	269	
Residence	Rural	5	63	41	X2 (3.476) D.F. (2) P-Value (0.17) NS
	Urban	13	250	228	
Total		18	313	269	

Cont.... **Table (1) Relationship between the Nursing Students' Self-Esteem and their Demographic Data `**

Father's Job	Unemployed	1	10	15	X2 (3.309) D.F. (6) P-Value (0.76) NS
	Earner	7	121	90	
	Employee	7	127	114	
	Retired	3	55	50	
Total		18	313	269	
Mother's Job	Housewife	16	271	232	X2 (4.284) D.F. (6) P-Value (0.63) NS
	Earner	0	5	5	
	Employee	2	36	27	
	Retired	0	1	5	
Total		18	313	269	
Marital Status	Single	16	278	236	X2 (1.751) D.F. (6) P-Value (0.94) NS
	Married	1	25	26	
	Divorced	1	8	6	
	Widowed	0	2	1	
Total		18	313	269	
Family Monthly Income	Plus	1	37	25	X2 (10.54) D.F. (6) P-Value (0.10) NS
	Barely Enough	13	133	112	
	Enough	3	121	120	
	Don't Enough	1	22	12	
Total		18	313	269	
The study at the College of Nursing was my desire	No	8	144	129	X2 (0.26) D.F. (2) P-Value (0.87) NS
	Yes	10	169	140	
Total		18	313	269	

Table (2) Overall Assessment of the Nursing Students' Self-Esteem

Self-esteem levels	**Frequency**	**Percentage**	**Prevalence rate of poor self-esteem**
Poor	18	3.0	3.0%
Average	313	52.2	
High	269	44.8	
Total	600	100.0	

Table (3) Mean Difference (ANOVA) in Nursing Students' Self-Esteem According to their Academic Year

Academic year	**N**	**Mean**	**Std. Deviation**	**F**	**Sig.**
First years	118	2.4322	.57704	3.377	0.018
Second years	259	2.4131	.55264		
Third years	129	2.3178	.51515		
Four years	94	2.5532	.54115		
Total	600	2.4183	.55121		

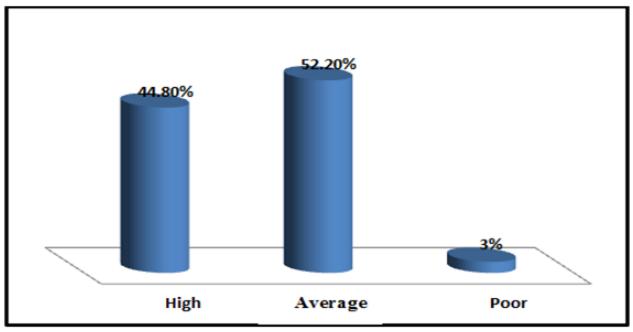

Figure (1) Overall Assessment of the Nursing Students' Self-Esteem

With the aim of assessing the level of self-esteem, the study evaluated the level of self-esteem among the study sample. The findings from the present study indicate that most of the students, amounting to 52.2 percent, had an average score of self-esteem. Also, 44.8 percent of the employees had a high level of self-esteem reflecting that low self-esteem level was not a major issue among the sample population investigated. Several authors have proved that when individuals have portrayed that persons high level of self-esteem are not susceptible to life stressors when compared to persons with low self-esteem. The focus by research in differentiating between participants with varying levels of self-esteem is focused on such aspects as their approaches towards perceiving a wide range of objects, their distinctive self-image, and an understanding of the personal strengths and weaknesses. Through these traits, the persons with high self-esteem levels are more likely to portray high acceptance to the different events they face thus minimizing the likelihood that they will be adversely affected by the nature of these effects. On the other hand, learners with low levels of self-esteem tend to be characterized by the feeling and perceptions of powerlessness, helplessness, and even ineptness. When faced by different tasks and challenges in their personal lives, the group with low self-esteem is highly susceptible to stress where they can perceive a normal challenge to be insuperable The findings from the present study align from the findings from a study by Fahran & Khan (2015) that identified that individuals with lower levels as compared to the average level of self-esteem portrayed lack of confidence and self-assurance. The study by Fahran & Khan (2015) emphasized that the lack of assertiveness is negative for these individuals, especially if they are nursing students. Such negativity is linked to contributing to the students accepting tasks that are beyond their capabilities. Due to the lack of the necessary skills and competencies required to complete these tasks, the students are more likely to demonstrate increased stress levels. Several aspects can be linked with the behavior of individuals with lower levels of self-esteem. These factors include challenges with alcohol usage, poor time management, study behavior and even reflecting self-defeating practices. Another research by Georgios et al. (2017) pointed out that self-esteem levels tend to decline when individuals are at the adolescent stage. Such a decline in self-esteem is linked with the growth in the cognitive ability of these individuals which makes them increasingly sensitive to social issues. The lowered self-esteem is also attributed to the widening gap between the real identity and the ideal selves. The gap between the two identities has been identified to be wider among the adolescent girls compared to the boys. These findings have also been contradicted by other studies that identify the female adolescents and having a more positive perception of themselves compared to the male adolescents and also those that identify no difference between the self-esteem levels across different genders.

Conclusion

Conclusions, despite that most of the students have good levels of self-esteem is still critical issue that needs close attention to minimize the expected impact on student's academic performance, because of the fact that self-esteem significantly in this study. There are gender differences in respect to self-esteem. In which that male had higher level of self-esteem than female students. Recommendations, it is important to raise awareness of faculty members about the alarming level of stress among students to help them find new ways of supporting their students. Teach and encourage students to use more effective stress management strategies to minimize the risk of academic stress. Maintain student's self-esteem to improve their academic wellness. Further, research is needed to target academic-related aspects (learning environments, teaching style, course workload, and tests) and their effect on the students' academic stress.

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Conflict of Interest: None to declare.

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Patterns of Haemoglobinopathies Diagnosed by High Performance Liquid Chromatography in Karbala Population and Correlations between Different Hematological Parameters

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Abstract

Aim: To know the patterns of hemoglobinopathies and their prevalence in Kerbala society by using high performance liquid chromatography and to conclude if there is any correlations between different hematological parameters in Hemoglobinopathic patients. A total of 70 cases studied from 16th of February 2018 to 20th of June 2018 for hemoglobin variant analysis at the teaching labrotarioes of Al- Hussseini Teaching hospital which were studied for patterns of hemoglobinopathies. When the samples were received, Complete Blood Counts including: Hemoglobin concentration, Total White blood cells count (WBC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Serum ferritin, Total Iron Binding Capacity (TIBC), Hemoglobin H (HbH) preparation were done. Out of total 70 patients screened for patterns of hemoglobinopathies the result of the study revealed that 48 Patients which represent(68.57 %) of total patients had B-thalassemia trait which form the predominant percentage of patients with haemoglobinopathy, 11.85% of patients had sickle cell trait, 5.71% of them had B-Thalassemia Major, 4.29% had Hb S/B+Thalassemia Trait, 2.86% had HbS/Alpha thalassemia trait, 1.43% of them had Hb S homozygosity, 1.43% Alpha thalassemia trait, 1.43% had D-Los Anglos, 1.43% had HbS/B+ Thalassemia trait and 1.43% had HbC/B-Thalassemia trait.

Key Words: *Hemoglobinopathies, Hb electrophoresis, Hemoglobin Variants, retention time, hemoglobin A2.*

Introduction

Haemoglobinopathies are groups of inherited disorders which result either by an abnormal structure of hemoglobin as in sickle cell anemia or by decreased formation of one or more globin chains as in thalassemia [1] There is worldwide increment of the prevalence of inherited hemoglobinopathies such as thalassemia and sickle-cell disorders due to people's migration [2,3]. Hemoglobin abnormalities are present as carrier states in About 5% of the world's population. [4] The normal structure of hemoglobin in healthy individual consisted of tetramers of 4 globin chains of polypeptide, every one of them connected to haem protein . The predominant hemoglobin in normal adults is HbA (forming about 97%) and it has 2 α and 2 β chains. A remaining part of hemoglobin consists of approximately 2-3% HbA_2 which has two α and two δ chains, and a few portion of hemoglobin of fewer than 1% represents HbF, which has two α and two γ chains. In the neonatal period, the major haemoglobin is HbF (approximately 80-90%), with a variable amount of HbA (approximately 10-20%). The switch from γ chain synthesis (which is the predominant form of hemoglobin in the fetus and neonate) to β chain synthesis (which predominates in children and adults), together with the production of δ chains, is usually finished by about one year from time of baby delivery. So, at that period of life, the infant

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get full maturation of HbA2 and Hb F levels.[5] HPLC is used for identification, and estimation of both normal and many abnormal Hb categories.[6] HPLC provides the clear benefits and priorities in comparison to traditional Hb electrophoresis because of it's ability to detect and estimate the quantities of different types of disrupted Hbs in very accurate and perfect manner.[7] It has been proved that HPLC is very rapid, sensitive, specific, and reproducible replacing technique to conventional Hb electrophoresis.[(8)] It's important to mention that every disordered haemoglobin (variant of hemoglobin) can represented in HPLC in specific area and had specific imprecision of retention time that can differentiate it from other types of hemoglobin's. Retention time is the time that extended from the moment of the person's sample injection to the appearance of the summit(apex) of a haemoglobin high point(peak) . The established values at which the well-known disrupted hemoglobin's (variants) were noticed to be eluted and separated by applying the "Variant Beta- thalassemia's short scheme or program are termed as "Windows". During printing the chromatogram's paragraph, there is existence of the following components: all portions of the separated disordered haemoglobin (variants), retention times, the reagons of of hemoglobin's peaks and the levels (%) of various haemoglobin elements. If hemoglobin peaked area was separated at an imprecision of retention time that is not pre-identified, it will be named as an "unknown" area.[(9)] Several structural Hemoglobin disorders and the rarest hemoglobinopathies that can't be identified and diagnosed by Hb electrophoresis, they were detected by HPLC which is preferred in such acases and gave very accurate results as it's the ideal, sensitive and gold standard technique.[(10)] Beside HPLC, there are numerous hematological tests and tools could be used in the assessment and diagnosis of many hemoglobin abnormalities, as example for these tests: sickling test, alkaline and acid electrophoresis, isoelectric focusing (IEF), capillary electrophoresis and DNA testing /protein testing and analysis. On the other hand, it is mandatory to correlate the results of all above mentioned tests when applied with the peripheral blood smears findings of the patients as there is pathognomonic picture of blood film for many hemoglobin abnormalities and disorders.[(11)]

Methodology

This Descriptive Cross Sectional study analyzed 70 blood samples received from February 2018 to June 2018 in Teaching Laboratories In Al-Hussaini teaching hospital in Kerbala governorate / Iraq. Patients with the following eligibility criteria included in the current study: anemia with Hb level extending from 9 to 11.2 g/dl , generalized weakness, patients from both genders with age limits from 17 to 30 years old with suspected haemoglobinopathy depending on the bases of clinical and family history and the results of blood film for those patients which show accordingly: hypochromic microcytic red cells, anisopoikilocytosis, target cells, sickle cells in sickle cell disease confirmed by sickling test were included for study on High Performance Liquid Chromatography (HPLC). Beside above mentioned Criteria, Patients with a recent blood transfusion and inadequate sample did not included in the current study. About Five milliliters of patient's blood was collected in dipotassium ethylenediaminetetraacetic acid vacutainers and was run in Sysmex autoanalyzer for hemogram and red cell indices; The initial laboratory tests done for the patients were: Comple Blood Picture CBP (RBC count, Hb level, Packed Cells Volume (PCV), Mean Cells Volume (MCV), Mean Cells Hematocrit (MCH), Mean Cells Hematocrit Concentration (MCHC), Total and differential white blood cells counts and Platelets Count in which the normal RBC count is **4.06-5.30 X 10^6/µl** and normal Hb level is **12.0-16.0 g/dl**. The manual blood film was performed using the method of Leishman stain[**(12)**]. The samples were run on High Performance Liquid Chromatography instrument HPLC manufactured by Bio-Rad Laboratories. Based on retention time and proportion of Hb variants, different hemoglobinopathies were diagnosed and their prevalence was analyzed. The assigned windows of Manufacturer for Bio-Rad variant HPLC system was shown in Table 1. The presence of Hb H was proved by the usage of brilliant crystal blue staining for the visualization of Hb H inclusions by the light microscope. Another parameters tested in present study are: Serum Iron, Serum ferritin, Total Iron Binding Capacity (TIBC) which were evaluated for all patients in this study.

Results and Discusion

This descriptive cross sectional study was attempted to see the difference in the prevalence of various hemoglobinopathies in Kerbala Governorate in Iraq Country and to conclude if there is any significant correlations between different hematological parameters. Different types of Hemoglobinopathies including thalassemia's and other groups of hemoglobin disorders are distributed worldwide and are known to be autosomal recessive in inheritance with defects in Hemoglobin synthesis.[**(14)**] Thalassemia is a haemoglobinopathy

that result from defect in the quantity and amount of hemoglobin formed in which there is reduction in the level of hemoglobin synthesized resulting in a form of anemia .with RBCs have reduction in their size and their hemoglobinization (hypochromic microcytic) RBCs. There are many forms of thalassemia which are classified according to which type of globin chain are mutated and depleted for example there is α and β thalassemia due to depletion of α and β chains synthesis. Also there is further clinical sub classifications of thalassemia's in to thalassemia major and minor(homozygous and heterozygous) respectively which is based on number of genes affected , either one gene mutated or two genes affected in respective manner. Thus the patients presented in different clinical manifestation. The patients who are of homozygous in type are so ill in which only very limited number of them can still alive to the adulthood period of life and this fatality is due to the severity of anemia and due to cardiopulmonary deteriorations. Regarding thalassemia of heterozygous gene defect, also categorized as thalassemia trait and the clinical finding in such type is solely very mild anemia with hemoglobin concentration not getting below 9g/dL.[15] The current research conclude that the predominant number of Hemoglobinopathic cases were of Beta thalassemia trait (68.57%) and were common in the age limit of 20-30 years and this is in consistent with Buch et al study . The high incidence of thalassemia trait require antenatal screening tests and screening of marriageable age groups. This will help in the limitation and prevention of thalassemia major in the offspring's. This may be the cause for detecting less number of cases of thalassemia major in our study group. We think that antenatal screening or screening of higher secondary school children to detect hemoglobinopathies, counseling of the individuals with hemoglobinopathies will definitely help in drastically reducing the incidence of the thalassemia major and sickle cell disease. This study found that the incidence of B-Thalassemia Major was (5.71%), Similar research done by Patel et al in Gujarat had reported the incidence of thalassemia major (5.63%).[16] The Current study concludes that the incidence of remaining Hemoglobinopathies was as Follows: Sickle/B+-Thalassemia trait(2.86%), sickle cell trait (10%), Hemoglobin S homozygosity (1.43%), Hemoglobin S/alpha thalassemia trait(2.86%), Hemoglobin C/B-Thalassemia(1.43%), Hemoglobin D-Los Anglos (1.43%). There was no gender difference in the different hematological parameters in the sample and this agreed with *Bhokare SB et al*[16] The present study shows that there were significant differences regarding some hematological parameters between the patients of different hemoglobinopathies including: Hemoglobin A, Hemoglobin F and White Blood Cell Count and this agreed with Patel et al. [8] Also there were significant differences between Thalassemia trait patients and Those with Thalassemia Major regarding HbA, HbA2, HbF, Hemoglobin Level, Mean Cell Hemoglobin Concentration (MCHC), Hb H preparation and Serum Iron and this is in consistent with Colah et al.[17] And Patel et al[8] HPLC also helped us in detecting various heterozygous states. Though these abnormal variants have less clinical significance but they can produce severe disease when coexisted with other variants. This once again highlights the significance of mass screening of the population. Although these states can be detected by both HPLC and gel electrophoresis, while there is further advantage of using HPLC further sub-classifying these syndromes based on detection and estimation the amount of various Hemoglobin's depending on their retention time. Regarding Hb D Los Anglos it had identical electrophoretic finding and existence at alkaline pH, on the other hand the average of its retention time in chromatography is creative and had variable results in predictable manner. This differentiation is in fact quite significant especially in a double heterozygous state with Hb S, as Hb SD [18, 19]

Table 1: Manufacturer assigned windows for Bio-Rad variant high-performance liquid chromatography system[13]

Hematoligical Parameters	Gender	Mean	Standard-Deviation	Significance (P value)
HemoglobinA(HbA)	Male	82.868	22.347	0.141
	Female	73.390	30.023	
HemoglobinA2(HbA2)	Male	6.265	10.350	0.315
	Female	4.358	1.774	

Cont... **Table 1: Manufacturer assigned windows for Bio-Rad variant high-performance liquid chromatography system[13]**

HemoglobinF (HbF)	Male	3.619	6.406	0.263
	Female	6.461	13.735	
HemoglobinS(HbS)	Male	42.614	23.200	0.472
	Female	52.313	26.946	
HemoglobinC(HbC)	Male	64.657	24.654	0.255
	Female	73.700	34.543	
Retic Count	Male	3.322	1.980	0.745
	Female	3.955	2.617	
HemoglobinConcentration(Hb)	Male	9.422	1.482	0.831
	Female	9.542	1.608	
Hemotocrit (HCT)	Male	28.919	4.175	0.615
	Female	29.145	4.647	
Mean Cell Volume(MCV)	Male	63.738	8.186	0.198
	Female	64.794	9.286	
Mean Cell Hemoglobin(MCH)	Male	20.908	3.452	0.781
	Female	22.031	3.720	
	Female	73.672	36.566	

Table 2: The mean level of different haematlogical parameters of hemoglobinopathy patients and its relation to gender in Al Hussieni Teaching hospital in Kerbala in Iraq in 2018 (n=70)

Hb Type		**Summation of Squares**	**Means of Squares**	**P value**
HbA	Between Groups	40636.56	3386.38	<.0.001
	Within Groups	5898.831	107.251	
	Total	46535.39		
HbA2	Between Groups	207.63	17.302	0.994
	Within Groups	3804.839	69.179	
	Total	4012.47		
HbF	Between Groups	4295.342	330.411	<.0.001
	Within Groups	3360.108	60.002	
	Total	7655.45		
HbS	Between Groups	6579.804	822.476	0.155
	Within Groups	2083.393	347.232	
	Total	8663.197		
Hb. H. Preparation	Between Groups	2.871	0.221	.0.8888
	Within Groups	0	0	
	Total	2.871		
Retic. Count	Between Groups	299.899	23.069	<.0.001
	Within Groups	67.333	1.202	
	Total	367.232		
Hb level	Between Groups	42.571	3.275	0.134
	Within Groups	119.486	2.134	
	Total	162.058		

Table 3. The Differences within and between groups of Different Hematological Parameters and their significance among Patients of different patterns of Hemoglobinopathies.

	Hemoglobinopathies Types	frequency
1	B-Thalassemia Trait	68.57%
2	sickle cell trait	11.85%
3	B-Thalassemia Major	5.71%
4	Sickle/B+-Thalassemia trait	4.29%
5	Hemoglobin S/alpha thalassemia trait	2.86%
6	Hemoglobin S homozygousity	1.43%
7	Hemoglobin C/B-Thalassemia	1.43%
8	Hemoglobin D-Los Anglos	1.43%
9	alpha thalassemia trait	1.43%

Table 4. The distribution of different types of Hemoglobinopathies in patients in Al Hussieni Teaching hospital in Kerbala in Iraq in 2018 (n=70)

Hematological Parameters	**Disease**	**Mean**	**Std. Deviation**	**Significance**
HbA	B Thal trait	92.534	3.441	<0.001
	B-Thal Major	52.125	12.809	
HbA2	B Thal trait	6.440	9.084	<0.001
	B-Thal Major	5.125	1.315	
HbF	B Thal trait	2.373	3.239	0.002
	B-Thal Major	35.200	25.130	
Retic. Count	B Thal trait	2.450	0.858	0.377
	B-Thal Major	8.200	1.166	
Hb level	B Thal trait	9.581	1.499	0.002
	B-Thal Major	7.200	0.963	
Hb H preperaion	B Thal trait	29.394	4.351	0.0022
	B-Thal Major	22.275	2.680	
MCV	B Thal trait	62.235	7.162	0.004
	B-Thal Major	59.000	2.582	
MCH	B Thal trait	20.533	2.728	0.06
	B-Thal Major	19.500	3.109	

Table 4. The distribution of different types of Hemoglobinopathies in patients in Al Hussieni Teaching hospital in Kerbala in Iraq in 2018 (n=70)

MCHC	B Thal trait	26.863	3.123	0.001
	B-Thal Major	24.500	3.416	
WBC	B Thal trait	8.923	3.405	0.74
	B-Thal Major	6.900	2.255	

Conclusion

The current study conclude that B-Thalassemia Trait represent the higher frequency or incidence of hemoglobinopathies among Al-Hussaini teaching Hospital Patients followed by Sickle cell/B+Thalassemia trait disease. There is no gender difference in relation to different hematological parameters among Hemoglobinopathic patients. There in significant differences regarding some hematological parameters among Hemoglobinopathic patients including: HbA, HbF,MCV,MCH, MCHC. There is significant differences between some hematological parameters between Patients of Thalassemia Major and those of Thalassemia Trait including: HbA, HbA2,
HbF, HbH preparation, MCV,Hb Level, MCHC and Serum Iron. We conclude, HPLC is an ideal method for routine diagnosis of hemoglobinopathies.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Pathology, College of Medicine, University of Karbala, Karbala, Iraq and all experiments were carried out in accordance with approved guidelines.

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Risk Factors of Acute Otitis Media among Infants Children in Kerbala Pediatric Teaching Hospital: A Case-Control Study

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Abstract

Objective of the study: To explore the most prevalent risk factors those are associated with AOM among infant children. A case-control study design was carried out in the outpatient clinic at Kerbala pediatric teaching hospital, from the period from January 2017 to February 2019. A purposive sampling method of 100 infants was included in this study, 50 of them were infected with AOM and the others are not. A data were collected directly from infant's parent by the researchers through the interviewing technique, then the collected data were analyzed by using the program of IBM Statistical Package of Social Sciences (SPSS) Version 24. The results of this study revealed that about 40%, 64% and 40% of infected infants were 7-9 months of age, females and urban residency respectively. The study showed a significant association between AOM and nasal obstruction at p-value 0.02, child's position during feeding at p-value 0.00, mother's position in case of bottle feeding at p-value 0.00, and using bottle feeding at night (for breast feeding infants) at p-value 0.002. Infants exposed to cigarette smoking, and that's were using pacifiers when sleeping also identified a significant risk factors for AOM at p-value 0.00, and 0.00 respectively.

Key Words: *Risk Factors, Acute Otitis Media, Infants.*

Introduction

Otitis media are well identifying as an infection and/or inflammation of the middle ear [1], it is the second most common childhood disease after upper respiratory tract infection [2] AOM is the most frequently recognized ear infection [3], and it is one of the most widespread communicable diseases of childhood that is most frequently occurs between 3 months to 3 years of age. In the United States of America (USA), 80-90% of AOM occur in children less than six years, the main incidence is between 6 months to 24 months of age [4]. Newborns and infants are more likely to infect with AOM because of the immature immune system [6] reported that the AOM is a disease of infancy; it can affect more than 33% of children less than one year of age. Ginkel et al., (2017) mentioned that about 25-36% of children less than one year have experienced at least one event of AOM and approximately 20% of the infants developed more than one event of AOM. It is an estimated that about 709 million new AOM cases annually worldwide, with greater than half in children less than five years of age [8]. A recurrent and prolonged episode of AOM may lead to many health problems such as hearing impairment, delayed speaking development, and delayed language and educational development [9]. In the United States of America, the annual cost of AOM is approximately 5 billion dollars [10]. Several influences have been identified to be accompanied with the development of AOM, some of these influences are unanimously recognized such as environmental tobacco smoke exposure and adenoids hypertrophy; other influences are more controversial such as gender or poor economic status [11] stated that's the possible risk factors that are associated with otitis media are family history of otitis media, recurrent upper respiratory infections, younger age, and second hand smoking. [12] reported other risk factors for ear infections involve low birth weight, younger motherly age, inadequate housing conditions, lack of access to health care, and mothers who smoke during pregnancy. Numerous studies have been documented that the pacifier use for some babies was determined as an additional risk factor for developing otitis media. Other risk factors that are associated with recurrent otitis media among infants are includes orofacial anomalies such as cleft palate, overcrowding, lack of breast feeding or shorter duration

of breastfeeding, and prolonged bottle-feeding while lying. Supine-feeding positions, immune deficiencies, and gastro-esophageal reflux are also identified as a risk factors of AOM. Identifying of otitis media risk factors can contribute to improving management and effective prevention plan. Pawathil, and Rajamma (2016) mentioned that are by recognizing the risk factors if AOM, the clinician can notify parents about preventive measures to avoid these factors. Ginkel et al., (2017) reported that the documented of preventable risk factors is a significant effort in the prevention of AOM. In this study, we aim to determine the impact of several risk factors that are associated with AOM among children less than 12 months of age in order to improve preventive methods of this disease.

Methodology

A case-control study design was carried out in the outpatient clinic at Kerbala pediatric teaching hospital, in the period from January 5th 2017 to February 1st 2019, in order to investigate the most prevalent risk factors that are associated with AOM among children less than one year of age. A purposive sampling method of 100 infants was included in this study, 50 of them were infected with AOM and the others are not. A verbal informed consent was obtained from the infant's parent to be enrolled in this study. A questionnaire form was prepared by the researchers in order to collect all the relevant data associated with the study sample. The questionnaire form contains of (11) items that are (Age, gender, residency, type of feeding, nasal obstruction condition, child position during feeding, mother's position during feeding, upper respiratory tract infection, using bottle feeding at night, exposed to second hand smoke, and using the pacifiers when sleeping). Data were collected directly from infant's parents by the researchers through the interviewing technique. Finally the collected data were analyzed by using the program of IBM Statistical Package of Social Sciences (SPSS) Version 24, both descriptive statistical analysis procedures (frequency, percentage) and inferential statistical analysis were used in order to analyze and assess the results of the study, a p value <0.05 was considered statistically significant.

Results and Discusion

As shown in table (1), there are a statistically significant association between female gender with AOM, and no significant association with the other listed factors. Analysis of the risk factors associated with AOM among infants as shown in table (3), indicates that the nasal obstruction, infant's position during feeding, mother's position in case of bottle-feeding, using bottle-feeding at night, second hand smoke, and using of pacifiers when sleeping were found to be a significant risk factor associated with AOM among infants. A total of (50) infants who are infected with AOM, and (50) non-infected infant were involved in this study to investigate the most prevalent risk factors that are associated with AOM. Data that listed in table (1 and 2) show that are many risk factors are contributing to the occurring of AOM in the examined group of infants. The results in table (1) revealed a significant association between female gender and AOM (at P value 0.005), this result was disagreement to the findings of the study which was done by Abed Alsalama, et al, (2018) revealed that the male gender were determined a significant risk factor for the occurrence of AOM. Another study, which was done by Sangeetha, et al., (2014) revealed that the boys infant were found to have a higher incidence of AOM than girls. Concerning to the association between nasal obstruction and AOM among the study subjects, the results in table (2) exposed a significant association between AOM and nasal obstruction (at p value of 0.02), this result was corresponding with the findings of a meta-analysis study which was done by Ilechukwu, et al., (2017) that identified the upper respiratory tract infection as an important risk factor for the occurrence of AOM. Regina, et al, (2017) reported that the AOM is mostly occurring secondary to viral upper respiratory tract infection and subsequent microbial colonization of the nasopharynx and the middle ear. Supine position of infants during feeding as shown in figure (2) also determined as a main factor contributing to AOM because this position lead to leakage of milk into the ear canal and therefore increase the chance for developing AOM, the result in table (2) show that are about 66% of infant infected with AOM were putting in supine position by their mothers during feeding, this finding was corresponding with the study of Regina, et al, (2017) emphasized that the use of supine position during feeding was significantly increases the risk of AOM in infant. Another studies that were done by Sangeetha, et al., (2014), Swamy, et al, (2017), and Abed Alsalama, et al, (2018) revealed that there is a significant association existed between supine feeding position and AOM in infants. Furthermore, the findings of the study of Regina, et al, (2017) illustrated that the using of supine or near so position by mothers for feeding their babies facilitates the reflux of milk into middle ear, with carriage of respiratory flora into

the middle ear space, thereby leading to otitis media. Pawathil, and Rajamma (2016) indicate that the supine feeding position was identified to be highly significant risk factor for otitis media in infancy. Bottle feeding as shown in figure (1) and the mother's position in case of bottle-feeding are considered as an important factor that are associated with AOM, the findings in table (2) show that about 68.5% of mothers are always away from their infants when using bottle-feeding. Many studies have been evaluated breast-feeding role in the prevention of AOM and showed that the breast milk protects against AOM. Some studies also have been exposed that the wrong position of feeding can increased AOM among children (Regina, et al, 2017). Abrahams and Labbok (2011) highlight that the breast milk feeding, unlike bottle feeding formula, because human milk is a containing antimicrobial, and anti-inflammatory substances, therefore infants with bottle feeding show a relative immunodeficiency compared with those receiving breast feeding, placing them at significantly greater risk of infections. In a case-control study that was done by Pawathil, and Rajamma (2016), confirmed that are a significant association between bottle-feeding and the occurrence of AOM.

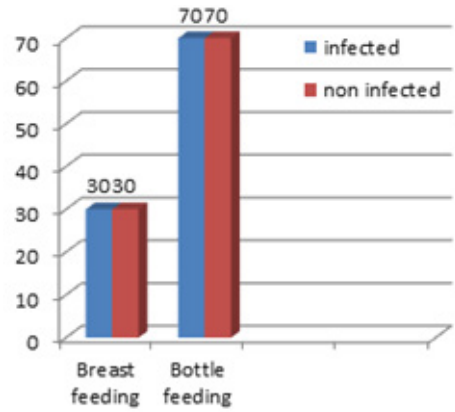

Figure1: Association of AOM with type of feeding

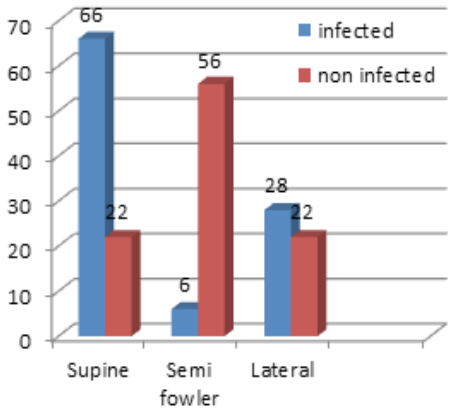

Figure 2: Association AOM with infant's position during feeding

Table (1): Demographic characteristics for infant infected and non-infected with AOM.

No.	Demographic characteristics		Case N=50		Control N=50		p. value	Significant level
		Frequency (F) Categories	Percent (%)	Frequency (F)	Percent (%)			
	Items							
1.	Age	1-3 months	5	10	5	10	0.5	N.S
		4-6 months	11	22	14	28		
		7-9 months	20	40	13	26		
		10-12 months	14	28	18	36		
		Total	50	100	50	100		
2.	Gender	Female	32	64	18	36	0.005	S
		Male	18	36	32	64		
		Total	50	100	50	100		
3.	Residency	Urban	30	60	24	48	0.2	N.S
		Rural	20	40	26	52		
		Total	50	100	50	100		

NS: Non-Significant (P value >0.05); S: Significant (P value < 0.05).

Table (2): Statistical result of the risk factors for infants infected and non-infected with AOM.

No.	Risk Factors		Case N=50		Control N=50		p. value	Significant level
	Items	Frequency (F) Categories	Percent (%)	Frequency (F)	Percent (%)			
1.	Type of feeding	Bottle feeding	35	70	35	70	1.0	N.S
		Breast feeding	15	30	15	30		
		Total	50	100	50	100		
2.	Nasal obstruction	Yes	37	74	26	52	0.02	S
		No	13	26	24	48		
		Total	50	100	50	100		
3.	Child's position during feeding	Supine	33	66	11	22	0.00	S
		Semi fowler's	3	6	28	56		
		Lateral	14	28	11	22		
		Total	50	100	50	100		
4.	Mother's position in case of bottle feeding	Away from infant	24	68.5	8	23	0.00	S
		Near of infant	11	31.5	27	77		
		Total	35	100	35	100		
5.	Upper respiratory infection	Yes	22	44	29	58	0.1	N.S
		No	28	56	21	42		
		Total	50	100	50	100		
6.	Using bottle feeding at night (for breast feeding infants)	Yes	12	80	4	26.7	0.002	S
		No	3	20	11	73.3		
		Total	15	100	15	100		
7.	Children exposed to passive smoking	Yes	42	84	22	44	0.00	S
		No	8	16	28	56		
		Total	50	100	50	100		

Furthermore, data in table (2) have been recognized that the using of bottle-feeding at night for breast feeding infants are also contributing to development of AOM in 80 % of infant, and determine statistically significant (at P value 0.002), this finding was corresponding with the results of the study of Sangeetha, et al., (2014) who stated a higher prevalence of otitis media were associated with bottle-feeding than with breast feeding. A study of Abrahams and Labbok (2011) emphasized that the introduction of infant bottle-feeding formula in the first 6 months of life is associated with increased risk of otitis media when compared with 6 months of exclusive breast-feeding. In a meta-analysis study which was done by Bowatte, et al., (2015) provided an evidence that the breast-feeding protects against AOM until two years of age.

Concerning the children exposed to passive smoking, the finding of the study indicates a statistically significant association (at P Value 0.00) for the development of AOM in 84% of infants who have exposed to second hand smoke, this was consistent with the findings of a meta-analysis study that identified reliable passive smoke are important risk factor for the occurrence of otitis media. The results in table (2) also demonstrate that are the using of pacifiers for infants was determine a significant risk factor for 82% of infants (at P value 0.00), its believes that the saliva may be leakage into the ear canal of infants due to the pacifiers use, that's leads to increase the chances for developing AOM. The results of the present study confirms the findings of previous studies regarding the association between pacifier use and otitis media, and largely agrees with other findings of the previous studies regarding otitis media occurrence and its risk factors.

Conclusion

Many risk factors that are associated with AOM, these include nasal obstruction, infant's and mother's position, using bottle-feeding formula, exposure to cigarette smoking, and using pacifiers when sleeping.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing/ Kerbala University and all experiments were carried out in accordance with approved guidelines.

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The impact of L-carnitine Supplement on Semen Variables and the Levels of Sexual Hormones (Serum LH, FSH, Testosterone, and Inhibin) in Males with Infertility

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Abstract

Background: It had been estimated that tenth of partners had infertility, and in approximately half of these cases, the imperfection can be ascribed to male related causes. Fecundity in men necessitate regular functioning hypothalamus, pituitary gland, and testes axis. aberrant spermatogenesis is habitually incorporated to permuted serum gonadotropins and testosterone. L-carnitine is an supreme antioxidant existing in a lofty concentration in epididymal excretions. It affords liveliness for sperms to earn their dynamism in the epididymis.

Aim: The study proposed to explore the effect of L-carnitine supplement on semen parameters, and sex hormones milieu in patients with primary or secondary infertility.

Patients and Methods: A clinical trial conducted on a 58 patients(mean age± SD: 30.6 ± 6.07) treated with 2gm of L carnitine, which prescribed orally at a daily dose of 2gm a day in divided doses for 3 months. Before, and after the end of the L-carnitine treatment, semen analysis and serum levels of LH, FSH, Testosterone, and inhibin were performed.

Results: The mean values of sperm count, total motility and normal morphology of and oligo-astheno-teratospermic were found notably changed ($p<0.05$) with 3 months carnitine supplement. The outcome of this trial exhibited that L-carnitine supplement can improve sperm concentration, and count, as well active motility. Additionally it has been noticed that the serum gonadotropin (FSH and LH) levels were noteworthy declined, while serum testosterone and inhibin levels were upraised in infertile males when compared with the levels before, the 3 months course of L-carnitine treatment.

Keywords: *L.Carnitne, semen variables, sex hormones, OAT.*

Introduction

Infertility is a failure to procure conceiving over a year of orderly unguarded coitus. It affects nearly 15% of sexually active partners; however, male factor accords to up to 40% of instances [(1)]. The male aspects of aberration marked by decrease sperm tally and/or sperm motility and/or raised anomalous sperm morphology. These changes are altogether named oligo-astheno-terato-zoospermia (OAT) and they are regarded noteworthy only if discovered in 2 sequential perusals of semen samples collected apart within one to four weeks. This accordant with the WHO guidelines (2010) [(2)]. Around 30% of sufferer have idiopathic (iOAT), which may be linked to aging or to non-inflammatory, or functional changes in epididymis or due to some viral or chlamydial infections [(3-4)] .

Semen analysis prevails the most trustworthy laboratory workup for the assessment of non-fertile male.[5] The remarkable elements that takes primacy in in-vivo and in-vitro fertilization are sperm total number

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and the percent of motile and morphologically normal sperms.[6-7] furthermore, experiences suggested that computer-assisted semen analysis (CASA) exactly and trustably evaluate kinematics of the sperms, which sequentially, highly related to the fertility rate in-vitro and in-vivo time to conceive.[8-9]

The therapy of male infertility particularly iOAT can be burdensome. Many medicines, dietary supplementations and antioxidants are tried but a verifying evidence reinforcing their efficacy is not yet proved [(10)] .

L. carnitine (LC) is a water-soluble, and a vitamin-like structured molecule. Three quarters of the body requirments of LC is of dietary source and only 25% is endogenously synthesized from lysine and methionine the two essential amino acids, and they are hoarded in striated muscles, cardiac muscles, brain tisues, and testicles.[(11)] The lofty level of LC is detected in epididymal excretions and sperm, with a level of about 2000 folds higher than that of the plasma giving a clue about its pivotal task in energy metabolism and sperm maturation.[12] Furthermore, LC convey the long-chain fatty acids from the cytosol into mitochondria and promote β-oxidation of long-chain fatty acids so it plays vital role in energy production.[13,14] LC have an essential duty as it act as an outsized non-enzymatic antioxidant and helps in cell, mitochondrial membrane, and DNA protection against structural disfigurement induced by oxygen free radicals. Many experiments have been conducted to assess the antioxidant role of carnitine, their results demonstrate upswing of antioxidant enzyme activities, and the curtailment of oxidative stress in diverse cells. [15]

The balanced endocrine interplay of hypothalamus, pituitary and the testis, determine the flourishing and utter development of male gametes. Hypothalamus excreted gonadotropin releasing hormone (Gnrh) evoke the libration of gonadotrophins i.e follicle stimulating hormone(FSH) and lutenizing hormone(LH) from the pituitary gland [(16)]. FSH ties up with the sertoli cells receptors, and triggers spermatozoa production. LH triggers the excreation of testosterone in Lydge cells, which seriatimly exploit on the Sertoli and peritubular cells of the seminiferous tubules and trigger spermatozoa production [(17)]. The failed secretion of FSH and LH by the hypophyseal stalk will result in turmoil of testicular function causing infertility. Testosterone, estradiol as well as inhibin orchestrate the excretion of gonadotropins [(18)]. The upraised FSH level in men with azoospermia or severe oligospermia (less than 5million sperm/ml) stipulate seminiferous tubules damage [(19)]. Moreover if inhibin, testosterone, dihydrotestosterone and estradiol secretion is changed, this will disorganize the negative feedback mechanism which lead to disturbance of the levels of FSH [(20)]. Customarily, the amount of FSH correspond to the production of sperms, when spermatogonia are non-existent or strikingly reduced, FSH level will be skywarded ; when the quantity of spermatogonia within normal limits, but their maturation is arrested at a specific stage (spermatocyte or spermatid), FSH values are in the ordinary range.[(21)]

The study proposed to explore the effect of L-carnitine supplement on semen parameters, and sex hormones milieu (serum LH, FSH, Testosterone, and Inhibin levels) in patients with primary or secondary infertility.

Patients and Method

A clinical trial conducted between November 2016 to the end of December 2018, a total number of 82 infertile male patients (aged 18-47 years) referred to the infertility clinic in Salahaldeen General Hospital, after the exclusion of 15 patients with varicocele, orchitis, cryptorchidism, and those males who taking herbals or medications that might affect seminal parameters in the last 3 months prior to the study, and 9 patient dropped out because of incompliance of treatment; 58 treated with L-carnitine 2 mg given orally in 2 divided doses a day. The treatment period was 3 months. Seminal fluid analysis and serum levels of LH, FSH, testosterone, and inhibin, was done prior to the onset of the study and after the end of the treatment course. Semen samples were collected after 3-5 days of sexual abstinence and the standard manual semen analysis was performed according to the WHO guidelines [(2)] . During the study period, the patients were interviewed monthly to assess their compliance with treatment.

Infertility assigned depending upon (WHO) guidelines. Infertile males, with more than 1year infertility, in spite of regular coitus with a normal female partner were chosen. The diagnosis was made after clinical assessment that includes: history; clinical examination especially for varicocelle and testicular volume which further evaluated by sonography of genitalia. Semen analysis; Follicular stimulating Hormones (FSH), Leutinizing Hormone(LH), testosterone, and inhibin

levels were done for each patient before and after treatment course. The semen parameters, including sperm concentration, total and forward sperm motility (percentage at one hour after ejaculation) and sperm morphology (percentage of atypical forms), according to WHO standard procedures. Data presented with simple tables and analyzed statistically to test significance at p value< 0.05

Result

In this prospective study we considered a total of 58 patients with infertility due to unexplained astheno, oligo, and/or teratospermia before and after treatment with L. carnitine for 3 months. The mean age was 29.7 ± 5.07 years. The mean duration of infertility was 3.9 ± 4.65 (1.5 to 11years). 58 patients given L carnitine for three months. The median seminal fluid volume, total perm count, normal appearance, the actively motile and Viabile sperm percentage of the treatment group before treatment were 2.1 *vs.* 3.1ml, 21 *vs.* 41million ,21% *vs.* 51% , 28% *vs.* 47% and 36% *vs.* 72% respectively, which a noteworthy increment (P<0.05).

Additionally a significant drop in sluggish and non-motile sperm percentage, as well the teratospermia percentage (47% *vs.* 23%, 43% *vs.* 26% and 58% *vs.* 29% respectively) was beheld. (P<0.05).

Table (1): Semen parameters before and after 3months treatment with L. carnitine

Parameters	Mean ± S.D. before	Mean ± S.D. after
Volume (ml)	2.10±0.39	3.12±0.74*
Count	21±2.3546	41±3.7651*
Active	28±5.887	47±6.4 16*
Sluggish	47±5.226	23±3.709*
Non Motile	43±5.472	26±3.756*
Normal	21±4.561	51±5.103*
Abnormal	58±5.082	29±4.110*
Viability	36.88±12.31	72.7±5.2*

P value<0.05

As it is clear in table 2, after 3 months of 2gm of L-carnitine for the selected infertile patient with hypofertility, a significant decline in the serum levels of gonadotropins(FSH, LH) 9 vs. 5 IU/L, and 6 vs. 4 IU/L respectively. On the other hand, a notable elevation in serum testosterone and inhibin B levels (4 vs. 7 ng/ml and 99 vs. 232 pg/ml respectively) have been recorded.

Table (2): Hormonal assay before and after 3months treatment with L. carnitine

	Before L-carnitine	After L. carnitine
FSH (IU/L)	9.534(±1.418)	5.650(±0.721)*
LH (IU/L)	6.346(± 9.112)	4.234(±1.109)*
Testosterone ng/ml	4.556(±1.167)	7.122(± 0.571)*
Inhibin B pg/ml	99.436(±6.472)	232.756(±5.945)*

P value<0.05

Discussion

In the near past time, male has not been accused as a cause of infertility. Male hypo-fertility has no distinct existence but it displays a diversity of pathophysiologic mechanisms. Sperms in the environment of Epididymis use a various substrates as sources of energy, but oxidation of fatty-acid which involves carnitine dependent system appears to be the crucial energy vouchsafing operation[22].

Mazzilli et al divulged a solid relation between spermatic cytoplasmic L-carnitine reserve with spermatic activity and viability in the mucus of uterine cervix of bovine. This correlation could be described by the fact that fats are prime energy resource for sperms in cervical mucus blug and to utilize these fats, it is important to have adequate cytoplasmic spermatic L-carnitine. L-carnitine not only aids in metabolism of lipids, but it adjusts the hoards of the free CoA as well, which is vital for tri-carboxylic acid cycle adjustment. That's why , L-carnitine reserves can be regarded as an index of spermatic activity extent in cervical mucus.[23]

The refinement in the fertility tally achieved by L-carnitine supplement allocated by the noteworthy upgrading in sperm concentration and total counting, volume of ejaculate and proportion of highly motile spermatozoa in our trial could be explained by the inkling

that L-carnitine is a vital cofactor that may enhance lipid metabolism and play a crucial role in mitochondrial beta-oxidation of long-chain fatty acids for cell life maintenance and energy production. (24,25)

A controlled trial conducted in (2004) by Lenzi et al. written account of a noteworthy relations between LC/LAC and ameliorations in all semenal indices in 56 infertile males with unexplained Oligo-Astheno-Spermia who treated by 2 g/day of LC and 1 g/day of L-acetyl carnitine (LAC) for a 180 days duration.26) An observational study of infertile men conducted by Haseen and his team research in (2017) approved that free LC seminal proportions were further down in hypo-fertile male than in control group, and a sturdy positive relations were noticed between semen plasma LC proportions and sperm number, activity, and normal morphology .(41)

A randomized trial conducted by Ramesh Babu and his team in (2004), described an increment with significant differences ($p< 0.05$) in the mean FSH and LH levels in all of non-fertile males studied when compared with the control arm of the study. Though, difference in the mean levels of testosterone in both arms was insignificant.(28) Reports as early as (1994) demonstrated by Zabul and followers when they established that gonadotropin (FSH and LH) levels were significantly higher in non-fertile men in comparism to the levels in controls with proved fertility. (29)

FSH is mandatory for initiation of production and evolution of spermatozoa. FSH is regarded as a reliable marker of damage of germinal epithelium, so it's found in higher concentration, in the sera of hypo-fertile men, and it was shown that this elevation is associated with azoospermia and severe oligozoospermia.(19)

Upswing of seminal fluid indices, after 3 months of 2gm daily dose of L-carnitine that has been noticed in our trial suggesting the boosted spermatogenesis and maturation of spermatozoa that seemed to be governed by the antioxidant power of L-carnitine, and by enhanced level of serum testosterone. While the induced spermatogenesis, and increased levels of serum testosterone and inhibin hormones may be the stand behind factors that resulted in reduction of LH and FSH hormones levels by its negative feedback effects.

Conflict of Interest - (nil – There are "No Conflict of Interest")**.**

Source of Funding - By both researchers **(self).**

Ethical Clearance: Committee members are approved to perform a study about "The impact of L-carnitine supplement on semen variables and the levels of sexual hormones (Serum LH, FSH, Testosterone, and Inhibin) in males with infertility" after discussion of study plan with researchers.

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Assessment of the Understanding of Medical Ethics Subject among Anbar Medical Group Students

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Abstract

Background: Future doctors and medical students are expected to learn about the important moral ethical communication with their patients and health care personnel.

Objectives: The study aimed to have opinion of medical students on medical ethics education.

Methodology: Cross sectional study carried out in both medicine and pharmacy colleges of at Anbar University for a period extended from 4th of November 2018 till the 28th of May 2019. The study was conducted on total sample size of 211 both males and females in medical and pharmacist students at Anbar University who age group ranging from 20-24 years old.

Results: About 86.3% of students under study believe that medical ethics subject is important in the curriculum of medical group colleges so as to learn basic knowledge of attitude (47.8%), and about 48% have the opinion of taking such subject in the first/second year of college. At the same time, 45.6% of students believe that the subject is boring and patient communication lecture was the highest percentage (47.9%) chosen by students as the most important item among the medical ethics subject.

Conclusion: Low perception and knowledge about the importance of medical ethics subject among medical group students in their near future career.

Keywords: *Understanding, Medical Ethics, Medical students*

Introduction

Huge social change is undergoing and people is aware of social determinants of health, so nowadays, the public has a negative view and low trust towards medical doctors because they believe that doctors are putting their own interests before those of their patients. These believe has resulted in reducing the compliance of the patient and increasing the communication conflict between doctor and his patient(1). For these reasons, doctors should be aware of these determinants that influence patients'; and a process of effective and good doctor-patient communication should be established to improve patient compliance and recall, adherent to treatment and even symptom reduction. In addition community participation, health services and social groups can share and built right communication between the two sides(2,3).

Healthcare professionals should understand ethical communication with their patient and begin to practice it in their career which includes, autonomy, justice, beneficence and non-maleficence(4). Hippocratic Oath and Helsinki declaration put these ethical philosophies to be practice in real medical life(5). Although in developing countries this item and matter it is still challenging, but future doctors should learn these moral ethical principles and such programme for medical professional ethics shouldn't be weak in such countries(6).

Incorporating ethical elements into the medical group education curriculums is an ongoing process in medical institutions and it gives an accreditation of medical schools(7-9).

Medical students as future healthcare professionals better to enhance their knowledge on professional practice and safeguarding ethics and their rights as well

as their patient's rights[10].

Assessment of the students is also required in medical ethics education, just like in other fields of medicine[10, 11]. Proper assessment of students should be performed in a way to ensure efficiency of ethics education and to help students recognize the humanitarian aspect of medicine and gain necessary skills to analyze ethical dilemmas in clinical practice[12].

Medical students in Iraq in the last three academic years in the college are in direct clinical practice experience with health care personal and with patients in both hospitals and primary health care centers, while in the last academic year, pharmacy students are in orientation and clinical practice with health care services and patients. The current study aims to assess the knowledge of medical and pharmacy students in Anbar University for medical ethics subject and practice.

Methodology

After taking the ethical approval from both medical and pharmacy colleges, informed consent and permission from students, this cross sectional study carried out in both college of medicine and pharmacy in Anbar University for a period extended from 4th of November 2018 till the 28th of May 2019. The study was conducted on total sample size of 211 both males and females in medical (4th,5th and 6th grades) and pharmacist (3rd, 4th, 5th grades) students at Anbar University who age group ranging from 20-24 years old. A non-response rate or incomplete information of 6% only.

All students in all grades included in the study have taken medical ethics subject in their curriculum "medical students at fourth, fifth, & sixth grade, while pharmacy students at third, fourth, & fifth grade".

The questionnaire include demographic characteristics of students (age, grade, type of study, and gender) in addition to questions related to the importance of medical ethics, about the year they prefer to take the subject, ethical attitude towards different aspects of the subject in college and hospital. Their relationship with medical staff in their future career. Questions about the intension to apply what they have learned in their career and the fields covered by the curriculum lectures in medical ethics were also included.

The questionnaire form has been distributed to students and was collected at the end of the day, some students have the time and opportunity to answer the questions immediately through closed and opened ended questions.

Data and variables were coded and entered in IBM SPSS Statistical Packages for Social Sciences version 25. Data were presented as frequency and percentages and descriptive results were done for each questionnaire in form of tables and charts.

Results

Table 1 describes the basic characteristic of study population of students. Male represent 43.6% in the study while female were 56.4% of the total sample size. 54.4% were medical students (fourth, fifth, & sixth grade) and 45.5% were pharmacy students (third, fourth, & fifth grade).

Table 1: The basic characteristic of study population (n=211)

		No	%
Gender	Male	92	43.6
	Female	119	56.4
College	Medicine	115	54.5
	Pharmacy	96	45.5
Grade	Third pharmacy	34	16.1
	Fourth pharmacy	38	18.0
	Fifth pharmacy	24	11.4
	Fourth medicine	46	21.8
	Fifth medicine	27	12.8
	Sixth medicine	42	19.9

Regarding medical ethics subject, table 2 shows that 86.3% of students agreed that medical ethics is important subject in medical and pharmacy colleges curriculum "and better to be taken at first/second years of study (47.9%)". 47.8% of students think that it is important as it provides basic knowledge to student's attitude while only 7.7% relates its importance as it is studied in all other universities and it provides students with guidelines (44.5%).

Table 2: The importance of medical ethics subject

		No	%
Think the subject is important	Yes	182	86.3
	No	29	13.7
Why it is important	Basic knowledge to attitude	87	47.8
	Important & studied by other universities	14	7.7
	Provide students with guidelines	81	44.5
At which year students prefer to take the subject	First/Second year	101	47.9
	Third year	64	30.3
	Fourth year/Higher	46	21.8

Results of the study revealed that 73.0% of the students like the subjects and finding it interesting (46.8%), easy (34.4%), good marks can be get in it (11.7%), taught in good aspect by teachers (2.6%), the subject focused on important ethical issues (1.3%), and finally help them to learn how deal with patients in good manner (3.2%). The rest (27.0%) who did not like the subject, they believe that the subject is boring (45.6%), has no clinical knowledge (35.1%), difficult to be understood (3.5%), and teacher couldn't deliver the subject properly (15.8%) (Table 3).

Table 3: Students like the subject or not and why.

		No	%
Do you like the subject	Yes	154	73.0
	No	57	27.0
Why do you like the subject	Interesting	72	46.8
	Easy	53	34.4
	Good marks can be get in it	18	11.7
	Taught in good aspect by teachers	4	2.6
	Focusing on important ethical issues	2	1.3
	Learn how to deal with patients in good manner	5	3.2
Why you did not like it	Boring	26	45.6
	No clinical knowledge	20	35.1
	Difficult to be understood	2	3.5
	Teachers could not deliver the subject properly	9	15.8

The intension of students was explored and showed that 38.9% would always apply what they have learned in their future career while only 8.5% reported that they rarely or never use it later on in practice life (Table 4).

Table 4: The intension of students to apply what they learned.

		No	%
Do you have the intension to apply what you have learned in your career	Always	82	38.9
	Not always	50	23.7
	May be	61	28.9
	Rarely	10	4.7
	Never	8	3.8

Regarding items provided by lectures, table 5 shows that patient communication item was among the highest percentage (47.9%) chosen by students, then confidentiality, respect patient, and relationship with other colleagues (27.0, 26.5 & 24.6% respectively) followed by euthanasia (16.1%).

Table 5: The opinion about the most important lecture in the subject.

		No	%
In students opinion, the most important lecture they took that will be of concern about in their career	Patient communication	101	47.9
	Confidentiality	57	27.0
	Respect patient	56	26.5
	Relationship with colleagues	52	24.6
	Euthanasia	34	16.1
	Medical report	16	7.6
	Anonymous	8	3.8
	Breaking Bad News	2	0.9

Table 6 describes how to deal with colleagues mistakes, 34.6% believed they should give him an advice after his mistake while 22.7% believe one should correct his mistake, tell other colleagues (14.2%), warn hospital management (11.4%) and even tell his patients about the mistake (10.9%).

Table 6: Dealing with colleagues' mistakes and deal with other health staff.

		No	%
How would you deal with your colleagues mistakes	Give him advice	73	34.6
	Try to correct the mistake	48	22.7
	Tell other colleagues about him	30	14.2
	Warn hospital management	24	11.4
	Tell his patient	23	10.9
	Do nothing	6	2.8
	Don't know	7	3.3

Discussion

In the recent years ministry of higher education in Iraq has been focusing on such subject to be enrolled in the curriculum of medical group colleges including medical, dentistry and pharmacy colleges because of its important for future doctors and pharmacist. In our study, although about (86.3%) of students agreed that the medical ethics is important subject in their curriculum and only (13.7%) of them consider it not that important. Also in our study (44.5%) of students think it gives students and future doctors guidelines for their career in medical practice while (45.6%) and (35.1%) they think medical ethics subject and practice are boring and lack of clinical knowledge respectively. These findings in our study are in agreement with the results reported by other studies that highlighted the presence of major important gaps in knowledge and attitude of doctors in recognizing autonomy, patients' right and ethical principles importance their profession[13-16]. Cruess *et al.* alerts the fact of medicine has lost the autonomy in addition to the influence throughout the world[17]. Although our study was mainly on students but it reflects their thinking and attitude towards medical ethics in the near future, as we found in the current study that only 38.9% of students will "always" apply what they have learned in medical college about medical ethics and commitments.

Saeed & Al-Jawadi (2017) in their article identify that 50% of participant in study was not sure about physician's commitment towards their patients and that there physicians are focusing mainly on clinical practice more than ethical practice [18]. In our study, about (48%) chose first/second grade in medical group colleges to give medical ethics subject and (30.3%) chose third year medical group colleges to give such subject. In a study done by Sadaf *et al*. suggested that it is important to give these components of medical ethics to third year medical students so as to build trust and respect relationships with patients[19]. In this study, (4.1%) of students claim that the teacher couldn't deliver medical ethics subject to students, this make us to think to change learning activities approach or tools such as role play exercises, programmers and interactive seminars[20].

In conclusion, low perception and knowledge about the importance of medical ethics subject among medical group students to their near future career. Still medical students thinking towards clinical practice side and view more than ethical side and this give us alarming sign to change learning tools used in teaching such essential subject in their medical curriculum. Patient communication was among the highest percentage chosen by students concern among other different lecture items. More efforts should be done by the college, department and lecturers to give rise to new teaching approach to this subject and to decide on which grade it should be given to medical group students.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Isolation and Identification of Facultative Anaerobic Bacteria from Feces of Pet Dogs

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Abstract

A fifty fecal swabs (25 from Anbar province and 25 from Salahuddin province) were collected from both sexes dogs with different global breeds, different ages. These swabs were cultured onto blood , nutrient and MacConkey agars, then the colonies were purified on nutrient and MacConkey agars. A gram stain and biochemical tests were done including catalase, oxidase and TSI. The results of isolation and identification showed that *Salmonella* spp. were isolated from 5 out of 14 puppies (35.7%), while *E. coli* from 9 (64.3%) and *Serratia* spp. from 1(7.1%), whereas, from adults , *salmonella* spp. isolated from 9 (25%) dogs and *E. coli* from 24 (66.7%) out of 36 adult dogs.

It is concluded that *Salmonella* spp. and *E. coli* are the most prevalent in asymptomatic dogs and puppies in Anbar province and Salahuddin which means that the dogs are a reservoir for these bacteria and it is considered as a bad indication for transmission of this pathogen to human being and other animals.

Key words: Facultative anaerobic, dog, E. coli, Salmonella, puppies.

Introduction

Dogs are the pet animals that have been kept from 14 centuries, numerous studies have shown that pets play an important role in human life, and the infected pets threaten the lives of individuals, especially those immunocompromised, it has been found that pet dogs that live in contact with human have a significant role in transmitting diseases to their own [1 ; 2].

Dogs transmit viral and bacterial diseases to humans being through saliva, contaminated feces and urine or via direct contact, and the most important bacteria that transmitted by dogs are *Salmonella spp*., *Yersinia spp.*, *Capnocytophaga spp*., *Coxiella burnetii*, *Staphylococcus intermedius* and Leptospira spp. , in addition to MRSA [3]. It has been documented that dogs are potential reservoirs of pathogenic *Salmonella* spp. and *E. coli* [4]. The most common sources of *Salmonella* spp. and *E. coli* in dogs are from chicken and meat that undercooked [5].

The subclinical carrier cases in dogs are regarded as the common form of salmonellosis seen in dogs which shed these organisms in feces without showing clinical signs and contaminate the surrounded environments [6].

It has been reported that the prevalence of dogs which carry *E. coli* is 26.9% [7], whereas [8] reported that the *Salmonella* prevalence in dogs varies greatly between 5% to 70%.

The most prevalent bacteria isolated from dogs were Salmonella [9; 10, 11, 12], E. coli [13, 14, 15; 16].

This study was aimed to isolate the facultative anaerobic bacteria from canine feces.

Materials and method

Animals

From March 2019 to the end of April 2019, A fifty dogs (25 dogs from Anbar province and 25 from Salahuddin province) from different global breed and both sexes at 2 months-6 years of age were used in this study.

Examination of animals

All animals were examined clinically for

temperature, pulse and respiratory rates and for presence of any abnormal clinical signs.

Collection of the Samples:

A fifty fecal swabs with transport media were collected and transported anaerobically by special containers to the college of veterinary medicine, universities of Fallujah and Tikrit lab's for bacterial isolation and identification.

Bacteriological examination:

The isolation and identification of bacteria from samples were done according to [17], all swabs were cultured on nutrient , blood and MacConkey agars and incubated for 24-48 hours. at 37℃. in anaerobic jar with 10% CO_2. The colonies were subjected to Gram stain, then suspected isolates subcultured on MacConkey, EMB and nutrient agars for purification and characterization of the colonies. Catalase, oxidase and other biochemical tests includes TSI, Indole and citrate were done for identification of the isolated bacteria.

Results

All animals that were clinically examined showed normal signs and parameters and appeared to be healthy.

1. **Bacteriological isolation:**

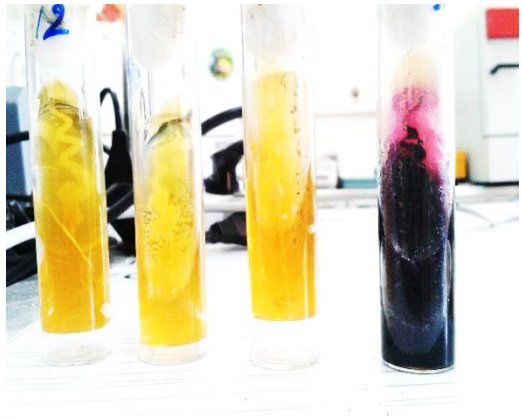
In the present study, culturing of faecal samples from 50 dogs and puppies revealed a presence of some gram negative bacteria including *E. coli*, *Salmonella* spp. and *Serratia* spp.

Cultural and biochemical characteristics of different species of bacteria isolated from faecal samples of affected dogs are depicted in figure 1.

a) *Salmonella spp*. on SS agar

b) *E. coli* on EMB agar

c) Indole for *E. coli*



d) TSI, *Salmonella* spp. on right and *E. coli* on left

Figure 1. Different cultural appearance and biochemical tests

2. **Rate of infection**

Out of 14 puppies, only 5 (35.7%) had a subclinical infection with *salmonella* , 9 (64.3%) with *E. coli* and 1(7.1%) with *Serratia* spp., while the adults revealed 9(25%) dogs with *salmonella* spp. and 24 (66.7%) with *E. coli*, while there was not infection with *Serratia* spp. (Table 1).

Table (1) Rates of subclinical infection with *Salmonella* spp., *E. coli* and *Serratia* spp. of puppies and adults.

Animals	No. of animals	Animals positive For Salmonella spp.	%	Animals positive For E. coli	%	Animals positive For Serratia spp.	%	Animals negative for culturing	%
Puppies	14	5	35.7	9	64.3	1	7.1	0	0
Adults	36	9	25	24	66.7	0	0	2	5.6
Total	50	14	28	33	66	1	2	2	4

Discussion

Many studies were done to estimate the incidence of *Salmonella* spp. in puppies and dogs, Carter and Quinn [(18)] and Sanchez *et al*. [(6)] found that the percentages of *Salmonella* in clinically appeared healthy dogs was ranged between 0- 44 % , this results were in agreement with our results.

At the opposite side, the current results were disagree with many researches, the study of Shimi *et al*. [(19)] in Iran was performed to detect *Salmonella* in feces of 181 dogs, they found that 28 (15.5 %) were carry the *Salmonella*. Also, Kocabiyik *et al*. [(20)] found that dogs positive for *Salmonella* were 11 % in Turkey, also , Fukushima *et al*. [(21)] examined stray dogs that appeared healthy and found that the percentages of carrying *Salmonella* was 5.9 % in Japan. While the percentages of *Salmonella* in feces of stray dogs found in Taiwan were 6.3 % [(22)].

The reason for presence a higher percentages of *Salmonella* as observed in our study may be due to the potentially higher exposure of dogs to this genus of bacteria.

The results of the current study are compatible with the results of Ojo *et al*. [(7)] who showed that the percentage of *E. coli* in apparently healthy dogs was 26.9% , while the presented results were disagree with Beutin ([23]) who examined the healthy dogs for presence of *E. coli* and they found that the percentages of this bacteria ranged from 3.2 to 12.3%. Also, it has been reported that the percentages of *E. coli* was 1.1% in healthy dogs [(24)], while in Japan was 0.16% [(25)].

Serratia spp. and *Escherichia coli* were also isolated from bacteriological culture of fecal swabs obtained from dogs [(26;27)].

Hancock *et al*. [(28)] reported that the higher percentages of *E. coli* and other bacteria is due to younger age susceptibility, transport, sudden alteration in feed and using of antimicrobial therapy which may lead to disorder in microflora of intestine.

Conclusion

These results refer to that large percent of dogs that appear clinically healthy may carry *E. coli*, *Salmonella* spp. or other bacteria and may be act as a source for infection to human being and other animals , and this is considered as a bad indication for transmission of these pathogens to human , and the dogs act as a good reservoir.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Molecular characterization of Eae and Stx_s genes for *E. coli* O157:H7 isolates from Calves

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Abstract

This study aimed to detect eae and Stx genes of thirty two *E. coli* O157:H7 isolates recovered from calves. These isolates were isolated and identify by traditional methods of culturing and latex agglutination test from fecal swabs of four diarrheic calves and twenty eight apparently healthy calves. The eae and Stx genes were detected by a realtime PCR applied on the extracted bacterial DNA. The results showed that 19 isolates had stx1 gene, 10 isolates had stx2 gene and 17 isolates had eae gene. It is concluded that molecular characterization by using real time PCR is a good test for confirming infection with *E. coli* O157:H7 and detection of their eae and Stx genes .

Keywords: *Real time, PCR, stx, eae, Calves, E. coli O157:H7.*

Introduction

Escherichia coli regarded as one of the main cause of calf diarrhea in newborn livestock, by causing high economic losses, the prevalence of enterohemorrhagic *E.coli* (ETEC) in calves with diarrhea ranged between 70 – 75.6 % [(1)]. It has been stated that cattle may act as reservoir for *EHEC* [(2)]. Also, Osman *et al*.[(3)] reported a prevalence of O157 serotype in 63.6% of their studied calves.

Escherichia coli strain O157:H7 is one of the most significant bacteria that convey by the food, it mostly cause a series of clinical signs , such as diarrhea, haemorrhagic colitis and hazardous problematics in other body organs or systems. It have many virulence factors, most significant one was *Stx*s [(4)], therefore, the strain nomenclatured as shigatoxic *E.coli* (STEC) [(5)].

It infects adult cattle even the calves [(6)]; human being [(7)]; ovine [(8)]; canine [(9- 11)] and other animals , and may cause fatal disease in human being and other animals,

The most significant confirmatory tests used for detection of ETEC and STEC are PCR [(12)] or realtime PCR (RT- PCR) [(13)] which is considered as an appropriate, dependable way for confirmatory diagnosis of *E. coli* O157:H7 [(9)].

The aim of the presented study was to corroborate the *eae* and *stx* genes of virulent *E. coli* O157:H7 isolated from calves by using realtime PCR.

Material and Method

Source of bacteria : A number of 32 isolates of virulent *E. coli* O157:H7 were used in this study and obtained from college of veterinary medicine / University of Fallujah and were confirmed previously by [(3)].

Bacteriological Method: All 32 isolates were identified as shown in a previous study [(14)] prior to the PCR test to confirm the presence of Eae, Stx1 and Stx2 genes.

Serotyping was made as mentioned in [(14)] and [(15)].

PCR assay:

This test was performed on the thirty two virulent *E. coli* O157:H7 strains, a bacterial DNA extraction was done by using Geneaid kit, USA. The assay of RT- PCR was done via using Applied Biosystems™ RapidFinder™ STEC Screening Assay in Sacace Real-Time PCR System. A twenty eight micron of water free nuclease was added to two micron of DNA. The conditions of RT- PCR were adjusted as follows: hold at

95 °C for 2 min., then denature at 95°C for 1 second and lastly, anneal/ extend at 60 °C for 20 seconds, for 40 cycles.

Results and Discussion

The RT- PCR assay of the examined virulent isolates exposed that nineteen isolates had stx1 gene and 10 isolates had stx2 gene , while 17 isolates had eae gene and this is compatible with previous results of Yousif and Hussein, 2015 who found the same results by using conventional PCR on the same isolates (Fig. 1).

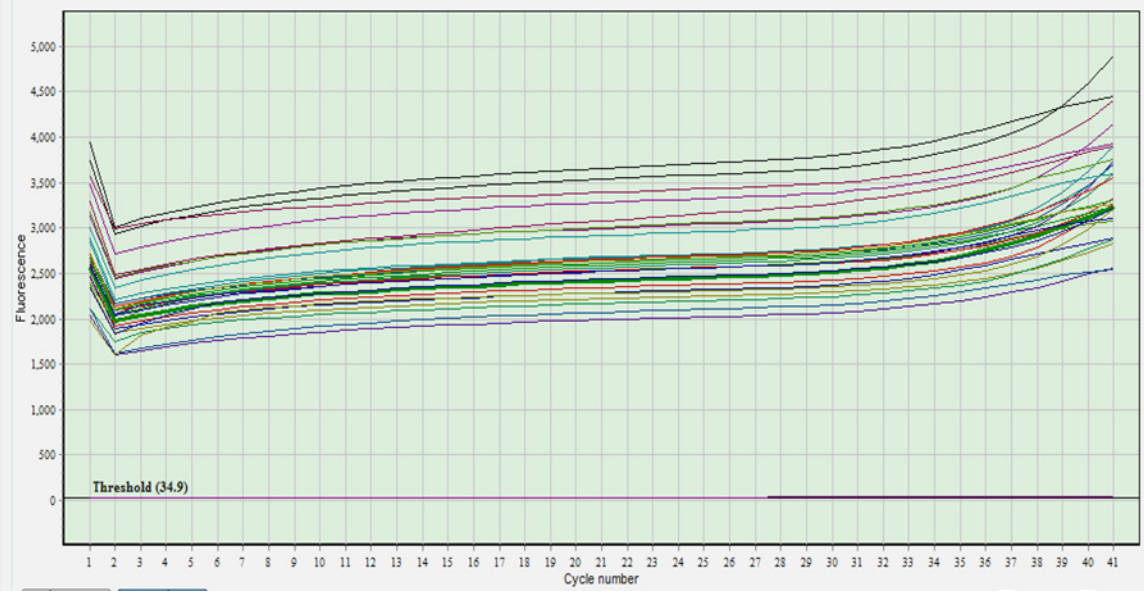

Figure (1) Results of RT-PCR, positive samples were Cycle Threshold (CT) less than 35

It has been reported that RT-PCR is a screening and effective method for detecting shigatoxigenic *E. coli* from fecal samples [16].

The current results were in compatible with results of Rebekka *et al*. [17] who reported that real-time PCR has a high sensitivity and accuracy for recognition of EHEC and they mentioned that RT- PCR is a quick method for detection presence or absence of EHEC.

RT- PCR method described as a "golden test" for identification of EHEC [18]. Other studies reported similar results, where Abbasi et al. [19] reported that the RT- PCR conventional form is the quicker and more accurate assay and it doesn't need any other ways for confirming the diagnosis when it used to detect the incidence of virulence factors of E. coli isolated from diarrheic children, as well as , Mondani *et al*. [20] used RT-PCR for detect STEC, they found that this test have many advantages from these: reduction the time and reducing the limitations which found in alternative traditional tests.

Also , a study done to estimate the efficacy of RT-PCR test in testing the presence or absence the virulence genes rfbO157*, Stx1*,*Stx2* from pediatric stool samples, out of fifty nine samples that positive for PCR, a twenty nine samples were +ve for *Stx* and they concluded that this test is quick way for diagnosis STEC in pediatric [21].

Conclusions

The Real time PCR test is an exact method for specific recognition of EHEC O157:H7 and their eae and Stxs genes.

Ethical Clearances: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Estimation of Serum Cystatin-C as early marker of Kidney Dysfunction in correlation with Serum Ferritin among β-Thalassemia Major Patients

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Abstract

The Cystatin C is early predictor for evaluation the kidney functions. The Serum Cystatin C is a cysteine proteinase inhibitor, that the nucleated cells produce it at stable rate. It is filtered through the glomerular filtration membranes of kidneys, and the filtration rate will be unaffected by external factors. A group of recessively inherited hemoglobin disorders called Major Beta-thalassemia and considered the most common genetic disorder all over the world, which could be noticed by the reduction in of β-globin chain synthesis.

Objective: To study Serum Cystatin-C estimation as early marker of renal function in relation with other parameters in beta thalassemia patients.

Materials and Method: A case-control study was executed on thalassemia patients between February and April 2019 at Al-Zahra'a Teaching Hospital in Najaf/Iraq.

Results: the results in this study showed there are significant differences ($p \leq 0.05$) between major beta thalassemia patients and control group in regarding to hematological parameters. The result of biochemical parameters showed there are variations between patients and control where there were significant statistical increases in our thalassemic patients comparing to the control about Cystatin-C (1.27±0.33and 0.7±0.24 ng\ ml respectively).

Conclusion: the cystatin –C is useful marker at major beta thalassemia patients that suffering from renal dysfunction.

Keyword: *cystatin –C, Ferritin, liver enzymes.*

Introduction

A group of recessively inherited hemoglobin disorders called Major Beta-thalassemia and considered the most common genetic disorder all over the world, which could be noticed by the reduction in of β-globin chain synthesis [(1)]. Most of the types come from severe anemia that requires even transfusion of the blood. For fifth decades of life, the grouping of chelation treatment and transfusion has lengthened the life of thalassemia cases [(2,3)]. The totally insufficient production of hemoglobin Hb A (α2β2) in human adult is the Thalassemia disease, which is produced from the absence or decrease in the synthesis of alpha α- or beta β- globin chain and thalassemia is categorized into α and β thalassemia based on those chain disorders. Important medical indications of these patients are severe anemia, hemolysis, and ineffective erythropoiesis [(4,5)]. The autosomal receding genetic anemia is Beta-thalassemia major (β-TM) in which there is a lack in creating of 1 globulin chain resulting hemoglobin (Hb) molecule. Generally, in the first year of life, blood transfusion is needed for patients of beta thalassemia [(6)]. For instance, renal difficulties are one of the new unrecognized difficulties appeared with the progresses in chelating

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and treatment agents of β-TM patients [7]. Numerous researches proved that common difficulties among β-TM patients are low urine osmolarity, aminoaciduria, proteinuria, dysfunction, and proximal tubular [8,9]. All human nucleated cells can secrete and synthesize the Cystatin-C, which is a low-molecular-weight non-glycosylated protein, and havs the ability to inhibit cysteine protease [10]. Cystatin-C is not reabsorbed back into the serum or secreted by the renal tubules due to its sensitivity as a biomarker for glomerular filtration rate (GFR). It is better than creatinine clearance in the renal function impairment diagnosis, because muscle mass, diet, sex, and height could not affect it [11]. Cystatin-C is a cysteine protease inhibitor that is secreted into the blood after it is synthesized from all human cells. In comparing to the creatinine clearance, the benefit of measuring Cystatin-C is that height, sex, diet, and muscle mass could not affect it [12].

Materials and Method

A case-control study conducted between February to April 2019 in the Thalassemia Center at the Teaching Hospital of Al-Zahra'a in Najaf/Iraq. A group of 40 β-thalassemia major intermedia patients (10 females and 30 males) have been enrolled in this research, as well as 40 healthy control, ranged between 4 and 20 years old. Both control and patients were matched in gender and age. 5ml of blood was taken from beta-thalassemia patients as well as from healthy control *for biochemical tests.*

The patients are employed for measuring of the following parameters:

1. *Determination of Human CST3 (Cystatin C) concentration in serum by using ELISA kit performed by (Abcam, USA).*

2. *Determination of Creatinine concentration in serum* using *automated chemistry instrument from LANDWIND Company.*

3. *Determination of urea concentration in serum using automated chemistry instrument from LANDWIND Company.*

4. *Determination of ALP concentration in serum using automated chemistry instrument from LANDWIND Company.*

5. *Determination of AST concentration in serum using automated chemistry instrument from LANDWIND Company.*

6. *Determination of ALT concentration in serum using automated chemistry instrument from LANDWIND Company.*

7. *Determination of hematological parameters (complete blood count) by using automated hematological analyzer instrument from MINDRY Company.*

Statistical Analysis

Statistical Package for Social Sciences (SPSS) program version 24 has been used for data analysis. T-independent test was used to find the variances between patients and control, the results were expressed as (Mean ± SD). Pearson correlation coefficient (r) were calculated to evaluate the correlation between parameters. A p-value of (<0.05) has been considered significant and (<0.001) has been considered highly significant.

Results and Discussion

Hematological Parameters of Study Groups

Our study included 40 beta thalassemia major patients their age ranging between 4-20 years (30 males and 10 females), and the control group included 40 healthy participants their ages and sex mated with patients group. There were statistically highly significant differences between beta thalassemia major patients and control as regarded with hematological parameters (CBC) included in this study, sever anemic presentation were seen in patients indicated by the levels of hemoglobin (7.7 ± 1.2) (13.8 ± 1.1) and RBCs count (3.83 ± 1.01) (5.22 ± 1.4) in comparison with the control. The hypochromic microcytosis observable in patients than control with MCH and MCV values (17.2 ± 2 fl) and (62.3 ± 6 pg) in patients compared to (30.7± 2.6 fl) and (84.7± 5.4 pg) in controls, with p-value 0.001 for both. In addition, a remarkable significantly increases in platelets count reported in patients (378 ± 80.3) as compared with control (246 ± 82.7) with p-value 0.001. Furthermore, a highly important leukocytosis also realized in patients than control (11.12 ± 1.18) and (7.04 ± 1.8) respectively (table 1).

Table 1. Hematological parameters of study groups

Parameters	Patients (n=40)	Control (n=40)	P-value
	Means ± SD	Means ± SD	
Hb g\dl	7.7± 1.2	13.8±1.1	<0.001**
MCV(fl)	17.2±2	30.7±2.6	<0.001**
MCH (pg)	62.3± 6	84.7±5.4	<0.001**
RBCX 1012/L	3.83±1.01	5.22±1.4	<0.001**
WBCX 109/L	11.12±1.18	7.04±1.8	<0.001**
PLT 109\ L	378±80.3	246±82.7	<0.001**

**: highly significant

The beta-thalassemic patients enrolled in the present study showed a significantly changed specially with red blood cell mass (RBC count and Hb) in association with RBCs indices (MCV and MCH) attendant to a significantly observable microcytosis, the plain anemic appearances have been seen in the patients where hemoglobin levels as comparing with the controls. The RBC related differences are also related to an important secondary leukocytosis and thrombocytosis, these results agrees with Hagag et al. [13], and Ayyash & Sirdah [14]. Arshad et al. [15], described that thalassemic patients have different problems related with less Hb level, because of the reduction of erythrocyte numbers and lessened values of RBC indices (MCV, MCH, MCHC, HCT). So, those patients undergo anemia resultant in less oxygen content in blood. The study carried out by Shanthi et al. [16], concluded that the decreasing in the RBCs count, PCV and levels of Hb, that detected in beta thalassemia patients are because of continuous breakdown of erythrocyte and increased early degradation, as the presence of abnormal globin molecule leads to erythrocytes ruptured before maturation. Regarding to leukocytosis and thrombocytosis in our thalassemia patients due to the severe anemia that complemented by hyper cellular (thrombocytosis and leukocytosis) that causing encouragement of erythropoietin hormone which acts on bone marrow to increase proliferation of blood cells, or resulting from the activation of immune system by receiving blood from various donors [17].

Biochemical Parameters of Study Groups

The result of biochemical parameters showed a variation in the serum level (Cystatin-C, ferritin, creatinine, urea and liver enzymes includes AST, ALT and ALP) in beta thalassemia major patients as compare with control. When compared to control, there were statistically significantly increase in the our thalassemic patients regarding Cystatin-C (mean=1.27±0.33and 0.7±0.24 ng\ml respectively), Ferritin (mean=3480± 232 and 90.6± 43.5ng\ml respectively), Creatinin (mean=1.02±0.4 and 0.5±0.16 mg\dl respectively), Urea (mean=25.9±4.8 and 17.4±3.9mg\dl respectively),AST(mean=67.47±26.3and 23.14±8.1U\L respectively), ALT (mean= 75.79± 28.6 and 21.49±8.81 U\L respectively), and ALP (mean=289.78±113.43 and 97±52.12 U\L respectively) table (2). There was statistically positive correlation between creatinine and cystatin (fig.1) (r=0.569) and urea (fig.2) (r=0.137), the result agrees with Behairy et al. [6]. In accordance with our results, Elbedewy et al., concluded that beta thalassemia major patients suffer from glomerular and tubular dysfunction due to poor chelation therapy and inadequate transfusion [12].

Table 2: Biochemical parameters of study groups.

Parameters	Patients (n=40)	Control (n=40)	P-value
	Means ± SD	Means ± SD	
Cystatin C (ng/mL)	1.27±0.33	0.7±0.24	<0.001**
Ferritin ng\ml	3480± 232	90.6± 43.5	<0.001**
Creatinine (mg/dl)	1.02±0.4	0.5±0.16	<0.001**
Urea (mg/dl)	25.9±4.8	17.4±3.9	<0.001**
AST U/L	67.47±26.3	23.14±8.1	<0.001**
ALT U/L	75.79±28.6	21.49±8.81	<0.001**
ALP U/L	289.78±113.43	97±52.12	<0.001**

**: highly significant

The DFO, iron overload, chronic hypoxia, and long-standing anemia implicated in the mechanisms of tubulopathy in patients withβ-thalassemia major [6]. In the current study, there were highly significance increases in the levels of urea, creatinine, ferritin, cystatin shown by thalassemic patients. These results were in agreement with Ali and Mohmoud, as they reported considerably higher levels of serum ferritin and serum creatinine in thalassemic group than controls [18]. Serum Cystatin-C is supposed to be a more potent endogenous marker of GFR than creatinine as it is believed to be produced at a constant level by all nucleated cells, generously filtered by the glomeruli, minimally bound to proteins, and completely reabsorbed and metabolized in the proximal tubule [12]. Our result show increase in serum level cystatin when compared with control. Some study found a positive association between serum ferritin and serum cystatin Papassotiriou et al. [19]. Serum ferritin in our result at high levels this indicate to iron overload and many studies concluded that cirrhosis of liver is associated with increase in serum ferritin levels [20], this accordance with the result of our study that show highly significant increases in the liver enzymes (AST, ALT and ALP) when compared with control group.

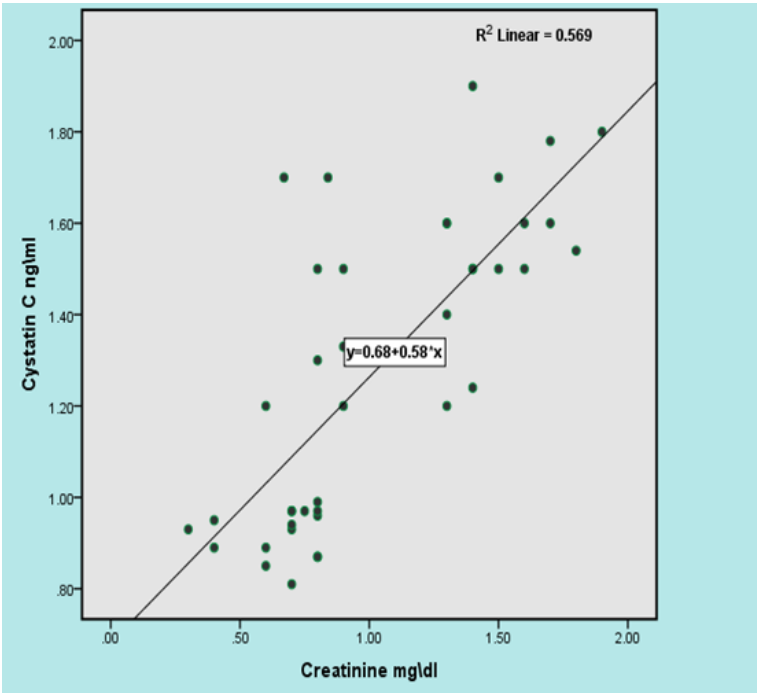

Figure 1: Correlation between Cystatin-C and creatinine in beta thalassemia patients.

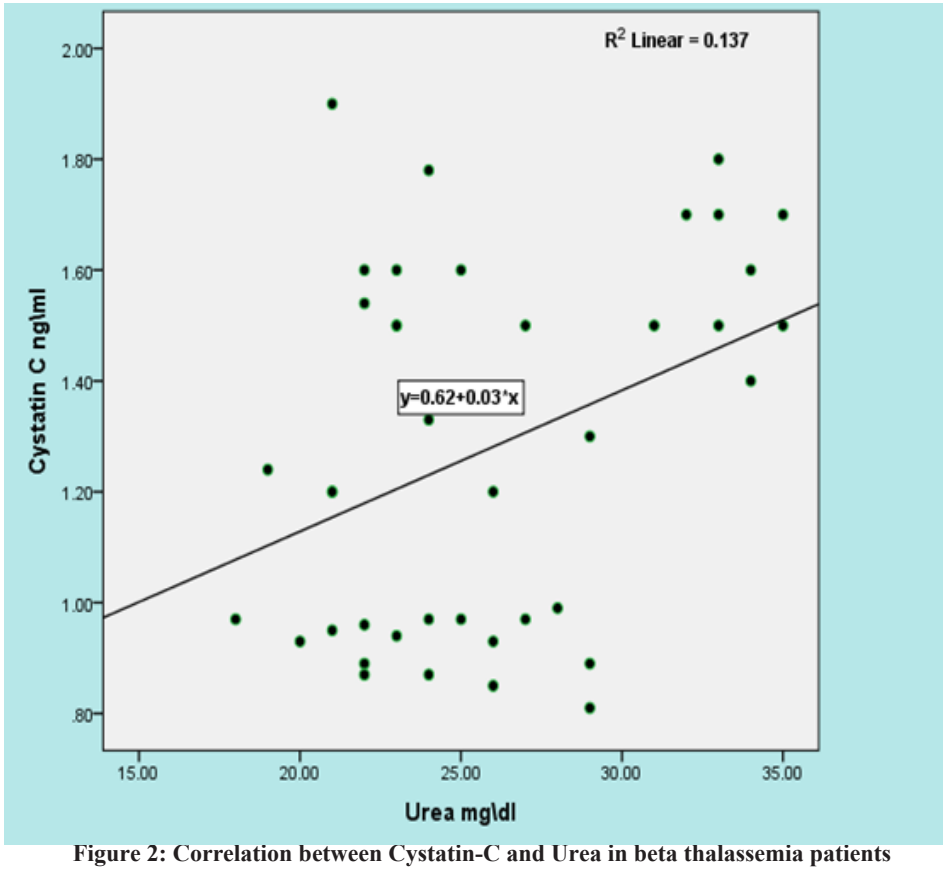

Figure 2: Correlation between Cystatin-C and Urea in beta thalassemia patients

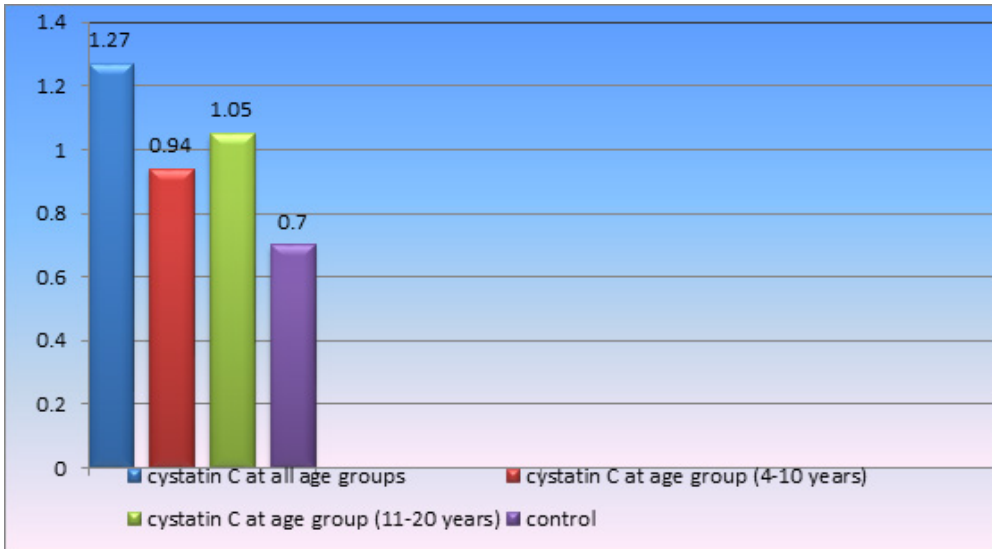

Figure 3: The Cystatin –C concentration distribution at age groups.

Conclusion

In this paper, we concluded that Cystatin C could be used as early predictor for major beta-thalassemia patients, which can be developed for renal dysfunction in the future.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Comparative Assessment of Patients' Satisfaction in Secondary and Tertiary Outpatients of Marjan Medical City in Babil Across Sectional Study

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Abstract

Introduction: Patient satisfaction is a basic measure by it the value of health care facilities is assessed. It a personal assessment of the service received and the person's prospects. This study would have significant input in assessing the level of patients'satisfaction in secondary and tertiary outpatient health care services. **Methods:** Across-sectional study of 300 patients random sample visit outpatients of Marjan medical city the interview was conducted at general and specialist outpatient only. The specialist outpatients include cardiac, gastrology, nephrology and diabetology outpatient. 150patients from secondary outpatient &150patients from tertiary outpatient. The purpose of study was to assessing the level of patients'satisfaction in secondary and tertiary outpatient health care services. **Results**: Majority of patients visit general and specialist outpatients with age31-50(45.5%,46%), female(57.3%,62.75), low income patients <500000ID (61%,53%), majority of patients visit general outpatients are rural and primary education (50.7%,49.3%) and visit specialist outpatients(54%,42%), most patients came for follow-up for both types of outpatient(56%,62%), and seen by consultants itself for both types of outpatients(78.7%,88%), majority referred by his family medicine(62%,48%), and came from far distance > 5 km(77%,79%), the majority of patients were not satisfied with health services produced to them(53%,63%) **Discussion:** showed no. of patients not satisfied health services more than patients satisfied, majority of patients with low income and just primary level of education, the majority of patients came to outpatients from rural area these data for both secondary and tertiary outpatient. Patients with bad satisfaction are18-50years, self-referral to tertiary outpatient, not satisfaction also in urban patients.

Keywords: *patient's satisfaction. Outpatients. health services. Marjan medical city*

Introduction

Patient satisfaction is a basic measure by it the rate of health maintenance facilities is assessed1,2. It well-defined as a personal assessment of the facility received contrary to the person's prospects3. Patients' decision of hospital facility class and their opinion are important in class of care checking and development4,5 Patient satisfaction information are usually saved and recycled for incessant quality development by health-care organizations and hospitals in developing states6,7 Patient satisfaction is dignified over a widespread of health facility scopes, including obtainability, accessibility and suitability of facilities, interpersonal skills, procedural ability of the workers, and the physical background where services are provided3,8,9 Patient opinions of quality are a lot subjective by their communication with the health worker; the care with which the suppliers observe and connect with them10. It has been presented that when patients are glad with the quality of facilities gotten from a hospital they agree medical recommending by taking their medications suitably and continue friendly relationship with the hospital workers11, Patient satisfaction studies in developing republics are progressively, being stimulated as a means of accepting wellbeing care quality. Asking patients what they reflect about the care and management they have expected is a vital step towards progressing the class of care, and confirm the health facilities are gathering patients' requirements12, Assessing patient satisfaction has become an essential part of hospital/ clinic management approaches crossways the world. Also, the quality guarantee and certification progression in most countries need that the satisfaction of patients

is measured on a systematic root13.The knowledge of satisfaction may also be related to pleasure, prosperity and class of a lifetime. Henceforth, satisfaction tends to reflect the quality of health facilities transported14.This study would assessing the level of patients'satisfaction in secondary and tertiary outpatient health care services, classify the elements affecting the patients 'satisfaction, and afford a recommendation on an enhanced health service supply that will be helpful to fill research awareness holes which eventually contribute to improve quality of patient services in the hospital and develop the level of patients' satisfaction.

Method

Study participants:

Across-sectional study of 300 patients random sample visit outpatients of Marjan medical city from January 2019 to March 2019, the interview was conducted at general and specialist outpatient only (for adult 18 years and above). The specialist outpatients include cardiac, gastrology, nephrology and diabetology outpatient. We take 150 patients from general outpatient and 150 patients from a specialist, comparison assessment about the satisfaction of patients for health services. The sociodemographic data include gender, the age of patients, income, place of living, education level if it is first to visit or follow up, see by a consultant or not, referral system and finally distance of the hospital. Satisfaction assessing by modify questionnaire (patients satisfaction questionnaire PQ1815,16 consist of 3 points Likert scale give agree=2, I don't know=1 and disagree=0. 27 and above mean patient satisfied on health services, the time of interview after taking permission from the patient was 15 minutes, The team managing the questionnaire contained an MBBS student posted in the Department of family and community medicine, was educated for the determination. Sample size thus produced was of nearly 300 patients. Statistical analysis done by SPSS 23 use chi-square and Fischer test for categorical variable and put p value < 0.05 mean significant.

Results

Among the 300 patients questioned during the data gathering, all patients included in the statistical and no missing data. Majority of patients visit general and specialist outpatients with age 31-50(45.5%,46%), females visit generally and specialist outpatients more than male(57.3%,62.75), low income patients <500000ID more than other patients visiting general and specialist outpatient(61%,53%), majority of patients visit general outpatients are rural and primary education (50.7%,49.3%)andvisitspecialistoutpatients(54%,42%), most patients came for follow-up for both types of outpatient(56%,62%), most patients see by consultants itself for both types of outpatients(78.7%,88%), majority of patients visit general and specialist outpatients refer by his family medicine(62%,48%), most of the patient came to general and specialist outpatients from far distance > 5 km(77%,79%), the majority of patients visit generally and specialist outpatients are not satisfied with health services produced to them (53%,63%), All showed in table 1, fig 1 and 2.

Table 1: frequency and percentage of study variable.

variable	General outpatient	%	Specialist outpatient	%
Age 18-30	33	22.0	28	18.7
31-50	68	45.3	69	46.0
above 50	49	32.7	53	35.3
Gender male	64	42.7	56	37.3
female	86	57.3	94	62.7
Income <500,000 ID	92	61.3	80	53.3
500000-1000000	47	31.3	57	38.0
above 1000000	11	7.3	13	8.7
Place rural	76	50.7	81	54.0
urban	74	49.3	69	46.0
Education primary	74	49.3	63	42.0
secondary	47	31.3	37	24.7
graduate	29	19.3	50	33.3
Visit first	66	44.0	57	38.0
follow up	84	56.0	93	62.0

Cont... **Table 1: frequency and percentage of study variable.**

Consultant yes	118	78.7	132	88.0
no	32	21.3	18	12.0
Referral self	51	34.0	67	44.7
family medicine	93	62.0	72	48.0
other	6	4.0	11	7.3
Distance near 5 km	34	22.7	31	20.7
far 5 km	116	77.3	119	79.3
Satisfaction no	79	52.7	95	63.3
yes	71	47.3	55	36.7

Comparison between satisfaction of patients according to studied variable in general and specialist outpatient show in table 2, according to the age there is positive correlation and significant association with age group 31-50y who are not satisfied, while in general outpatient age undependable, in place of living there is significant association between place and nonsatisfaction in general outpatient with negative correlation, also the level of education significant associated with nonsatisfaction in both types of outpatients with negative correlation, significant depending between nonsatisfaction and patients visit for follow up in specialist outpatient with positive correlation, and finally there is a significant association between referral variable and patients satisfaction with positive correlation. All other variables have not significant (undependable) association with patients satisfaction.

Table 2: comparison association and correlation between patients satisfaction and study variables

variable	**General outpatient**		**p-value**	**R***		**Specialist outpatient**	**p-value**	**R***
	***Sat.**	**No sat.**			**Sat.**	**No sat.**		
Age 18-30	16 10.7%	17 11.3%			3 2%	25 16.7%		
31-50	32 21.3%	36 24 %	0.98	N.S.	18 12%	51 34%	0.001	0.42
above 50	23 15.3%	26 17.3%			34 22.7%	19 12.7%		
Gender male	28 18.7%	36 24%	0.51	N.S.	20 13.3%	28 18.7%	1.000	N.S.
female	43 28.7%	43 28.7%			35 23.3%	43 28.7%		
Income <500000	47 31.3%	45 30%			25 16.7%	55 36.7%		
500000-1000000	19 12.7%	28 18.7%	0.48	N.S.	24 16%	33 22%	0.32	N.S.
above 1000000	5 3.3%	6 4%			6 4%	7 4.7%		
Place rural	43 28.7%	33 22%	0.02	-0.81	27 18%	54 36%	0.39	N.S.
urban	28 18.7%	46 30.7%			28 18.7%	41 27.3%		
Education								
primary	46 30.7%	28 18.7%			30 20%	33 22%		
secondary	18 12%	29 19.3%	0.001	-0.3	13 8.7%	24 16%	0.034	-0.21
graduate	7 4.7%	22 14.7%			12 8%	38 25.3%		
Visit first	26 17.3%	40 26.7%			13 8.7%	44 29.3%		
Follow up	45 30%	39 26%	0.1	N.S.	42 28%	51 34%	0.009	0.22
Consultant yes	57 38%	61 40.7%	0.6	N.S.	52 34.7%	80 53.3%	0.071	N.S.
no	14 9.3%	18 12%			3 2%	15 10%		
Referral self	20 13.3%	31 20.7%			10 6.7%	57 38%		
family medicine	47 31.3%	46 30.7%	0.26	N.S.	45 30%	27 18%	0.001	0.23
other	4 2.7%	2 1.3%			0 0%	11 7.3%		
Distance near 5 km	16 10.7%	18 12%	1.00	N.S.	15 10%	16 10.7%	0.14	N.S.
far 5 km	55 36.7%	61 40.7%			40 26.7%	79 52.7%		

***P value < 0.05 is significant. * R : correlation.**

Discussion

Patient satisfaction organizes an important element of the feature of health care facilities. . Since health care facilities cannot be 'kept for upcoming use' and they are predisposed by many issues, it is solid to quantity the class of service. The attendance of many powerful factors such as situations related to patients, medical staff, and organization complicate measurement of the class of the facility. This study was done to measure the satisfaction of patients with the health services produced in the secondary and tertiary outpatients in Marjan medical city, so in study showed that most patients not satisfied to the health services produced in secondary and tertiary outpatients but more no satisfied patients are in tertiary outpatient 95%it may be similar to study in Uganda "The mean patient satisfaction score was fairly little"[17] but another study in Delhi showed high satisfaction[18]. In our study there was significant association and positive correlation between age and satisfaction of patients towards health services in tertiary outpatient in age group 31-50 years majority not satisfied may be health services not suitable to them, the above than 50 years had most satisfaction it may be due to availability of health services more toward them similar to study done in Riyadh, and USA[1,19] while in secondary outpatient the age not significant association with satisfaction similar to study done in Nepal[20]. According to gender there was no significant association with satisfaction although there were no significant differences between men and women, we should not completely simplify the findings to all outpatients similar to paper in Uganda[17], the income of patients not significant association with satisfaction for both group of outpatients in compare to study do in Uganda and China the income and satisfaction is strongly association may be due to the health services in Iraq are semi-free[17,21]. According to place of living there was significant association between satisfaction and place of living in secondary outpatient with negative correlation mean more patients from urban area lead to decrease satisfaction may be due to decrease health literacy and lower socioeconomic are more in patients live in rural area compared to urban colleagues similar to study done in Nepal[20], while in tertiary outpatient there was no significant association between and living place it may be due to all cases referral from primary and secondary outpatient to tertiary so all patients well preoccupied about health services. In our study the education level had significant and important association with patients satisfaction in both types of outpatient with negative correlation mean when increase the level of education may lead to decrease in satisfaction so we see the primary education level more satisfy may be due to able to follow instructions compared with those with secondary and graduate education level those had more information about health services and not satisfy by little quality of services similar to study done in Uganda Malaysia and Riyadh[17,19,22].

In our study the association between satisfaction and types of visit was significant with positive correlation in tertiary outpatient mean patients with came for follow-up more satisfy than patients came for first visit possible description for improved satisfaction could be that chronic patients are self-selected for large observed satisfaction at this hospital but the insights of those who first visit maybe because of reduced satisfaction are not taken similar to study do in Uganda and South Africa[17], while in secondary outpatient the types of visit not associated with satisfaction.

Our study appeared the significant relation between referral system and satisfaction especially in tertiary outpatient with positive correlation mean patient referral by his family doctors more satisfied than patients came by itself or other way of referral may be due to family physician more orient to disease of all family members and health services and referral system and how to deal with health problem, similar to study do in Delhi, Poland[18] but referral system in secondary outpatient not association with satisfaction but still patients more satisfied when refer from family physician.

Conclusion

Our study showed no. of patients not satisfied health services more than patients satisfied, the majority of patients with low income and just primary level of education, the majority of patients came to outpatients from rural area these data for both secondary and tertiary outpatient.

Patients with bad satisfaction are 18-50 years old and self-referral system in only tertiary outpatient, not satisfaction also in urban patients visit the medical city.

Secondary and tertiary level of education had bad satisfaction in both secondary and tertiary outpatient. Further study is recommended to know the causes of all this bad satisfaction.

Good satisfaction appears in patients referred from his family physician also this may need farther study to

improve the health system in Iraq.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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High Levels of Tumor Necrosis Factor-Alpha and Interferon-Gamma in Patients with Gingival Recession

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Abstract

Background: Gingival recession considers as a common condition that associated with transposition of the margin of the gingiva in to the position apical to the cemento-enamel junction which consequently result in an exposure of the root . Inflammatory biomarker including interferon-gamma and tumor necrosis factor-alpha associated with a significant role in periodontal tissue destruction . The main goals of current study were to compare the levels of interferon-gamma and tumor necrosis factor-alpha in patients with different degree of gingival recession and control participants.

Material and method: Blood samples taken from twenty four male participants and they grouped in to three groups as: group 1(8 control subjects) , group 2 (8 patients with class 1 and 2 gingival recession)and group 3 (8 patients with class 3 and 4 gingival recession). Enzyme-linked Immunosorbent Assay was utilized to evaluate tumor necrosis factor-alpha and interferon-gamma levels.

Results : The levels of both biomarkers were greater in group 3 than group 1 and group 2 as well as group 2 had higher levels of these biomarkers than group 1.

Conclusion: Interferon-gamma and tumor necrosis factor-alpha could reflect periodontal destruction in patients with gingival recession.

Keywords*: gingival recession, interferon-gamma, tumor necrosis factor-alpha and Enzyme-linked Immunosorbent Assays.*

Introduction

The deposition of the gingival margin in an apical direction which consequently leads to revealing of the root surface to oral environment is called gingival recession [(1)]**.** Periodontal disease, tooth malposition, trauma, aberrant frenal attachment strongly considered as etiological factors of gingival recession [(2)].

It is a very common condition , about 50% of persons in the populations have at least one or more sites of 1 mm of root exposure or more [(3-5)] . Besides aesthetic shortcomings [(6,7)] , gingival recessions have a high predisposition to be associated with functional problems related to root exposure, such as dentinal hypersensitivity [(8-10)], plaque retention, gingival inflammation, root caries [(11-15)] , alveolar bone loss and eventually tooth loss [(15, 16)].

According to Miller (1985) , 4 classes of gingival recessions were proposed. In class I the recession not includes mucogingival junction (MGJ) , if the recession includes the MGJ the case considered as class II, both classes presenting no interproximal bone loss. In class III the recession extend to or include the MGJ with destruction of the interproximal bone and/or malpositioning of the tooth. Lastly, class IV presented with a severe destruction in the interdental bone and/or serious malpositioning of the tooth [(17)].

Proteins that are formed by different types of cells and represent a messenger to another cells are called

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cytokines. They start , control and mediate inflammatory and immune reactions; as well they control differentiation and growth of cells [18]. Cells of gingival epithelium secrete a wide variety of cytokines, like , tumor necrosis factor-alpha (TNF- α) , interleukin- 1a (IL-1a), interleukin-1b (IL-1b), interleukin-6 (IL-6) and interferon-gamma (IFN- γ) these are the pro-inflammatory cytokines while the anti-inflammatory cytokines include interleukin-10 (IL-10) and interleukin-4 (IL-4) [19-21].

In chronic inflammation, cytokines like TNF, IFN, IL-1, and IL-6 perform a significant role in bone destruction by activating osteoclasts [22–24].

Material and Method

A total twenty four male subjects with age range (30-40) were included in present study. Patients presenting at least two buccal gingival recession were enrolled while control showing no gingival recession . The participant were categorized as control (group 1) and patient groups furthermore patients divided in to two groups according to Miller gingival recession classification [17] . The first group include class 1 and 2 gingival recession (group 2) while the second group include class 3 and 4 gingival recession (group 3).

All patients were signed a written informed consent to participate in present study . Periodontal measurements involving plaque (PI) and gingival (GI) indices were recorded for each patients [25, 26].

Exclusion criteria include:

Patients with systemic diseases.

Patients with previous periodontal treatments or who take medication.

Tooth with restoration or crown involving cemento-enamel junction (CEJ).

Tooth with root abrasion at the CEJ.

Presence of periodontal pocket.

Blood collection and storage:

Blood were collected using sterile disposable syringes from each group. Clotting of the blood samples were gained by leaving the blood samples overnight at 4°C or for 2 hours at room temperature then centrifuge for 15 minutes at 1000×g. Supernatant were collected and stored at -20. °C, the assay was carried out during the first month after storage.

Quantitative determination of cytokines:

Quantitative determination of both biomarkers in serum of patients groups and control group using Sandwich- Enzyme-linked Immunosorbent Assays (ELISA) as in manufacture instructions (Elabscience Biotechnology Co., Ltd).

Statistical analysis .

Statistical analysis include mean and standard deviation (SD) also analysis of variance (ANOVA) test was utilized to estimate the differences among groups . *P* value less than 0.05 regarded as significant.

Results

Means of TNF were (17.48± 11.93) ,(52.76 ± 18.81) ,(72.27 ± 42.54) in control group ,group 2 and 3 respectively with significant difference between groups (P=0.003) as shown in table 1 and figure 1. Regarding IFN- γ control group had the lowest mean (47.59 ± 29.33) while group 3 had the highest mean (280.08 ± 121.35) also there was a high significant difference among groups as shown in table 1 and figure 2.

The results of present study revealed that there were highly significant differences in plaque and gingival indices among groups (P<0.001) as shown in table 1.

Table 1: Mean and standard deviation of plaque index, gingival index, TNF- α and IFN- γ of all groups.

	Group 1 Mean ± SD	**Group 2 Mean ± SD**	**Group 3 Mean ± SD**	**p-value**
PI	0.34±0.10	1.56 ±0.26	1.75 ± 0.24	0.000
GI	0.35±0.09	1.80 ±0.24	1.87± 0.24	0.000
TNF- α	17.48 ±11.93	52.76 ±18.81	72.27± 42.54	0.003
IFN- γ	47.59 ± 29.33	140.14 ± 81.76	280.08 ±121.35	0.000

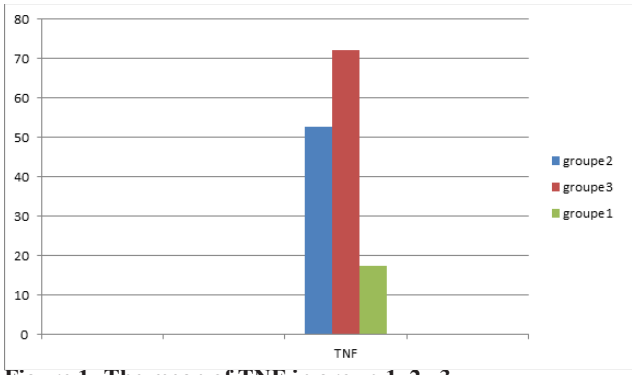

Figure 1- The mean of TNF in group 1, 2 , 3.

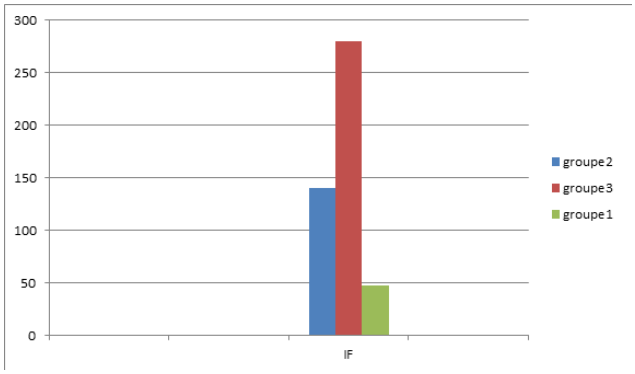

Figure 2- The mean of IFN- γ in group 1, 2, 3.

Discussion

Gingival recession may occur in dentitions with otherwise healthy periodontium . About the significance of gingival inflammation and dental plaque in progression of gingival recession, other researches have revealed that the most common cause of gingival recession was gingival inflammation . They proposed that the breakdown of connective tissue caused by a localized inflammatory process. Gingival recession results from of epithelial cells reproduction into the connective tissue which cause a collapsing of the epithelial surface [27,28]. Current study showed that the patients with gingival recession in both groups had higher gingival and plaque indices than control group and these results were in concurrence with other studies who revealed that the gingival recession was related to the high levels of both dental plaque and calculus as well as it related to the gingival bleeding on probing [27,29-33]. Also, a study conducted by Goutoudi et al. [34] shown that the gingival recession was concomitant with both high plaque scores and inflammation. One study [35] revealed a negative association between gingival recession and dental plaque.

The proinflammatory cytokine , TNF-α , induces the secretion of collagenase by the fibroblasts, bone and cartilage destruction . Tumor necrosis factor -α is part of the main proinflammatory cytokines which are usually produced on inflammatory sites by the mononuclear cells infiltration [36,37]. In consequence, this cytokine is part of the periodontal tissue destruction. Previous studies reported an elevated levels of TNF-α in serum of periodontitis patients [38,39]. Our results are corroborating with the previous data as we found great levels of TNF-α in serum of subjects with gingival recession [40].

Raised levels of numerous inflammatory mediators , like TNF-α, IFN-γ IL-1, IL-6 , and prostaglandinE2 (PGE2) have been identified in gingival crevicular fluid (GCF) and in gingival tissues of subjects presented with periodontal diseases [41,42].

The structure of bone tissue relies on the equilibrium between bone destruction and bone formation [43, 44]. As key factors that contribute to the breakdown of periodontal tissue , several proinflammatory cytokines were identified, involving IL-1, TNF-α, IFN-γ, and IL-6 [45,46]. It is possible that the higher levels TNF-α and IFN-γ found in our patients may have acted as an important osteoclastogenic factor by inducing the local osteoclast differentiation that could culminate in bone destruction.

Conclusion

Gingival recession presents an increase in inflammatory markers such as TNF-α and IFN-γ , which could be predictors of local bone destruction and disease spreading.

Ethical clearance : All participants received learned consent to join in current study , the study was accepted by Ethics team of Collage of Dentistry / Babylon University .

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Efficiency of Aspergillus Species to Produce the Lipase Enzyme from Various Types of Oil Seeds

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Abstract

Filamentous fungi can easily degrade agroindustrial wastes in solid- state fermentation processes synthesizing many important commercial biocompounds such as lipolytic enzymes. Lipases have various applications in industry so the aim of this study to evaluate the effect of the composition of the solid culture medium on the production of lipolytic enzymes by various species of Aspergillus, so in this study we use different types of of oil seeds to isolate different species of Aspergillus and study their effect on production of lipase enzyme by using of this different seeds as a substrate for culturing of this fungi and in this study *Aspergillus niger* that are isolated from *pistacia vera* seeds is more efficient for production of the lipase enzyme.

Keys word: *Pistacia vera (pistachio), Anacardium accidentae (kazoo) ,Prunus delicis(almonds) ,Arachis hypogavae(pistachio slaves), Juglanis regis(walnut)*

Introduction

The demand for microbial lipases is on the increase awing to their application in a wide variety of industrial process and it is used in food, dairy, cosmetic and pharmaceutical industries due to their hydrolytic reaction [(7)] .

Various types of microorganism, such as fungi are widely recognized as a good source of extracellular lipase [(2)]*Aspergillus*is the best candidate because it can produce more enzymes than other microbes in microbial engineering research development related to very high lipase [(3)] . The best species of fungi to produce the lipase enzyme is *Aspergillus niger* because it is classified as GRAS (Generally Regarded A Safe) by the FDA (Food and Drug Administration of the United States of America) [(4)]. So that the lipase from this fungi can be used in various fields including food and pharmaceutical industry [(5)] . Solid state fermentation is a fermentation technique that allows the use of simple substrates such as oil seeds, marine water, solid waste of agroindustrial products that are induced by using oil such as olive oil [(6)].

Lipase (triacylglycerol a cyl- hydrolases E C 3.1.1.3) are enzymes capable of hydrolyzing the ester bands of insoluble substrates in water at the substrate – interface [(7)] .

Many researcher worldwide direct their activities to the screening of new lipase producing microorganism, and subsequently to the optimization of the medium composition to increase the efficiency of the microorganism to produce the lipase enzyme because the composition of the medium can effect on the characteristic and efficiency of the lipase enzyme and change its properties [(8)] .

Material and Method

I-Isolation and diagnosis of the *Aspergillus* from oil seeds

The fungi used in this study was isolated from oil seeds of (pistaciavera(pistachio), Anacardium accidentae(kazo), Prunus delicis(almond),Arachis hypogavae(pistachio slaves),Juglanis regis(walnut)),various *Aspergillus* species was isolated from there various oil seeds by washing these seeds well by distilled water and sterilized by adding of 1% sodium hydrochloride solution (Naocl) for one minutes and then this seeds cultured on potato dextrose agar (PDA) that sterilized by adding of streptomycin, and this plates was incubated at (28°c ± 1) for 7 days. The species of *Aspergillus* was identified by macro and micro-morphological analysis [(9)] . The macro morphological characteristics observed werecolor, relief and edges of the colonies.

II-Isolation of the Aspergillus spp. from the soil

The samples that are used for isolation *Aspergillus* spp. from the soil that taken from 2-5 cm deep and cultured on (PDA) agar with added chloramphenicol in 10µg/ ml with 3 dilution for each sample and incubated at 28Ċ for (2-8 days), then *Aspergillus spp.* colonies was isolated depending on macro- morphological characteristics.

III-Screening for extracellular lipase production

Modified basal salt medium (BSM) containing (g/L): NH_4NO_3, (1) K_2HPO_4 (1.5) $MGSO_4$ 7 H_2O (0.025) $CaCl_2$ (0.025) $FSSO_4$ 7 H_2O (0.015) $ZnSo_4$, $7H_2O$(0.005), plus distilled water 1000ml and PH must be adjusted to 7. Sterilization of the medium is done and then adding of 250 mg of streptomycin and 1% (VIV) sterilized tween 80 were added.

Then I ml of the *Aspergillus* colonies suspension was transferred to 30 ml of the previously prepared medium. The flask that contain the fungi cultures were incubated at 28°c for 5 days, under shaking (150 rpm) and then filtrate was centrifuged under cooling at 1000 xg for 30 min at 4°c, and the supernatants was subsequently collected for lipase activity determination. Lipase activity was determined by using [10] .

IV-Screening of Aspergillus spp. for lipase production

Screening was carried out using mineral medium described by [7] with olive oil emulsion as the carbon source. Strains that showed by hydrolytic halos on this medium were selected for further lipase determination.

V- Extraction of pure lipase.

The production of lipase was studied in 250 ml flask containing 50 ml of mineral medium with olive oil as a carbon source, it also contain 3 gm of KNO_3, 1 gm of KH_2PHO_4, 1 gm of Mg So_4 7 H2o and 2% of sterile olive oil, then the strain of isolated Aspergillus was inoculated and incubated at 28°c on a rotatory shaker at 120 rpm up to 7 days, and then the growth was filtered through Whitman No.1 filter paper. The clear culture filtrate then was assayed for lipase activity.

VI- Effect of initial PH.

The fungi can grow on culturing medium at different initial PH ranging from 2 – 12. The initial PH was adjusted by 0.1 m HCL OR 0.1 M Na oH. After 6 days the fungi mass was grown and yield of lipase produced was determined.

VII- Effect of temperature on lipase activity and stability.

The effect of reaction temperature on the activity and stability of lipase was determined by incubation the enzyme at different range of temperature ranging from 30-70 °c. Immediately after incubation at each temperature, the enzyme activity was then determined at 30°c.

IX- Measuring of enzymes activity.

The enzymes activity was determined using the method standardized by [11] which is based on titration with NaoH of fatty acids released by the action of lipase on the extract on the triacylglycerol of olive oil was added to 250 ml flasks, 2 ml buffer prepared, 5 ml of olive oil and then 1 ml of enzyme extract was added to this system and it was incubated for 30 min. at 30°c. After incubation the reaction was stopped by adding 15 ml of acetone: ethanol: water (1:1:1) and released fatty acids were titrated with a solution of 0.01 mol/2 NaoH using phenolphthalein as indicator.

One unit of activity was defined as the amount of enzyme that releases 1 mol of fatty and per minute per ml of enzyme extract of the submerged fermentation under the test condition.

Results:

In this study 3 species of *Aspergillus* was isolated from soil and different oil seeds included *Aspergillus niger*, *Aspergillusflavus* and *Aspergillus fumigatus* and each one of these species was tested for its ability for production of lipase enzyme, and activity of this enzymes also was studied. The frequency of isolation of *Aspergillus spp*. from different types of substrate. We found that the most common isolate was the Aspergillus niger that was isolated from all types of oil seeds that are used in this experience in addition to soil.

As was showed in the table (1) the most active species for secretion of active lipase was Aspergillus niger isolated from (Pistacia vera) pistachio while (Pistacia vera) Aspergillusfumigates that are isolated from pistachios show no lipase activity, while Aspergillus flavus isolated from pistachios (Pistacia vera) showed

moderate lipase activity. Aspergillus flavus that were isolated from walnut (Jugans regis) also showed – ve activity for lipase production. While all species isolated from soil and pistachios slaves showed activity of lipase enzymes in various values. from this study we noticed that lipase enzymes can be produced by different species of Aspergillus and activity of these enzymes are depended on the substrate that are used for isolation of these enzymes, but the best one is Aspergillus Niger and the best substrate was the pistachios (Pistacia vera).

Table (1) Efficiency of enzyme lipases that are isolated from Aspergillus spp. according to types and substrates:

Types of Aspergillus spp	types of substrate	Efficiency of lipase
- Aspergillus niger	Soil	+++
- Aspergillus flavus	Soil	++
- Aspergillus niger	Pistacia vera	++++
- Aspergillus flavus	Pistacia vera	++
- Aspergillus niger	Arachis hypogavae	++
- Aspergillusfumigatus	Seasmum indicum	++
- Aspergillus niger	Seasmum indicum	++
- Aspergillus niger	Anacardium accidentae	+++
- Aspergillus flavus	Anacardium accidentae	+
- Aspergillus niger	Prunus delcis	+++
- Aspergillus flavus	Juglans regis	++
- Aspergillus niger	Juglans regis	-
- Aspergillusfumigatus	Juglans regis	+

Discussion

This study appears that the most common species that are isolated from soil and oil seeds were the Aspergillus niger and it was the most efficient producer of lipase enzyme by using of pistichios (Pistacia vera) as a substrate and this was gone with the study of Tim. [(1)] . The suitability of pistichios for lipase production may be attributed to the oil present in it. This oil may have induced the production of this enzyme without an additional of carbon source while supplementation of glycerol favored enzyme production [(12)] .

Also there are many studies appear that the enzymatic activity of Aspergillus niger that are cultured on solid state fermentation were higher than using other methods. As approved by studies done by [(13)] .

This study approves that substrate type can effect efficiently on the types of Aspergillus species and their efficiency for production of lipase enzymes and also the activity of these enzymes, that are based on the analysis conducted on the unite of lipase and approved also by study [(6)] .

Although the primary function of the substrate was to support the growth of Aspergillus but the lipid content in the substrate medium could increase level of lipase activity generated.

Study done by [14] agree with our study that using of solid lipid substrate that are served as the sole cource of nutrient for lipase production may have a combined benefit of utilizing a law- value waste material while producing a commercially valuable product due to using the inexpensive culture media comparison and stability of the enzymes over longer period.

Study of [7] approved that the best species of Aspergillus to produce lipase enzyme was Aspergillus niger, next to it was Aspergillus fumigatus, while Aspergillus flavus showed the least potential for lipase production.

We conclude from this study that oil seeds considered as a good substrate for lipase production because beside being a source of carbon and supporting the fungus has lipids that assist in the production of this enzyme. The addition of lipid substrate as oil is essential to obtain highest lipase production.

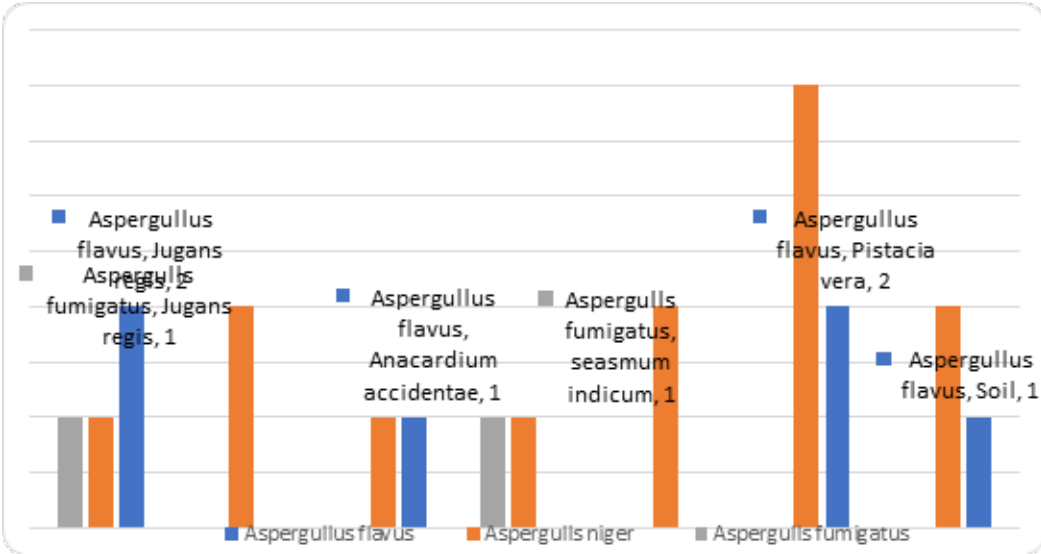

Figure (1)show frequency of isolation of Aspergillus from different source of substrate

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Seroepidemiology of *Toxoplasma gondii* Infection in Drivers Involved in Road Traffic Accidents in Baghdad City

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Abstract

In this study, a total of (200) people (170 men and 30 women), aged (20-60) years were enrolled during a period of 10 months in Baghdad city. The informed consent of the studied groups was first taken and the blood alcohol levels after accidents showed negative results. The control group consisted of 100 individuals (60 men and 40 women), residents of the same region and in the same age group with no clinical signs and symptoms of toxoplasmosis. The commercial IgG and IgM ELISA kits were used to examine the collected serum samples. The serological test results of the studied group (SG) showed that 92(21.90%) were IgG positive, 75(17.85%) were IgM positive when compared with the control group (CG) who showed 11(2.61%) IgG positive and 33(7.857%) IgM positive. We can conclude that there is a high traffic accident risk to the drivers because of the high seroprevalence of latent toxoplasmosis. Prospective studies are still recommended and latent toxoplasmosis of the car drivers should be considered while strategies are being designed to prevent traffic road accidents in Baghdad.

Key words: *Toxoplasma gondii, drivers, road traffic accidents.*

Introduction

Toxoplasmosis is a global disease caused by an obligate intracellular protozoan known as *Toxoplasma gondii*. The infection is usually caused by consumption of undercooked or raw pork or lamb meat that contains bradyzoites in addition to vegetables and fruits which contain the oocysts. The pregnant women can also transmit the infection to their newborn congenitally, and people can acquire the infection via blood transfusion or organ transplantation and it can also be transmitted to the butchers or people who handle infected tools or accidentally through their exposure to contaminated blood [1]. In about (80%) of the human population, toxoplasmosis develops with no symptoms since tissue cysts have the ability to persist for ever during the life of the host. In immunocompromised people, the tissue cysts act as reservoirs where local or disseminated infections may emerge [3], especially in patients with (HIV/AIDS) [4]. Many serious diseases can be caused by *T. gondii* such as the fatal encephalitis, pneumonitis, myocarditis and choreoretinitis. In Czech people infected with *Toxoplasma gondii*, personality changes were also detected [5,6]. Moreover, impaired motor performance and slow reaction times were shown in persons with latent infections [7,8]. The behavioral changes can be explained by the high levels of dopamine, because the genes of *T. gondii* encode for the aromatic amino acid hydroxylase enzymes, that result in metabolism of phenylalanine to tyrosine and tyrosine to L-DOPA, which is the dopamine precursor. It is suggested that the higher dopamine levels can lead to changes of neurotransmitters and as a result, to a change in the behavior [9,10]. Schizophrenia has been found to be correlated with latent toxoplasmosis [3]. The mental disease patients in Mexico showed (37.24%) anti-toxoplasma antibodies, which is (18.26%) higher than those in the whole population [4].

Motor cycle accidents were recorded as the leading cause of death by the Ministry of health within the (15-19) years age group, and as the second leading cause in the (20-50) years age group. *T.gondii* was found to cause choreoretinitis, due to their prolonged reaction times and the impaired motor performance. This study

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aimed to identify the IgM and IgG Anti-*Toxoplasma gondii* antibodies prevalence in drivers who had traffic accidents in Bab-Al-Muadham and Al-Nahdha public transport garages in Baghdad governorate. The study also aimed to determine ocular impairments and risk factors related to *T.gondii* seropositivity in such individuals in comparison with the control group.

Materials and Method

Study population

This group involved (170) men and (90) women drivers presented with injuries, and a positive alcoholmeter test was used as an exclusion criterion.

Study of the population who were not involved in traffic road accidents

This group was composed of (160) drivers, (100) of them were men and (60) were women. All of this study group resided in Baghdad city. They have never been previously involved in traffic road accidents, and were selected randomly from Bab-Al-Muadham and Al-Nahdha public transport garages in Baghdad governorate. These drivers were considered as a control group for comparison of results.

Sample and data collection

From all drivers**,** blood samples have been obtained during the period from October 2015 to March 2016. For all studied groups, data on sociodemographic, clinical and risk factors associated with *T.gondii* infection were collected through a structured questionnaire form. Information regarding consumption of raw vegetables and fruits, consumption of raw or undercooked meat, cats at home, organ transplantation and blood transfusion have been also taken, while clinical information involved presence of an underlying ocular impairment such as (Astigmatism, hypermetropia, presbyopia or myopia).

Serological test for *T.gondii* antibodies

Blood samples were sent to Biochemistry laboratory / Institute of medical technology / Baghdad – Bab Al-Muadham. Centrifugation of clotted blood has been used to obtain serum sample, which have been kept frozen at (-20C) until use. The commercially available enzyme linked immune sorbent assay (ELISA) kit (Platelia TM Toxo , BioRad, Marnes_la_Coquette_ from France) was used to detect IgG and IgM antibodies to *T.gondii* in the sera of all the studied groups. The wave lengths (450/620 nm) were applied for reading the results of the plates and the standard curve in (IU/ml) was used for plotting the optical density. For latent infections, a titer less than (6 IU/ml) was considered as negative, (6-9) as intermediate and more than (9-200) as a positive titer. A duplicate testing was done to samples with values more than 200 IU/ml. The manufacturer's guidelines were used to perform all investigations.

Results and Discusion

The results of our study revealed that 211(50.23%) out of 420 traffic accident participants and 33(20.62%) out of 160 controls had positive anti-*T.gondii* IgG antibody results. This finding in part explained the negative relationship between the presence of anti-*T. gondii* IgG antibody titers and the ages of the traffic road accident groups, a result that has not been observed in the control group. As a result, univariate ORs for the relationship between many categorical variables and the traffic road accidents showed a deviant protecting effects on the age group (40-49) years. Tables (1) and (2) show serum testing results in both the experimental and the control groups. As seen in table (2), IgM and IgG antibodies were found in both groups. It has been demonstrated that the number of IgG seropositive results was significantly higher in comparison with the control group. Therefore, it can be concluded that the incidence of chronic toxoplasmosis was three times higher than the control group among people involved in the traffic road injury accidents, and this finding indicates that there is a relation between the road accidents and acute and chronic toxoplasmosis. The anti-*T.gondii* IgG antibody titers were found to be correlated with the occurrence of traffic accident risks. The fact that seropositive status as a categorical variable is not associated with the traffic accident risk is proven when IgG antibodies are considered as continuous variables suggesting that the high antibody titers could be marker of an acute infection or a subclinical reactivation. However, this hypothesis is supported by the finding which revealed that increased IgG levels took place in people who showed seropositivity to anti-*T.gondii* (IgM) antibodies. In general. It is thought that the mechanism in such conditions is correlated with dopamine level modulations (decreased or increased) because of the existence of toxoplasma parasite in the brain tissues [11,12]. The prevalence of IgG anti-T. gondii antibodies was shown to be increased with the age of both groups. Similar results were demonstrated in the blood donor individuals where the prevalence of anti-*T. gondii* elevated after (20) years of age [4]. Regarding

the relationship between gender and anti-T. gondii antibodies, it was demonstrated that the percentage of positive males to those antibodies was more than those of females despite the statistical variation. These results were consistent with a previous study conducted by Hye Jin Ahn (2012) who indicated that there was a significant difference in the antibody concentration between males (20.6%) and females (13.1%) ($P < 0.05$) [13]. There was a statistical significant difference in the frequency of recent toxoplasmosis determined by IgM anti-*T.gondii* antibodies between traffic accident drivers (75(35.54%) and the control group 11(6.87%) [14].

There is an interrelation between socioeconomic strata variables and the educational levels. No significant statistical variation was found in our study between the demographic data and existence of anti-*T.gondii* antibodies. Similar results have been revealed within the same geographic region [4], as well as in the study conducted in Durango state, Mexico [15]. However, anti-*T. gondii* positive drivers with low and medium socioeconomic levels showed a trend for a risk in traffic road accidents in comparison with the high socioeconomic level drivers. The same results have been detected in Mexican population in Durango state when significant statistical difference was revealed in anti-Toxo antibody titers between low socioeconomic levels and workplace accident individuals [14].

In our study, it was shown that some toxoplasmosis risk factors like consumption of raw vegetables and fruits, consumption of raw or undercooked meat and cats at home are not correlated with anti-*T.gondii* antibody existence, and other risk factors like drinking of contaminated water may clarify the highly prevalence of anti-*T.gondii* antibodies. However, in our study, contaminated water was not included [16].

In regard to visual and ocular results determined by the questionnaire form designed in our study, both the involved groups showed the presence of ocular abnormalities. The control group revealed more frequency of astigmatism, presbyopia, myopia and glaucoma than the experimental group. No ocular injury due to toxoplasma infection in drivers with positive serum anti-toxoplasma has been shown in studying the ocular fundus. In immunocompetent persons, the toxoplasmosis ocular signs and symptoms did not result from the toxoplasma parasite itself but from the inflammatory immune responses. In the current study, all individuals positive to IgG anti-*T.gondii* antibodies had no signs and symptoms related to visual toxoplasmosis. Probably, the strains of Toxo which infect our study population differ from the type-1virulent toxoplasma, and this strain was found to be related to visual toxoplasmosis among healthy Brazilian populations [17,18].

Table (1): Prevalence of toxoplasmosis in the experimental and control groups by ELISA test according to gender

Group	Examined samples	Elisa test	
		Positive %	Negative %
Men	270	148 (54.81%)	122 (45.18%)
Women	150	63 (42%)	87 (58%)
Total	420	211 (50.23%)	209 (49.76%)

Table (2): Prevalence of IgG and IgM anti-*Toxoplasma gondii* antibodies in the experimental and the control groups

Group	Anti - T. gondii +ve				Anti – T.gondii -ve		Odd radio (1.95)	p-value
	IgM		IgG					
	Men	Women	Men	Women	Men	Women		
Experimental samples	45(38.4%)	30(33.33%)	72(61.52%)	20(22.22%	53(31.17%	40(44.44%)	0.566	0.001
Control	6(6%)	5(8.3%)	25(25%)	8(13.3%)	69(69%)	47(78.33%)	0.614	

Table (3): Prevalence of toxoplasmosis among the traffic accident participants in Baghdad city according to gender

Toxoplasmosis	Traffic accident / No				Odd radio (1.95)
	Men	Women	Men	Women	
Yes	53(35.81%)	43 (29.05%)	95(64.18%)	20 (42%)	0.259
No	62(50.81%)	62 (71.76%)	60 (49.18%)	25(28.73%)	0.416

Table (4): Association between (IgG) and (IgM) anti -*T.gondii* seropositivity and risk factors

	The traffic accident drivers / Anti-Toxo V+e (%)	The control group drivers / Anti-Toxo V+e (%)	The odd ratio
Age groups / years			
20-30	14/40 (53%)	5/30 (16.66%)	2.8
31-40	60/80 (75%)	30/50 (50%)	1.4
41-50	64/80 (80%)	7/50 (14%)	3.2
51-60	20/70 (28.57%)	2/20 (20%)	3.5
Age groups in men			
20-30	20/30 (66.66%)	7/15 (46.66%)	2.85
31-40	45/60 (75%)	15/35 (42.85%)	1.05
41-50	25/40 (62.5%)	7/30 (23.33%)	1.25
51-60	27/40 (67.5%)	2/20 (10%)	4.82
Age groups in women			
20-30	10/50 (66.66%)	2/10 (20%)	5
31-40	25/45 (55.55%)	8/25 (32%)	0.625
41-50	8/15 (53.33%)	1/15 (6.66%)	1.6
51-60	7/15 (46.66%)	2/10 (20%)	0.14
Educational level			
Illiterate	27/40 (67.5%)	7/20 (35%)	3.85
Primary school	70/120 (58.33%)	17/70 (24.28%)	1.06
Secondary and above	60/110 (32.0%)	20/70 (28.57%)	0.77
Socioeconomic level			
Low	45/60 (75%)	17/30 (56.66%)	2.64
Medium	102/180 (56.66%)	20.80 (25%)	0.18

Cont... **Table (4): Association between (IgG) and (IgM) anti -*T.gondii* seropositivity and risk factors**

High	10/30 (33.3%)		0.54
	No. of samples tested	Prevalence of T.gondii	
		Infection No.%	
Cats at home			
Yes	90	42.65	0.54
No	121	57.34	
Raw or undercooked meat consumption			
Yes	60	28.43	0.69
No	151	71.56	
Raw fruit and vegetable consumption			
Yes	150	71.09	0.87
No	61	28.90	

Conclusion

Toxoplasmosis may show no symptoms in approximately (80%) of immunocompetent host in the latent stage. Cysts may persist in the host's muscles and brain for a life span. However, the motor function may be diminished to represent a major factor of traffic road accident of toxoplasmosis among humans. In our study, we could not explain the relationship between traffic road accidents or toxoplasma infection and parameters like longer reaction times and impaired motor performance, because we did not evaluate these parameters. Further studies should be done in the future to detect the effect of neurological damage caused by toxoplasmosis on the driver's motor reflexes.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Institute of Technical Medicine/ Middle Technical University/ Baghdad – Iraq and all experiments were carried out in accordance with approved guidelines.

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Therapeutic Evaluation of the Partial Movement of the Center of the Nerve Bundle

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Abstract

Neuroscience in the human body is one of the basic components and any defect in it, although it has a simple effect in many aspects of organic or inorganic because it is the precise organizer of most movements and instructions and others, and the problem is based on the most important reasons that it was observed that most of the activities and activities carried out by An individual in his daily life must be in accordance with mechanical foundations such as load weights, sudden movements, sitting, abnormal sleep and other things that have a direct impact on the movement of the nerve beam from its exact position may be up or down, making the body in an abnormal state, And move them out of place as a result of these factors that we mentioned are natural factors and not satisfactory and also cause some of the problems of organic diseases, such as diarrhea, vomiting and inability to eat in addition to pain in the lower abdomen and the symptoms of inorganic.

Keywords: *Nerve bundle, Therapeutic evaluation*

Introduction

The combination of science between them in order to find appropriate solutions to the problems that hinder work wherever they are and topical treatments is one of the important procedures in this aspect, and the process [1] of appropriate diagnosis of the movement of the nerve bundle due to exposure to movement from its natural position in response to sudden movements or carrying some weights and other things Is important in the treatment methods, including topical treatment [2] of the follow-up procedures of one of the nerve endings associated with the nerve bundle, and this type is special to return this package to its natural position without medical interference, through the simple procedure [3] that does not have any side effect on the body . As a result of the therapeutic application of the partial motion of this package we obtained a clear amount of improvement of most of the injured by a very high rate [4]. The degree of scientific progress in obtaining better results and greater development in the process of acquiring information for development, which helps in the acquisition of information and development in which we achieve the requirements of success at the required level, and the concept of comprehensive measurement means the accuracy of accurate decision-making, measurements of tools Which can be identified on many indicators through the analysis or quantification of the components that indicate the knowledge of scientific [5] facts and the amount of prediction between them during what is required of the performance of the athlete, which achieves development rates in the side that works by this contribution to the formation of a scientific experience for coaches Or workers within their specialties and thus we may get to the desired optimum performance in the sports movement according to a scientific measure, which is a direct goal in this aspect [6]. The research in the field of tests and measurement in the field of sports has proven qualitative changes in order to secure the requirements of athletic achievement and raise the level from the health aspect, by giving us a precise description and analysis of this variable which has a simultaneous or cumulative health effect occurring to different body cells when practicing sports activity [7] The calendar is the program or measurements to determine the correct starting point, which determines the available characteristics or the objective treatment that is related to the subject of the reform, that this type of role after the diagnosis for the scientific interaction of microprocessors [8]. The importance of the research to some non-chemical procedures to treat the sudden movement of the movement of the nerve bundle, which

has no side effect or other complications. The researcher noted the lack of scientific research on the movement of the motor neuron, as well as the lack of interest by athletes or workers in this field to this type of injury, which may be an obstacle in sports performance, which result from the misuse of the optimal mechanical aspects of pregnancy Balances and sudden movements such as jumps or rapid fall from different places and other natural factors that help move the abdominal nerve bundle from its natural place [9], which has an effect in some aspects of organic nausea and diarrhea and lack of desire to eat and inorganic ones idle and lack of movement promise M sense of activity and other related things, hence we want to highlight the treatment of this injury without chemical interference [10].

Research Objectives:

1 - Application of scientific methods to address the movement of the nerve bundle in the members of the research sample.

2- To know the effect of the scientific methods in the treatment of this type of origin.

Research hypotheses:

1 - the presence of different rates of movement of the nerve bundle in the members of the research sample.

2 - the effect of the treatment methods used for this type of injury in the members of the research sample.

Research Areas:

a - The human field: - The players of the individual and the individual games in the province of Thi - Qar.

b. Time domain: 1/1/2015 to 23/6/2015.

c - Spatial field: - Laboratory physiology in the Faculty of Physical Education and Sports Sciences at the University of Thi - Qar.

Methodology

The choice of the curriculum usually depends on the exact solutions and objectivity through which to find a solution to the problem, so the researcher adopted the descriptive method in the survey method to suit the nature of the research.

Community and Research Sample:

The methods and methods of selection of the research sample are numerous and multiple, but there are things to consider when selecting the sample of the research. "The selected sample should represent the community of origin in a real and real representation so that the researcher can later circulate the results of his research to the community" Athletes with this type of injury were selected after their diagnosis by scientific methods and according to the precise measurements of the (20) players from various individual and individual events in the province of Dhi Qar.

Tools used: Stopwatch to calculate the pulse period, cotton, pot, water.

Exploration Experience:

The researcher conducted a survey of 2 members of the research community. The experiment was conducted within one day during which the measurements were conducted. The purpose of the experiment is as follows:

- Ensuring the total time to carry out the experiment.

- Ensure the work and field and the extent to which the implementation of measurements.

- Know the obstacles that appear and avoid the occurrence of errors and overlap in the work.

- Know the time needed to make measurements for each individual.

- Know the errors that occur in this experiment to avoid in the final experiment.

Procedural Methods:

The process of scientific evaluation should be objective so that we can accurately evaluate and the treatment method is appropriate. There are many ways and many, we do not take the scientific direction in this research has resorted to the researcher to choose the appropriate measurement method leads us to the diagnosis of appropriate and simple according to common symptoms such as nausea and loss Anorexia nervosa in the ventral nerve bundle area is close to the nerve center with a clear pulse appearance in the center. The measurements that have been adopted are:

A - Pulse Sensitivity Test [1] :

If the pulse is directly under the finger, such as the heartbeat, this indicates that the system is compatible and that the packet is controlled, or vice versa, you will

notice the movement of the pulse from the center of the region either up or Down or you may not find the throbbing at all and you may find a weak pulse, we show that the beam moving from its place and the period is calculated to feel the pulse of a steady non-oscillating within ten seconds.

B- Direct pressure measurement [2] :

The large finger is pressed at the center point of the leg joint from the back after lying on the abdomen. Both legs, which have a very strong pain in this area, indicate that the abdominal nerve bundle is moving from its normal position and can be treated from the leg which is

Where the source of the mother was calculated and the emergence of pain when the pressure on the region within a period of ten seconds.

Calendar: After confirming the scientific presence of a partial movement of the center of the nerve bundle according to the measurements that have been conducted, we do some of the following:

a - lying injured upright on the ground and the back facing the top and the arms stretched back.

b. The body is completely relaxed.

c. We take a piece of cotton with the size of the palm and put a quantity of water, we scan the leg from the middle of the knee to the end of the leg from the back, to facilitate the withdrawal of the nerve.

d - We press both thumbs on the center of the knee from the back and then drag down to the end of the leg.

e - Push the entire body through the feet forward in order to adjust the procedure for the withdrawal and return of the package.

Main experiment:

The main experiment was conducted on 23/6/2015 after completion of the exploratory experiments and the validity of the application and the time of the diagnosis, after which the researcher emptied the data with special forms for the purpose of conducting statistical treatments on them.

Statistical Methods

The researcher used the statistical bag (spss) in the extraction of the search results.

1. The arithmetic mean 2. The standard error, 3. The link box difference,

4. The simple correlation, 5. The degree (t) of the sample, 6. The contribution ratio.

Some statistical means of the sample of the research, which indicate the extent of achieving the objectives desired by the researcher according to the contribution rates and the amount of measurements that have been applied and the field of achieving its impact in the sample of the research chosen by the researcher, the researcher finds through these results there are a set of reasons that Led to this change in the level of therapeutic evaluation of individuals in this research, including us that the movement of any part of the nerve package from the original basis shows a defect in the functional aspects in addition to a defect in the physical aspects, and that the movement of the result of multiple causes, but weightlifting inappropriate and jump The sudden Or abnormal movement is one of the most important reasons for this move, and as a result of the diagnosis process, which most of the cases are dysfunction in the functional side as well as the motor side, the movement, although partially causing imbalance in the overall balance of the body because of its direct association with members of the body, The abdominal nerve bundle from its center has a clear effect on the various organs of the body and it is considered one of the important signs of moving this package from its place, because it is a center for mechanical distribution, but the main regulator of the process of balance in the body. The movement of the abdominal nervous system, although a simple movement has a significant impact in the spasms of the muscles of the diaphragm, which cause respiratory imbalance, where the diaphragm is one of the most important organs responsible for natural breathing, and believes "movement of the abdominal nerve plexus is a factor in the spasm of the surrounding muscles Diaphragm, which helps him in all his movements in order to complete breathing operations, and the connection of the nerve bundle abdominal through the abdominal artery near the stomach " [3] ·The nerves are one of the main receptors of the instructions and that the occurrence of free, but simple, it prevents the arrival of the orders required for the performance of the motor and where it emphasizes that "the nerves of multiple benefits directly and the other directly and that any disorder in the organization leads to the lack of access to the natural doubt Affects the desired movement performance. On the sporting side, the integration of performance requires

safety in all machines function fully, as the presence of symptoms of organic and inorganic affect directly on the progress of performance and the athlete cannot give any amount of normal to the real level, but it could lose several properties and potential due to the existence of such These obstacles, which confirms that "the injury, although minor, has to have a direct impact on the performance of the sport and have additional aspects, whether they are accumulated or accumulated" [4,6]. These are the injuries that have an effect on the level of sport through the loss of motor balance due to the movement of the nerve beam that causes the presence of abdominal pain continuously, and have no chemical treatments for pain relief because it is caused by the simple movement of this package and is considered inorganic pain to be Treatment of the chemical side, where he points out that "the movement of the nerve bundle, although partially by its natural position, is working on the imbalance so that this imbalance is evident, especially in sports performance." [5] .

Conclusions

The level of the two measures measured for diagnostic processes varied. There is a high correlation between the two methods of measurement that have been diagnosed. The presence of high contribution rates in achieving the level of the evaluation process on the members of the research sample. The presence of direct impact through the amount of differences obtained statistically.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Physical Education and Sports Sciences / Dhi Qar University, Iraq and all experiments were carried out in accordance with approved guidelines.

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Investigating the Level Of Vitamin E, C and Superoxide Dismutase in Patients with Allergic Asthma and Allergic Asthma with Helminths Infection

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Abstract

The aim of this study was to investigate the relationship of antioxidant activity, vitamin E, vitamin C and superoxide dismutase with the severity of allergic asthma by estimating their level in the patients' sera, as well as evaluating their association with the level of total and specific serum IgE in allergic asthmatics and allergic asthmatics with helminths infection. The study applied on 180 subjects divided into three groups, 100 patients with allergic asthma and 50 patients having allergic asthma with helminths infection. The group of patients included 50 males and 100 females age 15-60 years. The control group comprised of 30 healthy individuals, included 14 males and 16 females age range 16-60 years. For each participant, the medical history was considered; the total and specific IgE levels, vitamin E, vitamin C and superoxide dismutase levels were determined in the patient's sera using ELISA technique. The present study has demonstrated an increased level in serum total IgE in the patients' groups (allergic asthma group and allergic asthma with helminths) compare to the control group, mean 374.54 IU/mL; 472.24 IU/mL; 29.21 IU/mL respectively. The level differences among the three groups are highly significant when compared statistically.

Keywords*: Allergic asthma, vitamin E, vitamin C, superoxide dismutase, antioxidant, asthma with helminths infection, total IgE, specific IgE.*

Introduction

Asthma is a major public health problem affecting many people around the world; men and women, young and old all over the world [1]. Asthma is a chronic disease of airways with acute lung inflammation and bronchial hyperresponsiveness that characterized by different respiratory symptoms including wheezing, dyspnea, chest discomfort, cough and variable airflow limitation [(2, 3).] These symptoms are often caused by several factors such as allergen exposure, exercise, irritant exposure, viral respiratory infections or change in the weather [4] . Asthma is believed to be trigger by a combination of complex genetic and environmental interactions. It is divided into two types, both of them have the same signs. These types are allergic (or extrinsic) asthma and non-allergic (or intrinsic) asthma [5] . Allergens can activate the inflammatory cells in the airways and lungs. This activation leads to generate oxidants (free radicals) [(6,7)]. The antioxidant such as vitamin E, vitamin C and superoxide dismutase (SOD) terminate and inhibits oxidant reaction. As a result of this reaction, antioxidant reducing external attacks in the lung [8] . However, asthma's patients have been reported to have reduced antioxidant defences in their peripheral blood [9] . Vitamin E is an important antioxidant vitamin in the body, playing a protective role by which damage free radical and scavenge reactive oxygen species (ROS) [10] . Vitamin E becomes oxidized itself while preventing the oxidation of other factors more important metabolically [11]. In allergic asthma and allergic lung inflammation, vitamin E acts as an anti-inflammatory factor and blocks the airway hyperreactivity by regulating leukocytes'

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signals of trans-endothelial migration from the blood to the lung [12]. Vitamin E intake is associated with low serum IgE concentrations and low frequency of allergen sensitization [13] . Vitamin C is a critical water soluble vitamin that presents into two active forms, ascorbic acid which is oxidized derivative and dehydroascorbic acid. Vitamin C can act as a hydrogen donor to converse oxidation; therefore, it functions as a non-enzymatic antioxidant which deactivates and reacts with the free radicals [14, 15].

Materials and Method

Subjects

The current study included 150 allergic asthmatic patients, 100 with allergic asthma and 50 having allergic asthma with helminths infection confirmed by general stool examination (GSE). The group of patients comprised of 50 males and 100 females age 15-60 years. These cases were followed up in the Specialized Center of Allergy in Baghdad/ Al-Rusafah and Al-Zahraa Center of Allergy in Baghdad/ Al- Karkh for the period from July 2018 to January 2019.

Exclusion criteria were included: smoker, oral corticosteroids, respiratory tract infection, cancer, any chronic disease other than asthma, parasitic infections other than helminths and pregnant females.

The control group involved 30 healthy individuals 14 males and 16 females with age ranged between 16-60 years. Exclusion criteria were chronic respiratory disorders, current or past history of allergy, helminths infection and all chronic diseases mentioned in reference to the study group above.

Study design

All patients underwent a detailed clinical examination included total immunoglobulin E test (T.IgE), specific immunoglobulin E test (S.IgE), general stool examination (GSE) and serum level of vitamin E, vitamin C and superoxide dismutase (SOD).

Five milliliters of peripheral blood sample were taken from each subject using disposal 10ml syringes under sterile conditions. The blood collected in a gel tube to get the required serum for serological tests (T.IgE and S.IgE). Samples were centrifuged for 10 minutes at 2500 rpm, then serum divided onto five Eppendorf tubes, one for each test. All serum samples kept frozen at -20˚C until they used.

Determination of T-IgE, vit. E, vit. C and SOD

The level of serum T.IgE, vit. E, vit. C and SOD were determined using enzyme linked immunosorbent assay (ELISA) based on the manufacturer's instructions, where microtiter plates have pre-coated with antibodies. The standard and samples were applied on the wells, followed by adding the conjugate, then the TMB substrate to produce a blue color which will be changed to yellow when adding the stop solution. The concentration is proportional to the density of the produced yellow color, which is read using the microplate reader at 450nm to find the concentration of T.IgE, vit. E, vit. C and SOD.

Determination of S-IgE

The determination of S-IgE was performed using ELISA (polycheck kit, Germany). The polycheck strips were firstly coated with the allergens and calibrators, followed by adding the patients' and control sera which incubated on the rocker shaker. In the next step, the conjugate was applied followed by the substrate to quantify the IgE by the scanner and the computer. The inhalation profile kit that used to diagnose the types of allergens contains 20 parameters from the common allergens including: *Dermatophagoides pteronyssinus* (D1), *Dermatophagoides farina* (D2), Timothy Grass pollen (G6), Rye pollen (G12), Plantain pollen,(W9), Mugwort pollen (W6), *Cladosporium herbarum* (M2), *Aspergillus fumigatus* (M3), *Penicillium notatum* (M1), *Alternaria alternate* (M6), Dog epithelia (E2 / E5), Cats epithelia (E1), Horse epithelia (E3), Guinea epithelia (E6), Hamster epithelia (E84), Rabbit epithelia (E82), White Oak pollen (T7), Birch pollen (T3), Hazel pollen (T4) and Alder pollen (T2).

Investigation of helminth infections

The association of helminths infection in the group of allergic asthmatic patients were investigated using the GSE. Fecal samples were collected from the patients and control groups. The specimens were directly examined under 10X and 40X objectives.

Statistical Analysis

Statistical analysis was conducted with Statistical Program for Social Science software (IBM SPSS 23.0). Parametric quantitative data was presented as the mean ± standard error. Least significant difference test (L.S.D) was used to compare among the means of over than three (compare the concentration of T.IgE and vitamin

E, vitamin C and SOD in asthmatics, asthmatic with helminths infection and control groups). Correlation analyses between data were performed using Spearman's tests, with the correlation coefficients presented as "R". Level of significance was defined by using ANOVA test with probability $P \leq 0.05$ (significant), $P > 0.05$ (none significant) or $P < 0.01$ (highly significant).

Results and Discusion

Studying the relationship between the infection with allergic asthma and serum level of antioxidants vit. E, vit. C, SOD as well as its relationship with the level of total and specific serum IgE has been addressed in this study. In addition, the effect of the association of helminths infection with allergic asthma and the effect of this association on the immune response of allergic asthmatic patients was investigated for the first time in this study, particularly in regard with the biomarkers mentioned above.

The current study has observed a significant elevation in the levels of the total serum IgE parallel with declined serum level of antioxidants in allergic asthmatic subjects and allergic asthmatics with helminth infection compared with healthy individuals. The mean serum level of total IgE of asthmatics was 374.540 ± 10.394 IU/mL, which is highly significant than its level in healthy control, mean value 29.216 ± 1.786 IU/mL, $p < 0.01$. In addition, the mean T.IgE level in allergic asthmatic subjects with helminth infection is 472.242 ± 6.273 IU/mL which is highly significant than that of the control. However, the T-IgE level in the patients with allergic asthma and helminth infection was higher than its level in the patients having only allergic asthma, the full comparison is presented in Figure 1.

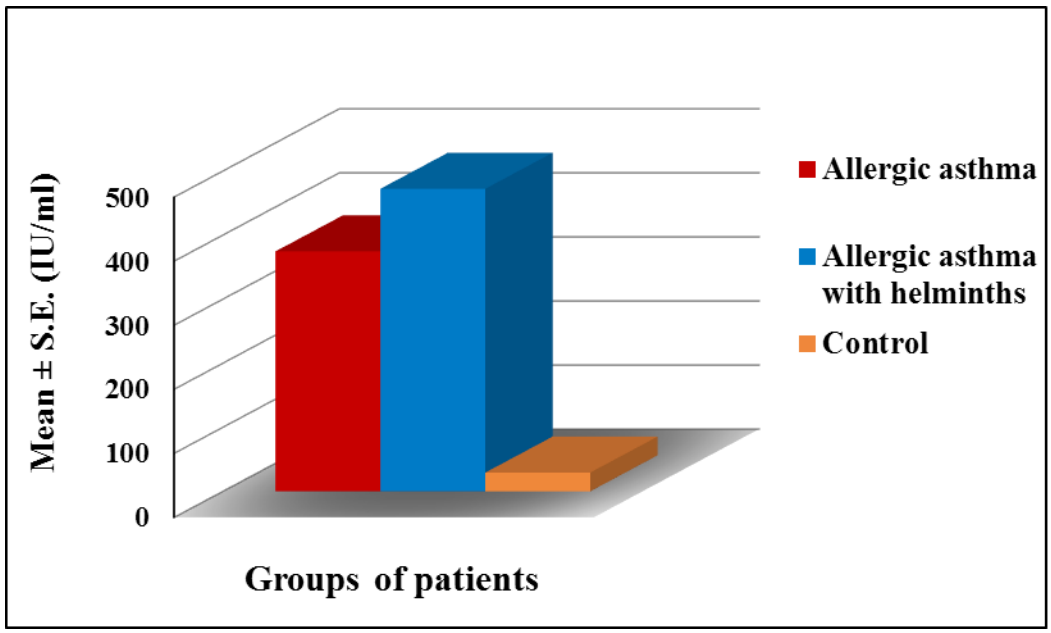

Figure 1: Serum levels of total IgE in the groups of allergic asthma, allergic asthma with helminths and control.

Allergens sensitization test was also performed in the current study by evaluating the level of serum S.IgE. The results have disclosed that the frequency of the most common allergens in 113 allergic asthmatic patients with positive specific IgE was as in the following: D1 (27%), D2 (26%), G12 (25%), T7 (23%), T2 (22%), W9 (21%), G6 (17%), E1 (17%), T3 (14%) , E2 / E5 (14%), W6 (14%), E3 (12%), T4 (11%), M3 (7%), M2 (4%), E82 (3%), M1 (2%), M6 (2%), E6 (1%), E84 (1%), as shown in Figure 2.

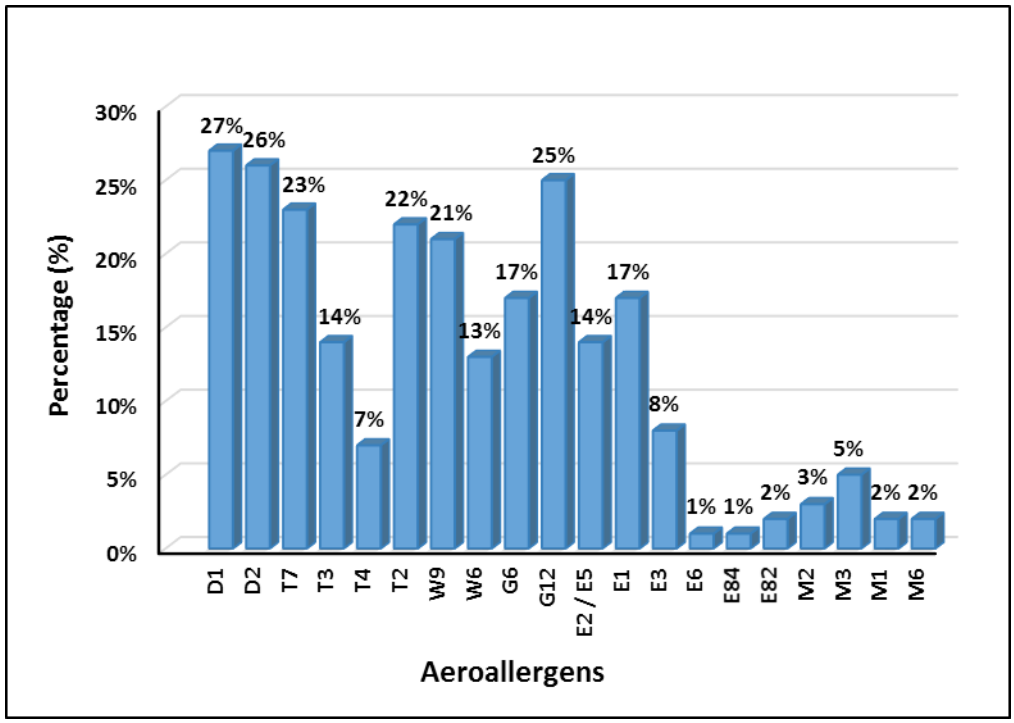

Figure 2: The percentage of aeroallergens that sensitize asthmatic patients' groups.

As shown in Table 1, the mean level of vitamin E in allergic asthma patients was 11.545 ± 0.320 IU/ml and it was 9.413 ± 0.180 IU/ml in allergic asthma with helminths as compared to its mean level in healthy control group that was 23.253 ± 0.726 IU/ml. Statistical analysis shown that there are a highly significant differences when compared between patients' groups and control group and also between allergic asthma and allergic asthma with helminths.

Table 1: Show the variation in vitamin E concentration among patients' groups and control.

Groups	Vitamin E (µg/mL) Mean ± S.E.	L.S.D. P-value
Allergic asthma	11.545 ± 0.320	P1= 0.00**
Allergic asthma with helminths	9.413 ± 0.180	P2= 0.00**
control	23.253 ± 0.726	P3= 0.00**

**Highly significant ($p < 0.01$)

P: probability, *P*1=Allergic asthma Vs Allergic asthma with helminths, *P*2=Allergic asthma Vs control, *P*3= Allergic asthma with helminths Vs control.

The serum level of vitamin C in allergic asthmatics was 36.040 ± 1.367 µg/ml, while the recorded mean level in allergic asthmatics with helminths infections was 21.163 ± 0.793 µg/ml. The statistical analysis reached that vit. C level in both patients' groups are significantly different from the level of vit. C in the sera of the control group which was 76.288 ± 2.708 µg/ml. On the other hand, there was a highly significant difference in the level of vitamin C between the two patients' groups as shown in Table 2.

Table 2: Show the difference in the level of serum vitamin C in the groups of allergic asthma,allergic asthma with helminths and control.

Groups	Vitamin C (µg/mL) Mean ± S.E.	L.S.D. P-value
Allergic asthma	36.040 ± 1.367	P1= 0.00**
Allergic asthma with helminths	21.163 ± 0.793	P2= 0.00**
control	76.288 ± 2.708	P3= 0.00**

**Highly significant ($p < 0.01$)

P: probability, *P*1=Allergic asthma Vs Allergic asthma with helminths, *P*2=Allergic asthma Vs control, *P*3= Allergic asthma with helminths Vs control.

Moreover, it has been found that superoxide dismutase is also decreased in both patients' groups. Statistically, there were highly significant differences between the both patients' groups (Table 3) and between the patients in the two groups and the control too. The level of SOD in allergic asthmatic group was 149.048 ± 6.447pg/mL, whereas, it was 78.271 ± 3.635 pg/mL in the group of allergic asthma with helminths. Nevertheless, the SOD level in the serum of the control group was 1034.157 ± 32.659.

Table 3: Demonstrate the difference in the level of superoxide dismutase (SOD) in the groups of allergic asthma, allergic asthma with helminths and control.

Groups	**SOD (pg/mL) Mean ± S.E.**	**L.S.D P-value**
Allergic asthma	149.048 ± 6.447	P1= 0.00**
Allergic asthma with helminths	78.271 ± 3.635	P2= 0.00**
control	1034.157 ± 32.659	P3= 0.00**

** Highly significant ($p < 0.01$)

P: probability, *P*1=Allergic asthma Vs Allergic asthma with helminths, *P*2=Allergic asthma Vs control, *P*3= Allergic asthma with helminths Vs control.

Table 4: Show the variations in the level of antioxidant according to different groups of T.IgE level.

Antioxidants		**T.IgE lower than (300) group**	**T.IgE between (300-500) group**	**T.IgE upper than (500) group**	**L.S.D. ANOVA (p-value)**
Vitamin C (µg/mL)	Mean ± S.E.	37.162±2.110	27.380±2.021	25.257±1.632	P=0.00**
Vitamin E (µg/mL)		11.179±0.348	11.101±0.602	9.821±0.240	P=0.01**
Superoxide dismutase (pg/mL)		138.149±8.281	116.647±12.328	100.637±8.320	P=0.03*

*Significant ($p < 0.05$) **Highly significant ($p < 0.01$)

S.E.= standard error

Allergic asthma is the most common phenotype of asthma among patients. The IgE is a well-known component of allergic reactions where it is produced during the process of sensitization which is triggered on the first exposure to the allergen. However, the level of T.IgE is increased with helminths infections too [22] . The results observed in current study indicating that the T.IgE playing a central role in the initiation and propagation of the inflammatory cascade of the allergic response and also in the immune response to the helminths infection, as shown in Figure 1.

Conclusion

The level of antioxidants, vit. E, vit. C and SOD are correlated with the severity of allergic asthma, where asthmatic patients having antioxidant insufficiency due to the increased oxidative stress. In addition, antioxidant activity has a negative correlation with pathogenesis parameters of allergic asthma such as total and specific IgE as well as with helminth infections.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science, Mustansiriyah University, Baghdad-Iraq and all experiments were carried out in accordance with approved guidelines.

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The Role of Cytomegalovirus (CMV) and Vitamin D in Multiple Sclerosis (MS)

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Abstract

Multiple sclerosis (MS) is one of the autoimmune demyelinating diseases involved in the central nervous system (CNS) and its etiological factors believed to be both environmental and genetic factors . The disease affected brain and spinal cord with various characteristic involved defect of myelination , chronic inflammation , pathological effect on axon and oligodendrocyte . The herpesvirus family is play a vital role in pathogenicity of disease . Cytomegalovirus (CMV) from these family members which detected to have a role in MS and our results investigated a significant relation of CMV with MS between patients and controls . Besides a previous data which approved the effect of the level of vitamin D on MS development and modifications in patients . In this study the results achieve significant differences in the levels of vitamin D between the two groups under study .

Key words: *multiple sclerosis (MS), Cytomegalovirus (CMV), (25- hydroxyvitamin D), Vitamin D.*

Introduction

One of the diseases involved in the central nervous system (CNS) is multiple sclerosis (MS) [1] . The disease affected brain and spinal cord with different clinical features such as blemish of myelination , chronic inflammation , pathological effect on axon and oligodendrocyte [2] . Generally , the disease pathogenesis may related to genetic factors of an individual or due to environmental conditions surrounding the patients [3] . The environmental factors can be suggested to related to distribution of the disease , in some population like Jordanians the disease incidence , is ranged from 20-60 cases per 100,000 persons [4] . In spite of World Health Organization , showed the incidence of MS reported to be 4 in 100,000 ; in South Asia reported to be region with a medium to high risk for MS [5] . While the prevalence in northern Europe or Canada is about 0.1 - 0.2% in population [3] . Multiple sclerosis is considered as idiopathic disease, and many factors can be associated with its progression ; from these are the genetic factors such as the role of the genes responsible of DNA repair in activation of MS and lesions formation [6] . As well as the role of polymorphisms in human leukocyte antigen (HLA) genes class II [7] . Numerous of genes associated with the disease are the regulatory genes affected the immune cells and their function which lead to dysregulation of the immune system [8] . Auto-reactivation of T cells against myelin may contribute to pathogenesis, and there is increasing attention of the role of human viruses in autoimmune diseases and its progression [9] . There is a significant increasing damage in nuclear and mitochondrial DNA in lesions of MS comparing with normal brain white matter and this highlights on the role of repair genes in DNA [6] .

Studies shown that multiple genes such as base excision repair genes involved in DNA repair may expressed in lymphocytes and lesions of MS when compared with tissues of control [10] . The environmental factors are strongly approved to be associated with the triggering of MS , like viral infection , high radiation of UV , climate and temperature , fat intake from animal sources , consumption of dairy products and levels of vitamin D [11] . genotoxic agents ,like cigarettes and nitric oxide may support and involve in MS induction [12].

The Role of Vitamin D :

Vitamin D conceders as essential nutrient for the bone homeostasis and plays a role in many disorders like cardiovascular and autoimmune diseases [13] . Although the deficiency of vitamin D is common in all ages but it hard to occur in the young and elderly ; and not only bone disorders , but many disorders can be related to

it , such as cardiovascular , autoimmune and neuro-related diseases such as schizophrenia [14] . In the immune system it is proposed to play a vital and supportive role in controlling of several diseases, as a cancer and neurodegenerative disease and possible relation with MS [15] .

Enhancement the function of vitamin D is by binding to the vitamin D receptor (VDR) on the cells ; by the ability to converting 25(OH)D to the active form 1,25(OH)2D. The VDR conceders as a mediator for the signaling, and its regulation is unclear until now [16] More than 2,000 genes can be regulated directly or indirectly by 1,25(OH)2D ; as well as there are at least eighty various metabolic processes regulated by these genes such as repair of DNA , apoptosis , alteration of function of the immune system , oxidative stress [17] .

Many factors and there accurate roles must be examined because they preventing and treating many disorders related to with VDR and its function by facilitating and improving strategies [18] . Many studies indicate the protective role of vitamin D on MS patients, and the risk of disease is reduced by Vitamin D supplements and exposure to sunlight [19] . Moreover, expression of VDR induced by 1,25(OH)2D in both resting and activated cells ; and for homeostasis of T cell during MS , vitamin D plays crucial role , and supplementation of it should be appropriate to treat the disease severity [20] .

Multiple Sclerosis and Viral Association :

Studies connect between viral etiology and MS; that due to the association of viral infection and encephalomyelitis , demyelinating processes in the nerves and axonal damage ; all that may precede the lesions of MS; as well as plaques of active demyelinating caused by T CD8 lymphocytes which involved in viral immunity [21] . Processes of cortical and axonal damage with lesions absence are detected in viral demyelinating , are seen generally in MS , and there is a resemblance of relapsing remitting MS form and periods of reactivation and latency of many viruses, such as the herpes virus [22] .

Cytomegalovirus (CMV) , Epstein- Barr Virus (EBV) , Human Herpes Virus 6 and 7 (HHV-6 and HHV-7) are members of the family *Herpesviridae*, and are from plausible viral agents associated with triggering of MS, as well as endogenous retroviruses also considered to be significant agents in MS [21] . The infection by CMV may activate the reaction of the immune system against the constituents of myelin of peripheral nerve [23] . A wide of inflammatory diseases and brain cancer , CMV was concerned to be as a co-etiological agent [24] . Furthermore a wide range strategies can be caused by CMV can be targeting the host defenses, from disturbing the pathways processing of antigen to modulating of cytokines ; and this establishing success coexistence of CMV during active infection , and in many tissues and organs this virus can be found , as well as in fluids of body [25] .

Types of MS :

There are 4 clinical types according to the course of disease; relapsing-remitting MS (RR-MS), secondary-progressive MS (SP-MS), primary progressive MS (PP-MS), and progressive-relapsing MS (PRMS) ; the first type is the major one and approximately 85% of patients are identified with it , in which exacerbations symptoms is noticeable and remission period is followed by , and the symptoms either progress or withdraw ; in a number of patients RR-MS types of disease may developed into the second type (SP-MS) in which the disease continues to be deteriorate with remission period or without [4] . The third types of disease (PP-MS) about 10 percent of total patients be affected ; and the symptoms gradually worsening without remissions or relapses, the rare types of MS is the last one (PR-MS) which affecting less than 5 percent of total patients and from the onset of disease it is progressive intermittent flareups with no remission periods and the symptoms are worsening the way [26] . In the adolescence the disease onset initiates and the peak reaches among the persons in late 20s and the beginning of early 30s , and the females developed the disease in doubling than males [27] . In Arabic populations , all ratio of gender designate that the females affected more over than males [28] .

Materials and Method

In this case control study sixty patients participating . They had MS from all types ,who attendance the MS center in medical city, their ages range between (19-53) years . And the confident diagnosis was confirmed according to examination clinically and neurologically by neurologists . Sixty persons ; females and males included in this study as a control with normal clinical and neurological status and with no history of MS , and their ages approximately matched to that of patients .venous blood samples were collected and the serum separated from patients and control . Vitamin D immediately

examined while the rest of serum stored in -4ºC until Anti-CMV antibodies examined . CMV IgM ELISA kit provided by (CALBIOTECH / USA company) .

Statistical Analysis

The statistical analysis of this study included the calculation of mean values and percentage. The association between Vitamin D and Anti-CMV antibodies was done by calculated of Chi square and P value with 95% confidence interval (95%CI). T-test was used to compare means of (Vitamin D and Anti-CMV antibodies) serum level between two groups (patients and controls) . A p-value ≤ 0.05 was considered as significant [29] .

Results and Discussion

In present study 60 for each of patients and control group taken . All the types of MS included in this study and 38 (63%) were detected with the first type (RR-MS) . The results obtained is illustrated in the Table (1) .

Table (1) : **The ages , mean of ages and gender of patients and control groups .**

Groups	Ages	Mean of ages	P value	Females	Males
Patients (n=60)	(19-53) years	34.73±8.9	0.9	43(72%)	39(65%)
Control (n=60)	(17-56) years	34.93±10.4		17(28%)	21(35%)

P-value ≤ 0.05 is significant

In this study we found that there is no significant differences of ages between two groups and , the prevalence of females is more than males , our results matched with previous studies who told that , generally MS presents in women higher than in men ; and this may be due to variations in the nervous or the immune system , differences in hormones , variations in the genetic , differences in the environment and modern lifestyle between men and women [30] . And there are markedly increasing of this disease in women than men in last years [31] .

This increasing possibly pointed to unclear changes in nutrition or environmental conditions which lead to predominance of this disease in women [32] . Also there is a different response of females and males against factors in the environment ; such as the sun light and supply of Vitamin D [33] . Moreover , increasing intake of dietary salt may be suggested to develop autoimmune diseases by activation of T-helper 17 cells [34] .

The role of CMV in MS patients :

The level of anti-CMV antibodies of patients and control groups were detected by ELISA kit and our results represented that , there is a significant differences of anti-CMV antibodies between two groups . And CMV showed to have a positive role in MS disease ; table (2) .

Table (2) : Anti-CMV antibodies of patients and control group .

Groups	Anti-CMV antibodies positive (number and %)	Anti-CMV antibodies negative (number and %)	Mean of Anti-CMV antibodies positive (IU/mL)	Border line (number and %)	P value
Patients (n=60)	40(66.66%)	16(26.66%)	2.21±1.5	4(6.66%)	0.005
Control (n=60)	26(43.33%)	29(48.33%)	1.46±1.2	5(8.33%)	

P-value ≤ 0.05 is significant

Many studies about *Herpesviridae* family , talk about the role of these family members in triggering and pathogenesis of MS ; and most of these studies recorded the significant role in induction of MS ; these viruses have periods of latency and exacerbations within CNS; Epstein-Barr, cytomegalovirus , human herpesvirus 6 and human herpesvirus 7 viruses from these viruses which are possibly triggering MS [21] . several studies proved a high rates of infection with CMV in MS patients ; but with more benign characteristic of this disease [23] . In comparison , the high titers of antibodies against CMV , the patients have disease reactivation in lower rates and cerebral atrophy in fewer signs with those patients who had lower antibodies titers [35] .

Sanadgol *et al* ,. support the hypothesis of contribution of CMV with MS and establishment of systemic infection by CMV in induction of immune response [23] . As well as induction of demyelinating lesions may be caused by myelin-specific lymphocytes which triggering by CMV [36] . In contrast of that Najafi *et al* ., revealed , there is no significant differences between the anti-CMV antibodies and MS based on statistical analysis [37] .

Many surveys correlate between the role of infection by CMV and some types of autoimmune disease ; and these studies demonstrated the role of CMV infection in attacking of the immune system to own cells of patient's by antigenic mimicry, and a high titers of IgG antibodies against CMV found in certain autoimmune disease [38] . In other hand probably CMV is capable to evade the immune system and induce the mechanisms of immune regulation, which may decrease the reactivity of MS disease in patients with MS [39] .

In general There is controversy about the role of pathogenesis of CMV in MS patients ; some studies suggested a positive relationship between MS activity disease and infection with CMV ; while others investigated negative role between MS and CMV infections [40] . Many studies needed to improve the role of CMV in MS induction by taking larger samples of patients of MS and healthy control .

The role of vitamin D in MS :

In this study the level of Vit. D is measured and the results of two groups shown in the following table .

Table (3) : the level of vitamin D of patients and control group .

Groups	Deficiency (number and %) of Vit.D level	Sufficiency (number and %) of Vit.D level	Mean of Vit.D deficiency (ng/ml)	P value
Patients (n=60)	47(78.33)	13(21.66)	21.78±13.1	0.000
Control (n=60)	19(26.66)	41(68.33)	36.43±14.1	

P-value ≤ 0.05 is significant

The result revealed that , there is a significant differences between two groups in levels of vitamin D . The patients with MS suffering from deficiency in vitamin D level and Sintzel *et al*. pointed to relationship between vitamin D levels in serum and development and activity modifications of MS in patients ; besides the lower level of vitamin D , the higher risk of MS development [41] .

Besides the levels of (25- hydroxyvitamin D) in many cases of MS are much lower when compared with healthy peoples , as well as the patients with active form of disease have lower levels of vitamin D than those with the disease with less active state , and this indicated a significant inverse relationship between serum 25(OH) D and the activity of clinical state of MS [42] .

Evaluation the relationship between the intake of vitamin D from diet or supplements and the risk of development of MS showed that , the women with highly intake of vitamin D supplement had a lower incidence with 33% less of MS development in comparison with women who taking low or no supplements of vitamin D ; moreover, the women that used supplements of vitamin D had a 41% reduction in development of MS disease in comparison with non-user women ; and that predict to

reducing the onset of MS risk [43] .

Furthermore , an individual etiological factor for MS , vitamin D reported to interact with other causes of MS like , genetic factor as HLA-DR1 to environmental factor as a smoking [42] . Decrease in serum level of vitamin D due to genetic causes supposed to increase the susceptibility to MS by fifty percent ; while increasing the level of 25(OH)D by 50% may decrease getting MS at about 50% [44] . On the other hand a vitamin D daily dose or a gestational dose per day ; may *"keeps the MS doctor away"* is not proven until now ; in addition in a large majority , the level of serum 25(OH)D may not prevent MS of many peoples [45] .

Runia *et., al*. told that risk of relapse may reduce significantly in individuals with medium (20-40 ng/mL) or high as (> 40 ng/mL) levels of vitamin D in comparison to those individuals with levels lower that [46] . The association between MS lesions development and the levels of vitamin D showed that individuals with high levels of vitamin D are developing much lower newly lesions and of enhancing lesions gadolinium (Gd+) [47] . Moreover incresed level of 25(OH)D related to less active rate of new lesions and relapse rate at 57% [48] .

The receptors of vitamin D especially for 25(OH)D found in many cells of immune system like macrophages and ; and stimulation of these cells through the receptors can inhibit the immune response and cytokine production ; and in patients with MS , vitamin D supplements can the level of IL-2 mRNA in mononuclear cells [49] . In patients with MS, the immunomodulation by 25(OH) D ,can increase the anti-inflammatory cells and inhibit the pro-inflammatory cells , which reduce the severity disease ; after the intake of vitamin D dietary(1000 IU/day) also the cytokine profile modification can reduce inflammation and this can be demonstrated in patients with MS [50] .

As well as in the genomic level vitamin D receptor (VDR) gene polymorphisms have been demonstrated to be link to numbers of diseases such as MS [51] . Furthermore a similar result showed the significant differences between the patients and controls of genotype distribution of (VDR) genes [52] . On the other hand , Cakina *et al,.* showed that , there is no significant differences between MS patients and control groups [53] .

Conclusion

In spite of the controversy role of CMV in triggering of MS ; this virus play a major role in this disease either by induction of disease by activation of immune system against the myelin of CNV by molecular and antigenic mimicry or by reduce the reactivity of disease and lesion formation due to evasion of immune system by periods of latency of these viruses. Vitamin D is not only act a vitamin it is a gene and cell regulator . It is play a crucial role in induction of many diseases like cardiovascular disease , diseases related with the nervous system and immune system . And its role in MS induction and activation is clear , and its role of it and its receptors need for more investigations.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kerbala – Veterinary college / Microbiology Dept. /Iraq and all experiments were carried out in accordance with approved guidelines.

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Potential Factors Associated with Epilepsy among Epileptic Patients Attending Middle Euphrates Neurosciences Center - Al-Najaf City

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Abstract

Objective: To identify the some factors associated with epilepsy. A Case-control study was carried out at Middle Euphrates Neurosciences Center in An-Najaf City- Iraq, the study was conducted from mid of October 2016 until mid of March 2017. Participants recruited in the study were selected as conveniently, (74) of them were already diagnosed by neurologist as epileptics patients and considered as cases, while (140) were non-epileptic from same setting considered as control group. instrument structured for this purpose, data collection was done using pretested questionnaire which consist of two parts, first one included: biographical data (age, gender, marital status, level of education, occupation, and resident). The cases and control groups are age and sex matched. Epileptic patients had a significantly low level of education (Not read and write) 34.4% as compared to healthy control group 13,3% this difference is statistically significant $p<0.05$ the Odds Ratio (OR) was 2.1.,the following possible risk factors were significantly associated with epilepsy in this study; unemployment OR= 2.7 , positive family history OR=1.8, central nervous system lesions OR= 4.3 and natal defects OR=8.3.

Keywords: *epilepsy, risk factors, Iraq*

Introduction

Epilepsy is a common health problem that effect peoples and it is featuring as a seizure attacks with differences in clinical events, that need to be treated by use of medications [6] .Epilepsy can occur at any age, race, ethnic [5]. More than 10 % of people around the world were effected with single attack of abnormal movement, generally single attack not classified as epilepsy if not affected at least two unprovoked seizures attack separated by 24 hours, but the person at risk for recurrent and appear as epileptic patient [8]. About 724,500 epileptic patients are distributed in Arab countries according to epidemiological review in Arab countries for epilepsy [1]. The risk evolving for epilepsy in life of person along life time is 1.4-3.3 % [13]. Many factors are associated with occurrence of secondary epilepsy or considered as risk factors for it if present in person like family history, any brain lesion (tumor, trauma, and cerebrovascular accident), and central nervous system infection. Otherwise if not associated with any mentioned factor consider primary (unknown cause) epilepsy [9]. People who are affected with epilepsy are interesting to identify the risk factors related to underling health condition [11], 25%-45% of epileptic patients, were affected with epilepsy related to many causes such as hereditary, brain structure lesion, or metabolically disturbances cause, while other peoples with epilepsy considered an idiopathic affect [4]. Family history according to antecedent studies consider one of major risk factors affecting young age [8]. It is important to predict the potential risk factor for epilepsy to take preparation of health planning contribution [3]. This study was conducted to identify the risk factors associated with epilepsy.

Methodology

A Case-control design was carried out at Middle Euphrates Neurosciences Center in An-Najaf City which is the single specialist center established in (2010) to cover the needs of neurological consultations for population in south region of Iraq, the study period from 16 October 2016 until 16 March 2017. Data were

collected from (214) patients selected as a convenience sampling, (74) of them were already diagnosed with epilepsy considered as cases group, while (140) were non-epileptic from the same setting considered as control group. This data were achieved verbally by direct interview with both who attend as outpatients of the center, structured questioner was constructed for this purpose, the using form consist of two parts, the first one includes: demographical characteristics (age, gender, marital status, level of education, occupation, and residence), while the second part consists of questions about potential risk factors for epilepsy such as family history, history of central nervous system lesion that result from (infection, congenital defect, trauma, tumor, and other defects for CNS), pregnancy and delivery factors, other potential risk factors, and unknown risk factors for epilepsy. Reliability of the questions was (0.68) of the form were calculated after carrying out a pilot study which included (20) participants who excluded from the study sample, the validity of the study tool was done through reviewing by panel of (10) experts. The selection of patients considered the clinical diagnosis of patients and using of chronic disease identification form which used for free medications and the diagnosis confirmed by electroencephalograph (EEG). Statistically analysis was done using Odds Ratio (OR) with measure the risk, OR more than one considered as a positive association.

Results and Discussion

The current case-control study includes 74(35.2%) epileptic patients and 140(64.8%) non-epileptic patients. Table (1) shows differences in distribution of socio-demographic characteristics among case and control group, males 117 (54.7%) more than females 97 (45.3%) in all sample. Most of them were married 128 (59.8%) and 86 (40.2%) were single. 45 (21.0%) were read and write, while 3 (1.4%) had higher academic level of education. The highest 77 (36.0%) were not working, 136 (63.6%) were living in urban area.

Table (1) Distribution of Socio-demographic characteristics of sample

Socio-demographic characteristics		**Cases**	**Control**
		No. (%)	**No. (%)**
(Age groups) years	(1-18)	40 (54%)	17 (12.2%)
	(19-36)	18 (24.3%)	60 (42.9%)
	(37-54)	9 (12.2%)	46 (32.8%)
	(55 and more)	7 (9.5%)	17 (12.1%)
Gender	Male	41 (55.4%)	76 (54.3%)
	Female	33 (44.6%)	64 (45.7%)
Marital status	Single	53 (71.6%)	33 (23.6%)
	Married	21 (28.4%)	107 (76.4%)
Levels of education	Not read and write	24 (32.4%)	20 (14.3%)
	read and write	14 (18.9%)	31 (22.1%)
	Primary school	19 (25.7%)	22 (15.7%)
	Secondary school	14 (18.9%)	30 (21.4%)
	Academic holder	3 (4.1%)	37 (26.4%)
Occupation	Not working	47 (63.5%)	30 (21.4%)
	Free working	10 (13.5%)	25 (17.9%)
	Office holder	15 (20.3%)	40 (28.6%)
	House wife	2 (2.7%)	45 (32.1%)
Place of Residence	Urban	40 (54.1%)	96 (68.6%)
	rural	34 (45.9%)	44 (31.4%)

Table (1) shows that (1-18) years were the highest age group in case, while in control group the age group (19-36) years was the highest.

In consideration of gender in case group the male were 41 (55.4%) more than female 33 (44.6%), like that in control group the male group was 76 (54.3%) more than female 64 (45.7%).

Regarding to marital status 53 (71.6%) were single case group, while in control group 107 (76.4%) were married.

The highest percentage were not read and write related to level of education in case group was 24 (32.4%), while in control group the highest were 34 (24.3%) for Academic holder.

Regarding to place of residence most of cases 40 (54.1%) were living in urban area in case group and control group 96 (68.6%).

Table (2) Distribution of the Associated Risk Factors Among the Study Group

Risk factors	Exposed	Cases	Control	OR
		F (%)	F (%)	
Family history	Not Exposed	39 (52.7%)	94 (67.1%)	1.83
	Exposed	35 (47.3%)	46 (32.9%)	
CNS lesion	Not Exposed	34 (45.9%)	110 (78.6 %)	4.31
	Exposed	40 (54.1%)	30 (21.4%)	
Antenatal def.	Not Exposed	45 (60.8%)	130 (92.9%)	8.37
	Exposed	29 (39.2%)	10 (7.1%)	
Others diseases	Not Exposed	70 (94.6%)	135 (96.4%)	1.54
	Exposed	4 (5.4%)	5 (3.6%)	

CI (confidence interval)=95%, OR= Odds Ratio

Table (2) presented the distribution of OR (1.83) for (74) epileptic patients in case group 35 (47.3%) patient exposed with family history and 39 (52.7%) are not exposed, in control group 46 (32.9%) exposed and 94 (67.1%) are not exposed, OR (4.31) for 40 (54.1%) exposed to CNS lesion and 34 (45.9%) are not exposed in case group, while in control group 30 (21.4%) exposed to same lesion and 110 (78.6 %) are not exposed, for 29 (39.2%) exposed and 45 (60.8%) not exposed to natal defects in case group, and 10 (7.1%) exposed and 130 (92.9%) not exposed the OR was (8.37). With history of other chronic disease potential risk factor the OR (1.54) for 4 (5.4%) exposed in case group and 70 (94.6%) are not exposed in same group, but in control group the exposed to these factors were 5 (3.6%) and not exposed were 135 (96.4%). The current study curried out to identify the risk factors for epilepsy in 74 participants who classified as disease compared with 150 participants no disease as control group. This study explain the association between different factors studies such as family history, brain lesion, natal and postnatal defects, the Odds ratio in all these exposure are more than 1.5 this means the associations are positive and the factors studies are related to the development of this disease. Family history is one of the major risk factors for epilepsy as that found by [10]. in their study in Iran at 2007, in current study family history had positive affect for occurrence of epilepsy. Central nervous system lesion included infection, congenital defect, trauma, tumor and other defects are positively associated with the study in occurrence of epilepsy in population, this finding is similar to Bhalla D. and Lotfalinezhad E. in their study at 2016 when presented that stroke is a significant risk factor for epilepsy. Wang H. et. al. in their study of Influential factors of epilepsy following aneurismal subarachnoid hemorrhage found that many aneurismal subarachnoid hemorrhage cases result in epilepsy that confirm the reality of brain lesion effect in occurrence of epilepsy. Prenatal, antenatal and post-natal defect history detect in this study have positive effects this defect distributed among (asphyxia, low weight, premature, trauma during delivery, jaundice, febrile seizure) this

finding agree with [7] who find in their study of Epileptic Encephalopathy in Children as Risk Factors for Brain Damage effect of prenatal and perinatal brain defect history on occurrence of epilepsy of many children. In view of point family history considered a risk factor in occurrence of epilepsy together with exposure to trauma and exposure to congenital anomalies and antenatal, post natal.

Conclusion

There are a strong association between epilepsy and family history, nervous system lesion, natal defect and other diseases.

Conclusion

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon –College of Nursing, Iraq and all experiments were carried out in accordance with approved guidelines.

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Acute Infective Bloody Diarrhea in Children Below Five Years Admitted to Children Welfare Hospital in Medical City - Baghdad During 2015

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Abstract

Bloody diarrhea in young children is usually a sign of invasive enteric infection that carry a substantial risk of serious morbidity and death. Objectives : to identify the proportion of infective bloody diarrhea out of total number of gastro-enteritis cases ,also to identify the prevalence of different etiological agents and to find the relation between the main infective etiological types of bloody diarrhea and some sociodemographic factors of this study. Retrospective study was conducted in Children Welfare Teaching Hospital / Baghdad during period extending from 1 st of January to 30 th of June 2016. Information was collected by reviewing the records of patients with acute infective bloody diarrhea under 5 years of age admitted during 2015. The study revealed that the proportion of infective bloody diarrhea was 10. 9% out of the total gastroenteritis (788) cases admitted during 2015, and Entamoeba Histolytica was the most common isolated pathogen in the study42 (48%) patients. followed by E.coli 15 cases (17.4%), shigella, salmonella 7(8%) and 4(4.7%) respectively . The most vulnerable age was those below 2 years (58%) and males were affected more than females (1.3:1), (37%) were bottle fed. The majority had chlorinated tap water supply (82.6%).

***Key wards**: bloody diarrhea/children/under 5/children*

Introduction

Diarrhea is a preventable and treatable disease however it is still a major public health problem particularly in developing world. It ranks as the third leading cause of both mortality and morbidity among infectious diseases, placing it above tuberculosis and malaria [1]. About 80% of deaths due to diarrhea occur in the first two years of life. The main cause of death from acute diarrhea is dehydration. Other important causes of death are dysentery, malnutrition and serious infections such as sepsis [2]. Bloody diarrhea in young children is usually a sign of invasive enteric infection that carries risk of serious morbidity and death. Non-infectious causes account for a very small proportion of episodes of bloody diarrhea [3] About 10% of diarrheal episodes in children under 5 years of age have visible blood in the stool and these account for about 15% of diarrhea-associated death in this age group world-wide [4]. Compared with the watery diarrhea, bloody diarrhea generally lasts longer, is associated with more complications, is more likely to adversely affect a child growth and has a higher case fatality rate [(5,6,7)]. There is also a synergism between bloody and persistent diarrhea accompanied by malnutrition; therefore the control of bloody diarrhea will prevent most of deaths attributed to malnutrition and persistent diarrhea [(2, 8, 9)]. According to WHO, in Iraq, the sanitary condition and general hygiene all over Iraq had been deteriorated during the last decade, particularly following the last war 2003 [(2,10).] However, only a few studies have described the epidemiology of bloody diarrhea among this age group.

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Aim of Study

1. To identify the proportion of infective bloody diarrhea cases and their different etiological factors out of total number of gastro-enteritis.

2. To identify the predisposing factors of bloody diarrhea.

3. To find the relation between the main infective etiological types of bloody diarrhea of the admitted patients and some sociodemographic factors of this study.

Patients & Method

Study design: A Cross-sectional retrospective study.

Study period: it is carried out during the period extending from 1st of January to 30th of June 2016.

Study population: Children under 5 years.

Study place: Children welfare Teaching hospital/ Baghdad.

Study sample: (86) cases of bloody diarrhea out of (788) gastro-enteritis cases admitted in the above mentioned hospital during 2015.

Data collection method:

Patients with bloody diarrhea (three or more loose bowel motions / day with visible blood in stool) [1]. Those with surgical conditions were excluded from the study (e.g. volvulus, intussusception), reports were reviewed and data collected from the case files of the patients (name, age, gender, address, type of feeding, type of water supply , boiling of drinking water, the presence of domestic animals).

Confirmation of diagnosis: for each patient the following was done by the same qualified laboratory personnel by stool culture and slide agglutination test was also done but it was not possible to identify the causative agents in 18 (21%), this could be due to infection with other organisms not routinely investigated in the stool samples or due to the lack of sensitivity of some laboratory procedures that used in this hospital or not present at all, this was similar to studies conducted in our country [10,11].

Statistical Analysis

The data were analyzed statistically by using computerized SPSS system version (20).Descriptive statistics including observed frequencies and percentages. And Chi square test was used to assess the association between two categorical variables. P-value less than 0.05 was considered as a statistically significant, and value less than 0.01 was considered to be highly significant .

Results

Number of patients with bloody diarrhea was (86) from total number of patients of gastroenteritis (788) admitted to Children Welfare Teaching hospital during 2015 so the proportion of bloody diarrhea cases was (10.9%).

Results of examination and stool culture (86) patients identified as follows

Table (1): Isolation rate of enteropathogenes in children with bloody diarrhea:-

Enteropathogenes Isolated	Number and percentages of cases.
E. histolytica	42 (48.8%)
E. Coli	15 (17.5%)
Shigella	7 (8%)
Salmonella	4 (4.7%)
No isolate	18 (21%)
Total	86(100%)

The proportion of causative agents in descending order, E. histolytica 42 (48.8%), E. Coli 15 (17.5%), Shigella 7 (8%) . Salmonella 4 (4.76%) , & 18 (21%) of cultures showed no isolates.

Table (2): Age Distribution of the patients according to etiological agents:

Age(month)	Etiological agent			Number of patients
	Bacterial	Amoebic	Unknown	
1-12	4 (18.1%)	13 (59%)	5(22.7%)	22(25.6%)
13-24	9(32.2%)	17 (60.7%)	2(7.1%)	26(32.6%)
25-36	4(40%)	3 3(30%)	3(30%)	10(11.6%)
37-48	7(46.6%)	4(26.7%)	4(26.7%)	15(17.4%)
49-60	2(18.1%)	5(45.5%)	4(36.4%)	11(12.8%)
ToTotal	26	4242	18	86

X^2= 20.95 ,P value = 0.001.

The majority of bloody diarrhea cases 50 (58.2%) found in those who were below 2 years. There is highly statistically significant difference (p value < 0.01) between different etiological agents and age group.

Table (3): Sex distribution of the patients according to the etiological agents :

Sex of child	Etiological agent			N. No of Patients
	Bacterial	Amoebic	Unknown	
Male	16(32%)	23(46%)	11(22%)	50 50(58.2%)
Female	10(27.8%)	19(52.8%)	7(19.4%)	36(41.8%)
Total	26	42	18	86

X^2= 1.97, P value = 0.04

Males were more affected 50 (58.2%) than females 36(41.8%) There is significant difference between total number of cases and gender of the patients p< 0.05.

Table (4): Distribution of the patients and their etiology according to the type of feeding

Type of feeding	Etiological agent			No. of patients
	Bacterial	Amoebic	Unknown	
Breast	12 (46.1%)	9 (34.6%)	5 (5(19.2%)	26(30.2%)
Bottle	6 (18.7%)	20 (62.5%)	6 (6(18.7%)	32(37.2%)
Diet	4 (30.7%)	6 (46%)	3 (3(23%)	13(15.1%)
Mixed	4 (30.7%)	7 46.6%)	4 (4(26.6%)	15(17.5%)
Total	26	42	18	86
X2 X2= = 12.47, P=0.002 .				

There are statistically significant differences (P value < 0.05). Between number of cases of bloody diarrhea and different types of feeding showed more patients admitted with bottle feeding 37.2%.

Table (5): Distribution of the patients and their etiology according to the types of water supply:

Water supply	Etiological agent			NO. of patients
	Bacterial	Amoebic	Unknown	
Tap water	21 (29.5%)	36(50.8%)	14(19.7%)	71(82.6%)
Others like river, irrigation, canal, wells	5 (33.3%)	6(40%)	4(26.7%)	15(17.4%)
Total	26	42	18	86
X2 X2= = 24.7., P value = 0.001				

There is a highly significant (P value < 0.01) relationship between total number of patients and different types of water supply. 71(82.6%) of patients used tap water. There is also a relationship between different etiological factors and type of water supply. Tap water associated with more amoebic diarrhea36 (50.8%) than bacterial 21(29.5%).

Table (6): Distribution of the patients and their etiology according to the habit of boiling water:-

Boiling of water	Etiological agent			NO. of patients
	Bacterial	Amoebic	Unknown	
Yes	7(46.7%)	5(33.3%)	3 (20%)	15(17.44%)
No	19(26.8%)	37(52.1%)	15 (21.1%)	71(82.56%)
Total	26	42	18	86
X2 X2= = 5.6, P value = 0.001 There is a highly significant P value < (0.01) relationship between total nunumber of patients and habit of boiling water , showed more patients of bloody bl diarrhea were using un boiled water 71(82.5%) .				

Discussion

The study revealed that the number of cases of bloody diarrhea was (86) cases from (788) cases of total gastroenteritis admitted during 2015, the percentage was (10.9%) this result is similar to [7] in Baghdad .

Entameoba histolytica_was the most common isolated pathogen as it was detected in stool sample of the patients. This finding was similar to that observed in studies conducted in Basrah by [12, 13 , 14] in Baghdad. Also in other studies done by [15,16,7].

The opposite finding was reported in many studies in different countries which showed that shigella species were the most common agent associated with bloody diarrhea [5,6,17 ,18,19] the reason for this difference could be explained by the fact that invasive amebiasis is an important public health problem and occur globally in endemic areas [3] . On the other hand, probably amebiasis is routinely over diagnosed and laboratory reports may be of little value, a study done by Walsh support that [20] .

Entameoba histolytica cyst was found in (38 %) of bacterial bloody diarrhea. This means that more than one pathogen may be isolated from patients with bloody diarrhea. This is in agreement with studies done by [21,16]. [22] found that association of E. coli with Entameoba histolytica infection will enhance amebic virulence.

It was not possible to identify the causative agent in 18 cases (21%). This could be due to infection with other organisms not routinely investigated in the stool samples or due to the lack of sensitivity of some laboratory procedures, This is in agreement with other studies [10,6,18].

The age of the most affected children was between (7-12) months. A similar result was also found by [8 , 23] . Susceptibility of this age group to bloody diarrhea may be explained by many factors such as declining level of maternal immunity , introduction of solid food which may be contaminated by enteropathogens , together with introduction of foreign material to the mouth of these children as they have learned to crawl up and pick subjects in their hands by this age ,which increases the risk of exposure to fecal pathogens [24,25] . Also most of the admitted cases were in this age group ,because of high worry of the families toward their children at this age group [2,10].

Male sex was affected more than female with ratio of 1.6:1. This is agreement with other studies [10,6,17], while the opposite found by [18].

The significant difference in sex distribution was in those amebic dysentery, male to female ratio 2.2:1. This was also found by [25,26,27] in Ethiopia .

A high percentage were bottle fed 32 (37%) while breast fed is less 26 (30%) and this strengthen protective value of breast milk against diarrhea, weather bloody or watery. Similar results were found by [2,23,25,28]

The majority of the patients included in the study had tap water supply 71 (82.6%) while other sources is just 15 (17.4%).However, even chlorination of water cannot kill amoebic cyst or some other enteropathogenes this was aided by some studies [1,10], or it may be due to contamination of water system by ground water responsible for outbreak of amoebic dysentery [29].

Conclusions

1. The proportion of infective bloody diarrhea out of total gastroenteritis cases was relatively low .

2. Entamoeba histolytica is one of the most frequent offending pathogen in patients with bloody diarrhea in our society.

3. Children below 2 years are the main affected group and male sex affected more than females .

4. Bottle feeding preference, non-boiling of drinking water make children more prone to have bloody diarrhea.

5. Tap water use was associated with cases of bloody diarrhea.

Source of Funding: Self-funding.

Ethical Consideration: Approval to conduct this study was obtained from concerned authorities (Children Wefare Hospital in Baghdad),as well as the name of the participants was kept hidden.

Conflict of Interest: Nil

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Comparison between Hypertensive and Non-Hypertensive Individuals in the Value of Response to Salt Administration

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Abstract

Background: consumption of,salt (sodium-chloride) causes,increment of blood pressure (B.P) in both normal and,hypertensive individuals, that is why,the use of diuretics which eliminate salts from the body decreases the B.P. and used as a line of treatment,in hypertension.

Objective: the study,aims to know whether there is any,difference in the increment of B.P. both systolic and diastolic between normal people and hypertensive people after giving salt orally.

Method: one hundred individuals were involved in the study, 50 patients were hypertensive,individuals (25 males and 25 females) and labelled as group A, and 50 persons (25 males and 25 females) were normal individuals and labelled as group B and played as control. After full physical examination including 3 times B.P. checking before and after giving 6 gm of sodium-chloride (table salt) orally ; after 2 hours the values of B.P. were recorded and statistical,analysis was done to know the difference,between the two groups.

Results: in all individuals of both groups, the B.P. increased,after salt consumption. In group A (the hypertensive group) the mean increment,of systolic B.P. (S.B.P.) was (15.98) mmHg and the mean diastolic B.P. increment (D.B.P.) was (9.4) mmHg , while the mean S.B.P. increment,in group B was (8.7) mmHg and mean D.B.P. increment was (8.0) mmHg.

Conclusion: the study showed significant difference in the increment of B.P., both systolic and diastolic between the two groups, showing that hypertensive group is more sensitive to salt consumption than normal individuals (the control group).

Keywords: *systolic B.P. , diastolic B.P., increment, sodium-chloride.*

Introduction

Hypertension (HTN) is a major health,problems world wide [1]. It is defined as chronically,elevated blood pressure (B.P.) and the British hypertensive society defined B.P. as :1- optimal if the B.P. > 120 mmHg systolic,and less than 80 diastolic, 2- normal B.P. as > 130/85 mmHg and 3- high normal of 130/89 mmHg [2].

Elevated,arterial B.P. is a major cause of premature vascular disease leading to cerebrovascular events, coronary heart disease,and peripheral vascular disease [3] .

In the past 1-2 decades, hypertension varied in prevalence,from western compared with eastern,countries e.g. it was very high in Germany and lowest in china. Now a days,the differences are less [4] .

Recently no much difference was found between developed,and developing countries, although it is still high,especially among black Africans (40-45-%) prevalence rate.

According to WHO reports in 2008, the prevalence,of high B.P. in Iraqi people was 40% among 25 years,old and above , in Oman also 40% (NCD/ Oman country WHO https:// www. WHO. Int/ beat – ncd / countries/ Oman/ enl). In Indian WHO 2008 also reported a prevalence,rate of (32-5)%. In November 16, 2006 the largest rise in prevalence of adults has been in Canada & U.K.

It is documented that HTN immediately,rises after the age of 60 years [5].

Essential hypertension,has no single or definite cause, there are multiple factors e.g. heredity, environmental,factors e.g. obesity, alcohol, smoking,and high salt intake and,probably other factors [3] .The effect of salt,consumption on B.P. differs among different,people and high potassium intake does the opposite effect.

Japanese who live north east of Japan eat more apple,which is rich in potassium suffer from low,cardio-vascular hypertension complications e.g. apoplexy compared with other regions in,Japan [6].

A positive linear correlation between,salt intake and B.P. which has been considered,since ancient times [7].

Experimental observations over the,few past decades have indicated that B.P. can significantly be reduced with salt restriction,and enhancing sodium excretion in urine,with the use of diuretics [8, 9, 10].

Diner stein in August. 9. 2018 wrote that 1 gm of salt consumed,increases B.P. by 3 mmHg [11] .

Patients and methods:

One hundred individuals were a dopted to this epidemiological,study. Fifty patient with hypertension,either having uncontrolled or controlled,B.P but should have BP less than 160/100 mmHg to avoid complication during the study.

This group is labelled as group A, they were 25 males,and 25 females. The same for normal individual, as control(group B) , for comparison they accepted to carry out the study,by their own wish (volunteers).

The study was conducted in the Al-Hussain teaching,hospital (Al-Hussain medical city), at the out patient medical,clinic. Hypertensive patients were labelled as group A and companions,of patient were labelled as control.

The study was conducted,from first February to first June 2019. For every individual after complete physical examination,including taking B.P at sitting position 3 times with 5-10 minutes,intervals in between, taking the mean B.P for both group, then 3 ml of blood was taken from each person for routine,blood test e.g.RBS, or (FBS) B. urea, S. creatine ,S. uric acid and CBP.

Blood pressure was taken,according to the strict B.P guide line, suspected white coat hypertension persons, pregnant,ladies and patient with renal failure were excluded from the study as well as patient,with B.P higher than 160/100 mmHg.

Then each individual was given 6 gm. of pure salt (measured by gold balance). Two,hours later the B.P was re-measured as before taken the average B.P. finally all data were collected,and statistically analyzed.

Results and Discusion

One hundred,individuals were studied. Fifty hypertensives and 50 normals as group A and as group B respectively. Each group,contained 25 males and 25 females they were matched for age and sex. Their ages ranged from 35-78 years with a mean of 60 $^{+}$_5 SD. The majority of individuals,lie in the age group 57-78 years (69%) , (table 1).

The prevalence rate of hypertension,for females and males differ slightly with 37.8% vs. 38.5% respectively – (table 2). The degree of increased,in S.B.P. among group A ranged from 1 to 20 mmHg with a mean of 15.98 - (table 3), while the degree,of increment of D.B.P. was also from 1-20 with a mean of 9.4 mmHg.- (table 4).

In group B the average,value of increment of S.B.P. was 8.2 mmHg and for D.B.P was 8.0 mmHg (table 6)

Table (1): Age distribution of both groups (n = 100)

Age groups in years	No. of individuals
35 – 45	11
46 – 56	20
57 – 67	33
68 – 78	36
Total no.	100

Table (2): Prevalence to Hypertension in both groups matched with age

Sex	Prevalence rate
Males	38.5
Females	37.8

Table (3): value of increment of Systolic B.P. in group A in mmHg (patients)

Value of increment	No. of patients	Average
0 – 5	1	15.98
6 – 10	8	
11 – 15	23	
16 – 20	15	
< 20	3	
Total no.	50	

Table (4): value of increment of Diastolic B.P.in group A in mmHg (patients)

Value of increment	No. of patients	Average
0 – 5	18	9.4
6 – 10	20	
11 – 15	11	
16 – 20	1	
Total no.	50	

Table (5): value of increment of Systolic B.P. in group B in mmHg (control)

Value of increment	No. of individuals	Average
0 – 5	25	8.7
6 – 10	15	
11 – 20	10	
Total no.	50	

Table (6): value of increment of Diastolic B.P.in group B in mmHg (control)

Value of increment	No. of individuals	Average
0 – 5	22	8.0
6 – 10	23	
11 – 20	5	
Total no.	50	

In industrialized,societies the high degree of inter-individual variability or difference of sodium intake compared to much smallesr inter individual,deference may obscure,potential biological association probably,better solved by quantitative,statistical method using,urinary sodium estimation [12] . The age distribution in the study (table 1) showed higher prevalence,value as the age advances. It is reported that the average systolic,B.P (SBP) rises with age while the diastolic,B.P. (D.B.P.) rises up to the age of 50 years and then declines [13] . The sex deference in prevalence,rate also showed little difference with slight male preponderance. After adjusting for age no significant,difference between sexes in mean prevalence except for higher prevalence among men in developed,countries [14]. The average male,prevalence rate in developed,countries is 40.8% VS 32.2% in developing countries , while in female the prevalence rate was 33.0% VS 30.5% respectively, these figures are not far away from the study figures (table2) . High salt intake is the major,cause of raised B.P and,accordingly leads to cardiovascular disease as well as,recently showed association with increased risk of obesity through sugar- sweetened beverage,consumption [15]. Uptill now the exact pathophysiology,of how increased salt intake,increases B.P is unknown, but the known fact is that high salt intake,increases B.P, while reducing daily,salt intake reduces B.P, as well as by using diuretics [16].

Conclusion

The study showed that salt intake,increases both S.B.P. and D.B.P., but the increment is different in the two groups (A & B) e.g the value of increment is more obviously higher in group A (the hypertension,group), than group B (the control group) as shown in,tables from 3 to 7 . especially noted by highly significant increment

of the S.B.P. in group A compared,with group B but to a lesser extent regarding,increment in DBP between the two groups.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the AL- Safwa University College, Karbala province, Iraq and all experiments were carried out in accordance with approved guidelines.

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Effect of Lactic Threshold Exercises using us Ginseng in the Development of Some Functional Variables of the Lungs and Lactic Tolerance and the Performance of Complex Offensive Skills in Basketball Youth

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Abstract

The aim of the study is to prepare the lactic threshold exercises using US ginseng in improving some functional variables of the lungs and the lactate tolerance and performance of the offensive skills of the young basketball players. The researcher used a curriculum Alter Webby B test kisses me and posttest of the two groups of experimental (Experimental and first trial) . Dosages of US ginseng were used in the form of capsules (400 mg) and 5% ginsosides, equivalent to 20 mg. Thus, the total US ginseng for the program is 60 g, Sexosides. The superiority of the first experimental group was determined by the second experiment with all the research variables. Effect of lactic threshold exercises using American ginseng in the development of some functional variables of the lungs, lactation and complex offensive skills in basketball for youth.

Keywords: *Lactic threshold exercises, Lungs, Lactic tolerance*

Introduction

The modern sports training depends on the concentration of its objectives for the development of energy production systems and the associated functional changes. The more aerobic or anaerobic exercise is possible, the more direct it will be on the level of physical and professional performance. Training also leads to many changes, whether physical , functional or chemical. The most important chemical changes that are affected by the training is the concentration of lactic acid in the muscles and blood as the anaerobic training, which lasts for (1-3) minutes, oxidizes the sugar to anaerobic, which leads to the production of acid The higher the duration of the anaerobic action, the higher the ratio of the accumulation of lactic acid in the muscle, which in turn leads to slower other chemical processes. As the game of basketball is a game in which the work of the anaerobic system (Lactic) high rate and this is a sign that during the games there will be accumulation of lactic acid in large quantities so players have to bear this accumulation in the muscle and blood and not stop the player and work fatigue early. This is also related to the functional variables of the lungs, which work on many important functions of runners, including oxidation of oxygen through the vesicles pulmonary. , Nutrition science is now one of the applied sciences that depend on it in the field of physical education and sports. Nutrition has been associated with exercise for health because it has an important role in weight control and control of body composition. Nutrition has also been linked to the competitive sports field during its various stages In training or in competition, nutrition plays an important role in the ability to tolerate training, delay the onset of fatigue, as well as the speed of recovery, as evidenced by its important role in the competition to raise the level of performance.

Methodology and field procedures

Research Methodology

The researcher used a curriculum Altger Webby B) Pre and post test for the experimental groups (first and second

experimental) to suit the nature of the research.

The research community and its design

Itwasidentifiedresearchsocietymyplayersbasketball youth belonging to the Sports Club MAHAWEEL ages (16-18 years) training for the season 2018-2019 and the 14 players. The sample was selected in a comprehensive inventory method and the sample was divided into two experimental groups with seven players per group.

Homogeneity and equivalence of the sample:

In order for the work of the researcher to proceed in the right direction and the objective of the work of the researcher , he found the homogeneity and equivalence between the two groups of research in terms of physical measurements (length, mass, and training age and time as well) on the functional variables of the lungs and lactic endurance and offensive skills complex using the appropriate statistical treatments to know the truth Differences between the two research groups as shown in Table (1, 2).

Table (1) Shows the homogeneity of the sample

T	Variables	measruing unit	Test Phrase Leven)		Indication of difference
			Calculated	Standard error	
1	Age	Year	1, 6 46	0, 4 22	Not significant
2	Training age	Year	0.3 9 2	0, 5 29	Not significant
3	Bloc	Kg	0, 089	0, 9 71	Not significant
4	Length	cm	1, 5 70	0, 4 20	Not significant

Table (2) It shows circles and standard deviations and the values of (t) And the significance of differences in the tests examined between the experimental groups in the pre- test

Variables	measuring unit	The second experimental group		First experimental group		Values t Calculated	mistake percentage	Significance of differences
		s	P	s	P			
Compulsive dynamic capacity	L / d	3.64	0.6 67	3.535	0.347	0.542	0. 5 31	random
Forced volume is one second	L / d	3.566	0.3 87	3.387	0.382	0. 1 87	0.3 22	random
Maximum lung ventilation volume	L / d	102.9	12.201	100.9	12.68	0. 2 83	0.3 43	random
Pick up and jump by 2 points	Degree	4.2 71	1.6 67	4.267	1. 318	0. 1 42	0.4 22	random
Pick up and jump 3 points	Degree	1.1 48	0.7 93	1.9 59	0:561	0.0 7 7	0.3 0 1	random
Receiving and high-pitched finished pacification	Degree	5.3 91	1.9 63	4. 891	0.8 29	0. 1 41	0.2 06	random
Hold the locket	Accurate	1. 331	0.1 19	1. 410	0.1 11	0.5 87	0. 4 73	random

* Significant at the level of significance (0.05) if the error level is less than (0.05).

Determination of measurements and tests used :

1/ measurement of lung function variables.

Functional variables of the lungs were measured through a device Vica test Spirometer. The variables are:

1- Compulsive dynamic capacity Forced Vital Capacity .

2- The expiratory volume is forced for one second Forced Explorative Volume in one Second

3- Maximum lung ventilation volume Maximum Voluntary Ventilation .

Measuring tolerances Lactic :

The researcher has access to a lot of scientific studies concerned with the physiological domain where Kojnham and Vlawkins test run for carrying lactic [2] . Where a questionnaire was distributed to a number of experts to clarify the validity of the tests and their suitability to the sample and after a statistical analysis proved to be valid for measuring the variables under study .

Measuring offensive basketball skills:

Through the researcher's knowledge of many sources and scientific studies in the field of basketball has been identified offensive skills compound is : (receipt + jump correction / two points) . (Receiving + jump correction / three points) and (receiving + high pitched + peaceful correction). And have been identified for offensive skills tests the vehicle surveyed by seeing a lot of references and scientific studies Ot three tests churned the nomination (Faris Sami) [3] . Where were distributed a questionnaire to a number of gentlemen experts form to clarify the extent of the validity of the tests and the suitability of the sample after it was found statistically analyzed in his favor to measure the variables under study.

Determination of US ginseng doses:

The US was determined Alzhinsnj doses used in research where the researcher reference studies, research and scientific references survey [4] Which dealt with the use of US ginseng to identify the permissible and effective doses in the human body, where it was found that:

1- Effective doses of adults aged 20-30 should be between 50-75 g and 2 to 8 g for a period of 8-12 weeks maximum.

2- American ginseng should be taken every 12 hours, twice a day.

3- It is best to take the American ginseng before eating to increase the absorption speed of the food and to take advantage of it more.

4- Use of US ginseng should not exceed 3 months because it leads to vitamin deficiency B6 In the body leading to feeling numbness and depression.

5- The good product for American ginseng is the one that contains ginsocide Ginsenoside By between 4 and 7 percent.

On this basis it was used doses of American Alzhinsnj in the form of capsules (400) mg and Psonbh Jnsosadat 5% is equivalent to 20 mg, bringing the total US Alzhinsnj program completely is (60 g), and the equivalent of (3 g) of Gansusaadat, table The following illustrates this.

Table (3) US ginseng dosages used in research

Subject	size Capsule	The dose Per day the one	The dose the college Of the program (10)weeks	The dose the college Of the program (G)
Ginseng American)capsule (	400milligrams	800 mg (2 capsules)	800 x 75 days = 60,000 mg	60 g
Sexesides	20 mg	40 mg	40 x 75 days = 3000 mg	3 g

8 - reconnaissance experience:

The researcher conducted the pilot experiment on a sample of (5) players on 5/12/2016 at 3:00 pm in the stadium of Al Mahaweel Sports Basketball Club. The exploratory experiment led the researcher to identify:

1- The validity of the equipment and tools used in the research.

2- The time taken to perform the tests.

3- Stand on the difficulties that may be exposed to it when the researcher giving a C of the President tests.

1/ scientific transactions for testing:

I. Honesty:

The researcher concluded the validity of the tests in question through the questionnaire distributed to the experts and specialists who indicated its validity to measure the (tactical endurance and complex offensive skills).

2 - Persistence: The researcher tried to find the coefficient of stability of the tests, by finding the correlation between the results of the first and second test after retesting on the survey sample after three days of the first test, and after calculating the coefficient of correlation (Seberman) of the ranks between the results of the first and second test and As shown in Table (4), the correlation coefficients were extracted by T-law. It was found that the correlation was significant at the degree of freedom (3) and the significance level (0.05). The calculated value (T) was greater than the maximum scale (2.11) The test has a high degree of stability.

III. Objectivity: The objective researcher concluded by finding the correlation between the results of two judgments. The correlation coefficient (Siberman) for the rank between the results of the two sentences confirms that the test is highly objective, since the value of (T) (2,11), at the degree of freedom (3), and the level of significance (0.05), see Table (4).

Table (4) Shows the value of the coefficient of stability and objectivity and statistical significance of the tests examined

the test	measuring unit	Stability coefficient	Values(T) calculated	Statistical significance	Subjectivity coefficient	Values(T) calculated	Statistical significance
Lactic endurance	Accurate	0, 897	4 , 221	moral	0, 878	3 , 998	moral
Pick up and jump by 2 points	Degree	0. 889	4.229	moral	0. 889	3. 987	moral
Pick up and jump 3 points	Degree	0. 889	5.129	moral	.873	4.228	moral
Receiving and high-pitched finished pacification	Degree	.858	3. 987	moral	0. 890	4.721	moral

Values (t) Table 2.11 at degree of freedom 3 and level of significance 0.05

9 / tribal tests: The researcher conducted tribal tests on Sunday , corresponding to 10/12/2018 three o'clock in the afternoon and after giving researchers a brief explanation of how to perform the tests and the aim of made and then taking measurements to height, weight, age and the time training and then conducted skills tests And physical research on the sample .

1. Presentation, analysis and discussion of the results of the two groups that are not involved in the variables investigated.

a- display the results of the differences between the pre and post tests experimental group first in the surveyed variables and analysis.

Table (5)Shows the difference in the computation and its standard deviation and the value of (t) And the significance of the differences between the results of the pre - and post - test of the first experimental group in the variables under consideration

the exams	measuring unit	Tribal Test		Post-test		Values t Calculated	Error level	Significance of differences
		s	P	s	P			
Compulsive dynamic capacity FVC	(L)	3.535	0.347	4.398	0.712	2.793	0.000	moral
Forced volume is one secondFEV 1	(liter)	3.387	0.382	3.787	1.397	2.561	0.021	moral
Maximum lung ventilation volume MVV	(L)	100.9	12.68	108.6	12.71	4.670	0.011	moral
Receiving +correction 2 points	Degree	4.276	1.218	7.876	2.132	7.761	0.000	moral
Receiving +correction 3 points	Degree	1.999	0 .541	3.761	1.897	5.776	0.023	moral
DOOR STEP +clapotement+ peaceful correction	Degree	4.221	0.869	7.359	2.111	7.136	0.000	moral
Lactic StressTest	Accurate	1.510	0.101	2.81	0.29	4.698	0.003	moral

* Significant at the level of significance (0.05) if the error level is less than (0.05)

Show me from the table (3) The level of error ranged from (0.00 to 0.023) For all search variables which is smaller than (0.05) b , indicating the significance of the differences between the tribal and remote tests at the level of error (0.05) in front of the degree of freedom (6) and for the post-test.

b- display the results of the differences between the pre and post tests of the experimental group second in the surveyed variables and analysis.

Based on the above results (Table 5 . 6 . 7) is clear we have significant differences in the number of lung function variables between pre and post tests for the two sets of research for the benefit of the post test, attribute the researcher the reason for this to the evolution in the level of players through the use of threshold training The importance of physiological abilities in improving construction and development as a natural result of training that contributed to the development of lung variables. The researcher also attributed this improvement to the improvement in the lungs by increasing the capacity of the vital, and increase the strength of breathing muscles and large volume of breath, To Zia The exchange of gases with the blood more after the training of the threshold of Latakia differential (8) weeks with (3) weekly training units. He confirmed (Mohamed Osman , 1990 "The world has started to rely entirely on energy systems in sports training. This has been demonstrated by the field experiments that have emphasized the need to match the special performance requirements during the exercise with the energy system working during the competition to ensure the largest percentage of the contribution of special exercises in refining the athlete and achieving the required achievement " [5].

CONCLUSION

Lactic threshold exercises using ginseng have contributed to the development of the investigated variables (combined offensive skills, lactation tolerance, and functional variables of the lungs) by: The results showed a superior superiority between the tribal and remote measurement in the (combined offensive skills) of the players of the first experimental group and for the benefit of the post measurement. The results showed a superior superiority between the tribal and remote measurement in the (non-tactical endurance) of the players of the first experimental group and for the benefit of the telemetry. The results showed a superior superiority between the pre - and post - natal measurement in the functional variables of the lungs for the first experimental group and for the post - measurement . The first experimental group is superior to the second experimental group in the telemetry of the investigated variables and for the benefit of the first experimental group .

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kerbala /college of Education for pure Sciences, Iraq and all experiments were carried out in accordance with approved guidelines.

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