الجامعة التقنية الوسطى

كلية التقنيات الصحية والطبية /بغداد

قسم تقنيات التحليلات المرضية

المادة: فايروسات

المرحلة:- الثالثة

Title:-

General properties of viruses Lecture 1

Name of the instructor:

Assist prof dr. Athraa Zaidan Hassan

استاذ مساعد د. عذراء زيدان حسن

Target population:

Three stage students in department Medical Laboratory Techniques

Introduction

Viruses are the smallest infectious agents (ranging from about 20 to 300 nm in diameter) and contain only one kind of nucleic acid (RNA or DNA) as their genome. The nucleic acid is encased in a protein shell, which may be surrounded by a lipid-containing membrane. The entire infectious unit is termed a virion. Viruses are parasites at the genetic level, replicating only in living cells and are inert in the extracellular environment. The viral nucleic acid contains information necessary to cause the infected host cell to synthesize virus-specific macromolecules required for the production of viral progeny. During the replicative cycle, numerous copies of viral nucleic acid and coat proteins are produced. The coat proteins assemble together to form the capsid, which encases and stabilizes the viral nucleic acid against the extracellular environment and facilitates the attachment and penetration by the virus upon contact with new susceptible cells. The virus infection may have little or no effect on the host cell or may result in cell damage or death.

`Pre-test

Define Virion ?

Scientific Content

Medical Virology :- The science that deal with the study of the medically viruses which infect human**.**

Virus is a broad general term for any aspect of the infectious agent and includes**:-**

- The infectious or inactivated virus particle.
- Viral nucleic acid and protein in the infected cell.

Virion:- is the physical particle in the extra-cellular phase which is able to spread to new host cells; complete intact virus particle. The whole virus particle is called (Virion)

General Properties of Viruses

1.Viruses are smaller than bacteria, they range in size between 20-300 nanometer(nm).(Table- 1-).

2. Viruses contain only one type of nucleic acid, either DNA or RNA, but never both.

3. Viruses consist of nucleic acid surrounded by a protein coat. Some viruses have additional lipoprotein envelope.

4. Viruses lack cellular organelles, such as mitochondria and ribosomes.

5. Viruses are obligate cellular parasites. They replicate only inside living cells.

6. Viruses replicate through replication of their nucleic acid and synthesis of the viral protein.

7. Viruses do not multiply in chemically defined media.

8. Viruses do not undergo binary fission.

Table (1) : Comparison between viruses and bacteria

No	property	Viruses	Bacteria
1	Size	20-300 nm	1000nm
2	Genome (type of nucleic acid)	DNA or RNA but not both	DNA and RNA
3	Cell wall	Envelope present in some viruses	Cell wall
4	Ribosomes	No Ribosomes	Ribosomes
5	Multiplication by binary fission	-	+
6	Sensitivity to antibiotics	-	+
7	Growth in culture media	Growth only in the living host cell	Grow in culture media

The structure of viruses:

1-Viral nucleic acid:

The viral nucleic acid is located internally and can be either single or double- stranded RNA or DNA. The nucleic acid can be either linear or circular. The DNA is always a single molecule, the RNA can exist either as a single molecule or in several pieces (segmented).

• Some RNA viruses are positive polarity and others are negative polarity.

• Positive polarity is defined as an RNA with same base sequence as the mRNA. (positive strand RNA)

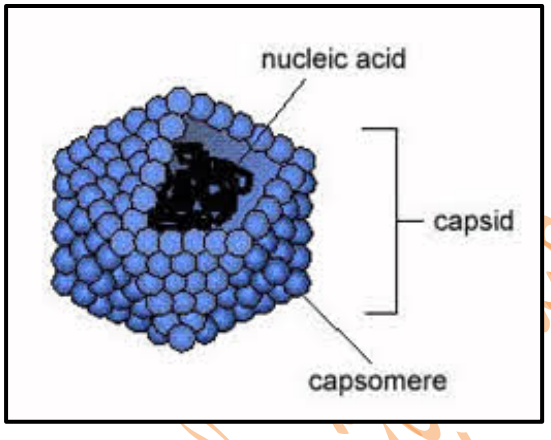
• Negative polarity has a base sequence that is complementary to the mRNA (Negative strand RNA) .

2- Capsid

The protein shell, or coat, that encloses the nucleic acid genome and mediates the attachment of the virus to specific receptors on the host cell surface.

3- Capsomeres

Morphologic units seen in electron microscope. Each capsomere, consisting of one or several proteins. Naked viruses are composed of nucleic acid + capsid (nucleocapsid). (Figure -1-)



Figure(1)Naked virus composition

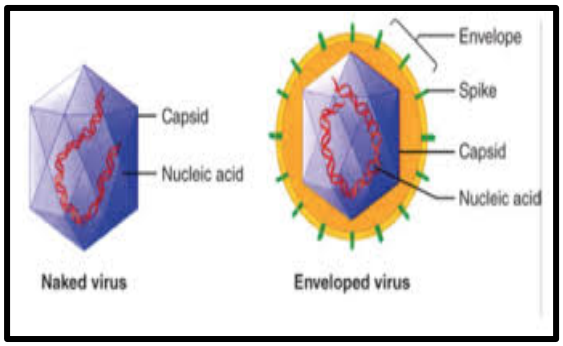
4- Viral Envelope

The envelope is a lipoprotein membrane composed of lipid derived from the host cell membrane and protein that is virus- specific. Furthermore, there are frequently glycoproteins in form of spike-like projections on the surface, which attach to host cell receptors.

Matrix protein mediates the interaction between the capsid proteins and enveloped .

The presence of an envelope confers instability on the virus.

Nucleic acid +capsid + envelope = enveloped Viruses (Figure (2).



Figure(2) illustrate the difference between Enveloped virus and Naked virus.

Types of symmetry of virus particles

Viruses are divided into three groups, based on the morphology of the nucleocapsid and the arrangement of capsomeres.

1- Icosahedral (Cubic) symmetry

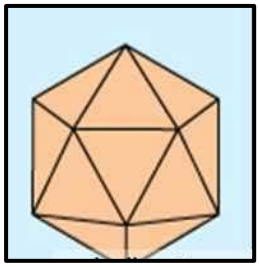
Composed of 12 vertices, has 20 faces (each an equilateral triangle) with the approximate outline of a sphere. e.g. Virus that cause yellow fever and Poliovirus.

2. Helical symmetry

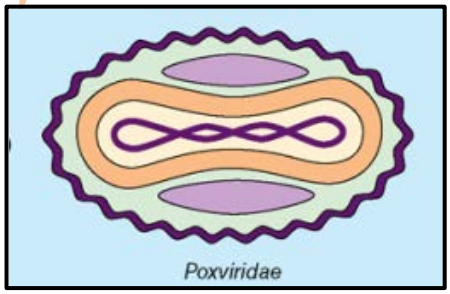
The virus particle is elongated or pleomorphic (not spherical), and the nucleic acid is spiral. Caposomeres are arranged round the nucleic acid. e.g. Rabies virus.

3. Complex structures

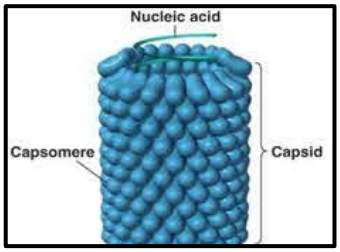
The virus particle does not confirm either cubic or helical symmetry e.g. Poxviruses.



Icosahedral



Complex



Helical

Reaction to physical and chemical agents:

1. **Heat and cold**

Viral infectivity is generally destroyed by heating at 50-60^{o}C for 30 mint., Viruses can be preserved at -90 ^{o}C or -196^{o}C (liquid nitrogens).

2. PH

Viruses can be preserved at physiological PH (7.3).

3. Ether susceptibility

Ether susceptibility can be used to distinguish viruses that possess an envelope from those that do not.

4. Detergents:

Nonionic detergents solubilize lipid constituents of viral membranes. The viral proteins in the envelope are released. Anionic detergents also solubilize viral envelopes; in addition, they disrupt capsids into separated polypeptides.

5. Salts

Many viruses can be stabilized by salt in concentrations of

1 mol/L. e.g. MgCl2, MgSO4, Na2SO4.

6. Radiation

Ultraviolet, X-ray, and high-energy particles inactivate viruses

7. Formaldehyde

Destroys viral infectivity by reacting with nucleic acid.

8. Antibiotics

Antibacterial antibiotics have no effect on viruses.

Classification of Viruses

Classification of viruses is based on the following characteristics:-

1- Virion morphology, including size, shape, type of symmetry, presence or absence of enveloped.

2. Virus genome properties, including type of nucleic acid (DNA or RNA), size of genome, strandedness (single or double), whether linear or circular, positive or negative sense (polarity), segments (number, size).

3.Physicochemical properties of the virion, including PH stability, thermal stability, and susceptibility to physical and chemical agents especially ether and detergents.

4. Virus protein properties, including number, size and functional activities of structural and non-structural proteins, amino acid sequences, and special functional activities (transcriptase,reverse transcriptase, neuraminidase, fusion activities).

5. Genome organization and replication , including gene order, strategy of replication (patterns of transcription, translation), and cellular sites (accumulation of proteins, virion assembly, virion release).

6. Antigenic properties

7. Biological properties, including natural host range, mode of transmission, vector relationships, pathogenicity, tissue tropisms, and pathology.

Baltimore classification

Viruses were divided into seven groups based on the their nucleic acid and m-RNA production.

1- Double strand DNA (ds-DNA viruses) for example (adenovirus , herpes viruses) .
2- Single strand DNA (ss-DNA viruses) for example (Parvoviruses).
3- ds- RNA viruses(e.g. Reo viruses).
4- (+) ssRNA viruses (+) sense RNA (e.g. Picornaviruses, Togaviruses).
5- (-) ssRNA viruses with (-) sense RNA (e.g. Orthomyxoviruses).
6- ssRNA-Reverse Transcriptase viruses (+) sense RNA with DNA intermediate (e.g. Retroviruses)
7- dsDNA-RT viruses (e.g. Hepadnaviruses).

Universal system of virus taxonomy:

Families – on the basis of virion morphology, genome structure and strategies of replication**.**

Virus family names have the suffix – viridae for example **Herpesviridae**

Genera – based on physicochemical or serological differences.

Genus names carry the suffix – virus for example Herpesviruses .

Post test

Q1:- Answer True or false ?

1-Viruses contain both types of nucleic acid (DNA and RNA).

2- Spike found on the envelope surface in some viruses.

Q2:- Choose the correct answer?

1- Which the viruses contain single strand negative sense :-

a- Adenoviruses b- Reo viruses c- Orthomyxo viruses d- Hepadna viruses

References

1- Themes, U. F. O. (2017-02-19). "6 Viruses–Basic Concepts". Basic medical Key. Retrieved 2020-05-29.

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قسم تقنيات التحليلات المرضية **المادة: فايروسات** **المرحلة:- الثالثة**

Title:-

Atypical virus –like agent (prion, defective viruses, pseudovirion and Viriods) **Lecture 2**

Name of the instructor:

Assist prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

1- Viroids very small ss RNA genomes (~300 nucleotides). No coat and RNA does not encode protein. Known viroids cause diseases in plants because host cells replicate the RNA.

2- Prions (protein infectious agent) do not have a nucleic acid genome. Prion diseases are often called spongiform encephalopathies because of the post mortem appearance of the brain with large vacuoles in the cortex and cerebellum. Prion diseases in humans are probably primarily a genetic neurotoxic disorder. Transmission of the disease to humans via infectious prions is likely to be rare.

• The prion is a modified form of a normal cellular protein known as PrPc (for cellular), found predominantly on the surface of neurons and thought to be involved in synaptic function. The modified form of PrPc (= prion) is known as PrPsc (for scrapie) which is relatively resistant to proteases and accumulates in cytoplasmic vesicles of diseased individuals. Prion protein may cause normal protein to fold abnormally.

3- Defective virus composed of nucleic acid & proteins but cannot replicate without a helper virus. for example Hepatitis D virus, adenovirus.

4- Pseudovirion contain host cell DNA instead of viral DNA within the capsid.

Pre-test

Define Pseudovirion , Defectve virus ?

Scientific Content

(1) <u>Defective Viruses</u> are composed of viral nucleic acid and proteins but cannot replicate without a "helper" virus, which provides the missing function. Defective viruses usually have a mutation or a deletion of part of their genetic material. During the growth of most human viruses, many more defective than infectious virus particles are produced. The ratio of defective to infectious particles can be as high as 100:1 .

For example certain **Adenoviruses** and **Hepatitis -D virus** are defective viruses.

<u>(2) Pseudovirions</u> contain host cell DNA instead of viral DNA within the capsid. They are formed during infection with certain viruses when the host cell DNA is

fragmented and pieces of it are incorporated within the capsid protein. Pseudovirions can infect cells, but they do not replicate.

(3) Viroid's :- Consist of a single molecule of circular RNA without a protein coat or envelope. There is extensive homology between bases in the viroid RNA leading to large double-stranded regions. viroids replicate but the mechanism is unclear. They cause several plant diseases. but are not implicated in any human disease.

(4) Prions are infectious particles that are composed of only proteins i.e, they contain no detectable nucleic acid.

Prion is a type of protein that can trigger normal proteins in the brain to fold abnormally. Prions are composed of a single glycoprotein with a molecular weight of 27,000-30,000. prion diseases are called spongiform encephalopathies (slowly progressive diseases) because of the post mortem appearance of the brain with large vacuoles in the cortex and cerebellum and Prion diseases in humans are probably primarily a genetic neurotoxic disorder which include Creutzfldt-**Jakob disease or Kuru** in humans and **scrapie** in sheep and bovine spongiform encephalopathy (BSE) in cattle and also called Mad cow in cattle.

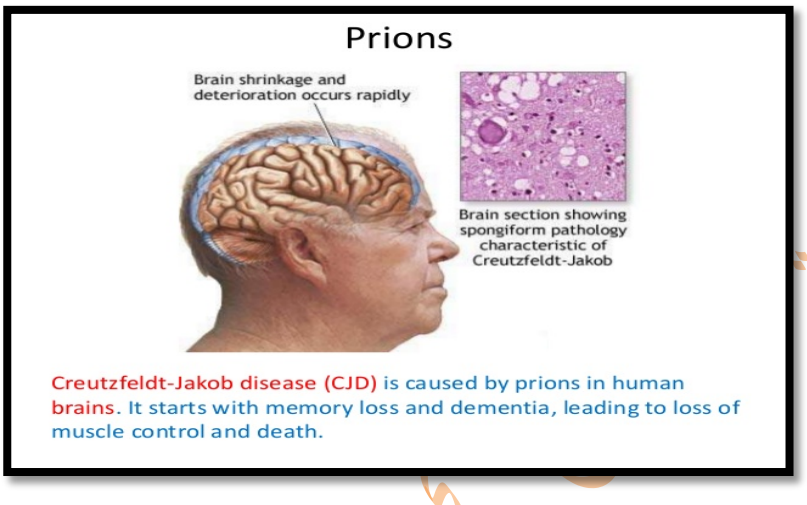
Because neither DNA nor RNA has been detected in prions, they are clearly different from viruses . Furthermore, electron microscopy reveals filament rather than virus particles. Prions are much more resistant to inactivation by ultraviolet light and heat than are viruses. They are remarkably resistant to formaldehyde and nucleases. However, they are inactivated by hypochlorite, NaOH, and autoclaving.

Comparison between prions and conventional viruses

Feature	Prions	Conventional viruses
Nucleic acid	No	Yes
Protein	Yes , encoded by cellular genes	Yes ,encoded by viral genes
Heat inactivation	No	Yes
Appearance	Amyloid- like	Icosahedral
Antibody response	No	Yes
Inflammatory responses	No	Yes

Causes of prion disease

Prion diseases occur when normal prion protein, found predominantly on the surface of neurons , becomes abnormal and clump in the brain, causing brain damage. This abnormal accumulation of protein in the brain can cause memory impairment, personality changes, and difficulties with movement. Experts still don't know a lot about prion diseases, but unfortunately, these disorders are generally fatal.



Post test

Write short notes on : Prion, viroid?

References

1- Jawetz, R., J.L. Melnick, and E.A. Adelberg,(2019). Review of Medical Microbiolog Twenty-Eighth Edition.

3- Microbiology, 5th edition, Lancin M. Prescott.

Title:-

Viral genetic , Molecular and Viral replication **Lecture 3**

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population:

Three stage students in department Medical Laboratory Techniques

Introduction

Viruses are simple entities, lacking an energy-generating system and having very limited biosynthetic capabilities. The smallest viruses have only a few genes; the largest viruses have as many as 200. Genetically, however, viruses have many features in common with cells. Viruses undergo subtle genetic changes through **mutation** and major genetic changes through **recombination.** Mutation occurs when an error is incorporated in the viral genome. Recombination occurs when coinfecting viruses exchange genetic information, creating a novel virus.

Replication of viruses: is the formation of viruses during the infection process in the target host cells.Viruses must first get into the cell before viral replication can occur. Most DNA viruses assemble in the nucleus while most RNA viruses develop solely in cytoplasm.

Pretest:

Define Replication of viruses ?

Scientific Content

Viral genetics

Viruses grow rapidly, there are usually a large number of progeny virions per cell. There is, therefore, more chance of mutations occurring over a short time period. The nature of the viral genome (RNA or DNA; segmented or non-segmented) plays an important role in the genetics of the virus. however , Viruses may change genetically due to mutation

or recombination. DNA viruses tend to more genetically stable than RNA viruses. There are error correction mechanisms in the host cell for DNA repair, but probably not for RNA.

Mutation in viruses

1- Spontaneous mutations

These arise naturally during viral replication: e.g. due to errors by the genome replicating **polymerase** or **a result of the incorporation of tautomeric forms of the bases**(resonance from keto to enol and from amino to imino forms).

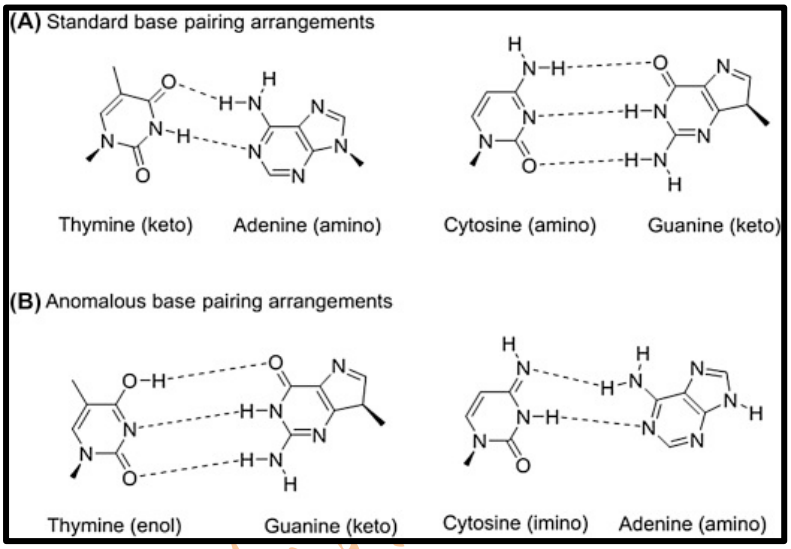


Figure (1)Tautomerization changes the base pairing abilities of the base

2- Mutations that are induced by physical or chemical means

a- Chemical:

Agents acting directly on bases, **e.g. nitrous acid**
Agents acting indirectly, e.g. **base analogs** which mispair more frequently than normal bases thus generating mutations.

b- Physical:

Agents such as UV light or X-rays can induce mutation in viruses.

Types of mutation

- Point mutation
- Insertion mutation
- Deletion mutation

Recombination: Exchange of genetic information occurs when viruses of two different parent strains coinfect the same host cell and interact during replication to generate virus progeny that have some genes from both parents.

Recombination generally occurs between members of the same virus type (e.g., between two influenza viruses or between two herpes simplex viruses)

"Classic" recombination: This involves breaking of covalent bonds within the nucleic acid, exchange of genetic information, and reforming of covalent bonds.

This kind of break/join recombination is common in **DNA viruses** or those **RNA viruses which have a DNA phase (retroviruses)**. The host cell has recombination systems for DNA. Recombination of this type is very rare in RNA viruses (there are probably no host enzymes for RNA recombination).

Reassortments

- Form of recombination (non classical)
- Very efficient
- Segmented viruses only.
- Can occur naturally.

- Used in some new vaccines : e.g for influenza and rotaviruses.

If a virus has a segmented genome and if two variants of that virus infect a single cell, progeny virions can result with some segments from one parent, some from the other.

This is an efficient process - but is limited to viruses with **segmented genomes** - so far the only human viruses characterized with segmented genomes are RNA viruses e.g. orthomyxoviruses, reoviruses, arenaviruses, bunya viruses.

Reassortment may play an important role in nature in generating novel reassortants and has also been useful in laboratory experiments.

(figure 2). For example, in a reassorted virus if one segment comes from virus A and the rest from virus B, we can see which properties resemble virus A and which virus B.

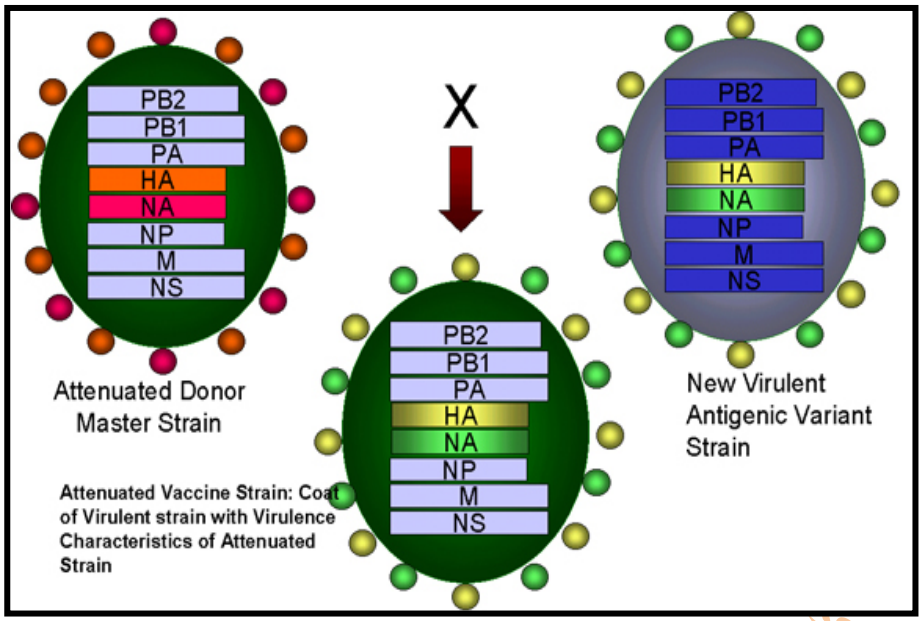


Figure 2:-Reassortment of genes between the attenuated strain of influenza virus and a new virulent strain in the formation of an attenuated influenza vaccine

Replication of viruses: is the formation of viruses during the infection process in the target host cells. Viruses must first get into the cell before viral replication can occur. Most DNA viruses assemble in the nucleus while most RNA viruses develop solely in cytoplasm.

Steps of viral replication

A. Attachment & adsorption : This is the first step in viral replication. Surface proteins of the virus interact with specific receptors on the target cell surface.

B. Penetration (Uptake)

After binding of virus, virus is taken up inside the cell which is referred as penetration or engulfment**. . penetration in viruses by :-**

A) Direct translocation across cell membrane

B) Fusion of viral envelope and cell membrane as in some enveloped viruses for example :- paramyxoviruses (measles) , retroviruses (HIV).

C) Receptor mediated endocytosis or Engulfed in a pinocytotic vesicle (viropexis) as in naked viruses and other enveloped viruses.in which The cell engulfs virus by invagination of the cell membrane then vesicles formation in the cell cytoplasm. Low PH made the virus fuse with the vesicles membrane, followed by release of the virus.

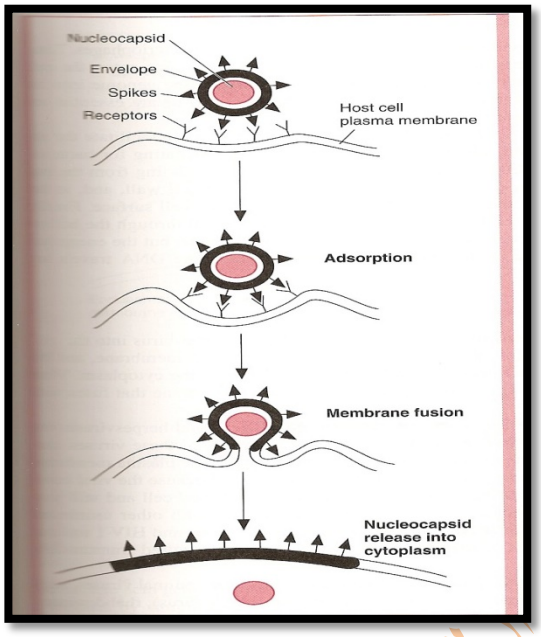


Figure (3) :- Entry of some enveloped viruses by fusion of the viral envelope.

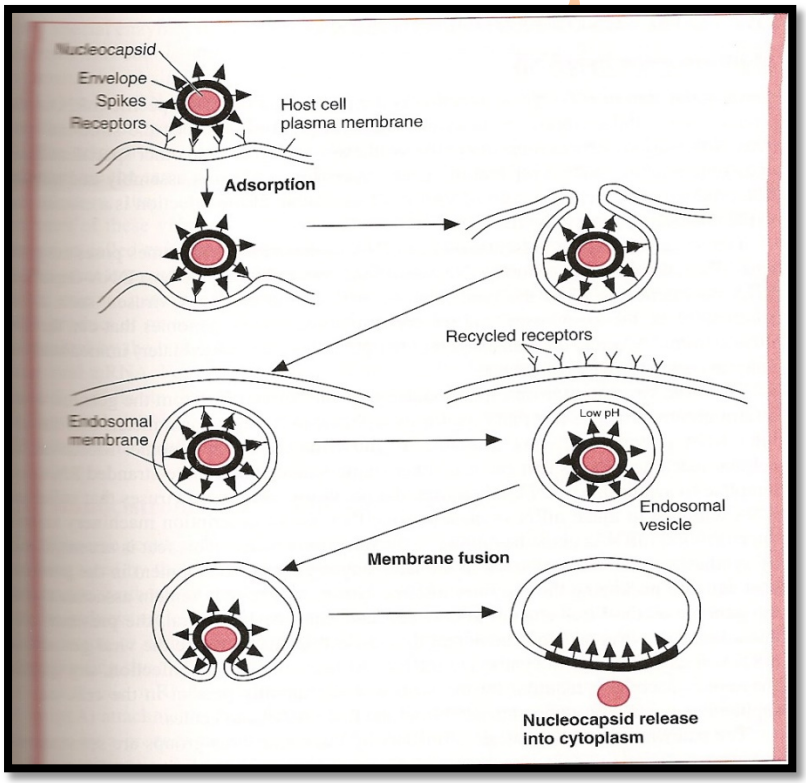


Figure (4) :- Uneveloped and some enveloped viruses enter the cell by endocytosis (viropexis).

C. Uncoating:

This process include release of the viral genome from its protective capsid to enable the viral nucleic acid to replicate. The period of the replication cycle between the end of

the uncoating stage and maturation of new viral particles is termed the **Eclipse period .** No virus is found inside the cell during this period .

D- Gene expression and genome replication: -

1-Early viral mRNA synthesis (transcription)

A. DNA viruses: Replicate in the nucleus and use the host cell DNA dependent RNA polymerase to synthesize their mRNA. The poxviruses are the exception because they replicate in the cytoplasm. They carry their own polymerase within the virus particle. The genome of all DNA viruses consists of double-stranded (ds) DNA, except for the parvoviruses, which have a single-stranded (ss) DNA genome.

B. RNA viruses: Fall into four groups with quite different strategies for synthesizing mRNA include :-

1.Single- stranded RNA of positive polarity. These viruses use their RNA genome directly as mRNA (e.g. Poliovirus).

2.Single- stranded RNA of negative polarity:- An mRNA must be transcribed by using the negative strand as a template. The virus carries its own RNA- dependent RNA polymerase (e.g. Influenza virus).

3.Double-stranded-RNA :- The virus carries its own polymerase for transcribing into mRNA (e.g. Reovirus).

4. Single- stranded RNA of positive polarity with DNA intermediate :- The RNA transcribed into double- stranded DNA by the RNA dependent DNA polymerase (reverse transcriptase), carried by the virus. This DNA copy is then transcribed into viral mRNA by the regular host cell RNA polymerase.(e.g Retroviruses).

Most RNA viruses undergo their entire replication cycle in cytoplasm. The two principle exceptions are **retroviruses and influenza viruses**, both of which have an important replication step in the nucleus.

2-Early viral proteins synthesis (Translation): Once the viral mRNA of either DNA or RNA viruses is synthesized, it is translated by host cell ribosomes into viral proteins. Some of which are early proteins, i.e. enzymes required for replication of viral genome, and others of which are late proteins, i.e. structural proteins of the progeny viruses. Early proteins: occurring before the replication of the genome. Late proteins: occurring after genome replication.

E- Assembly

New virus genomes and proteins are assembled to form new virus particles. The assembly occurs in nucleus or cytoplasm of host cell depending upon types of virus.

DNA virus assembled in nucleus **except Poxvirus** and RNA viruses assembled in cytoplasm **except Influenza virus and Reo virus.**

F- Release

Release of mature virus from host cell is the final event in virus replication. **enveloped viruses** are released by **budding** from the infected cells. **Unenveloped viruses** are released by **rupture or lysis** of the infected cells.

Post test

Mention of steps of viral replication

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2- Jawetz, R., J.L. Melnick, and E.A. Adelberg, Review of Medical Microbiology, 16th Edition, pp. 347, Figure 27-3. Reproduced with permission.

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قسم تقنيات التحليلات المرضية

المادة: فايروسات

المرحلة:- الثالثة

Title:-

Pathogenesis and Transmission **Lecture 4**

Name of the instructor:

Assist prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population:

Three stage students in department Medical Laboratory Techniques

Introduction:

Pathogenesis is the process by which virus infection leads to disease. **Pathogenic mechanisms include** implantation of the virus at a body site (the portal of entry), replication at that site, and then spread to and multiplication within sites (target organs) where disease or shedding of virus into the environment occurs. Most viral infections are subclinical, suggesting that body defenses against viruses arrest most infections before disease symptoms become manifest. Knowledge of subclinical infections comes from serologic studies showing that sizeable portions of the population have specific antibodies to viruses even though the individuals have no history of disease.

Pretest:

Define Pathogenesis of viruses ?

Scientific Content:

The viral pathogenesis **refers to ability of viruses to cause diseases.**

Pathogenic Properties of Viruses

1. Viruses avoid the host's immune response by growing inside cells.
2. Viruses gain access to host cells because they have attachment sites for receptors on the host cell.
3. Visible signs of viral infections are called **cytopathic effects (CPE).**
4. Some viruses cause cytocidal effects (cell death), and others cause noncytocidal effects (damage but not death).
5. Cytopathic effects include the stopping of mitosis, lysis, and the formation of inclusion bodies, cell fusion, antigenic changes, chromosomal changes, and transformation.

Cytopathic effects [CPE]

1.Cytocidal effects : are cytopathic effects that lead to host cell death.

♦ **Noncytocidal effects** are cytopathic effects that do not lead to cell death. Basically, viral infection can lead to cell abnormalities (biochemical and morphological) .

♦ **Syncytia [giant cells] or Big cells:** Syncytia are multi-nucleated , giant cells formed through the fusion of host cells .

2.Inclusion bodies:- Inclusion bodies are intracellular granules whose presence is a result of viral infection . The characterization of inclusion bodies is useful for the identification of some viral infections

The viral pathogenesis involves the following **stages :**

Stages of viral pathogenesis :

1. Transmission of virus from external source and entry into host.
2. Attachment of virus to host cell.
3. Replication of virus in target cell and damage to it.
4. Spread of the virus to other cells and organs.
5. Evasion of immune response.
6. Persistence and shedding of virus in some instances.

Sources of infection :

Main sources of infection are ;

1. Human : is common source of infection from patients or carriers. The **carrier** is person recovered from disease but harboring virus in his body. Fomites are inanimate objects of patients that may be contaminated and serves as source of infection. The infectious agent is transmitted from person to person by various ways such as direct contact, kissing, inhalation of aerosols, fecal-oral, venereal contact and arthropod-spread.

2. Animals :The animals can be serve either the source (reservoir)or made transmission (vector)of certain organism. The disease is called zoonosis. The virus can be transmitted from infected animals to human when direct contact with animals, and handling or consumption of their products ,or transmitted indirectly by bite of vectors.

3. Food: the foods are most important media for transporting.

4. Water: many viruses may be found in water. The infectious agents are transmitted to human by consumption of water or when swimming in it , therefore act as water-borne infection.

Transmission and Portal of entry :

♦ Portals of entry(routes of infection) :

Major :

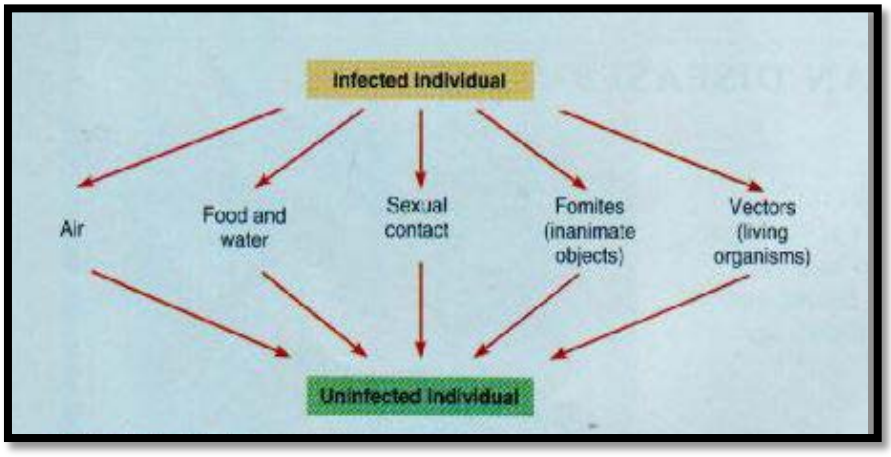
1. Respiratory tract(nose).
2. Elementary tract(mouth).

3. Skin, non-intact.
4.Genital tract(vagina).
Minor :
1. Eye.
2. Anus.
3. Ear canal.
4. Urethral canal.

♦ **Horizontal transmission :**

1. Inhalation : the pathogenic agents may be transmitted by inhalation of respiratory secretions of patients, or by inhalation of contaminated dust (air-borne) .
2. Ingestion: the infectious agents can be transmitted by consumption (during eating or drinking) of contaminated water or food.
3. Skin : The transmission from person to person may occur through direct contact (such as handshaking, kissing) with infected person , or during handling of fomites of patients. The virus can be introduces the skin through any small break, abrasion, and wound in skin that permit entry or during injection or blood transfusion. The transmission from infected animal to human also can take place either directly from bite of reservoir animal host, or indirectly through the bite of insect vectors.
4. Sexual intercourse: certain viruses can be transmitted by sexual contact in homosexual or heterosexual persons.

♦**Vertical transmission (from mother to her fetus):**
The infection of fetus can occur between mother and offspring across the placenta (prenatal) ,or at time of delivery from birth canal (perinatal), or during breast feeding (postnatal)



Route of disease transmission

Attachment of virus :-

The viruses tend to exhibit cell and organ specificities (cell tropism). The viral affinity for specific body tissue is dependent on :

1. Presence of specific cellular receptors on cell surface which interact with virus, and initial infection.

2. Ability of host cell to support viral replication .

3. Physical barriers ,local temperature , pH, oxygen tension are very important in tissue tropism.

Viral replication and dissemination:

The viruses replicate and produce diseases at site of entry or at site distant from their point of entry. In other word, the viral infections are either **localized on the portal of entry** or **spread systemically through the body** The best example of **localized infection is common cold** , which involves only the upper respiratory tract. The influenza virus is localized primarily to upper and lower respiratory tracts. **One of the best system viral infection is poliomyelitis (caused by poliovirus)** . The poliovirus spread from small intestine to central neural system (CNS) and cause damage to anterior horn cells resulting in muscle paralysis.

Evasion of host defenses: The viruses have several ways by which they evade host defenses:

1. Some viruses (such as: vaccinia virus) encode some proteins act as receptors for immune mediators such as interferon (IFN) and tumor–necrosis factor (TNF). these proteins bind to immune mediators and block their ability to interact with receptor on their intended target.

2. Some viruses (such as HIV and CMV) can reduce the expression of class-1 MHC protein . Thereby reducing the ability of cytotoxic T cell to kill virus-infected cell. Other viruses (e.g.: herpes virus) inhibit complement . Several viruses (HIV, EBV, adenovirus) reduce the ability of IFN to block viral replication.

These viral virulence factors are called **virokines.**

3- Some viruses (such as rhinovirus, influenza , HIV ,HCV) have **multiple antigenic types** (multiple serotypes)

Damage of host cell :

Multiplication of virus in host cell lead to disease. The time between exposure to virus and onset of disease or appearance disease symptoms is called **incubation period .**

The mechanisms of viral diseases are various:

1. Most viral diseases are result of cell death by shutoff macromolecules synthesis or by lysis of the cell membrane by lysozymes during viral replication.
2. Stimulate cellular cytokines .For example, diarrhea caused by rotavirus the rotavirus-infected cells (enterocytes) produce cytokines that stimulate the enteric neuron , resulting in excess fluid and electrolytes secretion into the bowel lumen.
3. Immunological attack. Both the **cytotoxic T-cell and antibodies play a role in immunopathogenesis. Example , the HAV, HBV, and HCV ,** they don't cause a cytopathic effect and the damage to hepatocytes is result of recognition of viral antigens on hepatocyte surface by cytotoxic T-cell.

Persistent viral infection: in most viral infection, the virus does not remain in the body for significant period after clinical recovery. In certain, the viruses persist for long periods either intact or in form of subviral component (eg: genome).

Three types of persistent viral infections:

1. Chronic infection such as HBV, HCV.
2. Latent infection such as herpes viruses.
3. Slow virus infections such as HIV.

The mechanisms that may play a role in persistence of viruses include:

1. Integration of provirus into chromosome of host cell without viral replication, as occur in retroviruses.
2. Spread from cell to cell without extracellular phase , so that is not exposed to immune response .
3. Occurrence of rapid antigenic variation in some viruses .
4. Location of virus within immunological sheltered e.g. : brain.
5. Immune tolerance may occur in some patients, because neutralizing antibodies are not formed.
6. Some patients suffer from immunosuppression , as in AIDS.

Virus shedding

The last stage in pathogenesis is the shedding of infectious virus into environment. The shedding usually occurs from the body surface involved in viral entry.

Post test

Write types of persistent viral infections?

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1- Themes, U. F. O. (2017-02-19). "6 Viruses–Basic Concepts". Basic medical Key. Retrieved 2020-05-29.

2- Jawetz, R., J.L. Melnick, and E.A. Adelberg, Review of Medical Microbiology, 16th Edition, (2019).

3- Knipe DM, Howley PM, (editors-in-chief): *Fields Virology*, 5th ed. Lippincott Williams & Wilkins, 2007.

الجامعة التقنية الوسطى

كلية التقنيات الصحية والطبية /بغداد

قسم تقنيات التحليلات المرضية

المادة: فايروسات

المرحلة:- الثالثة

Title:-

Immunity and laboratory diagnosis of viruses Lecture 5

Name of the instructor:

Assist prof dr. Athraa Zaidan Hassan

استاذ مساعد د. عذراء زيدان حسن

Target population:

Three stage students in department Medical Laboratory

Immunity against viral infection

Immunity that is developed against virus infections is known as viral immunity. It is developed by a variety of specific and non-specific mechanisms.

Pretest:

Define viral immunity ?

Immunity against viral infection include :-

1. Innate (non-specific response) immunity refers to those elements of the immune system that can clear virus or virus infected cells immediately upon or shortly after viral exposure and which are not dependent upon immunologic memory. Non-specific immunity may include:-

a. Phagocytic cells (neutrophils and monocyte/macrophages).
b. Interferons
c. Natural killer cells.
d. Complement proteins which formed membrane attack complex (MAC) can disrupt the viral envelope.

The most effective mechanisms of the innate response against viral infections are mediated by **interferon(IFN) and by the activation of NK cells.**

Interferons (INFs) are proteins with **three different types: α, β** and **γ.** that **α, β** are mainly produced by monocytes-macrophages and to a lesser extent by fibroblasts but **interferon- γ** is produced by T helper (CD 4) and T cytotoxic (CD 8) lymphocytes and NK cells. Interferon has a strong anti-viral action.

Virally infected cells produce and release Interferon which prevent replication of viruses, by directly interfering with their ability to replicate within an infected cell.

IFN can control viral infections by
- Binding receptors on infected or uninfected cells
- Prevents further spread of the virus

IFN binding to IFN-R can lead to :-
–Inhibits synthesis of viral proteins
– Increase expression of MHC class I molecules by viral infect cell which enhances the destruction of infected cells by cytotoxic T Lymhocyte (CTLs).
– Prevents uninfected cells from being killed by NK cells

Interferon work by two main mechanisms :-

i) Activate an RNA endonuclease causing mRNA degradation lead to blocks translation of mRNA of the virus.

ii) Cause phosphorylation of eIF2 (Eukaryotic Initiation Factor 2) essentially turning off cellular protein synthesis.

Natural Killer (NK) cells :- it another part of innate immunity and play important role against virus infected cells by:-

a) Viral infected cell induce MHC class I down regulation which triggers NK cells to kill the infected cells by release toxic substances **(perforin & granzymes)** inside the target cell , they initiate a process known as programmed cell death or **apoptosis** causing the target cell to die**.**

b) NK recognize infected cells coated with antiviral antibodies using Fc receptor (FcR) present on the surface of it and kill them through antibody dependent cell mediated cytotoxicity (ADCC).

c) NK cell produce increased amounts of IFN which prevent production of virus.

Adaptive immunity (specific response) acts against both viral particles and infected cells**.** The most important mechanisms against viral particles are antibodies **(Humoral immunity).** while the cytotoxic mechanism are the most important against infected cells. This is mediated by **cytotoxic T lymphocyte cell (CTLs).**

Viruses can be removed from the body by **antibodies** before they get the chance to infect a cell. Antibodies are proteins that specifically recognize invading virus and bind to them this binding serves many purposes in the eradication of the virus:

• **Firstly**, the antibodies neutralize the virus (**IgG, IgM and IgA**), prevents the virus from entering the cells. IgA give protection against viruses that enter through the respiratory and gastrointestinal mucosa while IgM and IgG protect against viruses that enter or are spread through the blood.

• **Secondly**, many antibodies **(IgM)** can work together, causing virus particles to stick together in a process called **agglutination**. Agglutinated viruses make an easier target for immune cells than single viral particles.

• **A third** mechanism used by antibodies to eradicate viruses, is the activation of phagocytes. A virus-bound antibody binds to receptors, called Fc receptors, on the

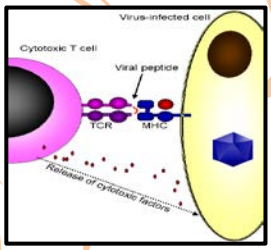
surface of phagocytic cells and triggers a mechanism known as **phagocytosis**, by which the cell engulfs and destroys the virus.

• **Finally,** antibodies can also activate the complement system, which opsonizes and promotes phagocytosis of viruses.

Acquired Immunity (Cell dependent control of viruses)

Effector and most important cell in acquired immunity are **Cytotoxic T lymphocytes (CTLs)** or called **CD8 positive T- cells.**

Cytotoxic T cells have specialized proteins on their surface that help them to recognize virally-infected cells. These proteins are called **T cell receptors** (**TCRs**). Each cytotoxic T cell has a TCR that can specifically recognize a particular antigenic peptide bound to an MHC molecule class I . If the T cell receptor detects a peptide from a virus, it warns its T cell of an infection. The T cell releases **cytotoxic factors (**Perforin/granzyme pathway **)** which degrade the cell contents and kill the infected cell by **apoptosis** (programmed cell death).



Cytotoxic T cell Mechanism

Laboratory diagnosis of viruses
A variety of methods exist to diagnose viral infections with the recent trend being toward molecular diagnostics**.**

Diagnostic Methods in Virology
1. Direct Examination
2. Indirect Examination (Virus Isolation)
3. Serology

❖ **Direct Examination**

1- Antigen Detection in body fluids with specific immune sera linked to fluorescence or enzyme detection immunoassay (**immunofluorescence, ELISA**).

2. Examination of tissue samples by light microscopy for viral induced cytopathology .

3. Examination of body fluids or tissues by electron microscopy.
This is not an efficient method and is dependent upon sufficient numbers of virions being present to permit detection.

4- Viral Genome Detection
PCR amplification , hybridization with specific nucleic acid probes to detect viral nucleic acid in body fluids or tissues.

❖ **Indirect Examination (Virus Isolation)**

Generally three methods are employed for the virus cultivation or isolation:-

a) Cell Culture [cytopathic effect (CPE) , haemadsorption , immunofluorescence]

b) Chicken embryo Egg **[** pocks on chorioallantoic membrane **(**CAM**)** **,** haemagglutination **,** inclusion bodies**].**

c) Animals (disease or death).

❖ **Serology**

Demonstration of significant increase in viral Ab in Patient serum during the course of illness. It includes the following tests:

1. Complement fixation test (CFT)
2. Immunoflourescent assay (IFA)
3. Radioimmunoassay (RIA)
4. Enzyme linked immunoassay (ELISA)

Post test

Q1:- Choose the correct answer :-

1- Strong anti-viral action in innate immune-response is:-

a) complement proteins b) Interferon c) Phagocytic cells d) Antibodies

e) Natural killer cell.

2- Effector and most important cell in acquired immunity is :-

a) helper T lymphocyte b) cytotoxic T lymphocyte c) Natural killer cell

d) phagocytic cell e) Non of them .

Q2:- A) Mention how interferon work against virus infection ?
B) Write methods used for Viral Genome Detection?

References:

1- Themes, U. F. O. (2017-02-19). "6 Viruses–Basic Concepts". Basic medical Key. Retrieved 2020-05-29.

2- Jawetz, R., J.L. Melnick, and E.A. Adelberg, Review of Medical Microbiology, 16th Edition, (2019).

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قسم تقنيات التحليلات المرضية

المادة: فايروسات

المرحلة:- الثالثة

Title:-

Herpes viruses **Lecture 6**

Name of the instructor:

Assist prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population

Three stage students in department Medical Laboratory Techniques

Introduction:

Herpes Viruses

- The herpes virus family contains several of the most important human pathogens. clinically the virus exhibit human pathogens with spectrum of diseases. Some have a wide host range, where are others have a narrow host range

- All herpesviruses can establish **latent infection** within specific tissues, which are characteristic for each virus which mean outstanding property of herpes viruses is their ability to establish lifelong persistent infections in their hosts and to undergo periodic reactivation.

- Herpes viruses that are commonly infect humans include:-
  1) Herpes simplex viruses type 1&2.
  2) Varicella- Zoster virus .
  3) Cytomegalovirus(CMV).
  4) Epstein- Barr viruses.
  5) Human herpes viruses 6,7
  6) Kaposi's sarcoma virus or human herpesvirus 8.
  7) Herpes B virus of monkey can also infect human.

Pretest

Enumerate Herpes viruses that are commonly infect humans?

Scientific Content:

Properties of Herpes viruses :-

1) Virion: Spherical,
2) Genome: dsDNA, Linear.
3) Protein: more than 35proteins in virion .
4) Envelope:- contain viral glycoprotein and Fc receptor .
5) Replication:- in the nucleus and bud from the nuclear membrane.
6) All Herpes viruses have **Four structural :-**

a) Genome

b) Capsid

c) Envelope

d) Tegument (The space between the envelope and the capsid is the tegument. This contains virally-encoded proteins and enzymes involved in the initiation of replication.

Classification:

Classified into three sub families – based on physical & genetically properties:-
1- **Alpha Herpes :** They have rapid replicate cycle (12-18 hrs). It is variable host range. These viruses tendency to form **latency in sensory ganglia.** Produced rapid

CPE & release virus from the infected cells Ex: **HSV-1, HSV-2 , VZV (Varicella-Zoster Virus).**

2- **Beta Herpes :** They have narrow host range. Has slow replication cycle (more than 24 hrs). **Ex: CytomegalovirusVirus (CMV) , HHV-6, HHV-7.**

3- **Gama Herpes :** They infected lymphoid tissue & causes latency in lymphocyte. Ex : Epstein-Barr Virus (EBV), HHV-8

❖ **Herpes simplex viruses (HSV)**

HSV is a wide spread in human, it has a wide host range which can infect many different animals in addition to human. It has Two types 1&2 , there genome are similar in organization and share 50 - 70% homology and two viruses can be distinguished most reliably by DNA composition .

♦ Mode of Transmission

HSV-1 transmitted by direct contact with infected saliva , skin lesions or respiratory secretions.

HSV-2 is transmitted sexually (Venereal disease) and also from maternal genital to newborn (Perinatal**).**

♦ Pathology

HSV is cytolytic and lead for the formation of ballooning of infected cells and the formation of intracellular inclusion bodies.

♦ **Pathogenesis**

a) Primary infection

Herpes simplex is one of the most common viral infections in humans. Primary infection is usually acquired in early childhood , between two and five years of age. Humans are the only natural hosts. Asymptomatic carriers form the more important source of infection.

The virus enters through defects or broken in the skin or mucous membranes and multiplies locally with cell-to cell spread. The virus enters cutaneous nerve fibres and is transported intra-axonally to the dorsal root ganglia where it replicates. migration of the virus can take place from the ganglia to the skin and mucosa to cause cutaneous and mucosal lesions. The virus remains latent in the ganglia **(HSV-1 in trigeminal ganglia** while **HSV-2 in sacral ganglia**) to be reactivated periodically in some individuals causing recurrent oral and genital lesions. **HSV-1** it is limited to the oropharynx but **HSV-2** occurred in the genital tract.

b) Latent infection

HSV have ability to stay as latent virus in infected cells for life time. The stimuli or trigger which is lead for the reactivation of the latent virus are :-

1) Axonal injury
2) Fever
3) Physical or emotion al stress.
4) Exposure to ultraviolet , sunlight
5) infection especially pneumococcal and meningococcal.

♦ **Clinical Features**

- **HSV-1 cause Herpes Labialis - Cold sore** is Painful ulcerating vesicles occurs on the lips which spontaneously resolves in < 2 weeks. **Herpes Labialis** is a recurrence of oral HSV.
- **HSV-2 cause of genital herpes** include :

- Genital herpes is painful vesicular lesions in genitals area.
- Neonatal herpes from contact with maternal genital to newborn.

♦ Laboratory diagnosis :-

1- Direct Detection

- Take a scraping from the base of the vesicles and stain it with Giemsa stain and examine under light microscope or Electron microscope to see the multinucleated giant.

- Electron microscopy of vesicle fluid - rapid result but cannot distinguish between HSV and VZV, Immunofluorescence of skin scrapping - can distinguish between HSV and VZV.

2- PCR - Now used routinely for the diagnosis of herpes simplex encephalitis

3- Virus Isolation

HSV-1 and HSV-2 are among the easiest viruses to cultivate. It usually takes only 1 - 5 days

3- Serology use to detect (IgM&IgG) after 4-7 days by ELISA test.

❖ Varicella-Zoster Virus (VZV)/ Human Herpes virus 3

Transmission:- Direct contact, Respiratory droplet.

Diseases :- Two disease caused by **Varicella-Zoster Virus:-**

1-Varicella(Chicken Pox) :- It is the acute disease that occurred by the primary contact with the virus which include Erythematous ulcerating encrusting vesicles beginning on the face and trunk and then progressing towards the extremities, as well as mucous membranes and

Presents fever, lymphadenopathy. Spontaneously resolves in < 1 week.

2- Zoster (shingles) it is disease occur in response to the reactivation of latent VZV in neurons in sensory ganglia.

Pathogenesis and clinical features:-

a)Varicella

The infection is occur through the mucosa of the conjunctiva and upper respiratory tract followed by initial replication in the regional lymph node then after primary and secondary viremia the virus transported by the mononuclear cells to the skin this associated with typical vesicles of chicken pox.

b) Zoster

The lesions of Zoster are histopathologically identical to those of varicella . the lesion is closely to the areas of innervation of dorsal root ganglia . the reactivation is occurred as a result of **lowering of immunity** which allows for replication of the virus and then it travel down with the nerve to the skin and induce vesicle formation .

Laboratory diagnosis :-

1-Direct detection

staining of the smear which has been taken from the base of the vesicle and examine under the microscope to see a multinucleate giant cell**.**

2- Virus Isolation:-

culture of the vesicle fluid in human cell .

3- serology

Elisa or Immunofluorescence **(IF) test by detect antibodies .** the presence of VZV IgG is indicative of past infection and immunity. The presence of IgM is indicative of recent primary infection.

❖ **Epstein-Barr Virus (EBV) /Human Herpes virus 4**

Transmission:- contact with the saliva of an infected person.

Characteristics:- Latency in B lymphocytes.

Viral EBVantigens are :

(a)Viral capsid antigen (**VCA)**

(b) Early antigen **(EA)**

(c) Epstein-Barr nuclear antigen **(EBNA)**

(d) Viral membrane antigen **(VMA)**

Diseases associated with EBV :-

1- Infectious Mononucleosis is a self-limited disease which consists of Fever, headache, severe pharyngitis, splenomegaly and generalized Lymphadenomegaly. primarily occurs in children and young adults.

2- Nasopharyngeal Carcinoma.

3- Burkitt Lymphoma.

4- Oral hairy leukoplakia. (awhitish , nonmalignant lesions with an irregular (hairy) on the surface of the lateral side of the tongue. More common this disease in AIDS patients .

♦ Pathogenesis

A) Primary infection

EBV is transmitted by saliva (the kissing disease) .Infection starts in the oropharynx then spreads to the blood. In blood , the virus infects B lymphocytes by binding of the virus with the **CD21 receptor** of the B lymphocyte. In B lymphocytes, viral DNA integrates in cell genome. After primary infection, EBV maintains a steady low grade latent infection in the body. Primary infection in children are usually subclinical but if they occur in young adults develop Infectious Mononucleosis (I.M) and the typical antibody produce with disease is the heterophil antibody that react with antigen on sheep RBC **(Monospot test)**

B) Reactivation from Latency.

C) Tumors :- EBV is the tumor virus number one as has been reported by the WHO.

♦ **Laboratory diagnosis :-**

1- Monospot test used for detection Heterophil antibody in cases of Acute EBV infection .

2- Serology by (ELISA, IF test) for detection anti-EBV VCA IgM in acute EBV infection and anti-EBV VCA IgG and anti- EBNA IgG in Past or chronic EBV infection .

3- Cases of Burkitt's lymphoma should be diagnosed by Histology.

4- Cases of Nasopharyngeal Carcinoma (NPC) should be diagnosed by Histology.

♦ **Prevention & treatment :-**

No vaccine to EBV available . Acyclovir reduce EBV shedding but has no effect on symptoms.

❖ Cytomegalovirus (CMV) / Human Herpes virus 5

CMV are ubiquitous herpes viruses that are common causes of human disease. The name for classic cytomegalic inclusion disease, derive from the massive enlargement of

CMV infected cells. CMV produces characteristic cytopathic effect nuclear inclusion form in addition to the cytoplasmic inclusion typical of herpes viruses.

♦ **Transmission**:-

1- Direct contact by the infected person's saliva , blood , urine, semen, cervical/vaginal secretions or breast milk.

2- Perinatal.

♦ **Pathogenesis , Pathology , clinical findings**

1) Normal host

CMV may be transmitted from infected person to person by close contact with virus bearing material **(**saliva , blood , urine, semen, cervical/vaginal secretions or breast milk and Perinatal. After 4-8 weeks the virus produce systemic infection . primary CMV infection of older children and adults usually asymptomatic but occasionally causes spontaneous **Infectious Mononucleosis (I.M) syndrome.**

B) Immuno compromised host

The primary infection is more severe than in normal immune host. pneumonia is the most common complication . CMV associated with leukopenia is common in solid organ transplant recipients also CMV related rejection of renal transplants .

C) Congenital and Perinatal infection

Fetal and newborn infections with CMV may be sever. A high percentage of babies with this disease will exhibit developmental defects and mental retardation . the virus can be transmitted in uteri with both primary and reactivated maternal infection. generalized cytomegalic inclusion disease results most often from primary maternal infection . fetal damage seldom results from reactivated maternal infections. The infection of the infant remain subclinical though chronic.

Mortality rates can reach up to 30% , Majority of the survivor with develop significant CNS defects within 2years. About 10% will develop deafness.

♦ **Diagnosis of CMV**

Most infections are asymptomatic and therefore go undiagnosed but in immunocompromised patients CMV can cause severe diseases and diagnosis by the following :-

1-Direct detection

- Biopsy specimens may be examined histologically for CMV for the presence of CMV antigens and also Multinucleated (cytomegalinic) cells with characteristic inclusions can be seen in biopsies of many tissues.

- The pp65 CMV antigenaemia test is now routinely used for the rapid diagnosis of CMV infection in immunocompromised patients.

- PCR for CMV-DNA.

2- Virus Isolation

• Conventional cell culture is regarded as gold standard but requires up to 4 weeks for result. Human fibroblast are used for virus isolation.

3-Serology

- The presence of CMV IgG antibody indicates past infection.

- The detection of IgM is indicative of primary infection .

❖ **Human Herpes virus 6**

Human herpes viruses 6 has two forms (A and B) first isolated from the peripheral blood of patient with ADIS. The virus is spread by the **saliva.** this virus associated with **exanthema subitum (roseola infantum)** this illness is characterized by 3 – 5 days of fever, followed by the appearance of a maculopapular "slapped cheek" rash In addition, there has been an association between human herpesvirus 6 and rejection of transplanted kidneys, fulminant hepatitis and infections of the central nervous system. The pathogenesis is poorly understood.

❖ **Human Herpes virus** 7

The virus has been associated with some cases of **exanthema subitum.** It is found in saliva of the majority of the adult (75%).the first isolated from a culture of CD4T-cells that developed a cytopathic effect from a healthy person.

❖ **Human Herpes virus 8**

Human herpes virus 8 has been found associated with **Kaposi's sarcoma** in AIDS patients as well as intra-abdominal solid tumors.

Post test

Q1:- Choose the correct answer :-

1- Shingles caused by :-

a) EBV b) HSV-1 c) HSV-2 d) CMV e) HHV-6

2- :- PP65 antigenaemia found in :-

a) HSV-1 b) EBV c) HSV-2 d) HHV-6 e) CMV

Q2:- Mention diseases associated with EBV ?

References:

1- Microbiology and immunology .5th Ed chapter 54 . 2014.
2- Jawetz, R., J.L. Melnick, and E.A. Adelberg, Review of Medical Microbiology, 16th Edition, (2019).
3- Knipe DM, Howley PM, (editors-in-chief): *Fields Virology*, 5th ed. Lippincott Williams & Wilkins, 2007.

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Title:-

Hepatitis Viruses **Lecture 7, 8**

Name of the instructor:

Assist prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Hepatitis, inflammation of the liver that results from a variety of causes. One of these causes are viruses. There are medically important viruses that are called hepatitis viruses because their main site of infection is liver . These viruses are **Hepatitis A virus(HAV) Hepatitis B virus (HBV), Hepatitis C virus(HCV), Hepatitis D virus (HDV), Hepatitis E virus (HEV) .** These viruses infect the liver and cause distinct clinical pathology by producing characteristic symptoms of jaundice and release of liver enzymes in the serum .

most of these disease spread very fast because are contagious not only during stage of manifestation of the disease but also during the phase of incubation.

Pretest:

Define hepatitis ?

Scientific Content:

Hepatitis A Virus

Hepatitis A virus (HAV) is a picornavirus that is most commonly transmitted by fecal-oral route (enterically) . It has a relatively short incubation period of 3-5 weeks after which jaundice starts suddenly. It is unique in that it does not cause chronic disease or fatal disease .

Properties of the HAV :-

1) Family picornaviridae.genus Hepatovirus.

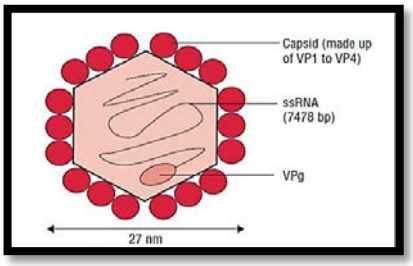
2) Non enveloped virus measuring 27nm in diameter, icosahedral symmetry.

3) It is also called as (enterovirus) because HAV lives to multiply in intestine.

4) It has single strand positive-sense RNA genome.

5) It heat and acid stable but it can be destroyed by autoclaving and by boiling in water for 5 min by dry heat in 180c for 1 hour .

6) Hepatitis A virus replicates in the cytoplasm of the infected cell



Structure of Hepatitis A Virus

Pathogenesis of hepatitis A virus infection

The virus appears to replicate first in the gastrointestinal tract and then spreads to the liver . the viruses in the liver infect hepatocytes and cause damage to hepatocytes. but the mechanism by which HAV causes cytopathic effect is not known . Cytotoxic T

cells appear to cause damage to hepatocytes ; hence once the infection is cleared , the cell damage is repaired and no chronic infection occurs.

Clinical feature of HAV

In general the **clinical feature of viral hepatitis in the start are the same** including fatigue, nausea, vomiting , anorexia and mild fever following by jaundice which is appear within few days of the prodromal period also associated with passing of dark-colored urine, pale feces and elevated serum transaminase level.

The incubation period of HAV is 10-50 days, with average of 4 weeks . the virus after infection stay in the blood 2 weeks before and one week after jaundice , but in stool it is appear 2 weeks before and 2 weeks after jaundice . it is rarely present in the urine and saliva. never pass to a chronic stage and it is not oncogenic. HAV infection is a self-limited disease and in most cases resolves spontaneously in 2-4 weeks. Hepatitis A virus infection confers life long immunity to HAV infection.

Laboratory diagnosis of HAV

Specimen: faces , blood , urine .

1- Direct detection by:-

a) Electron Microscopy

b) PCR

2- Isolation of virus

- Culture of HAV is difficult and takes up to 4 weeks to get result**.** and can be isolated on human and monkey cell line .

3- Serological Tests

Detection **HAV IgM (anti-HAV)** indicated recent or acute infection.while detection of HAV **IgG** indicated past infection .

Treatment

- No specific treatment for Hepatitis A**.** but supportive treatment include by rest and intake of food of low lipid and high carbohydrate .Vaccine available. This vaccine is given in a series of two injections, 6 to 12 months apart.

Hepatitis B Virus (HBV) .

Hepatitis B is an infectious disease called **serum hepatitis** caused by the hepatitis B virus (HBV) which affects the liver . It can cause both acute and chronic infections**. HBV is transmited by parenteral route (blood transfusion , use of HBVcontaminated needle , sexual intercourse, perinatal**).Many people have no symtoms during the initial infection

Properties of HBV

1- HBV is a member of the **Hepadnavirus family, genus Orthohepadnavirus**

2- It is a small **enveloped DNA virus.**

3- The genome is a small, **circular, double – strand DNA.the negative strand** is full length but the positive strand is incomplete, this gap must be complete at the beginging of the replication cycle .

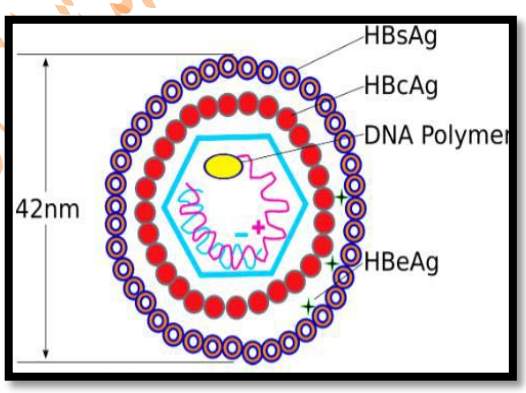
4- The viral particle is spherical and measure 42-47 nm in diameter.

5- The genome is contained in an **icosahedral nucleocapsid** of 22-25 nm in diameter surrounded by a lipid associated protein surface.

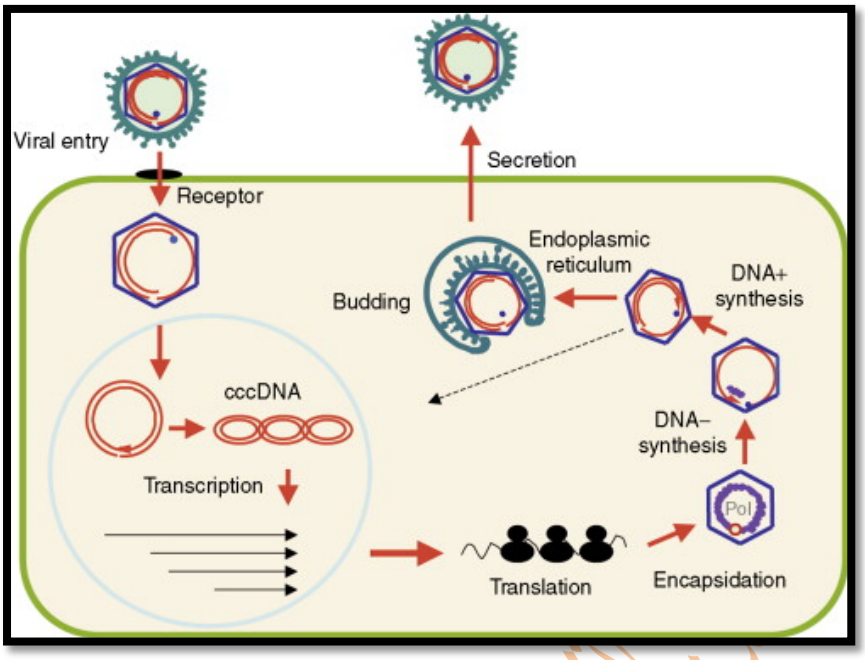
6- It is acid sensitive .

7- The replication by intermediate RNA copy of DNA genome in the nucleus and budding from the cell membrane.

8- Humans are the only natural hosts of HBV. There is no animal reservoir.



Morphology of HBV



HBV Replication cycle

Types of HBV antigens :-

1) HBsAg :- it is the surface antigen of the HBV , it is detected in large quantity in the serum, it is results from over production of HBVAg.

2) HBe Ag :- this antigen is assoicated with HBV nucleocapsid . it is usually circulated as soluble antigen in the serum and when it is prsent it indicate the occurance viral replication.

3) HBc Ag :- Hepatitis B core antigen.

Against the above antigens **antibodies will form in the blood of patients and the presence of such antibodies have the following indications:-**

a) Anti HBs antibodies :-

1- Past infection with formation of immunity to HBV.

2- Presence of passive antibody from HB immunoglobulins.

3- Immuno respons from taken vaccine .

b) Anti HBe antibodies :- presence of such antibodies indicate that this individual is a carrier.

c) Anti HBc IgM antobodies :- indicate recent infection to HBV**.**

d) Anti HBc IgG :- indicate past infection with HBV.

Incubation Time

The incubation period varies from 45to 180 days , but the average is to 60 to 90 days, duration may increase or decrease depending on the severity of the infection.

Pathogensis of HBV infection

Hepatitis B virus , after entering the blood , infects the hepatocytes in the liver with the expression of viral antigen on the surface of infected cells. Cytotoxic T cells , such as activated CD4 and CD8 lymphocytes, recognise various HBV- derived proteins present on the surface of hepatocytes resulting in immunological reaction.

1- Acute Hepatitis B virus infection:

The prodromal or pre icteric phase begins approximately two weeks after exposure is characterized by gradual onset of anorexia , malaise and fatigue , vomiting , headache and low-grade fever . during the icteric phase , the liver become tender and enlarge with development of jaundice, abdominal pain , pruritus with passing of dark-colored urine are the symptoms noted in this phase. Clinical manifestations of acute hepatitis B are similar to that of hepatitis A but with the difference that the symptoms tend to be more severe and life- threating with HBV infection.

2- Chronic Hepatitis B virus infection:-

Chronic HBV infection is one of the major complications of HBV infection The risk of chronic infection is also higher in those infected at birth (90%) and in patients who are immunocompromised . only 5-10% older children or adults progress to develop chronic infection. and the patient may become as chronic carriers and may become with chronic active hepatitis , cirrhosis and hepatocellular carcinoma may develop so it is **oncogenic virus**.

Laboratory diagnosis

Initial diagnosis of HBV infections is usually determined **clinically** if signs and symptoms are present. Evaluation of the patient's blood for elevation of liver enzymes. **Specimen : Serum** is an important specimen because definitive diagnosis of HBV depends on serological testing for HBV infections. In the diagnosis of HBV depend on the appearance of the markers in different stages of the disease as follow :-

1) Incubation period :-

- Can detect DNA of the virus by PCR

- HBs Ag and HBe Ag either at the same time or HBe Ag may appear shortly after HBs Ag by ELISA.

2) Acute phase

- HBV DNA by PCR

- HBs Ag increase in level and disappear after 6 months.

- Anti HBc : IgM antibodies followed by IgG.

- HBe Ag :- it is decrease and disappear and replaced by antibodies to HBe (Anti HBe antibody). Which indicate the end of the disease and it is usuallydisappear after 6 months.

3) Convalescence :-

During this period can detect the presence of HBs antibodies.

4) Chronic Carriers :-

It is defined that patient who have HBs Ag persist for more than 6 months in the presence of HBe Ag or anti HBe.

Treatment

- α- interferon , lamivudine antiviral drug.

- Vaccine available and use HBsAg in this vaccine.

Hepatitis C Virus (HCV)

Hepatitis C is a viral infection that affects the liver. It can cause both acute (short term) and chronic (long term) illness. It can be life-threatening.

Properties of the Virus

1- Is member of the **genus Hepacivirus , family Flaviviridae.**

2- Genome contain **single-stranded RNA , positive-sense.**

3- HCV consists of a lipid membrane enveloped . enveloped contain two viral glycoproteins (E1 & E2).

4- Most new infection (**acute infection) with HCVare subclinical (asymptomatic)** but majority of HCV infection (80%- 90%) develop **chronic hepatitis** and many at risk of progressing to chronic active hepatitis ,cirrhosis which may lead to hepatocellular carcinoma

5) Incubation period **8 weeks**.

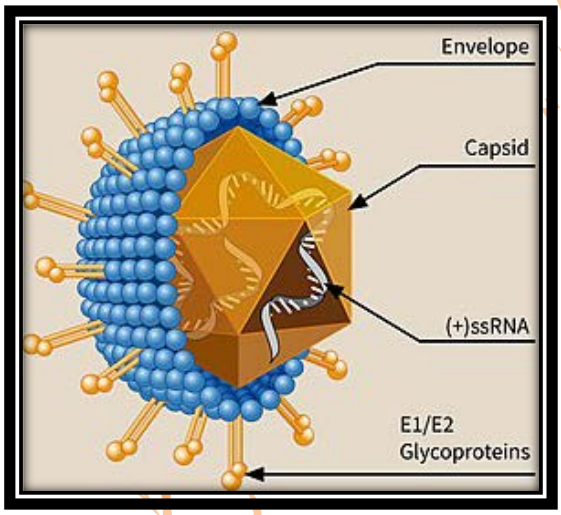
6) It is most commonly occurred in adults.

7) The root of HCV infection or transmission is **parenteral (blood borne virus)** This can happen through sharing contaminated needles or syringes an **injection drug** use accounts for almost all new HCV infection , **blood transfusions with unscreened blood products**.**Transmission from mother to child during birth** is another very common mode of transmission.

8) The virus present in the **blood and saliva** but **absent in stool and urine.**

9) It can pass to chronicity.

10) It is oncogenic virus.



Structure of HCV

Clinical feature of HCV

It is similar to other viral Hepatitis. **Acute HCV infections are usually asymptomatic** and when present symptoms usually occur a few weeks after infection and it is non specific include **Fever, anorexia, nausea, vomiting, and jaundice are common. Dark urine, pale feces, and elevated transaminase levels are seen**. The virus persists in the liver, becoming chronic in about 80% of the patients with HCV .early on chronic infection typically has no symptoms and after many years it often leads to liver disease and cirrhosis .in some cases cirrhosis will develop to hepatocellular carcinoma . Liver biopsy is often done in patients with chronic infection to evaluate the extent of liver damage and to guide treatment decisions.

Diagnosis of HCV

1) Serological assay detecting antibodies to HCV by ELISA.

2) PCR to detect the presence of Viral RNA (viral load) which are useful for monitoring patient on antiviral therapy.

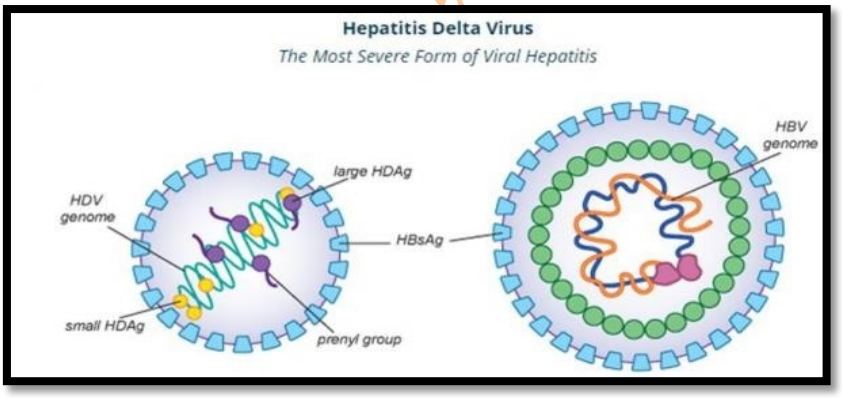
Treatment

- α –interferon , Lamivudine antiviral drugs.

- No vaccine is available.

Hepatitis D Virus (HDV)

HDV is **a defective virus** that is required the HBs Ag coat for transmission and it is only present with HBV infection .Transmission of HDV by **parenteral route.** The genome of the virus is **ss RNA with a negative sense.** HD virus antigen is the only protein coded by the HDV RNA and it is antigentically distinct from HBV antigens. HDV is the **smallest human pathogens and it is associated with most sever forms of hepatitis in the HBs Ag positive patients.**



Clinical features of HDV

The incubation period of HDV infection is **14-60 days**. clinical feature for HDV is similar to that of HBV, but because HDV is dependent on a coexistent of HBV infection ,the acute HDV infection occurs in two clinical forms :-

a) Co infection

When the two viruses HBV& HDV infect the body at the same time.

b) Super infection:-

When hepatitis D virus infect a patient who is infect with HBV with chronic infection.

Diagnosis of HDV

1) Coinfection

Can find the following antibodies and antigens:-

a) Ab to HD Ag develops late in the acute phase of infection and may be of low titer.

b) Assay for the presence of HD Ag , HD RNA, IgM to HDV.

2) Super infection

Detect the presence of **IgM and IgG Abs to HDV Ag and HDV RNA** , to gather with HBs Ag and anti HBc IgG.

Treatment

Similar to HBV treatment and vaccine. α- interferon , lamivudine antiviral drug. Vaccine available and use HBsAg in this vaccine.

Hepatitis E (HEV)

HEV is a member of the **Hepeviridae family**, genus **Hepevirus** which causes acute hepatitis in the normal host and chronic hepatitis in immunosuppressed patients. it has **ssRNA with a positive sense** , **non envelope virus** . HEV is transmitted **enterically** and occur in epidemic form in developing countries.

Clinical features of HEV

Similar to HAV. But if the infection occur in pregnancy it may has a high mortality rate reach to **20-30%.**

Diagnosis

Detection the presence of antibodies to HEV antigen IgM & IgG.

Treatment

No drug but supportive treatment . No vaccine present .

Post test

Q1:- Choose the correct answer :-

2- One of viral hepatitis lead to high mortality rate reach to 20-30% in pregnant women .

a) HAV b) HBV c) HCV d) HDV e) HEV

2- Enveloped contain two viral glycoproteins (E1 & E2) found in:-

a) HAV b) HBV c) HCV d) HDV e) HEV

Q2:- Mention laboratory diagnosis of HBV ?

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الجامعة التقنية الوسطى

كلية التقنيات الصحية والطبية /بغداد

قسم تقنيات التحليلات المرضية المادة: فايروسات المرحلة:- الثالثة

Title:-

Human Immune Deficiency Virus (HIV) **Lecture 9**

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Human Immune Deficiency virus (HIV)

Many retroviruses infect vertebrates One genus of retrovirus, Lentivirus, includes the subspecies HIV-1 and HIV-2, which cause AIDS.

- Human immunodeficiency virus (HIV) Is a retrovirus that causes human AIDS.
- HIV infects mainly CD4+ T cells, macrophages, and dendritic cells which express the surface receptor CD4.
- Destroying CD4+ T cells leads to opportunistic infection.
- Acquired immunodeficiency syndrome (**AIDS)** is the end stage of the disease that is associated with CD4+ T cell depletion, multiple or recurrent opportunistic infections, and unusual cancer (Kaposi sarcoma).
- HIV patient is different from an AIDS patient. AIDS is an end stage of HIV virus.
- **HIV-1:** most common cause of AIDS which :-
  - ►Causes HIV infection worldwide.
  - ►Highly virulent.
  - ►Highly susceptible to mutations.
- **HIV-2: -**
  - ►Causes the infection in specific regions e.g. West Africa, Mozambique.
  - ►Relatively less virulent so less pathogenic .
  - ►Relatively less susceptible to mutations.

Pretest:

Define AIDS ?

Scientific Content:

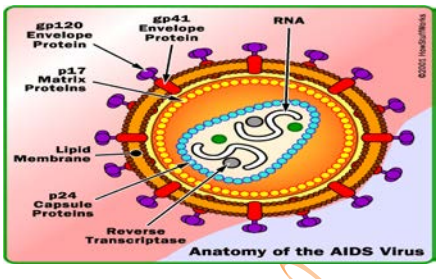
Human Immunodeficiency Virus (HIV):

Family: Retroviridae
Genus: Lentivirus
Species: Human Immunodeficiency Virus (HIV)

Characteristics of HIV

- Icosahedral (20 sided), enveloped virus and Virion consist of :-

1- Glycoprotein envelope (gp120, gp41).
2- Matrix layer (p17).
3- Capsid (p24).
4- Two copies of ss-RNA.
5- Enzymes:
• Reverse transcriptase
• Integrase,
• Protease
6- The genome consists of 9 genes:
● **3 structural genes** (gag, pol, env) required for the replication of retroviruses and 6 non-structural genes **(tat, nef, rev, vif, vpr, vpu**) regulate viral expression and are important in disease pathogenesis in vivo.



Viral Replication

First step, HIV attaches to susceptible host cell. Site of attachment is the CD4 antigen found on a variety of cells include helper T cells, macrophages. The gp120 protein on virus binds specifically to CD4 receptor on host cell with high affinity Gp41 causes fusion of the virus to the cell membrane. After fusion virus particle enters cell the Viral genome exposed by uncoating particle. Reverse transcriptase produces viral DNA from RNA becomes a provirus which integrates into host DNA then acts as a template for viral genomic and messenger RNA transcription by the host cell's nucleic acid replicating machinery.

Virus receptors

The virus use CD4 molecule which is expressed on macrophages and T- lymphocytes in addition to that HIV required a co receptor which is CCR5 a co receptor for the macrophage strains of HIV-1, whereas CXCR4 is the co receptor for the lymphocyte strain of HIV-1.these co receptors are acting normally as a chemokines receptors on the cell, and required for fusion of the virus with the cell membrane . the virus first bind to CD4 and then to the co receptors. These interactions causes conformational changes in the viral envelope activating gp41 fusion protein and triggering membrane

cell fusion. Individual who possess homozygous deletions in these co receptors may be protected from infection by HIV-1.

Transmission of HIV

a) Sexually (unprotected sex):

- ✓ Mainly in homosexual.
- ✓ The virus is present in blood, semen and vaginal secretions.

b) Parenteraly:

- ✓ Direct exposure to infected blood or body fluids (e.g. receiving blood from infected donor).
- ✓ Using contaminated or not adequately sterilized tools in surgical or cosmetic practice (dental, tattooing, body piercing).
- ✓ Sharing contaminated needles, razors, or tooth brushes .

c) Perinatally (from mother to baby):

- ✓ Infected mothers can transmit HIV to their babies transplacentally (25%). Virus spread to child perinatally mainly (50%) during delivery
- ✓ Breastfeeding is also an important way of perinatal transmission (25%).

Acute phase:

●Incubation period 2 weeks and lasts for about 12 weeks.

●Mostly asymptomatic, but in about 25-65% of the cases, patients may develop symptoms resemble infectious mononucleosis or Flu (fever, headache, anorexia, fatigue, lymphadenopathy, skin rash) which resolved in 2 weeks.

● Rapid viral replication (high viral load $>10^6$ copies/mL) .

● Gradual decrease in CD4+ T cell count.

● Blood markers in the acute stage:

● Normal to slightly decrease number of CD4+ T cells.

● Appearance of the viral RNA, and then the core antigen (p24 antigen) which indicate active viral replication.

● Appearance of two antibodies, Anti-envelop (Anti-gp120) & Anti-core (Anti-p24).

● The 1st choice marker for detection HIV in the acute phase is HIV RNA.

● Antibody tests may give false negative (no antibodies were detected despite the presence of HIV) results during the window period, an interval of three weeks to six months between the time of HIV infection and the production of measurable antibodies to HIV seroconversion.

Chronic phase:

Lasts for about 10 years in adults, and 5 years in children.

●Totally asymptomatic but the patients is still contagious.

●Relatively low viral load (500 cells/mm3).

●At the end of this stage, two syndromes appear:

1.Persistent generalized lymphadenopathy (PGL).

2.AIDS-related complex (ARC).

● Persistent generalized lymphadenopathy (PGL):
→Is defined as enlargement of lymph nodes for at least 1 cm in diameter in the absence of any illnesses or medications that known to cause PGL.
→ Clinical features: • In two or more lymph nodes out of the inguinal area.
→ Persists for at least 3 months.

● AIDS-related complex (ARC):
►Is a group of clinical symptoms that come before AIDS and may include the following:
► Fever of unknown origin that persists > 1 month.
► Chronic diarrhea, persisting > 1 month.
►Weight loss > 10% of the original weight (slim disease).
►Fatigue, night sweating, and malaise.
►Neurological disease as myelopathy and peripheral neuropathy.

●**Blood markers in the chronic stage:**
✓ Viral load (HIV RNA) increases gradually, and HIV core antigen (p24) may appear in blood.
✓ Anti-envelop (Anti-gp120) & Anti-core (Anti-p24) are positive.
✓ CD4+ T cell count gradually decreased .

AIDS phase
The end stage of the disease.
-Continuous viral replication (high viral load).
-Marked decrease in CD4+ T cell count < 200 cell/mm3.
-Defects in cellular immunity. Persistent or frequent multiple opportunistic infections.
-Unusual cancer (i.e. Kaposi sarcoma).
- Blood markers in AIDS stage:
►High viral load (HIV RNA), and HIV core antigen (p24) appears in blood
►Detection of both HIV RNA & the antigen p24 indicative of active viral replication.
►Anti-envelop (Anti-gp120) & Anti-core (Anti-p24) are positive.
►CD4+ T cell count decreased to very low levels (<200 cells/mm3).

Diagnosis
♦ Patients history with or without clinical symptoms provides hints for a physician whether the patient has ever exposed to HIV or not.
♦ Detection of both HIV Ag & Ab in the patient serum by ELISA.
→If result is positive, repeat the screening test in duplicate.
♦ If repeatedly reactive (positive), do confirmatory tests (Western blot, recombinant immunoblot assay (RIBA), or PCR).
♦Blood viral load by PCR is also used to monitor HIV replication and follow up patients treatment.

♦The western blot is an antibody detection test. The viral proteins are separated first and immobilized. In subsequent steps, the binding of serum antibodies to specific HIV proteins is visualized.

Treatment

● Is a combined therapy known as High Active Antiretroviral Therapy (HAART).

●HAART does not clear (eradicate) the virus from the body, and should be taken all life. But it prevents its duplication.

Post test

Q1:- Answer True or false :-

1- HIV patient is different from an AIDS patient. AIDS is an end stage of HIV virus.

2- HIV belong to the lentiviridae .

Q2:- Multiple choice :-

1- Co receptor for the macrophage strains of HIV-1 is :-

a- CD4 b- CCR5 c- CD8 d- CXCR4

2- The 1st choice marker for detection HIV in the acute phase is :-

a- p24 antigen b- Anti p24 c- HIV RNA d- Anti-gp120

3- AIDS stage in HIV infection include :-

a- Normal CD4+ T cell count b- high CD4+ T cell count c- decrease CD4+ T cell count d- None of them.

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كلية التقنيات الصحية والطبية /بغداد

قسم تقنيات التحليلات المرضية

المادة: فايروسات

المرحلة:- الثالثة

Title:-

Orthomyxoviruses Lecture 10

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan

استاذ مساعد د. عذراء زيدان حسن

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

The Orthomyxoviridae (influenza viruses) .

Ortho =True or real , Myxo = Affinity to mucins.

True influenza is an acute infectious disease caused by a member of the orthomyxovirus family: influenza virus **A, B** or, to a much lesser extent, influenza virus **C**. However, the term (**flu)** is often used for any febrile respiratory illness with systemic symptoms which may be caused by many of bacterial or viral agents as well as influenza. Influenza outbreaks usually occur in the winter in temperate climates. Orthomyxoviruses are divided into four types: influenza A, B , C and D but only A, B, and C infect humans. Human influenza A and B are the virus types responsible for the seasonal flu epidemics, whereas influenza type C infections generally cause mild illness. Influenza A viruses are the only influenza viruses known to cause flu pandemics and are divided into subtypes .

Pretest:

1- Orthomyxoviruses include:-

a- Pramyxo viruses b- Herpes viruses c- Influenza viruses d- Retroviruses

2- One of influenza viruses responsible for flu pandemic is :-

a- Influenza A b- Influenza B c- Influenza C d- Influenza D

Scientific Content:

Structure & Composition

Virion :- Spherical, pleomorphic, 80–120 nm in diameter. (helical nucleocapsid).

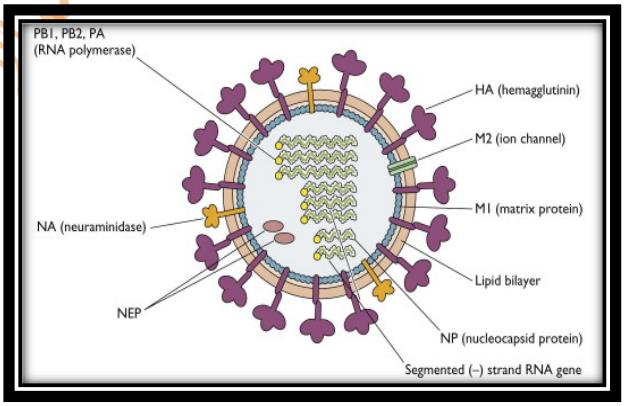
Composition: RNA (1%), protein (73%), lipid (20%), carbohydrate (6%) .

Genome: single stranded, negative sense RNA genomes of influenza A and B viruses occur as eight separate segments while influenza C viruses contain seven segments of RNA, lacking a neuraminidase gene and an outer lipoprotein envelope.

Proteins: eight structural proteins, two nonstructural protein .

Envelope: Contains 2 different spikes include viral hemagglutinin (HA) and neuraminidase (NA) proteins.

Replication: Nuclear transcription , particles mature by budding from plasma membrane.



Structure of influenza virus

The function of the HA is to bind to the cell surface receptor usually a neuraminic acid or sialic acid to infect the cell. The HA is highly antigenic and a target of the neutralising antibody.

- The hemagglutinin functions at the beginning of infection.
- The hemagglutinin agglutinates red blood cells, and this is the basis of diagnostic Hemagglutination inhibition test.

The function of the Neuraminidase (NA)

- The antigenicity of NA, the other glycoprotein on the surface of influenza virus particles, is also important in determining the subtype of influenza virus isolates.

- **The NA functions** at the end of the viral replication cycle include :-
a) It is a sialidase enzyme that removes sialic acid from glycoconjugates.
b) It facilitates release of virus particles from infected cell surfaces during the budding process
c) helps prevent self-aggregation of virions.

Influenza A virus have **Two** matrix proteins **M1** and **M2.**

M1 is located between the internal nucleoprotein and the envelope and provides structural integrity.

M2 protein forms an ion channel between the interior and the exterior of the virus. It transports protons into the virion causing the disruption of the envelope. This leads to the uncoating of the virus and frees the nucleocapsid containing the RNA genome and allows it to migrate to the nucleus.

A non structural protein called NS-1, by its ability to inhibit the production of interferon mRNA reduces the host innate defense and is an important

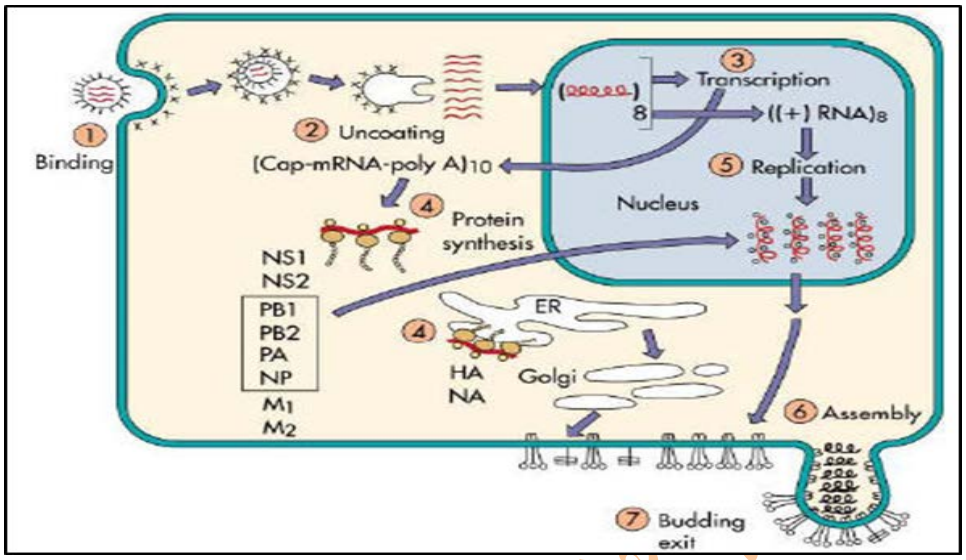
Influenza virus life cycle

Replication of influenza A virus include after binding to sialic acid-containing receptors, influenza is endocytosed which fuses with the vesicle membrane and uncoats mediated by the M2 proteins and is facilitated by the low pH within the endosome/vesicle .

- The viral nucleocapsid enters the cytoplasm and migrates to the nucleus where the genome RNA (8 segments) gets transcribed into mRNA by the viral RNA polymerase (transcriptase). Unlike for most other RNA viruses, transcription and replication of the genome occur in the **nucleus** were Viral proteins synthesized

- Most RNA's move to cytoplasm, some remain in the nucleus to serve as a template for the synthesis of negative polarity strand RNA genomes for the progeny, by a different subunit of viral RNA polymerase (replicase).

▸ Helical nucleocapsid segments form and associated with the M1 protein-lined membranes containing M2 and the HA and NA glycoprotein's . The virus buds from the plasma membrane .



Influenza virus life cycle

Antigenic Changes of Orthomyxoviruses:

Changes in the antigenicity of hemagglutinin and neuraminidase confers on the Influenza A virus the ability to cause pandemics.

Two types of antigenic changes are known

1- Antigenic drift refer to a minor change based on accumulate mutations during virus replication in the genome RNA. Thus, Influenza viruses have many serotypes.

2- Antigenic shift that involves a major change based on the reassortment of segments of the genome RNA.

Antigenic shifts can result from mechanisms Genetic reassortment between subtypes. Reassortment is possible whenever two different influenza viruses infect a cell simultaneously; when the new viruses (the progeny) are assembled, they may contain some genes from one parent virus and some genes from the other .

Types of influenza viruses

There are four types of influenza viruses: **A, B, C and D**

1. Influenza A viruses

Influenza A viruses include the avian, swine, equine and canine influenza viruses, as well as the human influenza A viruses. Influenza A viruses are classified into subtypes based on two surface antigens, the hemagglutinin (H) and neuraminidase (N) protein. There are 18 different known H antigens (H1 to H18) and 11 different known N antigens (N1 to N11). H1N1, H1N2, and H3N2 are the only known influenza A virus subtypes currently circulating among humans.

2. Influenza B viruses

Influenza B viruses are mainly found in humans. These viruses can cause epidemics in human population, but have not, to date, been responsible for pandemics**.**

3- Influenza C viruses

Influenza type C infections generally cause mild illness and are not thought to cause human flu epidemics.

4- Influenza D viruses primarily affect cattle and are not known to infect or cause illness in people.

Viral Transmission

Influenza viruses are transmitted in aerosols created by coughing and sneezing, and by contact with nasal discharges, either directly or on fomites. Close contact and closed environments favor transmission . Person-to-person transmission occurs with the H1N1 virus that is currently circulating in humans.

Clinical findings

Incubation Period :

The incubation period for human influenza is usually short; most infections appear after one to four days .The incubation period for the novel H1N1 virus circulating in humans appears to be 2 to 7 days .

Clinical Signs &Pathogenicity

Uncomplicated infections with human influenza A or B viruses are usually characterized by upper respiratory symptoms, which may include fever, chills, anorexia, headache, myalgia, weakness, sneezing, rhinitis, sore throat and a nonproductive cough . Nausea, vomiting and otitis media are common in children, and febrile seizures have been reported in severe cases. Most people recover in one to seven days, but in some cases, the symptoms may last up to two weeks or longer.

More severe symptoms, including pneumonia, can be seen in individuals with chronic respiratory or heart disease. Secondary bacterial or viral infections may also occur.

Laboratory Diagnosis of Human Influenza

Specimen collection

Respiratory specimens: Respiratory specimens obtained within four days of onset of symptoms and different types of respiratory specimens can be used such as nasal washes and nasopharyngeal aspirates tend to be more sensitive than pharyngeal swabs.

Blood specimens : Acute and convalescent serum samples 14 − 21 days should be collected to demonstrate a significant (at least fourfold) rise in strain-specific antibody titer.

Laboratory Tests

1- Isolation methods (Viral Culture)

- Embryonated egg culture

- Cell culture :- Various cell-lines are utilized to isolate influenza viruses, most commonly primary monkey kidney cells. infection of cells gives a visible cytopathic effect (CPE).

2- Direct methods

- Immunofluorescence
- Enzyme immuno assays
- Reverse transcription polymerase chain reaction (RT-PCR).

3- Serology

Different serological techniques are available for influenza diagnosis include haemagglutination inhibition (HI), complement fixation (CF), enzyme immunoassays (EIA) and indirect immunofluorescence**.**

Post test

Q :- Multiple choice :-

1- Replication of the genome in influenza viruses take place in:-

a-Cytoplasm b- nucleus c- cytoplasm and nucleus d- Golgi apparatus

2- One of influenza virus protein which has ability to inhibit interferon mRNA is :-

a- M1 b-M2 c- HA d- NS1

3-Antigenic shift in influenza virus refer to :-

a- Minor change in genome b- Major change in genome c- Intermediat change in genome d- Non of them

4- All of the following include Neuraminidase (NA) functions except one :-

a- It is a sialidase enzyme that removes sialic acid from glycoconjugates.
b- helps prevent self-aggregation of virions.
c- It facilitates release of virus particles from infected cell surfaces during the budding process
d- Agglutinates red blood cells, and this is the basis of diagnostic Hemagglutination inhibition test.

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1- Medical Microbiology . Jawetz . 2016.

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4- Richard Hunt ,Microbiology and Immunology On-line. Update 2021.

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كلية التقنيات الصحية والطبية /بغداد

قسم تقنيات التحليلات المرضية

المادة: فايروسات

المرحلة:- الثالثة

Title:-

Paramyxoviruses Lecture 11

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan استاذ مساعد د. عذراء زيدان حسن

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

The paramyxoviruses include the most important agents of respiratory infections of infants and young children (respiratory syncytial virus [RSV] and the parainfluenza viruses) as well as the causative agents of two of the most common contagious diseases of childhood (mumps and measles). The World Health Organization estimates that acute respiratory infections and pneumonia are responsible every year worldwide for the deaths of 4 million children younger than 5 years. Paramyxoviruses are the major respiratory pathogens in this age group.

All members of the **Paramyxoviridae** family initiate infection via the respiratory tract. Whereas replication of the respiratory pathogens is limited to the respiratory epithelia, measles and mumps become disseminated throughout the body and produce generalized disease.

Classification

The family Paramyxoviridae consists of three important genera

1- Paramyxovirus includes parainfluenza and mumps virus.

2- Morbillivirus includes the measles virus.

3-Pneumovirus includes respiratory syncytial virus (RSV), which is responsible for majority of acute respiratory infections in infants and children

PARAMYXOVIRUS FAMILY

GENUS	MEMBERS	GLYCOPROTEINS
Paramyxovirus	mumps human parainfluenza viruses (HPIV 1-4)	HN, F
Morbillivirus	Measles	H, F
Pneumovirus	Respiratory syncytial virus	G, F

Pre-test

Which the following of paramyxoviruses belong to pneumovirus ?

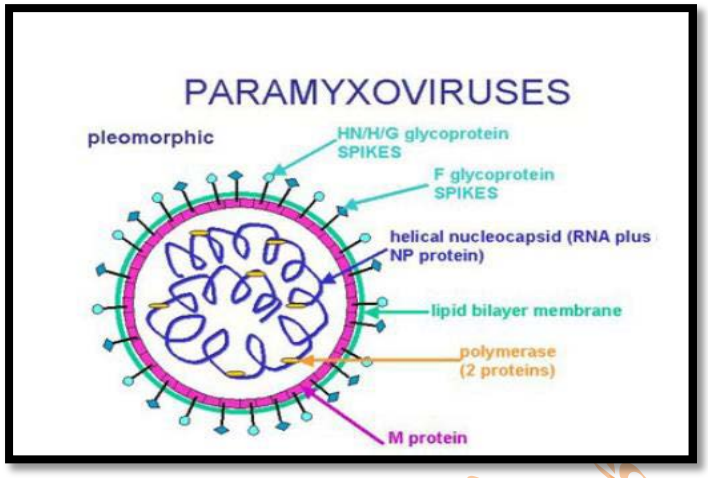
measles virus, parainfluenza , mumps virus , respiratory syncytial virus (RSV)

Scientific Content

Properties of Paramyxo viruses

- **Virion:**Spherical,pleomorphic,150 nm or more in diameter (helicalnucleocapsid,13–18nm)
- **Composition:** RNA(1%),protein (73%),lipid (20%), carbohydrate(6%)
- **Genome:** Single- stranded negative RNA, linear,non segmented ,about15kb, no reassortment .
- **Proteins:** Six to eight structural proteins.
- **Envelope:** Contains viral glycoprotein(G ,H, or HN)(which sometimes carries hemagglutinin or neuraminidase activity) and fusion(F) glycoprotein
- **Replication:** Cytoplasm ; particles bud from plasma membrane. . A large excess of nucleocapsids are produced in infected cells, which form characteristic cytoplasmic inclusion bodies.
- **Outstanding characteristics :** Antigenically stable Particles are labile yet highly infectious.

Transmission :- spread by droplets from the nose and mouth to close contacts. Many of them are highly infectious and go around the community in epidemics- often seasonal, eg. Winter coughs and colds. Fomites might also assist spread.



Structure of Paramyxovirus

♦ **Human parainfluenza viruses (HPIVs)**

HPIVs are single-stranded, enveloped RNA viruses of the Paramyxoviridae family. There are four serotypes(1-4) which cause respiratory illnesses in children and adults. HPIVs bind and replicate in the ciliated epithelial cells of the upper and lower respiratory tract and the extent of the infection correlates with the location involved. Seasonal HPIV epidemics result in a significant burden of disease in children and account for 40% of pediatric hospitalizations for lower respiratory tract illnesses and **75% of croup cases.**

Pathogenesis of human parainfluenza virus (HPIV) infection

The virus adsorbs to the respiratory epithelial cells by specifically combining with neuraminic acid receptors in the cell through its hemagglutinin. Subsequently, the virus enters the cells following fusion with the cell membrane, mediated by F1 and F2 receptors. The virus replicates rapidly in the cell cytoplasm and causes formation of multinucleated giant cells. The virus also causes the formation of single and multilocular cytoplasmic vacuoles and basophilic or eosinophilic inclusions.

The virus causes inflammation of the respiratory tract, leading to secretions of high level of inflammatory cytokines, usually 7–10 days after initial exposure. Airways inflammation, necrosis, and sloughing of respiratory epithelium, edema, and excessive mucus production are the noted pathological features associated with HPIV infections.

Clinical feature

Human parainfluenza viruses cause **croup** (a heterogeneous group of illnesses that affects the larynx, trachea, and bronchi. The condition manifests as fever, cough, laryngeal obstruction), pneumonia, bronchiolitis and tracheobronchitis, and Otitis media, pharyngitis, conjunctivitis. The severity of the disease occurred in infant less than 6 months.

- **Laboratory Diagnosis**
- **Clinical feature**
- **Respiratory specimens** include nasopharyngeal aspirations nasal washings, and nasal aspirations.

1- Direct antigen detection

The ELISA, immunofluorescence assay are used to detect HPIV antigen

2-Molecular Diagnosis :

polymerase chain reaction (PCR) has been developed for detection of HPIV-1, HPIV-2, and HPIV-3 genome in clinical specimens.

3- Isolation and identification

Nasal wash are good specimens, culture in monkey kidney cell line , the diagnosis depending on hem adsorption

- **Prevention and Control**

Currently there is no vaccine against infection by HPIV, However, researchers are trying to develop one.

Mumps

Mumps is an acute contagious disease characterized by nonsuppurative enlargement of one or both salivary glands. Mumps virus mostly causes a mild childhood disease, but in adults complications including meningitis and orchitis are fairly common. More than one-third of all mumps infections are asymptomatic.

Pathogenesis & Pathology

Humans are the only natural hosts for mumps virus. Primary replication occurs in nasal or upper respiratory tract epithelial cells.

Viremia then disseminates the virus to the salivary glands and other major organ systems. Involvement of the parotid gland is not an obligatory step in the infectious process. The incubation period may range from 2 to 4 weeks but is typically about 14–18 days. Virus is shed in the saliva from about 3 days before to 9 days after the onset of salivary gland swelling. About one-third of infected individuals do not exhibit obvious

symptoms (in apparent infections) but are equally capable of transmitting infection. Virus frequently infects the kidneys and can be detected in the urine of most patients. Viruria may persist for up to 14 days after the onset of clinical symptoms. The central nervous system is also commonly infected and may be involved in the absence of parotitis.

Clinical Findings

Fever, malaise followed by rapid enlargement of the parotid gland and it is painful . mumps may be associated with aseptic meningitis . testis and ovaries may be infected especially after puberty and it may pass to sterility in man but it is rare (not more than 1%).

Laboratory diagnosis of Mumps virus

1) Clinical feature

2) Isolation and identification

The most appropriate clinical samples for viral isolation are saliva, cerebrospinal fluid, and urine collected within a few days after onset of illness. Virus can be recovered from the Culture in monkey kidney cells urine for up to 2 weeks**.**
and diagnosis by using mumps specific antiserum by immunofluorescence method, hemadsorption test can also be used.

3) Nucleic acid detection:- by PCR test.

4) Serology

IgM and IgG Abs detection by ELISA and Heamagglution inhibition test.

Treatment , Prevention

- There is no specific therapy .
- Immunization with attenuated live mumps virus vaccine is the best approach to reducing mumps-associated morbidity and mortality rates. Mumps vaccine is available in combination with measles and rubella (MMR) live-virus vaccines.

Measles

Measles is an acute, highly infectious disease characterized by fever, respiratory symptoms, and a maculopapular rash. Complications are common and may be quite serious.

Pathogenesis & Pathology

Humans are the only natural hosts for measles virus. The virus gains access to the human body via the respiratory tract, where it multiplies locally; the infection then spreads to the regional lymphoid tissue, where further multiplication occurs. Primary viremia disseminates the virus. Finally, a secondary viremia seeds the epithelial surfaces of the body, including the skin, respiratory tract, and conjunctiva, where focal replication occurs. The described events occur during the incubation period, which typically lasts 8–12 days but may last up to 3 weeks in adults. involvement of the central nervous system is common in measles.

Clinical Findings

Infections in non immune hosts are almost always symptomatic. After an incubation period of 8–12 days, measles is typically a 7-11days illness. The prodromal phase is characterized by fever, sneezing, coughing, running nose, redness of the eyes, **Koplik spots**, and lymphopenia. The conjunctivitis is commonly associated with photophobia. **Koplik spots** are small, bluish-white ulcerations on the buccal mucosa opposite the lower molars. These spots contain giant cells and viral antigens and appear about 2 days before the maculopapular rash. . The most common complication of measles is **otitis media** (5–9% of cases**). Pneumonia** is the most common life-threatening complication of measles, caused by secondary bacterial infections.

Subacute sclerosing panencephalitis (SSPE) is a very rare, but fatal disease of the central nervous system that results from **a measles virus infection** acquired earlier in life SSPE generally develops 7 to 10 years after a person has measles**.**

Laboratory Diagnosis

1) Clinical feature

2) Isolation & Identification of Virus

● Nasopharyngeal and conjunctival swabs, blood samples, respiratory secretions, and urine collected from a patient during the febrile period are appropriate sources for viral isolation. culture in monkey and human kidney cells , diagnosis by cytopathic effect , multinucleated and intra nuclear and intra cytoplasmic inclusion bodies.

3- Antigen detection

Measles antigen can be directly detected from specimen include respiratory secretion , nasopharynx and conjunctiva by Immunofluorescence test.

4- Serology

IgM and IgG antibodies by ELISA and Heamagglution inhibition test (HI) test.

5- Detection of viral RNA by RT-PCR

Is a sensitive method that can be applied to a variety of clinical samples for measles diagnosis.

Treatment, Prevention, & Control

No treatment . A highly effective and safe attenuated live measles virus vaccine has been available since 1963.

Respiratory syncytial virus(RSV)

It is the most common cause of lower respiratory tract illness in infant and young children.

Pathogenesis and pathology

Replication of the virus occurred initially in the nasopharynx , then the virus may spread to the lower respiratory tract and produce bronchiolitis and pneumonia. The incubation period 3-5 days and virus shedding for 1-3 weeks.

Clinical findings :-

Common cold , pneumonia in infant and may bronchitis and bronchiolitis which Life-threatening disease in infant especially under 6, and can lead to chronic lung disease in later life. Reinfection is common in both children and adult with less severity. This virus are a common cause of otitis media about 30% of otitis media cause in infant .

Laboratory diagnosis of Respiratory syncytial virus (RSV)

1- Clinical feature

2- Antigen detection

Nasal wash or aspirate are good sample .Virus antigens detection by immunofluorescence test .

3- Isolation and identification of the virus

By culturing the specimen into human heteroploid cell line (Hela) and Hep-2, the diagnosis is depend on the cytopathic effect and appearance of giant cells.

4- Nucleic acid detection

Diagnosis by detection of the RNA of the virus by PCR.

5-Serology

Detection of serum antibodies which include IgM and IgG Abs by using immunofluorescence test .

Treatment

Supportive care , Ribavirin may be used in the treatment of severe cases by aerosol for 3-6 days . No vaccine is available today but passive immunization immunoglobulin can be given for infected premature infants.

Post test

Q :- Multiple choice :-

1- Croup caused by :-

a- Measles virus b- Mumps virus c- RSV d- Parainfluenza virus

2- Koplik spots occur in :-

a- Mumps b- RSV c- Measles d- Parainfluenza virus

3- **SSPE** is complication of :-

a- Parainfluenza virus b- Measles c- Mumps d- RSV

4- Orchitis caused by :-

a- Measles virus b- RSV c- Mumps virus d- Parainfluenza virus

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قسم تقنيات التحليلات المرضية

المادة: فايروسات

المرحلة:- الثالثة

Title:-

Enteric viruses (Polio, Rota) Lecture 12

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan

استاذ مساعد د. عذراء زيدان حسن

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Enteric viruses represent a wide spectrum of viral genera that invade and replicate in the mucosa of the intestinal tract .Enteric viruses are the commonest causes of gastroenteritis worldwide, they are most often transmitted via the fecal-oral route, with transmission by direct human contact and via fomites being common. Enteric viruses may also be present in contaminated water supplies.Enteric viruses can be grouped as follows:

1) Viruses causing localized inflammation at any level of the intestinal tract, predominantly in small intestinal mucosa, resulting in acute gastroenteritis, for example, rotaviruses, caliciviruses, adenoviruses, astroviruses .

2) Viruses that multiply at any level of the intestinal tract, causing few enteric symptoms prior to producing clinical disease at a distant site, for example, measles virus, reoviruses , enteroviruses (including polioviruses, coxsackieviruses, hepatitis A and E).

3) Viruses that spread to the intestinal tract during the later stages of systemic disease, generally in an immunocompromised host, for example, human immunodeficiency virus (HIV), cytomegalovirus.

Pre-test

Define Enteric Viruses ?

Scientific Content

Polio Virus

Polio virus is the causative agent of polio (also known as poliomyelitis) which is a highly contagious disease caused by a virus that attacks the central nervous system. Children younger than 5 years old are more likely to contract the virus than any other group **.**

Structure and properties of polio Virus

1- Virus classification : Picornaviridae ,Genus Enteroviruses .
2- Virion :- Viral particle is spherical in shape about 30 nm in diameter with icosahedral symmetry .
3- Genome :- is a single-stranded positive-sense RNA (+ssRNA) , linear with 7500 nucleotides long .
4- The particles are simple in that they are composed of **a protein shell** surrounding the **naked RNA genome**.
5- The virus particles **lack a lipid envelope**, and their infectivity is insensitive to organic solvents.
5- Humans are the only susceptible hosts.
6- Three serotypes of poliovirus, PV-1, PV-2, and PV-3.
7- Capsid contains 60 copies each of the four viral polypeptides VP1, VP2, VP3, and VP4.
8- Replication of Polio Virus occur in the **cytoplasm** . viral particles are release from the host cell through **lysis** of the cell.

Pathogenesis of Polio Virus

The mouth is the portal of entry for the virus, transmitted by fecal oral route on ingestion of contaminated water. **The incubation period is 9-12 days.**
Following ingestion, the virus multiplies in the oropharyngeal and intestinal mucosa. The lymphatic system , in particular the tonsils and the Peyer's patches of the ileum are invaded and the virus enters the blood resulting in primary viraemia . Antibodies to the virus appear early in the disease, usually before paralysis occurs. The antibodies are produced to prevent infection from spreading. In a minority of cases, On continued

infection and multiplication of virus in the ReticuloEndothelial System (RES), it invades the blood stream causing secondary viremia **.** During this period of viremia, the poliovirus crosses the blood brain barrier and gain access to the brain. The virus shows tissue tropism by specifically combining with neural cells. The virus recognizes the receptor present on the anterior horn of spinal cord, dorsal root ganglia and motor neurons. The destruction of motor neurons leads to paralysis.

Clinical Manifestations of Polio Virus

There are 3 possible outcomes of infection:

1- Subclinical infection (90 - 95%) : – in apparent subclinical infection account for the vast majority of poliovirus infections.

2- Abortive infection (4 - 8%) :- a minor influenza-like illness occurs characterized by fever, headache, sore throat, loss of appetite, vomiting, and abdominal pain. **Neurological symptoms are typically absent.**

3- Major illness (1-2%) :- the major illness may present 2 - 3 days following the minor illness or without any preceding minor illness. Signs of aseptic meningitis are common. Involvement of the anterior horn cells of spinal cord lead to flaccid paralysis. Involvement of the medulla may lead to respiratory paralysis and death.

Laboratory Diagnosis of Polio Virus

Specimen:

Stool, rectal swab, throat swab, CSF (rare)

1- Microscopy

Virus can be detected in stool specimens by direct electron microscopy or also by immune electron microscopy.

2- Virus isolation

Virus may be recovered from pharyangeal aspirations and feces. Virus isolation from feces and throat swab is carried out by cultivation on monkey kidney, human amnion, HeLa cells, Hep-2 and other cell cultures. Cytopathogenic effects appear in 3–6 days. An isolated virus is identified and typed by neutralization with specific antiserum .

3-Serodiagnosis

Demonstration of antibody titer in the serum sample collected at the time of acute illness and time of convalescence. **Neutralization test** and **complement fixation** test is carried out to demonstrate antibodies presence.

Treatment of Polio Virus
No antiviral treatments are available for the treatment of poliomyelitis.

Prevention
The disease may be prevented through Vaccination. There are two vaccines available :-
1- Intramuscular Poliovirus Vaccine (IPV):- consists of formalin inactivated virus of all 3 poliovirus serotypes. Produces serum antibodies only: does not induce local immunity and thus will not prevent local infection of the gut.

2- Oral Poliovirus Vaccine (OPV)
Consists of live attenuated virus of all 3 serotypes. Produces local immunity through the induction of an IgA response as well as systemic immunity.

Rotavirus

► Classification of Rotavirus:

- Family: **Reoviridae**
- Genus: **Rotavirus**
- Classified into seven distinct groups (A to G) based on structural antigen VP6.

Group A, B, and C Rotaviruses are found in Human infection as well as animal infection. Group A Rotaviruses are most frequent Human pathogen.

► Structure, composition and properties of Rotavirus

- Characteristics **"wheel"** like appearance (Rota-means wheel).
- **Size:** 65nm-100nm in diameter.
- **Shape:** Spherical shape.
- **Symmetry:** Icosahedral.
- **Genome:** 11 segments of **double stranded RNA (ds RNA**).
- **Protein:** 6 structural protein (VP) and 6 Non-structural protein (NSP).
- **Envelope:** Absent

Nucleic acid is surrounded by two layer of capsid- **inner capsid (VP6)** and **outer capsid (VP7)**.
VP4 is the spike protein, it is a cell surface receptor.
Replication: Occurs in cytoplasm of infected cell.
Rota virus contain an **RNA-dependent RNA polymerase** and other enzymes capable of producing capped RNA transcripts.
Rota virus do not **grow in cell line culture**.

► Mode of Transmission:

- Ingestion of contaminated food and water.
- Directly from faces contaminated fingers.
- Occasionally by droplet infection.
- Children below 5 years are mostly affected.
- Adults are infected by contact with pediatric cases.

► Pathogenesis:

Incubation period: 2-3 days

● Rota virus replicates in enterocyte near the tip of villi destroying enterocytes.

● Viral encoded toxin: early profuse, secretory diarrhea is caused by enterotoxin, NSP4.

● Disruption of intestinal epithelium due to virus replication

● Histologic changes of enterocytes that triggers enteric nervous system, intestinal secretion and immune response.

● The acute infection and diarrhea normally resolves within 7 days in immunocompetent hosts.

Clinical symptoms:

1- Local infection:

● Acute Gastroenteritis, severe in case of infants aged 6-24 months.

●Infected Infants are unable to digest milk due to lactase deficiency caused by destruction of enterocytes

● Diarrhea, nausea and vomiting

● Malabsorption of Na+, water and disaccharides.

● Symptoms of Dehydration: decrease in urination, dry mouth and throat and feeling dizzy when standing up.

2. Systemic infection:

High grade Fever

Lymphocytosis and transient neutropenia.

Laboratory diagnosis:

Specimen: faces in early infection,

1- Viral antigen detection: by solid phase agglutination , ELISA (it is sensitive for detected virus in stool sample , Electron microscopy.

2-PCR: For genotyping of Rotavirus.

3-Virus culture: No cell line culture.

Treatment:

- Oral rehydration
- Other supportive rehydration therapy to control loss of water and electrolytes.

Vaccine: Two Oral rotavirus vaccines are currently licensed for use in infants:-
1- RotaTeq (RV5) is given in 3 doses at ages 2 months, 4 months, and 6 months.
2- Rotarix (RV1) is given in 2 doses at ages 2 months and 4 months.

Post test

Q1:- Mention Clinical Manifestations of Polio Virus?
Q2 :- Answer True or false
1- Polio virus contain envelope membrane.
2- Group C Rotaviruses are most frequent Human pathogen.

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قسم تقنيات التحليلات المرضية **المادة: فايروسات** **المرحلة:- الثالثة**

Title:-

Rabies Virus

Lecture 13

Assist Prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

The family Rhabdoviridae consists of more than 100 single-stranded, negative-sense, non segmented viruses that infect a wide variety of hosts, including vertebrates, invertebrates, and plants. Common to all members of the family is a distinctive **rod- or bullet-shaped** morphology. Human pathogens of medical importance are found in the genera Lyssavirus and Vesiculovirus.Only **rabies virus**, medically the most significant member of the genus Lyssavirus.

Rabies virus causes acute infection of the central nervous system .

Pre-test

Family of rabies virus called :-

1- Retroviridae b- Rhabdoviridae c- Hepdnaviridae d- Reoviridae

Scientific Content

► This virus transmitted by Zoonosis.

► Rabies virus is the most important member of the rhabdoviridae

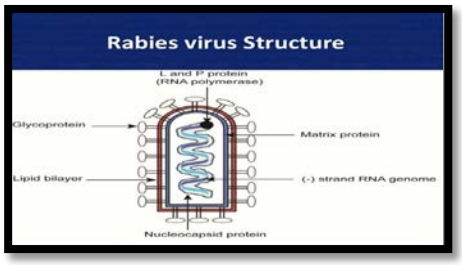
family, which causes disease in humans.

►This virus affects the central nervous system (cases inflammation in the brain).
► Primary reservoirs are wild mammals; it can be spread by both wild and domestic mammals by bites, scratches, and inhalation of droplets

►Multiplication of the virus occurs in cytoplasm of infected cells , viral proteins together with the viral RNA aggregate in the cytoplasm of virus infected neurons and compose Negri bodies.

Morphology:

● Bullet – shaped virus, size (180-75nm),with one end rounded. Enveloped RNA virus .
● Genome :-, linear , negative-sense ,SS RNA virus, unsegmented.
● Host Range **:-** Animal: Domestic dogs, cats and wild animals.



● **Antigenic Properties:**

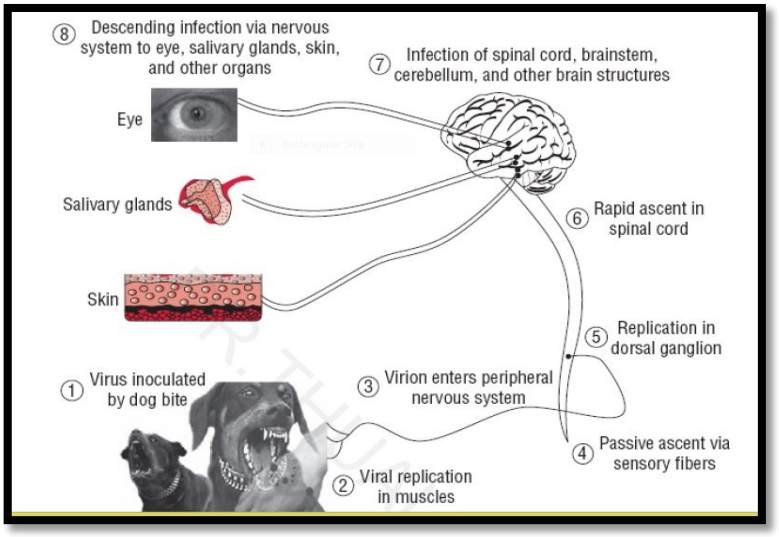
❖ **G protein:** The glycoprotein or G protein is present on the surface spikes present on the outer lipoprotein envelope of the virion, helps to absorb receptor on the nerve tissues.
❖ **N protein:** Nucleoprotein or N protein is a group-specific antigen. It shows cross-reaction with some rabies-related viruses.
❖ **Other antigens:** These include membrane proteins, glycolipid and RNA-dependent RNA polymerase
❖ Ab produce against these Ag-are protective.

Pathogenesis:

● Human infection is usually caused by the bite of dogs or other animals.

● Virus present in the saliva of the animals also can caused by licks or aerosols.

Entry in the body
(once it is bites)
⇓
Rabies is virus deep inside the muscle (saliva).
⇓
Multiple in muscle tissue, connective tissue.
⇓
Reach nerve or neural cell
⇓
Finally reaches the brain and produce Negri bodies in human.



Clinical Manifestations

Five general stages of rabies are recognized in humans:

1- incubation period :- usually 30 to 90 days but ranging from as few as 5 days to longer than 2 years after initial exposure.

2- Prodromal period, which usually lasts from 2 to 10 days. These symptoms are often nonspecific include fever, nausea, vomiting, headache, fatigue, sore throat, cough.
3- Acute neurological period (2 to 3 days, rarely up to 6 days).
4- coma
5- death

Lab Diagnosis:-

►Specimen – saliva , CSF, Brain biopsy, skin biopsy, cornea.

1. Direct detection by Microscope (immunofluroscent staining technique (for Ag detection).

2. Isolation of virus (cell lines, egg yolk, mice) to detect Negri bodies and viral Ag.

3- Detection of rabies virus-neutralizing antibody.

4- Molecular method for detection viral RNA.

Treatment:

No specific treatment for this virus .

Prevention:

By vaccination – Both active & passive

Post test

Q1 :- Multiple choice :-

1- Multiplication of Rabies virus take place in :-

a- Nucleus b- cytoplasm c- Golgi apparatus d- Non of them

2- Diagnostic characteristic for Rabies in infected cell is :-

a- inclusion bodies b- Negri bodies c- giant cell d- Round cell

References

1- Review of Medical Microbiology and Immunology. Warren Levinson, 12th May 2012. Mc Graw-Hill (Lange).

2- Jawetz, R., J.L. Melnick, and E.A. Adelberg,(2019). Review of Medical Microbiology, Twenty-Eighth Edition Edition,.

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المادة: فايروسات

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Title:-

Pox virus **Lecture 14**

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Poxviruses are the largest and most complex of viruses. Infections with most poxviruses are characterized by a rash, although lesions induced by some members of the family are markedly proliferative. The group includes variola virus, the etiologic agent of smallpox—the viral disease that has affected humans throughout recorded history.

Pre-test

Q :- Answer True or false :-

1- Poxviruses are the smallest and most complex of viruses ?

Scientific Content

Properties of Poxviruses

- **Virion:** Complex structure, oval or brick-shaped, 400 nm in length x 230 nm in diameter; external surface shows ridges; contains core and lateral bodies.
- **Composition:** DNA (3%), protein (90%), lipid (5%).
- **Genome :** Double-stranded DNA, linear; size 130–375 kbp; has terminal loops; has low G + C content (30–40%) except for *Parapoxvirus* (63%).
- **Proteins:** Virions contain more than 100 polypeptides; many enzymes are present in core, including transcriptional system
- **Envelope:** Virion assembly involves formation of multiple membranes
- **Replication:** Cytoplasmic factories
- **Outstanding characteristics:**

→ Largest and most complex viruses; very resistant to inactivation .

→ Virus-encoded proteins help evade host immune defense system.

→ Smallpox was the first viral disease eradicated from the world.

Classification

Poxviruses are divided into two subfamilies based on whether they infect vertebrate or insect hosts. The vertebrate poxviruses fall into eight genera, with the members of a

given genus displaying similar morphology and host range as well as some antigenic relatedness.

Most of the poxviruses that can cause disease in humans are contained in the genera Orthopoxvirus and Parapoxvirus .

Poxvirus Replication

Virus particles establish contact with the cell surface and fuse with the cell membrane. Viral cores are released into the cytoplasm. The mRNAs are transcribed within the viral core and are then released into the cytoplasm. The "uncoating" protein that acts on the cores is among the more than 50 polypeptides made early after infection. The second stage uncoating step liberates viral DNA from the cores; it requires both RNA and protein synthesis.

Viral DNA replication occurs in the cytoplasm and appears to be accomplished by viral coded enzymes. Viral DNA replication occurs 2–6 hours after infection in discrete areas of the cytoplasm.

Mature virions appear in electron micrographs as a DNAcontaining core encased in double membranes, surrounded by protein, and all enclosed within **two outer membranes**. Some of the particles are released from the cell by budding, but the majority of poxvirus particles remain within the host cell.

Poxvirus Infections in Humans: Vaccinia and Variola.

Comparison of Vaccinia and Variola Viruses

Vaccinia virus, the agent used for smallpox vaccination, is a distinct species of Orthopoxvirus.

Variola has a narrow host range (only humans and monkeys), whereas **vaccinia** has a broad host range that includes rabbits and mice. Some strains of vaccinia can cause a severe disease in laboratory rabbits that has been called rabbitpox. Vaccinia virus has also infected cattle and water buffalo, and the disease in buffalo (buffalopox). Both vaccinia and variola viruses grow on the chorioallantoic membrane of the 10- to 12-day-old chick embryo, but the latter produce much smaller pocks. Both grow in several types of chick and primate cell lines**.**

Pathogenesis & Pathology of Smallpox

The portal of entry of variola virus was the mucous membranes of the upper

respiratory tract. After viral entry, the following are believed to have taken place: (1) primary multiplication in the lymphoid tissue draining the site of entry; (2) transient viremia and infection of reticuloendothelial cells throughout the body; (3) a secondary phase of multiplication in those cells, leading to (4) a secondary, more intense viremia; and (5) the clinical disease.

By the sixth to ninth days, lesions in the mouth tended to ulcerate and discharge virus. Later, pustules broke down and discharged virus into the environment of the smallpox patient.

Clinical Findings

The incubation period of variola (smallpox) was 10–14 days. The onset was usually sudden. One to 5 days of fever and malaise preceded the appearance of the exanthems, which began as macules, then papules, then vesicles, and finally pustules. These formed crusts that fell off after about 2 weeks, leaving pink scars that faded slowly.

Immunity

An attack of smallpox gave complete protection against reinfection. Vaccination with vaccinia induced immunity against variola virus for at least 5 years and sometimes longer.

Laboratory Diagnosis

Several tests are available to confirm the diagnosis of smallpox.

1- **Isolation and Identification of Virus**

Skin lesions are the specimen of choice for viral isolation.

2- **Serology**

Antibody assays can be used to confirm a diagnosis.

Treatment

Vaccinia immune globulin is prepared from blood from persons vaccinated with the vaccinia virus. **Methisazone** is a chemotherapeutic agent of some value against poxviruses.

Time of Vaccination

Complications of vaccination occur most commonly under the age of 1 year. Therefore, vaccinating between 1 and 2 years of age is preferable to vaccinating in the first year of life. Revaccination has been done at 3-year intervals.

Post test

Q1 :- Multiple choice :-

1- Multiplication of pox virus take place in :-

a- Nucleus b- cytoplasm c- Golgi apparatus d- Non of them

2- The etiologic agent of smallpox is :-

a- Vaccinia b- Variola c- Orthopoxvirus d- buffalopox

Q2**:- Mention Comparion of Vaccinia and Variola Viruses ?**

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1- Review of Medical Microbiology and Immunology. Warren Levinson, 12th May 2012. Mc Graw-Hill (Lange).

2- Jawetz, R., J.L. Melnick, and E.A. Adelberg,(2019). Review of Medical Microbiology, Twenty-Eighth Edition Edition,.

الجامعة التقنية الوسطى

كلية التقنيات الصحية والطبية /بغداد

قسم تقنيات التحليلات المرضية **المادة: فايروسات** **المرحلة:- الثالثة**

Title:-

Coronaviruses **Lecture 15**

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Coronaviruses are large a family of viruses , enveloped RNA viruses and they are called “corona” because of crown-like spikes on the surface of the virus . Coronaviruses are known to cause disease in humans, other mammals, and birds. In humans coronaviruses cause common colds, may cause lower respiratory tract infections and have been implicated in gastroenteritis in infants. Novel coronaviruses have been identified as the cause of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) and the new strain of coronavirus — SARS-CoV-2 — was first reported in Wuhan, China in December 2019. It has since spread to every country around the world.

The human viruses are difficult to culture and therefore are more poorly characterized.

Pre-test

Q :- Mention strains of coronavirus that cause diseases in human ?

Scientific Content

Important properties of coronaviruses

►**Virion :-** Spherical, 120-160 nm in diameter, helical nucleocapsid

►Genome:- Single-stranded RNA linear, non segmented , positive-sense,27-32kb, infectious.

►**Protein :-** virions contain three or four structural proteins: a major spike glycoprotein (S), trans membrane glycoproteins (M and E), a nucleoprotein (N), and in some viruses, a hemagglutinin esterase (HE).

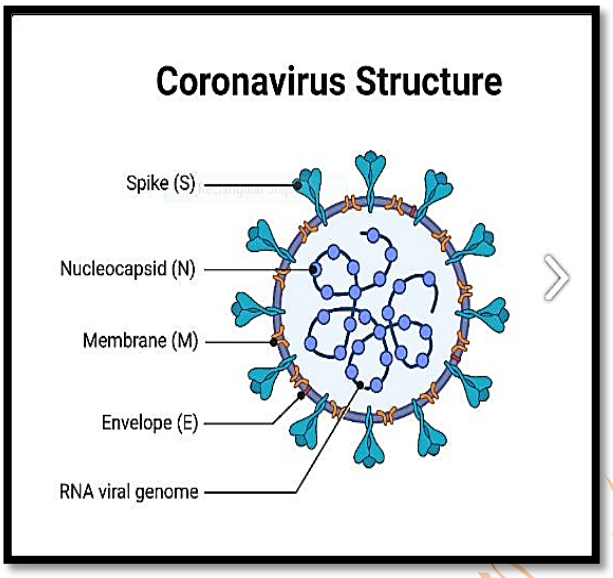
►Envelope :- contain large ,widely spaced, club-shaped spikes.

► Replication :- in cytoplasm, particles mature by budding into endoplsmic reticulum and Golgi apparatus .

►Outstanding characteristic:-

- Cause colds and SARS
- Display high frequency of recombination

- Difficult grow in cell culture



Classification

Coronaviruses belong to the family Coronaviridae ,subfamily Coronarivinae , and order Nidovirales were classified depend on the basis of the crown or halo-like appearance of the envelope glycoproteins and on characteristic features of chemistry and replication .

Depending on the serotype they are divided in 4 genera: α, β, γ and δ-CoVs. α and β-CoVs infect human and γ and δ-CoVs infect birds . Seven serotypes infect the human: 229E, NL63 (α-CoVs); OC43, HKU1 (β-CoVs, lineage A); SARS (β-CoV, lineage B); MERS (β-CoV, lineage C) and the most recent SARS-CoV-2 (β-CoV, lineage B).

Coronavirus Replication

The entire cycle of coronavirus replication occurs in the cytoplasm.

The virus attaches to receptors on target cells by the glycoprotein spikes on the viral envelope (either by S or HE). The receptor for human coronavirus 229E is aminopeptidase N, whereas a functional receptor for the SARS virus is **angiotensin-converting enzyme 2.**

after uncoating translation of the viral genomic RNA to produce a virus-specific RNA-dependent RNA polymerase.The viral polymerase transcribes a full length complementary (minus-strand) RNA. which are used to synthesize full-length genomic RNA and subgenomic mRNA. new virions form by budding from host cell membranes.

Transmission:

The virus is usually transmitted via **inhalation of contaminated droplets**, but it may also be **transmitted by the hands to the mucosa of the nose or eyes** . also **contaminated surfaces and fomites.**

Clinical Findings

♦ The human coronaviruses produce "colds," usually a febrile in adults. The symptoms are similar to those produced by rhinoviruses, typified by nasal discharge and malaise. The incubation period is from 2 to 5 days.

♦ The SARS coronavirus causes severe respiratory disease. The incubation period averages about 6 days. Common early symptoms include fever, malaise, chills, headache, dizziness, cough, and sore throat, followed a few days later by shortness of breath and may lead to pneumonia and death rate highest among the elderly from progressive respiratory failure occurs in almost 10% of cases.

Pathogenesis

Transmission is usually via airborne droplets to the nasal mucosa.Virus replicates locally in cells of the ciliated epithelium. Infected cells become vacuolated, show damaged cilia, and may form syncytia . Cell damage triggers the production of inflammatory mediators, which increase nasal secretion and cause local inflammation and swelling. These responses in turn stimulate sneezing, obstruct the airway, and raise the temperature of the mucosa.

Laboratory Diagnosis

- Specimens:- Nasopharyngeal swabs, throat swab, saliva, other lower respiratory tract secretions, blood , stool.

A. Antigen and Nucleic Acid Detection

● Coronavirus antigens in cells in respiratory secretions may be detected using the **ELISA test** if a high-quality antiserum is available.

● Enteric coronaviruses can be detected by examination of stool samples by electron microscopy.**Polymerase chain reaction (PCR) assays** are useful to detect coronavirus nucleic acid in respiratory secretions and in stool samples.

- **Virus RNA** was detectable in plasma by **PCR** with viremia most readily detectable between days 4 and 8 of infection.

B. Serology

Because of the difficulty of virus isolation, serodiagnosis using acute and convalescent sera is the practical means of confirming coronavirus infections. ELISA, indirect immunofluorescent antibody assays, and hemagglutination tests may be used.

C. Computed tomography (CT) examination is plays an important role in the diagnosis of SARS-CoV-2 pneumonia .

Treatment

The treatment of coronavirus colds remains symptomatic. No specific treatment for SARS-CoV-2 . most people with mild COVID-19, rest and drinking plenty of fluids are the best approach. severe cases require hospital care, including breathing support, mechanical ventilation, or other medical treatments. The transmission can be reduced by practicing hygienic measures.

Post test

Q1 :- Multiple Choice :-

1- Receptor for the SARS virus is :-

a- Aminopeptidase N b- angiotensin-converting enzyme c- hemagglutinin esterase d- Non of them

2- Genome of Coronavirus belong to the:-

a- ss RNA negative sense b- ds DNA c- ss RNA positive sense d- ds RNA

Q2 :-Mention strains or serotypes of coronavirus that cause diseases in human?

References

1- Review of Medical Microbiology and Immunology. Warren Levinson, 12th May 2012. Mc Graw-Hill (Lange).

2- Jawetz, R., J.L. Melnick, and E.A. Adelberg,(2019). Review of Medical Microbiology, Twenty-Eighth Edition Edition,.

3- Christopher J. Burrell, ... Frederick A. Murphy, in Fenner and White's Medical Virology (Fifth Edition), 2017

الجامعة التقنية الوسطى

كلية التقنيات الصحية والطبية /بغداد

قسم تقنيات التحليلات المرضية المادة: فايروسات المرحلة:- الثالثة

Title:-

Adenovirus and Parvovirus Lecture 16

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan استاذ مساعد د. عذراء زيدان حسن

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Adenoviruses most commonly cause respiratory illness; however, depending on the infecting serotype, they may also cause various other illnesses, such as gastroenteritis, conjunctivitis, cystitis (bladder infection), and rash illness. Symptoms of respiratory illness caused by adenovirus infection range from the common cold syndrome to pneumonia, croup, and bronchitis. Young infants and especially patients with

compromised immune systems are more susceptible to severe complications of adenovirus infection. Acute respiratory disease (ARD), which was first recognized among military recruits during World War II, can be caused by adenovirus infections. Adenoviruses were first isolated in human adenoids (tonsils), from which the name is derived. Adenoviruses represent the largest non enveloped viruses, because they are the maximum size able to be transported through the endosome. Adenoviruses are medium sized (90-100 nm), non enveloped icosahedral viruses containing double- stranded DNA.

Pretest

Genome of Adenovirus contain the following:-

a) dsDNA b) dsRNA c) ssDNA d) ssRNA

Scientific Content

Adenovirus

Family: Adenoviridae

Classification Contain two genera:-

1- **Masta adenovirus**. Infect the human .

2- **Avia adenovirus** Infect birds.

3- More than 60 serotypes infect human.

4- Adenoviruses are divided into seven Subgroups or species **(A to G)** based on physical, chemical, biological properties.

-Subgroup A: serotype12, 18 and 31: highly oncogenic and cause sarcoma when injected into new born hamsters.

Properties of adenoviruses

● Composition: DNA(13%), Protein(87%).

● Genome: Linear, double stranded DNA, 26-45 kbp ,

● The surface of its capsid consisting of three major proteins; hexon, penton base and a knobbed fiber .

● Hexon & penton capsomeres are the major components on the surface of the virus particle

- Penton base with toxin-like activity
- Fibers – with type – specific antigens; associated with hemagglutinating activity.
- Adenoviruses are unusually stable to chemical and physical agents and to adverse PH conditions, thus allowing for prolonged survival outside of the body that adenovirus **resistant** Acid , Detergent, Dry environment **while Inactivated** by Heat, Formaldehyde, Bleach.

Transmission

Adenoviruses are transmitted by several mechanisms

• **Direct contact,** Adenoviruses causing conjunctivitis are very infectious and spread by direct contamination of the eye.

• **Respiratory droplets** . Respiratory adenoviruses are spread by the respiratory route (aerosol droplet, direct and indirect).

• **Feco-oral route.** Enteric adenoviruses are spread via the fecal-oral route.

Viral replication:

1- Adenoviruses attach to surface of the cells by their fibers, then penetrate the cell, and once inside the cell, uncoated the viral DNA.

2- The viral DNA is then transported into the nucleus of the cell and initiates replication cycle.

3- Host cell DNA-dependent RNA polymerase transcribes the early genes leading to formation of functional m RNA.

4- Then the cytoplasm, the early m RNA is translated to nonstructural proteins.

5- In the nucleus , after viral DNA replication, late m RNA is transcribed and then translated into structural virion proteins.

6- This is followed by assembly of virions in the nucleus and release of virions by lysis of the cells, but not by budding.

Pathogenesis

Adenoviruses are transmitted mainly by respiratory or feco-oral contact from humans. They infect the conjunctiva or the nasal mucosa. They may multiply in conjunctiva, pharynx, or small intestine, where epithelial cells are infected.

The site of entry generally dictates the type of infection; 2 processes can occur:

These are (a) lytic infection, (b) latent infection.

(A) Lytic infection: Adenoviruses infect muco epithelial cells in the respiratory tract, gastrointestinal tract, and conjunctiva or cornea, causing damage of these cells directly(cell lysis) . After local replication of the virus, viremia follows with subsequent spread to visceral organs. Dissemination occurs more commonly in immunocompromised patients than in the immunocompetent individuals.

(B) Latent infection: The adenovirus has unique ability to become latent in lymphoid and other tissues such as adenoids, tonsils, and payer s patches. The exact mechanism of latency of adenovirus in these tissue is not known. These latent infections can be reactivated in patients infected with other agents or in the patients who are immunocompromised.

Clinical syndrome: -

Various syndromes are associated with particular serotypes:-

- Respiratory diseases (Phrayngitis and tonsilitis)
- Pharyngioconjunctivitis
- Eye disease (Conjunctivitis)
- Pneumonia: in preschool children
- Gastroenteritis (Gastrointestinal disease)
- Acute hemorrhagic cystitis (bladder infection)
- Cervicitis and urethritis

Laboratory Diagnosis

Specimens: from throat, eye, urine, feces.

1- Isolation of virus

- Inoculation into cell cultures; human embryonic kidney/ Hela/Hep
- CPE: cell rounding and agglutination into grape like clusters. Others tests: HA, Neutralization, CF.

2- Serology: detection of adenoviral antigens by ELISA, Haemagglutination inhibition test, Neutralization tests and Immunofluorescence for antigen detection in nasopharyngeal/ ocular specimens.

3- Direct detection of Virus particle by Electron microscopy (EM) in stool Sample .

4- PCR indicates adenovirus infection by use type-specific primers can be used to distinguish between different types of adenoviruses.

Treatment

There is no antiviral therapy. Limited efficacy of antivirals (Ribavirin).

Post test

Mention Laboratory Diagnosis of Adenovirus ?

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1- Review of Medical Microbiology and Immunology. Warren Levinson, 12th May 2012. Mc Graw-Hill (Lange).

2- Jawetz, R., J.L. Melnick, and E.A. Adelberg,(2019). Review of Medical Microbiology, Twenty-Eighth Edition Edition,.

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قسم تقنيات التحليلات المرضية المادة: فايروسات المرحلة:- الثالثة

Title:-

Parvovirus Lecture 16

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Parvovirus, commonly abbreviated to **parvo**, is a genus of the Parvoviridae family linear, non-segmented s**ingle stranded DNA** viruses with an average genome size of 5 kbp. Parvoviruses are some of the smallest viruses found in nature (hence the name, from Latin **parvus** meaning *small*).

Pretest

Genome of Parvovirus contain the following:-

a) dsDNA b) dsRNA c) ssDNA d) ssRNA

Scientific Content

Properties of Parvoviruses :-

- Parvoviruses are the smallest DNA.
- Icosahedral, non enveloped particles are 18–26 nm in diameter.
- Virions are extremely resistant to inactivation.
- They are stable between a pH of 3 and 9 and withstand heating at 56°C for 60 minutes, but they can be inactivated by formalin, and oxidizing agents.
- Virions contain two coat proteins ; VP1 and VP2.
- The genome is about 5 kb, linear, single-stranded DNA.
- Virions contain either positive or negative-sense strands. They need help from other viruses or from rapidly dividing host cells in order to replicate.

Classification:

There are two subfamilies of Parvoviridae :-
1- Parvovirinae, which infect vertebrates, which have three genera:

a) Parvovirus :- which takes its name from that of the family, and infects only animals and birds;
b) Dependovirus :- named for dependence on a helper virus, usually an adenovirus, but occasionally a herpesvirus, to assist in replication.
c) Erythrovirus :- which has only one member, known as B19, the only parvovirus causing significant disease in humans; Erythema infectiosum, Fetal infections and Aplastic crisis .

2- Densovirinae which infect insects.

Transmission:

1- Respiratory route.
2- Transmitted by blood transfusions or by infected blood products.
3- Vertically from mother to fetus.

Parvovirus Replication:

Only primary erythroid progenitors are known to be permissive for B19 infection. The cellular receptor for B19 is blood group P antigen which is expressed on mature erythrocytes, erythroid progenitors, megakaryocytes, endothelial cells, placenta, and fetal liver and heart, which helps explain the narrow tissue tropism of B19 virus.

Clinical features:

1- Erythema infectiosum (Fifth Disease)

This manifestation is most common illness in children of early school age and occasionally affects adults. Fever and mild constitutional symptoms may accompany the rash, which has a typical “slapped cheek” appearance followed by a maculopapular rash on the trunk and limbs, which may persist for 2 or 3 weeks Joint involvement due to immune complex deposition is a prominent feature in adult cases; joints in the hands and the knees are most frequently affected. The symptoms mimic rheumatoid arthritis. The incubation period is usually 1–2 weeks but may extend to 3 weeks.

2- Transient Aplastic Crisis: Parvovirus B19 is the cause of transient aplastic crisis (with very low hemoglobin and disappearance of circulating reticulocytes) that may complicate chronic hemolytic anemia, such as in patients with sickle cell disease,

thalassemia, and acquired hemolytic anemia in adults. Transient aplastic crisis may also occur after bone marrow transplantation.

3-B19 Infection During Pregnancy (Fetal infection) : Maternal infection with B19 virus may pose a serious risk to the fetus, resulting in hydrops fetalis and fetal death due to severe anemia. Fetal death occurs most commonly before the 20th week of pregnancy.

Laboratory Diagnosis:

Specimen; blood cells, tissue samples, and respiratory secretions.

1- **Polymerase chain reaction (PCR),** in situ hybridization. PCR is the most sensitive assay.

2-**Immunohistochemistry** has been used to detect B19 antigens in fetal tissues and bone marrow.

4-Detection of IgM antibody by **ELISA** indicates a current or recent infection, whereas the much more prolonged presence of IgG is a sign of past infection.

Treatment and Prevention:

There is no vaccine against human parvovirus and there is no antiviral drug therapy.

Post test

Q1:- Multiple choice ?

1- The only parvovirus causing significant disease in humans is :-

a- Dependovirus b- Parvovirus c- Erythrovirus d- non of them

2- Virion of parvovirus contain :-

a- ss DNA b- ds DNA c- ss RNA d- ds DNA

References

1- Review of Medical Microbiology and Immunology. Warren Levinson, 12th May 2012. Mc Graw-Hill (Lange).

2- Jawetz, R., J.L. Melnick, and E.A. Adelberg,(2019). Review of Medical Microbiology, Twenty-Eighth Edition Edition,.

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قسم تقنيات التحليلات المرضية

المادة: فايروسات

المرحلة:- الثالثة

Title:-

Arbovirus

Lecture 17

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan استاذ مساعد د. عذراء زيدان حسن

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Arboviruses is a term used to describe a group of RNA viruses transmitted to humans by blood-sucking arthropods from one vertebrate host to another. There are many strains of arbovirus.

The viruses range in severity from no symptoms to mild flu-like symptoms to very severe symptoms. Avoiding insect bites is key to preventing these nasty viral infections. Avoiding insect bites is key to preventing these nasty viral infections.

Insects that can infect humans with arboviruses include fleas, ticks, gnats, and mosquitoes. There are over 130 different arboviruses that affect humans.

Pretest

Define Arboviruses?

Scientific Content

General properties The arboviruses share some common biological

1- They can multiply in the tissues of the arthropod without evidence of disease or damage

2- The vector acquires a lifelong infection through the ingestion of blood from a viremic vertebrate.

3- All arboviruses have an RNA genome, and most have a lipid-containing envelope.

4- All members produce fatal encephalitis in suckling mice after intracerebral inoculation

5- They possess haemagglutinin and agglutinate erythrocytes of goose or day-old chicks

6- They can be grown in tissue cultures of primary cells like chick embryo fibroblasts or continuous cell lines like vero, and in cultures of appropriate insect tissues

7- They may also be isolated in the yolk sac or CAM of chick embryo

8. In general, arboviruses are readily inactivated at room temperature and by bile salts, ether or sodium deoxycholate and other lipid solvents.

Common types of Arbovirus

There are many types of arboviruses. The different types of arbovirus are broken down into specific genera.

The three main genera for arboviruses that cause infections in humans are as follows:

1- flavivirus
2-togavirus
3- bunyavirus

Types of flavivirus include the following:

- Yellow fever
- West Nile virus
- Zika virus
- dengue fever

- Japanese encephalitis

Types of togavirus include the following:

- Ross River virus
- Eastern equine virus
- Western equine virus

Types of bunyavirus include the following:

- California encephalitis
- La Crosse virus
- Jamestown Canyon virus

Transmission

The arboviruses spread mainly through insect bites. The most common insect that spreads arboviruses is the mosquito. However, other arthropods such as ticks, fleas, and gnats can also spread these diseases if they bite a human. While insect bites are the most common way arboviruses are transmitted, the viruses can also spread through blood transfusion , organ transplant , sexual contact, pregnancy and childbirth from mother to child.

Pathogenesis

→When an infected vector bites a suitable host, the virus is injected into the capillary circulation.

→Virus comes in contact with susceptible target cells such as endothelial cells of capillaries, monocytes, macrophages and cells of RES.

→After replication in endothelial cells and RE cells, a secondary viraemia usually results leading to infection of target organs such as brain, skin, musculature and liver, depending on the tissue tropism.

→The virus reaches the brain by infecting small blood vessels of the brain or choroid plexus.

Clinical feature

Most infections caused by arboviruses asymptomatic .however , symptom when present can range from a mild flu-like illness to encephalitis, a potentially life-threatening inflammation and swelling in the brain.

The clinical characteristics and symptoms are divided into two subgroups:-

1- Neuroinvasive diseases indicating that the disease can infect the nervous system, often cause meningitis or encephalitis. Symptoms of neuroinvasive arboviruses include the sudden onset of fever , headache , stiff neck, muscle pain, confusion or disorientation, weakness in the arms and legs, seizures.

2- Non-neuroinvasive diseases in this disease arboviruses differ slightly in their symptoms. The nervous system is not affected, so they do not typically cause altered mental state, such as confusion or seizures.

non-neuroinvasive arboviruses can cause a fever in addition to headache , muscle aches , upset stomach, joint pain , nausea, vomiting or diarrhea, rash.

Laboratory Diagnosis of Arbovirus

Specimen: Blood, CSF (Cerebrospinal fluid) and Brain may be used for isolation of virus. All Arboviruses are viremia – blood is collected during the acute phase of the disease. CSF is useful in encephalitis cases but the best specimen is the brain.

Diagnosis may be established by virus isolation or serology.

Isolation of the virus :

a) Suckling mice – specimens are inoculated intracerebrally into suckling mice. The animal may develop fatal encephalitis.

b) Tissue culture – Arboviruses may also be isolated in tissue cultures – Vero, BHK-21 and mosquito cell lines are inoculated with specimens. The growth of virus in cell cultures is identified by immunofluorescence, haemagglutination inhibition, complement fixation, ELISA or neutralization tests.

Serology: Using ELISA, serotype-specific IgM antibody may be detected in patient serum within 1-3 days after the onset of illness.

Prevention

While effective vaccines are available for some arboviruses, including Japanese encephalitis and yellow fever, there is not a vaccine for all arboviruses. Many other vaccines for arboviruses are currently being developed, however. The best way to prevent arboviral infections is by preventing insect bites particularly in areas that have high incidences of arboviruses.

Post test

Q1:- Multiple choice ?

1- dengue fever belong to the :-

a- togavirus b- b- bunyavirus c- flavivirus d- Non of them e- All of them

2- Susceptible target cells of Arboviruses are :-

a- Endothelial cells of capillaries b- monocytes c- macrophages d- cells of RES. e- All of them

References

1- Review of Medical Microbiology and Immunology. Warren Levinson, 12th May 2012. Mc Graw-Hill (Lange).

2- Jawetz, R., J.L. Melnick, and E.A. Adelberg,(2019). Review of Medical Microbiology, Twenty-Eighth Edition Edition,.

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Title:-

Oncogenic viruses (Human cancer viruses) Lecture 18

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Cell growth: is the cell proliferation (the increase in cell numbers that occurs through repeated cell division).

• Cell growth is regulated by **two groups of regulatory genes:**

A. Proto-oncogenes (cellular oncogene, c-onc)

Are normal genes which control cell proliferation, but which have the potential to contribute to cancer development if their expression is altered (changed into oncogenes). So Oncogenes are genes that cause cancer.

B- Tumor Suppressor Genes include genes that inhibit cell growth , fixing broken DNA or causing a cell to die. Examples: P53, Rb (retinoblastoma) .

An important difference between oncogenes and tumor suppressor genes is that oncogenes result from the activation **(turning on) of proto-oncogenes**, but tumor suppressor genes cause cancer when they are inactivated **(turned off).**

In normal cells, oncogenes are ‘‘switched off’’or down-regulated by antioncogeneproteins.

An **oncovirus** is a virus that can cause cancer.

• It refers to any virus with a DNA or RNA genome causing cancer and also called "tumor virus" or "cancer virus".

• Most viruses are non-transforming - however, they may play a role in reducing the host cell’s ability to inhibit apoptosis.

Pretest

Define oncogenic viruses ?

Scientific Content

A virus that is able to cause cancer is known as an **oncogenic virus**. Evidence that a virus is oncogenic includes the regular presence in the tumor cells of virus DNA, which might be all or a part of the virus is possible that the virus is just one of a number of carcinogenic factors that can give rise to these cancers.

At least **15-20%** of all human tumors worldwide have a viral cause. The viruses that have been strongly associated with human cancers. Viruses are etiologic factors in the development of several types of human tumors, including two of great significance worldwide cervical cancer and liver cancer. They include **human papillomaviruses** (HPVs), **Epstein-Barr virus** (EBV), **human herpesvirus 8**, **hepatitis B virus**, **hepatitis C virus**, and two **human retroviruses** plus several **candidate human cancer** viruses. Many viruses can cause tumors in animals, either as a consequence of natural infection or after experimental inoculation.

CLASSES OF ONCOGENIC VIRUSES: There are two classes of tumor viruses:

- **DNA tumor viruses**
- **RNA tumor viruses, the latter also being referred to as Retroviruses.**

DNA tumor viruses :-

1- **Papovaviridae** include human papilloma virus causes uterine (cervical) cancer.

2- polyomaviridae include JK,BK causes solid tumor in rodents and Merkel Cell Polyomavirus cause Merkel Cell Carcinoma (rare skin cancer).

3- Herpesviridae include :-

a) EBV infection increases the risk of Burkitt lymphoma, Nasopharyngeal carcinoma and some types of Hodgkin's and non-Hodgkin's lymphoma also stomach cancer.
b) Kaposi's sarcoma-associated herpesvirus (KSHV or HHV-8) is associated with Kaposi's sarcoma, a type of skin cancer.
c) Human cytomegalovirus (CMV or HHV-5) is associated with mucoepidermoid carcinoma and possibly other malignancies.

4- Hepadnaviridae include Hepatitis B virus causes hepatocellular carcinoma.

5- Adenoviridae include adenovirus causes various solid tumor in rodents .

6- Poxviridae include Smallpox; cowpox causes various solid tumor.

RNA tumor Viruses

1-Retroviridae include Human T-cell leukemia virus (HTLV-1; HTLV-2) which causes Adult T-cell leukemia, Lymphoma.

2-Flaviviridae include Hepatitis C Virus causes Hepatocellular carcinoma.

General Features of Viral Carcinogenesis

1- Viruses can cause cancer in animals and humans.

2- Tumor viruses frequently establish persistent infections in natural host.

3- Viruses are seldom complete carcinogens.

1- Host factors are important determinants of virus-induced tumorigenesis

5. Virus infections are more common than virus-related tumor formation.

6. Long latent periods usually elapse between initial virus infection and tumor appearance.

7- Viral strains may differ in oncogenic potential.
8- Viruses may be either direct- or indirect-acting carcinogenic agents.
9- Oncogenic viruses modulate growth control pathways in cells.
10- Animal models may reveal mechanisms of viral carcinogenesis.

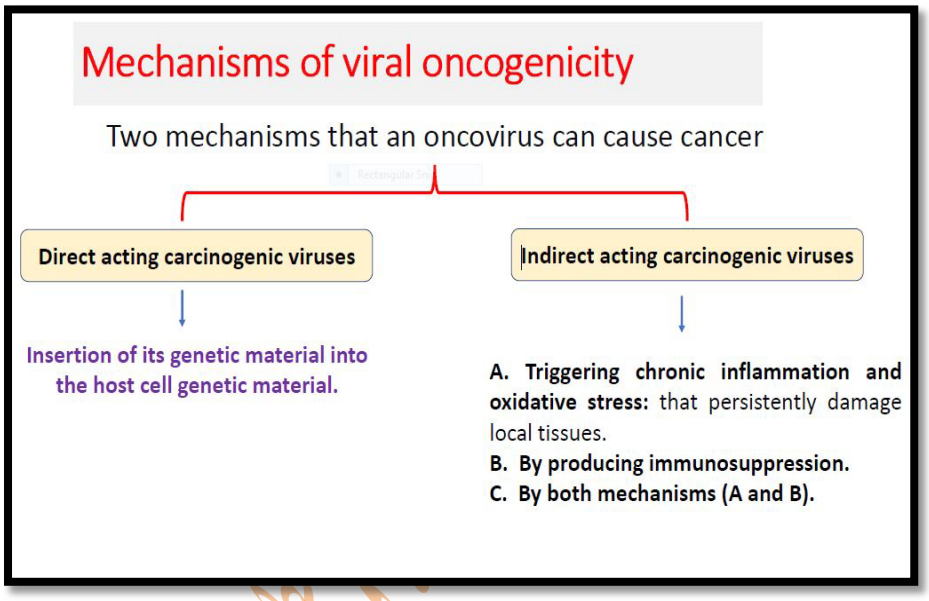
How do viruses cause cancer?

Viruses typically initiate cancer development by suppressing the host's immune system, causing inflammation over a long period of time, or by altering host genes. Most virus-induced cancers develop after a long period of persistent infection with an oncogenic virus; for adult T cell leukaemia this period is exceptionally long (around 60 years). The virus infections persist in their hosts in spite of immune responses, such as the production of virus-specific antibodies.

As cancer develops in only small percentages of virus-infected hosts it is clear that the virus infections alone do not cause cancer. several factors influence the progression from viral infection to cancer development. These factors include host's genetic makeup, mutation occurrence, exposure to cancer causing agents, and immune impairment.

Mechanisms of Action by Human Cancer Viruses

Tumor viruses mediate changes in cell behavior by means of a limited amount of genetic information. There are two general patterns by which this is accomplished: The tumor virus introduces a new "**transforming gene**" into the cell (**direct-acting**), or the virus alters the expression of a preexisting cellular gene or genes **(indirect-acting).** In either case, the cell loses control of normal regulation of growth processes. DNA repair pathways are frequently affected, leading to genetic instability and a mutagenic phenotype.



Papillomavirus-linked cancers

Cervical carcinoma is the third most common cancer in women, with approximately half a million new cases and 280 000 deaths in the world each year. Most, if not all, of these cancers result from infection with a papillomavirus. The papillomaviruses are small DNA viruses of mammals and birds .

They enter the body through small abrasions and infect keratin-making cells (keratinocytes) in skin or a mucous membrane. Each HPV type infects a preferred site, such as the hands or the genitals, and infection may result in a benign wart (papilloma) or a carcinoma.

Most papillomavirus infections do not become persistent, but in a minority of hosts the infection is not cleared by the host's immune response. In individuals who harbour persistent infection there is a small risk of cancer developing. This risk is associated

with about 15 of the HPV types; these '**high-risk**' types include **HPV-16 and 18**. Infection with other HPV types that infect the genitals (Warts) carries little or no risk of cancer; these '**low-risk**' HPV types include **HPV-6 and 11.**

Human Retroviruses

The human T-lymphotropic (HTLV) group of retroviruses has probably existed in humans for thousands of years. HTLV-1 has been established as the causative agent of **adult T cell leukemia-lymphomas (ATL**) as well as a nervous system degenerative disorder called tropical spastic Para paresis. It does not carry an oncogene. A related human virus, HTLV-2, has been isolated and associated with Some cases of **hairy cell leukemia**.

Transmission of HTLV-1 seems to involve cell-associated virus. Mother-to-child transmission via breast feeding is an important mode. Such early-life infections are associated with the greatest risk of ATL. Blood transfusion is an effective means of transmission, as are sharing blood-contaminated needles (drug abusers) and sexual intercourse.

Damage to immune defenses

Interactions between cell proteins and proteins produced by oncogenic viruses can lead to breakdown of immune defenses that may allow the development of a cancer. Papillomavirus proteins interfere with apoptosis, and hence prevent the death of virus-infected cells.

Post test

Q1:- Multiple choice ?

1- Human cytomegalovirus (CMV or HHV-5) is associated with :- :-

a- mucoepidermoid carcinoma b- Kaposis carcinoma c- hairy cell leukemia

d- Burkitt lymphoma

2 High-risk types of HPV is associated with - :-

a- Stomach cancer b-Warts c- cervical carcinoma d- Hodgkin's lymphoma

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1- Review of Medical Microbiology and Immunology. Warren Levinson, 12th May 2012. Mc Graw-Hill (Lange).

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الجامعة التقنية الوسطى

كلية التقنيات الصحية والطبية /بغداد

قسم تقنيات التحليلات المرضية المادة: فايروسات المرحلة:- الثالثة

Title:-

Bacteriophages **Lecture 19**

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan **استاذ مساعد د. عذراء زيدان حسن**

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Bacteriophages are viruses that infect bacteria. Replicating within the bacterial cell therefore they are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery. The term is commonly used in its shortened form, phage. Bacteriophages are much smaller than the bacteria

they destroy . Infection with bacteriophages is restricted to particular strains within a single bacterial species. Phages are ubiquitous and can be found in all reservoirs populated by bacterial hosts, such as soil or the intestines of animals. One of the densest natural sources for phages and other viruses is sea water. They occur widely in nature and can readily be isolated from feces and sewage. There are at least 12 distinct groups of bacteriophages, which are very diverse structurally and genetically.

Typical phages have hollow heads (where the phage DNA or RNA is stored) and tunnel tails, the tips of which have the ability to bind to specific molecules on the surface of their target bacteria. The viral DNA is then
injected through the tail into the host cell, where it directs the production of progeny phages often over a hundred in half an hour. These "young" phages burst from the host cell (killing it) and infect more bacteria.

Pretest

Define Bacteriophages ?

Scientific Content

Composition:

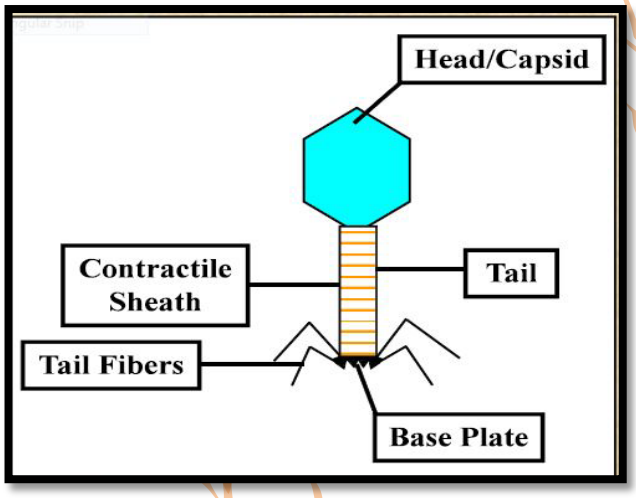
Depending upon the phage, the nucleic acid can be either **DNA or RNA** but not both. The nucleic acids of phages often contain unusual or modified bases, which protect phage nucleic acid from nucleases that break down host nucleic acids during phage infection. Simple phages may have only 3-5 genes while complex phages may have over 100 genes**.** The phages majority contain **double strand DNA (dsDNA),** while there are small phage groups with **ssRNA, dsRNA, or ssDNA.** There are three morphological forms of phages: filamentous phages, isosahedral phages without tails, phages with tails, and even several phages with a lipid-containing envelope or contain lipids in the particle shell.

Structure of bacteriophages

- **Size:-** Most phages range in size from 24-200 nm in length. **T4** is among the largest phages; it is approximately 200 nm long and 80-100 nm wide.

● **Head or capsid** :- All phages contain a **head structure,** which can vary in size and shape. Some are icosahedral (20 sides) others are filamentous. The head or capsid is composed of many copies of one or more different proteins. Inside the head is found the nucleic acid. The head acts as the protective covering for the nucleic acid.

● **Tail:-** Some phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. T4 tail is surrounded by a contractile sheath, which contracts during infection of the bacterium. At the end of the tail, phages like T4 have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers.



Bacteriophage Replication Cycle

All known bacteriophages can be divided into **two groups** according to **the type of infection** :-

One group is characterized by a **lytic infection** and the other is represented by **a lysogenic**, or **temperate**.

1- Lytic or virulent phages are phages, which multiply in bacteria and kill the cell by lysis at the end of the life cycle. Soon after the nucleic acid is injected, the phage cycle is said to be in **eclipse period**. During the eclipse phase, no infectious phage particles can be found either inside or outside the bacterial cell. Eclipse phase represents the interval between the entry of phage nucleic acid into bacterial cell and release of mature phage from the infected cell occurs. The phage nucleic acid takes over the host biosynthetic machinery and phage specified m-RNA's and proteins are

made. Nucleic acid is then packaged inside the head and then tail is added to the head. The assembly of phage components into mature infective phage particle is known as maturation. In Lysis and Release Phase the bacteria begin to lyse due to the accumulation of the phage lysis protein (holins and endolysins) and intracellular phage are released into the medium.

2- Lysogenic or temperate phages

Temperate phage has the ability to enter a lysogenic cycle and become a dormant state in the cell, in which the phage DNA is integrated into the host genome. The DNA is replicated along with the host genome. This integrated state of phage DNA is termed **prophage**. This process is known as lysogeny and the bacteria harboring prophage are called **lysogenic bacteria.** Since the prophage contains genes, it can confer new properties to the bacteria. Such transition of viral DNA could take place through several generations of bacterium without major metabolic consequences for it. Eventually the phage genes, at certain conditions impeding the bacterium state, will revert to the lytic cycle, leading to release of fully assembled phages.

Anytime a lysogenic bacterium is exposed to adverse conditions, the lysogenic state can be terminated. This process is called **induction**. Conditions which favor the termination of the lysogenic state include: desiccation, exposure to UV or ionizing radiation, exposure to mutagenic chemicals.

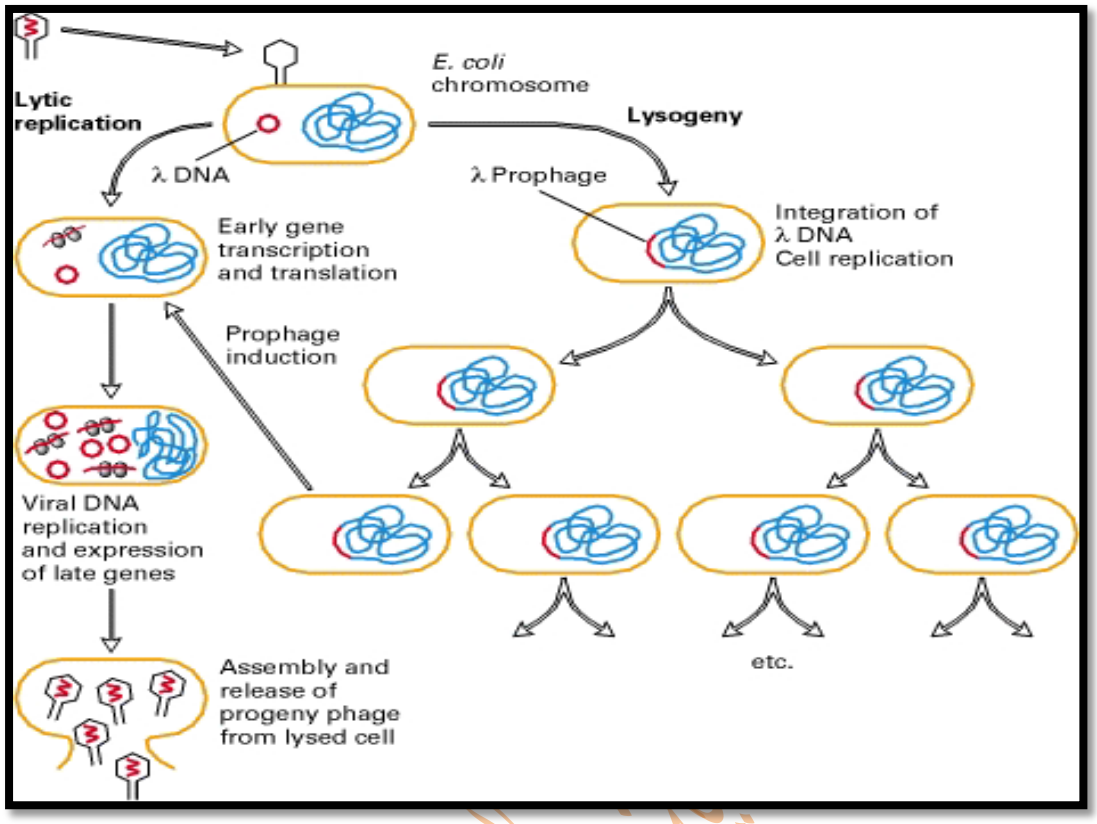
Significance of lysogenic conversion includes:

• Lysogenic phages have been shown to carry genes that can modify the Salmonella O antigen. which is one of the major antigens to which the immune response is directed.

• Toxin production by Corynebacterium diphtheriae is mediated by a gene carried by a beta phage. Only those strains that have been converted by lysogeny are pathogenic.

• Clostridium botulinum, a causative agent of food poisoning, makes several different toxins, 2 of which are actually encoded by prophage genomes.

• Lysogenised bacteria are resistant to super infection by same or related phages.This is known as super infection immunity.



Lytic and Lysogenic Cycle

Phage Therapy:

Phage therapy involves clinical treatment of bacterial infections with phages (bacteriophages). The method, which has gained a renewed interest because of increasing frequency of infections by multidrug-resistant bacteria, has potential benefits.

Phages are highly effective in killing their targeted bacteria (their action is bactericidal). Phages may be considered as good alternative for patients allergic to antibiotics.

Phage therapy benefits

- Phages work against both treatable and antibiotic-resistant bacteria.
- They may be used alone or with antibiotics and other drugs.
- Phages multiply and increase in number by themselves during treatment (only one dose may be needed).
- They only slightly disturb normal “good” bacteria in the body.

- Phages are natural and easy to find.
- They are not harmful (toxic) to the body.
- They are not toxic to animals, plants, and the environment.

Phage therapy disadvantages

- Phages are currently difficult to prepare for use in people and animals.
- It's not known what dose or amount of phages should be used.
- It's not known how long phage therapy may take to work.
- It may be difficult to find the exact phage needed to treat an infection.
- Phages may trigger the immune system to overreact or cause an imbalance.
- Some types of phages don't work as well as other kinds to treat bacterial infections.
- There may not be enough kinds of phages to treat all bacterial infections.
- Some phages may cause bacteria to become resistant.

Post test

Q1:-Enumerate phage therapy benefits ?

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1- Review of Medical Microbiology and Immunology. Warren Levinson, 12th May 2012. Mc Graw-Hill (Lange).

2- Jawetz, R., J.L. Melnick, and E.A. Adelberg,(2019). Review of Medical Microbiology, Twenty-Eighth Edition Edition,.

3- Christopher J. Burrell, ... Frederick A. Murphy, in Fenner and White's Medical Virology (Fifth Edition), 2017.

4- M. B. Dion, F. Oechslin, and S. Moineau, "Phage diversity, genomics and phylogeny," *Nature Reviews Microbiology*, vol. 18, no. 3, pp. 125–138, 2020.

الجامعة التقنية الوسطى

كلية التقنيات الصحية والطبية /بغداد

قسم تقنيات التحليلات المرضية

المادة: فايروسات

المرحلة:- الثالثة

Title:-

Antiviral drugs and viral vaccine **Lecture 20**

Name of the instructor:

Assist Prof dr. Athraa Zaidan Hassan استاذ مساعد د. عذراء زيدان حسن

Target population

Three stage students in department Medical Laboratory Techniques

Introduction

Anti-viral Drugs:

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics for bacteria, specific antivirals are used for specific viruses. Designing safe and effective antiviral drugs is difficult, because viruses use the host's cells to replicate. This makes it difficult to find targets for the drug that would interfere with the virus without harming the host organism's cells. Moreover, the major difficulty in developing vaccines and anti-viral drugs is due to viral variation.

The number of antiviral drugs is very small because:

1. The virus is obligate intracellular parasite, difficulty in obtaining selective toxicity against virus.
2. Relatively ineffective, because many cycle of viral patients is well. by the time the patients have systemic viral disease .

3. Some virus remain latent in cell e.g. Herpes virus family
4. The emergence of viral drug resistance viral mutates.

Pretest

Define Antiviral drugs?

Scientific Content

Antiviral drugs are available to treat **only a few viral diseases**. The reason for this is the fact that viral replication is so intimately associated with the host cell that any drug that interferes significantly with viral replication, is likely to be **toxic to the host**. Most of the antiviral drugs now available are designed to help deal with HIV, herpes viruses, the hepatitis B and C viruses, and influenza A and B viruses. Researchers are working to extend the range of antivirals to other families of pathogens.

Two useful antiviral are: One way of doing this is to develop nucleotide or nucleoside analogues (**Nucleotide analogues:**These are **synthetic compounds which resemble nucleosides** , but have an incomplete or abnormal deoxy-ribose /or ribose group) that look like the building blocks of RNA or DNA, but deactivate the enzymes that synthesize the RNA or DNA once the analogue is incorporated. This approach is more commonly associated with the inhibition of reverse transcriptase (RNA to DNA) .

Stages in virus replication which are possible targets for chemotherapeutic agents:

- Attachment to host cell
- Uncoating - (Amantadine)
- Synthesis of viral mRNA
- Translation of mRNA (Interferon)
- Replication of viral RNA or DNA (Interferon)
- Maturation of new virus proteins (Protease inhibitors)
- **Assembly**, release :- Protease inhibitors can be developed to prevent the final maturation of viral proteins in viruses that use a polyprotein expression strategy **Rifampicin and Tamiflu.**

vaccine:- is a biological preparation that provides active acquired immunity to a particular disease. A vaccine typically contains an agent that **resembles** a disease-causing micro-organism and is often made from **weakened** or **killed** forms of the microbe, its **toxins** or **one of its surface proteins**. Vaccines can be prophylactic or therapeutic (e.g., vaccines against cancer)**.**

Vaccines are very effective on stable viruses, but are of limited use in treating a patient who has already been infected. They are also difficult to successfully deploy against rapidly mutating viruses, such as influenza (the vaccine for which is updated every year) and HIV. Antiviral drugs are particularly useful in these cases.

Attributes of a good vaccine

1. Ability to elicit the appropriate immune response for the particular pathogen.

2. Long term protection.

3. Safety.

4. Stable.

5.Inexpensive.

Types of Vaccines

1- Live, attenuated vaccines

2- Inactivated vaccines (killed vaccine)

3- Subunit vaccines

4- Toxoid vaccines

5- DNA vaccines

6- Recombinant vector vaccines

1- Attenuated Live Vaccines vaccines contain **live**, **attenuated** microorganisms. Many of these are active viruses that have been cultivated under conditions that **disable their virulent properties**, and become less dangerous organisms to produce a broad immune response. Although **most** attenuated vaccines are **viral**, some are bacterial in nature. **Examples** include the viral diseases measles, rubella, and mumps, and the bacterial disease typhoid.

2- Killed Viral Vaccines

Vaccines contain **inactivated** virus, but previously virulent, micro-organisms that have been **destroyed** with chemicals, heat, radiation, or antibiotics **without** destroying the **antigenicity** of the virus. **Examples** are influenza, cholera, hepatitis A, and rabies.

3- Subunit vaccines:

viral **proteins** or groups of proteins are used. These proteins can be **purified** directly from viral particles. However this is **expensive**, since it is difficult to prepare virus in large enough quantities for protein purification, and potentially **dangerous** since there is the possibility of contaminating virulent virus.

4- DNA- Based Vaccines

genes (DNA) encoding specific viral proteins are injected into an animal (either in muscle or skin). The DNA is then taken up by cells, where it is **transcribed** into **mRNA** which is then **translated to give rise to the viral protein**. This protein is **expressed** on the surface of cells, either alone or in association with MHC molecules. It is **recognized** as a foreign molecule by the immune system, and **elicits an immune response**.

5- Toxoid Vaccines:

For bacteria that secrete toxins, or harmful chemicals. These vaccines are used when a bacterial toxin is the **main cause** of illness. they can **inactivate** toxins by **treating** them with **formalin** Such "detoxified" toxins, called toxoids, and are **safe for use in vaccines.**

6-Recombinant vector vaccines

Immunogenic proteins of virulent organisms may be synthesized artificially by introducing the gene coding for the protein into an expression vector, such as E-coli or yeasts.

Post test

Q1:-Enumerate types of viral vaccine ?

References

1- Review of Medical Microbiology and Immunology. Warren Levinson, 12th May 2012. Mc Graw-Hill (Lange).

2- Jawetz, R., J.L. Melnick, and E.A. Adelberg,(2019). Review of Medical Microbiology, Twenty-Eighth Edition Edition,.

3- Christopher J. Burrell, ... Frederick A. Murphy, in Fenner and White's Medical Virology (Fifth Edition), 2017.

Professor Dr. Ali Abdulhusain Mahdi **الاستاذ الدكتور علي عبدالحسين مهدي**
Assist. Prof. Dr. Abdulameer J. Mohammed **الاستاذ المساعد الدكتور عبدالامير جاسم محمد**
Lecturer Dr. Omar Sadiq Shalal **المدرس الدكتور عمر صادق شلال**

Target population:

Three stage students in department Medical Laboratory Techniques

Lecture 1 : Introduction

MYCOLOGY: Is the study of fungi and their multiple functions in nature.

Introduction:-

- ***Mykes***(Greek word) : Mushroom
- Fungi are eukaryotic protista; differ from bacteria and other prokaryotes.
    1. Cell walls containing chitin (rigidity & support), mannan& other polysaccharides .
    2. Cytoplasmic membrane contains ergosterols .
    3. Possess true nuclei with nuclear membrane & paired chromosomes.
    4. Divide asexually, sexually or by both.
    5. Unicellular or multicellular.

Taxonomy:

Kingdom	Characteristic		Examples
Monera	Prokaryocyte	Bacteria	E. coli
Protista	Eukaryocyte	Protozoa	E.histolytica
Fungi	**Eukaryocyte**	**Fungi**	Mushroom , Candida sp.
Plants	Eukaryocyte	Plants	Moss
Animals	Eukaryocyte	Arthropods	Arthropods,Mammals**,Man**

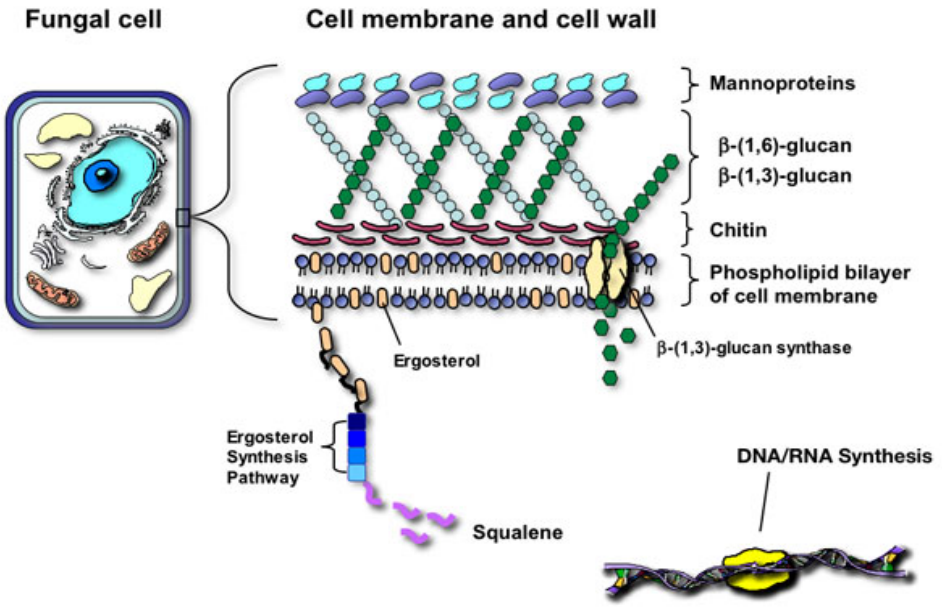

WHAT ARE FUNGI?

Fungi are not plants. Fungi form a separate group of higher organisms, distinct from both plants and animals, which differ from other groups of organisms in several major respects :-

First: fungal cells are encased within a rigid cell wall, mostly composed of chitin and glucan. These features contrast with animals, which have no cell walls, and plants, which have cellulose as the major cell wall component.

Chitin : Is a long-chain polymer of a N- acetyl glucosamine, a derivative of glucose, and is found in many places throughout the natural world.

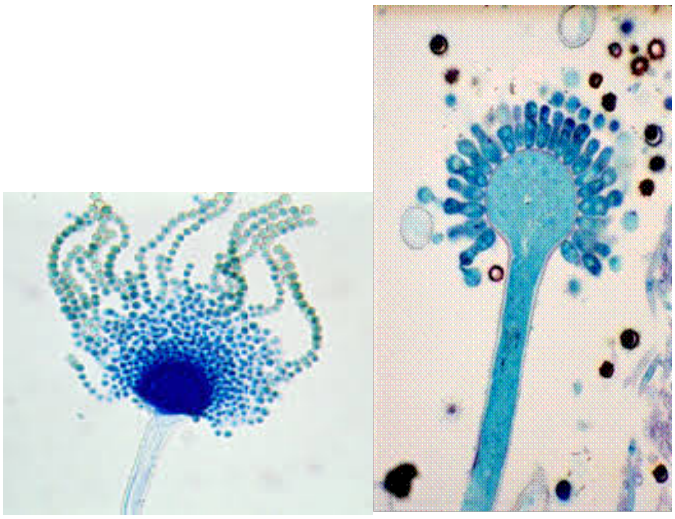
glucan molecule:-Is a polysaccharide of D-glucose monomers, linked by glycosidic bonds. Many beta-glucans are medically important. They represent a drug target for antifungal medications .



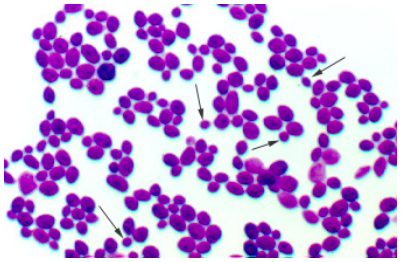
Second: fungi are heterotrophic. This means that they are lacking in chlorophyll and cannot make their organic food as plants can, through photosynthesis. Fungi live embedded in a food source or medium, and obtain their nourishment by secreting enzymes for external digestion and by absorbing the nutrients that are released from the medium.

Third: fungi are simpler in structure than plants or animals. There is no division of cells into organs or tissues. The basic structural unit of fungi is either a chain of tubular, filament-like cells, termed a hypha or hyphae (plural) or an independent single cell.

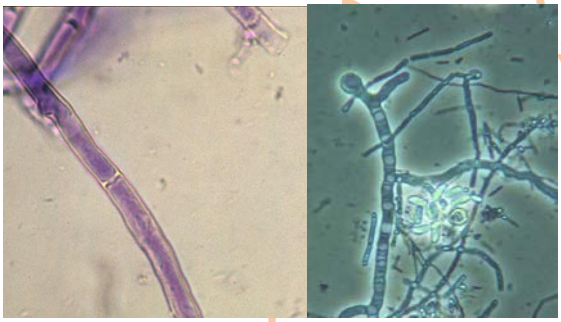
Fourth: fungi reproduce by means of microscopic propagules called *spores*. Many fungi produce spores that result from an asexual process .Many fungi are also capable of sexual reproduction. Some species are homothallic and able to form sexual structures within individual colonies.



- **Simplest fungus** :- Unicellular budding yeast



- **Hypha** :- Elongation of apical cell produces a tubular, thread like structure called hypha , Hyphae may be septate or non-septate



- **Mycelium** :- Tangled mass of hyphae is called mycelium. Fungi producing mycelia are called molds or filamentous fungi.

General properties of fungi:

1. They are eukaryotic; cells contain membrane bound cell organelles including nuclei, mitochondria, golgi apparatus, endoplasmic reticulum, lysosomes etc. They also exhibit mitosis.
2. Have ergosterols in their membranes and possesses 80S ribosomes.
3. Have a rigid cell wall and are therefore non-motile, a feature that separates them from animals. All fungi possess cell wall made of chitin.

4. Are chemoheterotrophs (require organic compounds for both carbon and energy sources) and fungi lack chlorophyll and are therefore not autotrophic.
5. Fungi are osmiotrophic; they obtain their nutrients by absorption.
6. They obtain nutrients as saprophytes (live off of decaying matter) or as parasites (live off of living matter).
7. All fungi require water and oxygen and there are no obligate anaerobes.
8. Typically reproduce asexually and/or sexually by producing spores.
9. They grow either reproductively by budding or non-reproductively by hyphal tip elongation.
10. Food storage is generally in the form of lipids and glycogen.

Many fungal pathogens of humans and animals change their growth form during the process of tissue invasion. These ***dimorphic*** pathogens usually change from a multicellular hyphal form in the natural environment to a budding, single-celled form in tissue. In most multicellular fungi the vegetative stage consists of a mass of branching hyphae, termed a mycelium. Each individual hypha has a rigid cell wall and increases in length as a result of apical growth. In the more primitive fungi, the hyphae remain aseptate (without cross-walls). In the more advanced groups,however, the hyphae are septate

Beneficial Effects of Fungi:

1. Decomposition(تحلل)) - nutrient and carbon recycling.
2. Biosynthetic factories. The fermentation property is used for the industrial production of alcohols, fats, citric, oxalic and gluconic acids.
3. Important sources of antibiotics, such as Penicillin.
4. Model organisms for biochemical and genetic studies. Eg: *Neurosporacrassa*
5. *Saccharomyces cerviciae*is extensively used in recombinant DNA technology, which includes the Hepatitis B Vaccine.
6. Some fungi are edible(صالح للاكل)) (mushrooms).
7. Yeasts provide nutritional supplements such as vitamins and cofactors.
8. Penicillium is used to flavor Roquefort(نوع من الاجبان) and Camembert cheeses.

9. Ergot(مرض يصيب الحبوب) produced by Clavicepspurpurea contains medically important alkaloids that help in inducing uterine contractions, controlling bleeding and treating migraine.
10. Fungi *(Leptolegnia caudate* and *Aphanomyceslaevis*) are used to trap mosquito larvae in paddy(الشلب) fields and thus help in malaria control.

Harmful Effects of Fungi:

1. Destruction of food, lumber(الاخشاب), paper, and cloth.
2. Animal and human diseases, including allergies.
3. Toxins produced by poisonous mushrooms and within food (Mycetism and Mycotoxicosis).
4. Plant diseases.
5. Spoilage(تلف) of agriculture produce such as vegetables and cereals(حبوب) in the godown(مخازن الحبوب).
6. Damage the products such as magnetic tapes and disks, glass lenses, marble statues(تماثيل المرمر), bones and wax.

The differences between bacteria and fungi:

1. Fungi are eukaryotes while bacteria are prokaryotes.
2. Bacteria are single celled whereas most fungi are multicellular except for yeast.
3. The compositions within their cell walls are different.
4. Fungi are heterotrophs while Bacteria can be autotrophs or heterotrophs.
5. Bacteria have 3 distinct shapes while fungi have various shapes.
6. Bacteria reproduce asexually via binary fision whereas fungi are capable of reproducing both sexually or asexually.

EPIDEMIOLOGY OF MYCOLOGY

Fungi are ubiquitous in nature and human beings are constantly exposed to them. Most mycotic agents are soil saprophytes and mycotic diseases are generally not **communicable from person-to-person (occasional exceptions: Candida and some** dermatophytes). Outbreaks of fungal disease may occur, but these are due to a common environmental exposure, not communicability.

The establishment of a mycotic infection usually depends on the size of the inoculum and on the resistance of the host. The severity of the disease seems to depend mostly on the immunologic status of the host. Thus, the demonstration of fungi, for example, in blood drawn from an intravenous catheter can correspond to colonization of the catheter, to transient fungemia (i.e., transitory dissemination of fungi through the blood stream without disease), or to a true infection. The physician must decide which is the clinical status of the patient based on clinical parameters, general status of the patient,

laboratory results, etc. The decision is not trivial, since treatment of systemic fungal infections requires the aggressive use of drugs with various degrees of toxicity.

GEOGRAPHICAL DISTRIBUTION

Most of the fungi, which cause systemic infections, have a peculiar, characteristic ecologic niche in nature. This habitat is specific for several fungi which will be discussed later. In this environment, the normally saprophytic organisms proliferate and develop. This habitat is also the source of fungal elements and/or spores, where man and animals, incidental hosts, are exposed to the infectious particles. It is important to be aware of these associations to diagnose mycotic diseases. The physician must be able to elicit a complete history from the patient including occupation, avocation and **travel history**.

Lecture 2 : Morphology ,Classification and Reproduction of fungi

Morphology of fungi:

Fungi exist in two fundamental forms; the filamentous (hyphal) and single celled budding forms (yeast). But, for the classification sake they are studied as moulds, yeasts, yeast like and dimorphic fungi.

Moulds:

The thallus of mould is made of hyphae, which are cylindrical tube like structures that elongates by growth at tips. A mass of hyphae is known as mycelium. It is the hypha that is responsible for the filamentous nature of mould. The hyphae may be branched or unbranched. They may be septate or aseptate. Hyphae usually have cross walls that divide them into numerous cells. These cross walls, called septa have small pores through which cytoplasm is continuous throughout the hyphae. Therefore all hyphal fungi tend to be coenocytic (multinucleate). With exception of zygomycetes (Rhizopus, Mucor), all moulds are septate. Non-septate hyphae are considered to be more primitive because if a hyphal strand is damaged the entire strand dies. When a septate hyphal strand is damaged, the pores between adjacent compartments can be plugged, thus preventing death of the whole hyphal strand.

Mycelium are of three kinds:

1. Vegetative mycelium are those that penetrates the surface of the medium and absorbs nutrients.
2. Aerial mycelium are those that grow above the agar surface

3. Fertile mycelium are aerial hyphae that bear reproductive structures such as conidia or sporangia.

Since hypha is the structural unit of mould, the mycelium imparts colour, texture and topography to the colony. Those fungi that possess melanin pigments in their cell wall are called phaeoid or dematiaceous and their colonies are coloured grey, black or olive. Examples are species of Bipolaris, Cladosporium, Exophiala, Fonsecaea, Phialophora and Wangiella Those hyphae that don't possess any pigment in their cell wall are called hyaline. Hyphae may have some specialized structure or appearance that aid in identification. Some of these **are:**

a) Spiral hyphae: These are spirally coiled hyphae commonly seen in Trichophyton mentagrophytes.

b) Pectinate body: These are short, unilateral projections from the hyphae that resemble a broken comb. Commonly seen in Microsporum audouinii.

c) Favic chandelier: These are the group of hyphal tips that collectively resemble a chandelier or the antlers(قرن الوعل) of the deer (antler hyphae). They occur in Trichophyton schoenleinii and Trichophyton violaceum.

d) Nodular organ: This is an enlargement in the mycelium that consists of closely twisted(مجدول) hyphae. Often seen in Trichophyton mentagrophytes and Microsporum canis.

e) Racquet hyphae: There is regular enlargement of one end of each segment with the opposing end remaining thin. Seen in Epidermophyton floccosum, Trichophyton mentagrophytes.

f) Rhizoides: These are the root like structures seen in portions of vegetative hyphae in some members of zygomycetes.

Yeasts:

Yeasts are unicellular spherical to ellipsoid cells. They reproduce by budding, which result in blastospore (blastoconidia) formation. In some cases, as the cells buds the buds fail to detach and elongate thus forming a chain of elongated hyphae like filament called pseudohyphae. This property is seen in Candida albicans. The same species also have the ability to produce true hypha, which is seen as germ tube. The difference between the two is that there is a constriction in psueudohyphae at the point of budding, while the germ tube has no constriction. Some yeast such as Cryptococcus and the yeast form of Blastomyces dermatatidis produce polysaccharide capsule. Capsules can be demonstrated by negative staining methods using India ink or Nigrosin. The capsule itself can be stained by Meyer Mucicarmine stain.

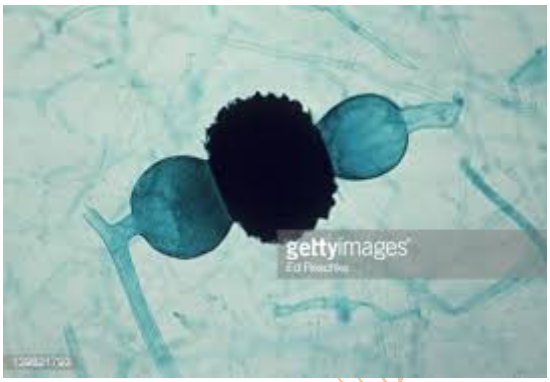
Some yeasts are pigmented. Rhodotorula sps produces pink colonies due to carotenoid pigments while some yeasts such as Phaeoannellomyces werneckii and Piedraia hortae are dematiaceous, producing brown to olivaceous colonies. True yeasts such as Saccharomyces cerviciae don't produce pseudohyphae. Yeast-like fungi may be basidiomycetes, such as Cryptococcus neoformans or ascomycetes such as Candida albicans.

Classification of fungi:

Fungi were initially classified with plants and were a subject of interest for botanists; hence the influence of botany can be seen on their classification. In 1969 R.H Whittaker classified all living organisms into five kingdoms namely Monera, Protista, Fungi, Plantae and Animalia. Traditionally the classification proceeds in this fashion:
Kingdom - Subkingdom - Phyla/phylum - Subphyla - Class - Order - Family - Genus-Species .This classification is too complicated to be dealt here.
There are alternate and more practical approaches, one based on sexual reproduction and the other based on morphology of the thallus (vegetative structure).

Based on Sexual reproduction:

1. Zygomycetes: which produce through production of zygospores.



2. Ascomycetes: which produce endogenous spores called ascospores in cells called asci.

3. Basidiomycetes: which produce exogenous spores called basidiospores in cells called basidia.

4. Deuteromycetes (Fungi imperfecti): fungi that are not known to produce any sexual spores (ascospores or basidiospores).

Based on Morphology:

1. Moulds (Molds): Filamentous fungi Eg: Aspergillus sps, Trichophyton rubrum

2. Yeasts: Single celled cells that buds Eg: Cryptococcus neoformans, Saccharomyces cerviciae

3. Yeast like: Similar to yeasts but produce pseudohyphae Eg: Candida albicans

4. Dimorphic: Fungi existing in two different morphological forms at two different environmental conditions.

They exist as yeasts in tissue and in vitro at 37C° and as moulds in their natural habitat and in vitro at room temperature. Eg: Histoplasma capsulatum, Blastomyces dermatidis, Paracoccidiodes brasiliensis, Coccidioides immitis Some 200 "human pathogens" have been recognized from among an estimated 1.5 million species of fungi.

Biased on the site of infection (Clinical Classification):-

1- **Superficial infection.**
2- **Cutaneous infection .**
3- **Subcutaneous infection.**
4- **Systemic infection .**
5- **Opportunistic infection .**

Reproduction in fungi:

Fungi reproduce by asexual, sexual and parasexual means. Fungi can reproduce asexually by *fragmentation*, *budding*, or *producing spores*, or sexually with *homothallic or heterothallic mycelia*.

Asexual reproduction is the commonest mode in most fungi with fungi participating in sexual mode only under certain circumstances. The form of fungus undergoing asexual reproduction is known as anamorph (or imperfect stage) and when the same fungus is undergoing sexual reproduction, the form is said to be teleomorph (or perfect stage). The whole fungus, including both the forms is referred as holomorph.

importance of Spores:

A. Biological

1) Allows for dissemination
2) Allows for reproduction
3) Allows the fungus to move to new food source.
4) Allows fungus to survive periods of adversity.
5) Means of introducing new genetic combinations into a population

B. Practical

1) Rapid identification (also helps with classification)
2) Source of inocula for human infection
3) Source of inocula for contamination

Lecture 3 : Superficial mycosis, tinea types and dematiaceuos (black fungi)

DEFINITION AND GENERAL CHARACTERISTICS OF CUTANEOUS MYCOSES

Fungal diseases that affects the skin, hair and nails. They are generally restricted the keratinized tissue.They cause inflammatory response.

DERMATOPHYTOSES

Etiological fungi are called “dermatophytes” (They are keratinophilic fungi)

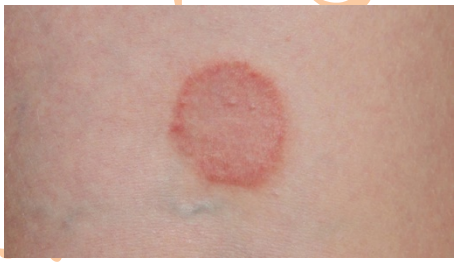
• There are 3 genera:-

Microsporum

Trichophyton

Epidermophyton

• Dermatophyte infections are called Tinea (= Ringworm)



Ring worm

DERMATOPHYTES ARE CATEGORIZED INTO 3 TYPES ACCORDING TO SOURCES OF INFECTION

1. Geophilic dermatophytes

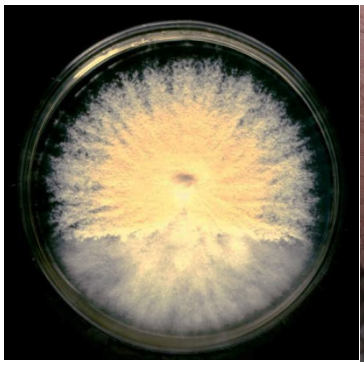
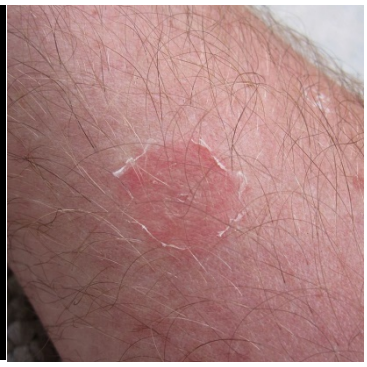
2. Zoophilic dermatophytes

3. Anthropophilic dermatophytes

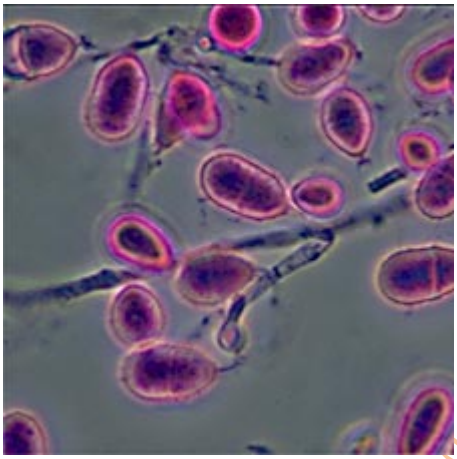
GEOPHILIC DERMATOPHYTES

Inhabit soil where they decompose keratinaceous debris of dead animals

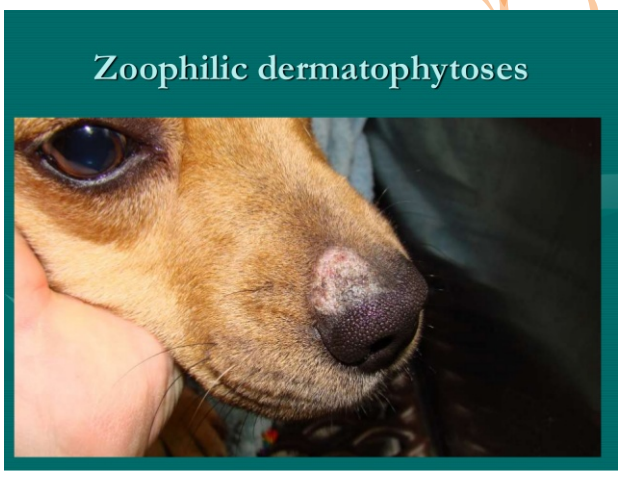
1-Microsporum gypseum :-

2- Microsporum nanum:-



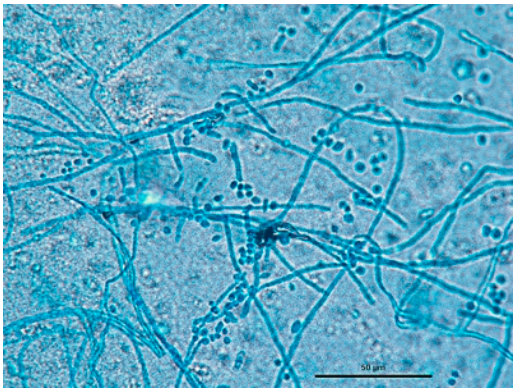

ZOOPHILIC DERMATOPHYTES:- Parasitic on animals:-



1-Trichophyton equinum

2-Microsporum canis

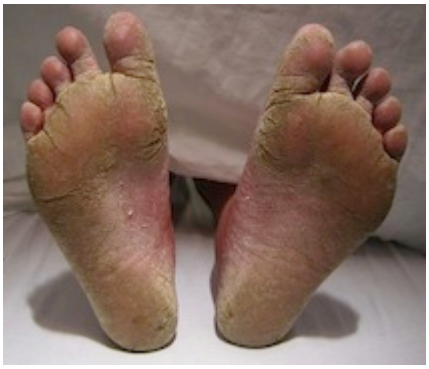
3-M. mentagrophytes var Mentagrophytes



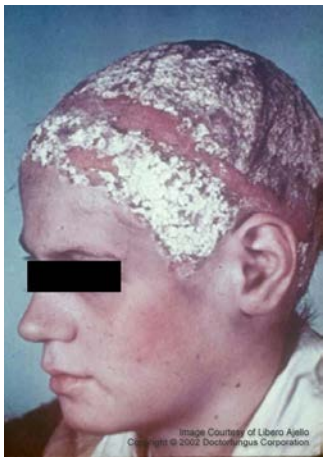
ANTHROPOPHILIC DERMATOPHYTES:-

Primarily parasitic to man. Man as exclusive host for maintenance and dissemination of species

1-Trichophyton rubrum



2-Trichophyton schoenleinii

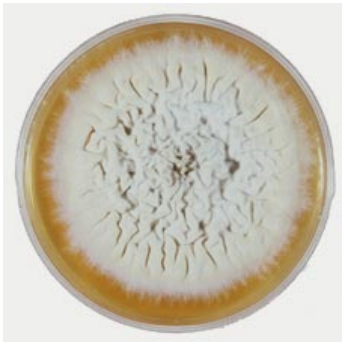


3-Trichophyton tonsurans

4-Trichophyton mentagrophytes var interdigitale

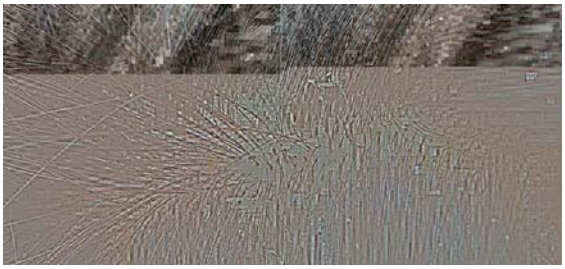
5-Microsporum audounii

6-Epidermophyton floccosum:-



Clinical manifestations of dermatophytoses:

A. Skin invasion = ringworm
B. Hair invasion



Favic type (inside, with oil deposits and air)
Ectothrix type (outside; the hyphae are accumulated around the hair shaft)
Endothrix type (inside)
Tinea capitis Scalp, eyebrow, eyelashes
Tinea favosa Cup shaped crusts

Tinea corporis Rings with scaly centers
Tinea imbricata Concentric rings caused by T. concentricum
Tinea barbae Bearded area of face and neck
Tinea cruris Jock itch, moist groin area
Tinea pedis Athlete's foot Toe webs, soles and nails
Tinea manuum Interdigitate areas and palmar surfaces
Tinea ungium (Onychomycosis)Invasion of nail plate Thickened, discolored and brittle nails

Laboratory diagnosis of dermatophytoses

A-Skin scraping, + infected hair

↓

KOH preparation

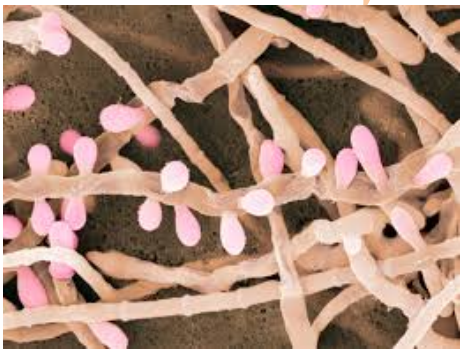
B- Potassium hydroxide (KOH): dissolves keratin and free hyphae from the cell
C- Calcofluor white (CFW) stains chitin at the cell wall
Need fluorescent microscopy. Improve the sensitivity and specificity of diagnosis

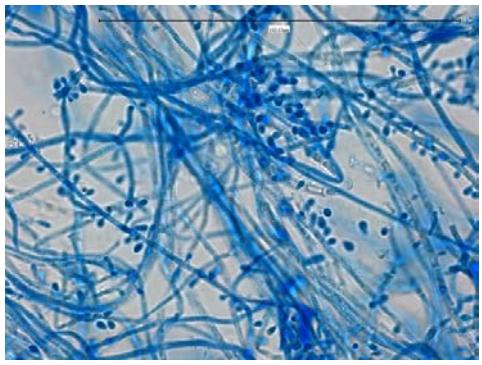
Culture: SDA or SDA with chloramphenicol and cycloheximide (mycosel agar) at room temperature at least 2 weeks Identification:

Microscopic characteristics:- Gross colors and textures

Trichophyton rubrum:-
White, cottony colony. Wine red pigment on reverse side.

• Pencil-shaped macroconidia

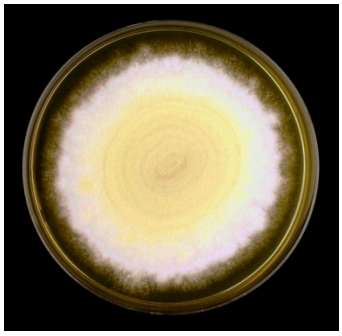


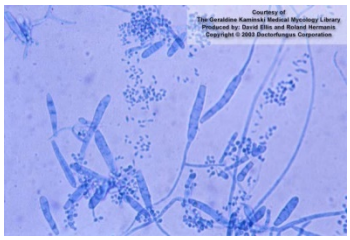


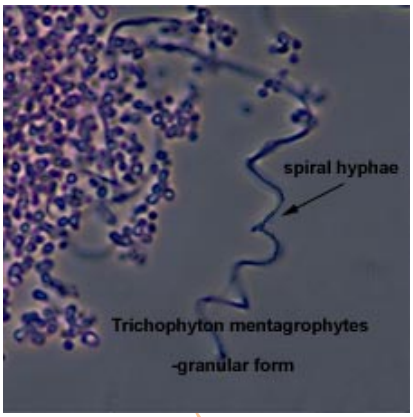
• Microconidia (club-shaped, tear drops)

Trichophyton mentagrophytes:-

Flat, white to cream color, powdery to granular surface







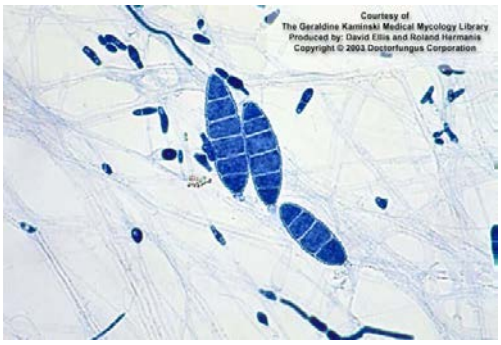
• Cigar-shaped macroconidia

• Microconidia present

• Coiled or spiral hyphae

Microsporum gypseum:-

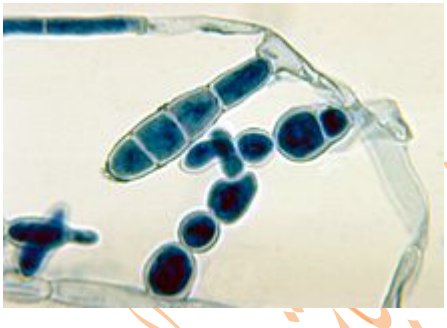
Light brown, powdery colony:



Spindled-shaped macroconidia, Microconidia present

Epidermophyton floccosum:-

Fluffy colony 

• Club-shaped macroconidia 

• Microconidia ABSENT

Dermatophytes (Cutaneous mycosis):

1-Microsporum:- Infect skin, hair and nails

Microscopic appearance : Macroconidia: Rough walled

Microconidia: Present : Macroconidia more than microconidia

2-Trichophyton:- Infect skin and hair

Microscopic appearance : Microconidia: Present:- Microconidia more than macroconidia

Macroconidia: Smooth walled

3- Epidermophyton:- Infect skin and nails

Microscopic appearance

Macroconidia: Smooth walled:- Microconidia: ABSENT: Chlamydoconidia

Black fungi:

Black Fungus or mucormycosis is a rare but dangerous invasive fungal infection caused by a group of molds called mucormycetes. Black fungus commonly affects the sinuses and lungs but can affect skin and brain. People can get infected when they inhale the mold spores or touch the mold spore. A skin infection can occur after the fungus enters the skin through a scrape, burn, or other type of skin injury. Mucormycosis is not contagious from person to person. You cannot get it from an infected person.

Most cases of mucormycosis are sporadic (meaning they occur infrequently) but outbreaks do occasionally occur. Most outbreaks are associated with water leaks, poor air filtration, building construction, and natural disasters. Healthcare providers who are concerned about an unusual number of cases should contact their state or local public health department

Symptoms depend on where in the body the fungus is growing and can include facial swelling, fever, skin ulcers, and black lesions in the mouth.

Lecture 4 : Subcutaneous mycosis :

These are infections confined to the dermis, subcutaneous tissue or adjacent structures. infection may arise following the wounding of the skin and the introduction of vegetable matter. these mycoses are rare and confined mainly to tropical regions. they tend to be slow in onset and chronic in duration. an example is **sporotrichosis** caused by *sporothrix schenckii*. the fungus is dimorphic, being a mould that can convert to a yeast form at 37°c on rich laboratory media or in infection.

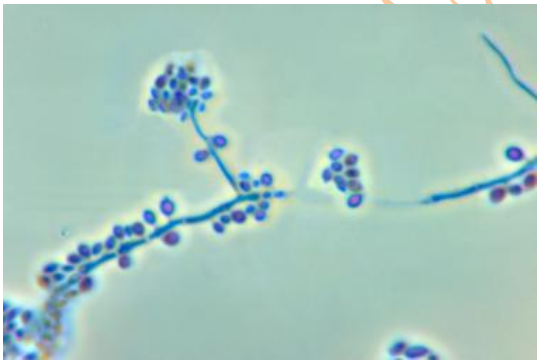
Sporotrichosis:-

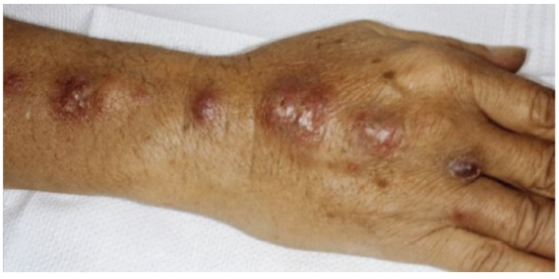
Is a disease caused by the infection of the fungus *Sporothrix schenckii*. This fungal disease usually affects the skin, although other rare forms can affect the lungs, joints, bones, and even the brain. Because roses can spread the disease, it is one of a few diseases referred to as *rose-thorn* or *rose-gardeners' disease*.

Because *S. schenckii* is naturally found in soil, hay, sphagnum moss, and plants, it usually affects farmers, gardeners, and agricultural workers. It enters through small cuts and abrasions in the skin to cause the infection. In case of sporotrichosis affecting the lungs, the fungal spores enter through the respiratory pathways. Sporotrichosis can also be acquired from handling cats with the disease; it is an occupational hazard for veterinarians.

Pathophysiology

Infection with the dimorphic soil fungus *S schenckii* is usually acquired from organic matter through cutaneous inoculation. The mycosis has also been transmitted from animals through bites or scratches. Cats have been responsible for cases among veterinarians and for a large outbreak in Rio de Janeiro, Brazil. See the image below.





Types of sporotrichosis

- **Cutaneous (skin) sporotrichosis** is the most common form of the infection. It usually occurs on a person's hand or the arm after they have been handling contaminated plant matter.
- **Pulmonary (lung) sporotrichosis** is very rare but can happen after someone breathes in fungal spores from the environment.
- **Disseminated sporotrichosis** occurs when the infection spreads to another part of the body, such as the bones, joints, or the central nervous system. This form of sporotrichosis usually affects people who have weakened immune systems, such as people with HIV infection (see Risk & Prevention).

Mycetoma: is a suppurative and granulomatous subcutaneous mycosis, which is destructive of contiguous bone, tendon, and skeletal muscle. Mycetoma is characterized by the presence of draining sinus tracts from which small but grossly visible pigmented grains or granules are extruded. These grains are microcolonies of fungi causing the infection.

Diagnosis of Mycetoma

Specimen collection: Aspiration (best),drainage, tissue biopsy /section • Examination of grains

• Examination of grains:-

• Record size, color, shape and consistency of grains

• Direct microscopic examination: KOH/LPC preparations (LPC = lactophenol cotton blue)

• Culture Standard mycological media or aerobic/anaerobic bacterial culture condition

Treatment

Treatment is difficult due to inability of drugs to infiltrate lesions, combination of medicine and surgery is the best Eumycotic mycetoma: Amphotericin B

Actinomycotic mycetoma: Antibiotics

Lecture 5 : infection due to filamentous fungi (Aspergillosis):

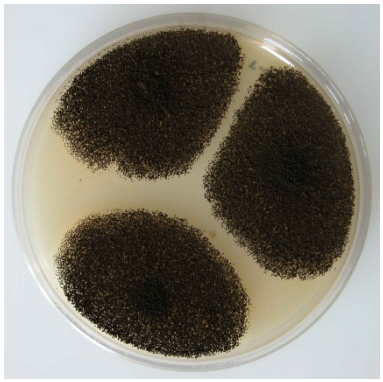
Aspergillosis :-

Aspergillus spp. are widely distributed fungal moulds found in soil and other organic matter. They have also been isolated in air-conditioning systems. There are more than a hundred different species but most human disease is caused by *Aspergillus fumigatus* or *Aspergillus niger*. Occasionally, *Aspergillus clavatus* and *Aspergillus flavus* cause human illness.

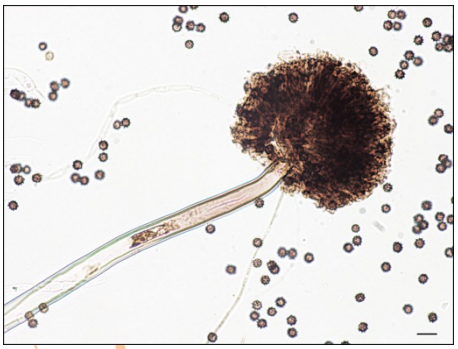
What Is Aspergillosis?

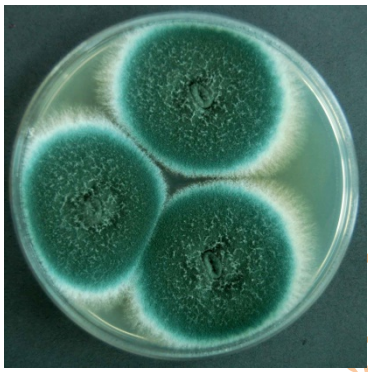
Aspergillosis is an infection, allergic reaction, or fungal growth caused by the *Aspergillus* fungus. The fungus usually grows on decaying vegetation and dead leaves. Exposure to the fungus doesn't necessarily guarantee to get aspergillosis. Almost everyone encounters the fungus on a daily basis and never contracts the illness. It's more likely to infect people with a weak immune system or a lung disease.

Aspergillus flavus

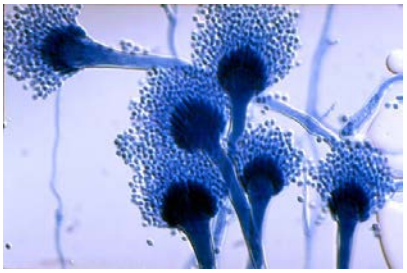


Aspergillus niger





Aspergillus fumigatus



What Are the Types of Aspergillosis and Their Symptoms?

Different types of aspergillosis affect the body in different ways. Certain conditions and medications increase the risk for developing each type. Different types of aspergillosis have different symptoms.

Allergic Bronchopulmonary Aspergillosis (ABPA):-

In allergic bronchopulmonary aspergillosis (ABPA), the fungus causes allergic reactions such as coughing and wheezing. The patient`s more susceptible to this type

of aspergillosis if he have lung problems such as cystic fibrosis or asthma. ABPA also causes shortness of breath, and general feelings of being unwell.

Investigation:-

Diagnosis is made on the basis of a deterioration in the patient's clinical condition (the underlying asthma or CF symptoms worsen), being a susceptible patient and the presence of the following:

- Eosinophilia.
- Positive skin test to *Aspergillus* spp.
- Elevated serum immunoglobulin E (IgE).
- Positive serology for *Aspergillus* spp.
- New infiltrates on CXR or CT scan.
- Sputum microscopy and culture may also reveal the presence of *Aspergillus* spp.

Invasive Aspergillosis:-

The more likely to have an invasive type of aspergillosis if the immune system is weakened by chemotherapy and conditions such as leukemia, cancer, and AIDS.

A weakened immune system makes it more difficult to fight off infections. This type of aspergillosis invades the lung tissues and can spread to the kidneys or brain. If invasive aspergillosis goes untreated, it can cause infectious pneumonia. Infectious pneumonia can be life-threatening in people with compromised immune systems.

Invasive aspergillosis often occurs in people who already have other medical conditions, so it can be hard to separate the symptoms of invasive aspergillosis from those of the other conditions. Known symptoms of invasive aspergillosis include:

- a cough (sometimes with blood)
- pain in the chest
- shortness of breath
- fever

Also, an infection of the lungs can spread throughout the body, causing new symptoms.

Investigations:-

- Invasive aspergillosis is a difficult condition to diagnose and must be specifically sought in symptomatic patients who are severely immunocompromised.
- CXR may show nodules, cavitary lesions or pulmonary infiltrates.

- CT scanning may show characteristic changes in the lungs, including the 'halo sign' (a haziness surrounding a nodule or infiltrate).
- The sputum, lung tissue from biopsy, or bronchoalveolar lavage (BAL) fluid may show the characteristic hyphae, using appropriate special stains. *Aspergillus* spp. may also be cultured from these sources.
- There is an assay to detect a component of the cell wall of *Aspergillus* spp., called galactomannan. This has the potential to be used as screening in those at high risk of invasive aspergillosis. Serum levels can be monitored on a regular basis. Galactomannan can also be detected in BAL fluid. Serum galactomannan can be detected several days before the presence of clinical signs, an abnormal chest radiograph, or positive culture.
- Another fungal cell wall constituent, B-glucan, can also be detected in the serum and has a potential role in diagnosis.
- Polymerase chain reaction (PCR) techniques are also being studied to detect *Aspergillus* spp. in blood and BAL fluid.
- Results may be negative and empirical therapy is often started on clinical grounds in deteriorating patients.

Aspergilloma:-

The tuberculosis patient`s or another lung disease, exposure to the fungus can cause to develop a fungus growth. Also called a fungus ball, this type of growth usually consists of fungus, clots, and white blood cells. The growth doesn’t typically spread to other areas of the body. However, the ball can become larger and damage the lung tissues.

With an aspergilloma, the patient`s may have a cough, with or without blood, and shortness of breath.

Other symptoms of different types of aspergillosis can include:

- pain in the chest and bones
- vision difficulties
- blood in urine
- less urine
- headaches
- chills
- difficulty breathing
- skin sores
- bloody phlegm

Investigations

- CXR shows a mass within a pulmonary cavity, often in the upper lobe. A crescentic outline of air may be seen to surround a solid mass.
- CT scanning can reveal the structure of the mycetoma in more detail. Supine and prone CT scans should be performed to demonstrate the mobility of the mass, which is a highly suggestive sign.
- Most show elevated serum precipitin levels to *Aspergillus* spp.

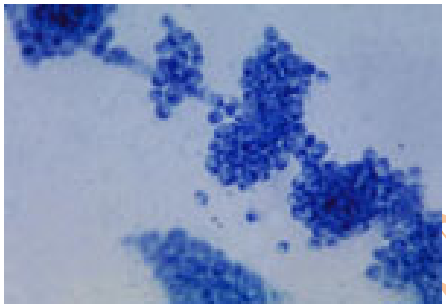
Lecture 6: Infection caused by yeasts :

Candidiasis:- is a fungal infection caused by yeasts that belong to the genus *Candida*.

Species:-

There are over 20 species of *Candida* yeasts that can cause infection in humans, the most common of which is ***Candida albicans***. *Candida* yeasts normally live on the skin and mucous membranes without causing infection; however, overgrowth of these organisms can cause symptoms to develop. Symptoms of candidiasis vary depending on the area of the body that is infected.

There is an increasing incidence of infections caused by ***C. glabrata*** and ***C. rugosa***, which could be because they are frequently less susceptible to the currently used azole antifungals. Other medically important *Candida* species include ***C. parapsilosis, C. tropicalis,* and *C. dubliniensis*.**



Photomicrograph of the fungus *Candida albicans*

Candida species : general feature:

- Normal flora
- Yeast like fungi
- Reproduction by budding
- Culture morphology : white to opaque on SDA
- Only candida albican has germ tube feature

Type candidiasis:

1- Oropharyngeal / Esophageal Candidiasis ("Thrush"):-

Candidiasis that develops in the mouth or throat is called "thrush" or oropharyngeal candidiasis. The most common **symptom** of oral thrush is white patches or plaques on the tongue and other oral mucous membranes. This infection is uncommon among healthy adults.

Risk of oral Candidiasis:-

Candida infections of the mouth and throat are uncommon among adults who are otherwise healthy. Oral thrush occurs most frequently among babies less than one

month old, the elderly, and groups of people with weakened immune systems. Other factors associated with oral and esophageal candidiasis include:

- HIV/AIDS
- Cancer treatments
- Organ transplantation
- Diabetes
- Corticosteroid use
- Dentures
- Broad-spectrum antibiotic use

Lab. Diagnosis of Oral Candidiasis:-

By taking a scraping of the affected areas to examine under a microscope. A culture may also be performed; however, because *Candida* organisms are normal inhabitants of the human mouth, a positive culture by itself does not make the diagnosis.

2- Genital / vulvovaginal candidiasis (VVC):-

Genital / vulvovaginal candidiasis (VVC) is also sometimes called a **"yeast infection,"** and it occurs when there is overgrowth of the normal yeast in the vagina. *Candida* is always present in and on the body in small amounts. However, when an imbalance occurs, such as when the normal acidity of the vagina changes or when hormonal balance changes, *Candida* can multiply. When that happens, symptoms of candidiasis may appear.

Symptoms of Genital / Vulvovaginal Candidiasis:-

Women with VVC usually experience genital itching, burning, and sometimes a "cottage cheese-like" vaginal discharge. Men with genital candidiasis may experience an itchy rash on the penis.

Risk of Genital / Vulvovaginal Candidiasis:-

Nearly 75% of all adult women have had at least one "yeast infection" in their lifetime. On rare occasions, men can also get genital candidiasis. VVC occurs more frequently and more severely in people with weakened immune systems. Other conditions that may put a woman at risk for genital candidiasis include:

- Pregnancy
- Diabetes
- Long-term use of broad-spectrum antibiotics
- Use of corticosteroid medications

Lab.Diagnosis of Genital / Vulvovaginal Candidiasis:-

Usually the diagnosis is made by taking a sample of the vaginal secretions and looking at the sample under a microscope to see if an abnormal number

of *Candida* organisms are present. A fungal culture may not always be useful because *Candida* species are normal inhabitants of the body.

3- Invasive Candidiasis:-

Invasive candidiasis is a fungal infection that can occur when Candida yeasts enter the bloodstream. Candidemia (a bloodstream infection with Candida), is extremely rare in people without risk factors.

Symptoms of Invasive Candidiasis:-

The symptoms of invasive candidiasis are not specific. Fever and chills that do not improve after antibiotic therapy are the most common symptoms. If the infection spreads to other organs or parts of the body such as kidneys, liver, bones, muscles, joints, spleen, or eyes, additional symptoms may develop, which vary depending on the site of infection. If the infection does not respond to treatment, the patient's organs may stop working.

Risk of Invasive Candidiasis:-

Candidemia (a bloodstream infection with *Candida*), is the fourth most common bloodstream infection among hospitalized patients in the United States. People at high risk for developing candidemia include:

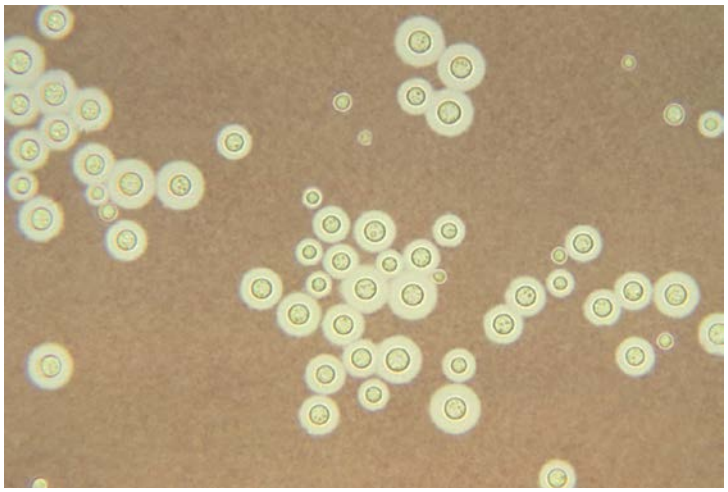
- Intensive care unit (ICU) patients
- Surgical patients
- Patients with a central venous catheter
- People whose immune systems are weakened (such as people with HIV/AIDS)
- Very low-birth-weight infants

Lab.Diagnosis of Invasive Candidiasis:-

Invasive candidiasis is primarily diagnosed through blood culture.

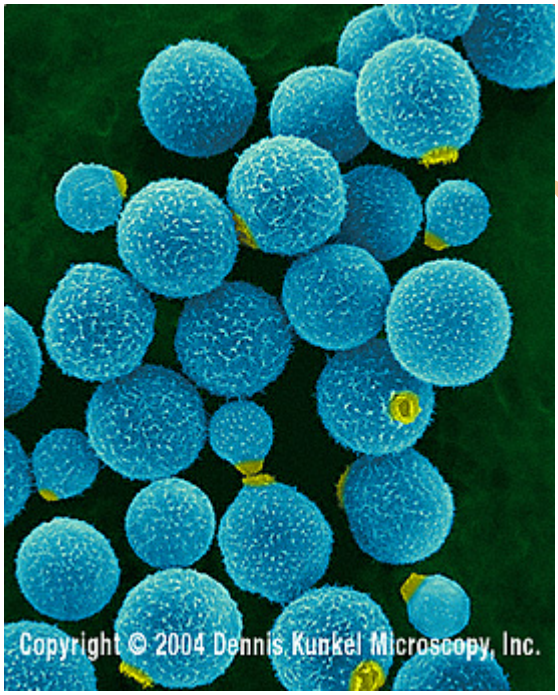
Cryptococcus:-

Cryptococcus (Greek for "hidden sphere") is a genus of fungus. These fungi grow in culture as yeasts. The sexual forms or teleomorphs of *Cryptococcus* species are filamentous fungi in the genus *Filobasidiella*. The name *Cryptococcus* is used when referring to the yeast states of the fungi.



General characteristics

The cells of these species are covered in a thin layer of glycoprotein capsular material that has a gelatin-like consistency and that, among other functions, serves to help extract nutrients from the soil. However, the *C. neoformans* capsule is different, in being richer in glucuronic acid and mannose, having O-acetyl groups, and functioning as the major virulence factor in cryptococcal infection and disease.



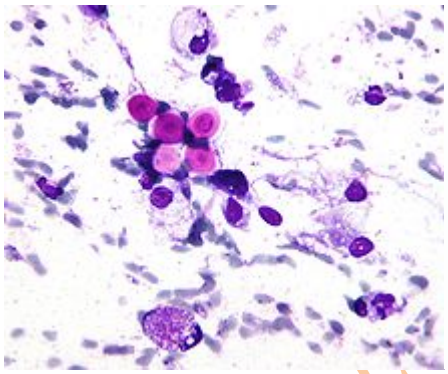
Infectious species

There are about 37 recognized species of *Cryptococcus*, but the taxonomy of the group is currently being re-evaluated with up-to-date methods. The majority of species live in the soil and are not harmful to humans. Very common species include *Cryptococcus laurentii* and*Cryptococcus albidus*. Of all species, *Cryptococcus*

neoformans is the major human and animal pathogen. However, *Cryptococcus laurentii* and *Cryptococcus albidus* have been known to occasionally cause moderate-to-severe disease, to be specific meningitis, in human patients with compromised immunity (owing to HIV infection, cancer chemotherapy, metabolic immunosuppression, *et cetera*).

C. neoformans

Cryptococcus neoformans is the most prominent medically important species. It is best known for causing a severe form of meningitis and meningo-encephalitis in people with HIV/AIDS. It may also infect organ transplant recipients and people receiving certain cancer treatments. *C. neoformans* is found in the droppings of wild birds, often pigeons; when dust of the droppings is stirred up it can infect humans or pets that inhale the dust. Infected humans and animals do not transmit their infection to others; they are not infectious. When plated on Niger or birdseed agar, *C. neoformans* produces melanin, which causes the colonies to have a brown color, and it is believed that this melanin production may be an important virulence factor.



Field stain showing *Cryptococcus* species in lung tissue

Other species of Cryptococcus which cause moderate infections :-

C. gattii

C. albidus

***C. uniguttulatus*:**

Antigenic Structure:-

The capsular polysaccharides, regardless of serotype, have a similar structure: They are long, unbranched polymers consisting of an -1,3-linked polymannose

backbone with -linked monomeric branches of xylose and glucuronic acid. During infection, the capsular polysaccharide is solubilized in spinal fluid, serum, or urine and can be detected by agglutination of latex particles coated with antibody to the polysaccharide. With proper controls, this test is diagnostic of cryptococcosis. Patient antibodies to the capsule can also be measured, but they are not used in diagnosis.

Pathogenesis:-

Infection follows inhalation of the yeast cells, which in nature are dry, minimally encapsulated, and easily aerosolized. The primary pulmonary infection may be asymptomatic or may mimic an influenza-like respiratory infection, often resolving spontaneously. In patients who are compromised, the yeasts may multiply and disseminate to other parts of the body but preferentially to the central nervous system, causing cryptococcal meningoencephalitis. Other common sites of dissemination include the skin, eye, and prostate gland. The inflammatory reaction is usually minimal or granulomatous.

Clinical Findings:-

The major clinical manifestation is chronic meningitis with spontaneous remissions and exacerbations. The meningitis may resemble a brain tumor, brain abscess, degenerative central nervous system disease, or any mycobacterial or fungal meningitis. Cerebrospinal fluid pressure and protein may be increased and the cell count elevated, whereas the glucose is normal or low. Patients may complain of headache, neck stiffness, and disorientation. In addition, there may be lesions in skin, lungs, or other organs.

The course of cryptococcal meningitis may fluctuate over long periods, but all untreated cases are ultimately fatal. About 5–8% of patients with AIDS develop cryptococcal meningitis. The infection is not transmitted from person to person.

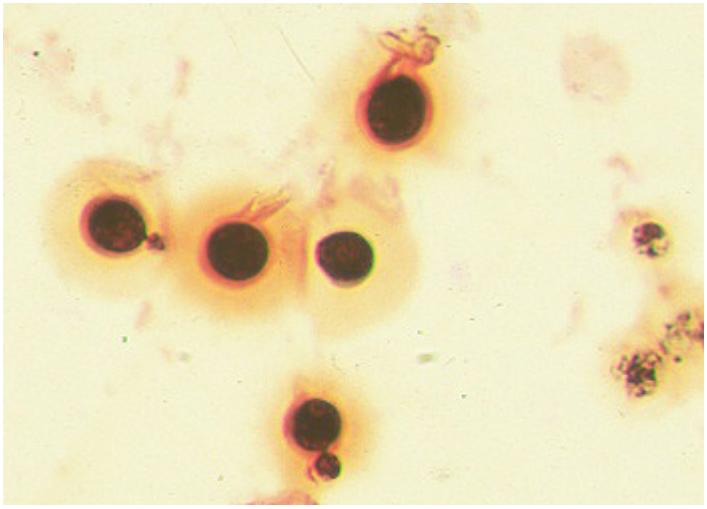
Diagnostic Laboratory Tests

Specimens:-

Specimens include spinal fluid, tissue, exudates, sputum, blood, and urine. Spinal fluid is centrifuged before microscopic examination and culture.

Microscopic Examination:-

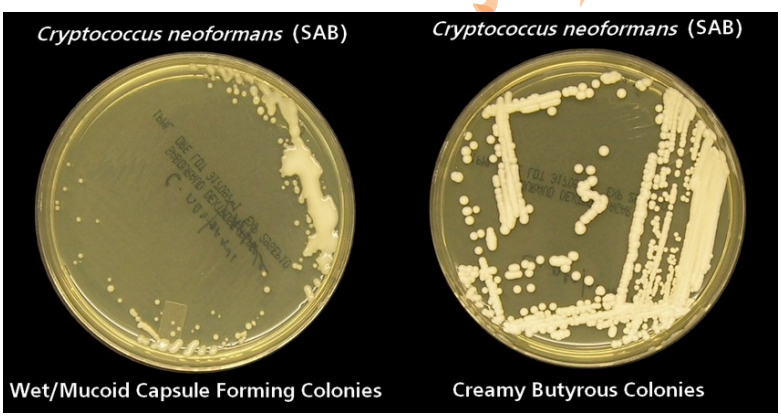
Specimens are examined in wet mounts, both directly and after mixing with India ink, which delineates the capsule.



Cryptococcus spp. can be distinguished under Gram staining - the India Ink method is just a confirmatory test.

Culture:-

Colonies develop within a few days on most media at room temperature or 37 °C. Media with cycloheximide inhibit *C neoformans* and should be avoided. Cultures can be identified by growth at 37 °C and detection of urease . Alternatively, on an appropriate diphenolic substrate, the phenol oxidase (or laccase) of *C neoformans* produces melanin in the cell walls and colonies develop a brown pigment.



Serology:-

Tests for capsular antigen can be performed on cerebrospinal fluid and serum. The latex slide agglutination test for cryptococcal antigen is positive in 90% of patients with cryptococcal meningitis. With effective treatment, the antigen titer drops—except in AIDS patients, who often maintain high antigen titers for long periods.

Treatment:-

Combination therapy of amphotericin B and flucytosine has been considered the standard treatment for cryptococcal meningitis, though the benefit from adding flucytosine remains controversial. Amphotericin B (with or without flucytosine) is curative in most patients. Since AIDS patients with cryptococcosis will almost always relapse when amphotericin B is withdrawn, they require perpetual suppressive therapy with fluconazole. Fluconazole offers excellent penetration of the central nervous system.

Epidemiology & Control:-

Bird droppings (particularly pigeon droppings) enrich for the growth of *C neoformans* and serve as a reservoir of infection. The organism grows luxuriantly in pigeon excreta, but the birds are not infected. In addition to patients with AIDS or hematologic malignancies, patients being maintained on corticosteroids are highly susceptible to cryptococcosis.

Lecture 7: Opportunistic mycosis : Penicillosis

Penicillium :-

is a genus of ascomycetous fungi of major importance in the natural environment as well as food and drug production.

Some members of the genus produce penicillin, a molecule that is used as an antibiotic, which kills or stops the growth of certain kinds of bacteria inside the body. Other species are used in cheesemaking. According to the *Dictionary of the Fungi* (10th edition, 2008), the widespread genus contains over 300 species.

Species:-

- *Penicillium marneffei*, a thermally dimorphic species endemic in Southeast Asia, which presents a threat of systemic infection to AIDS patients
- *Penicillium camemberti*, which is used in the production of Camembert and Brie cheeses
- *Penicillium candidum*, which is used in making Brie and Camembert. It has been reduced to synonymy with *Penicillium camemberti*
- *Penicillium chrysogenum* , which produces the antibiotic penicillin

- *Penicillium roqueforti*, which is used in making Roquefort, Danish Blue cheese, and also recently Gorgonzola
- *Penicillium verrucosum* produces ochratoxin A
- *Penicillium viridicatum* produces ochratoxin

Characteristics:-

The thallus (mycelium) typically consists of a highly branched network of multinucleate, septate, usually colorless hyphae. Many-branched conidiophores sprout on the mycelia, bearing individually constricted conidiospores. The conidiospores are the main dispersal route of the fungi, and often are green in color.

Sexual reproduction involves the production of ascospores, commencing with the fusion of an archegonium and an antheridium, with sharing of nuclei. The irregularly distributed asci contain eight unicellular ascospores each.

Economic value:-

Several species of the genus *Penicillium* play a central role in the production of cheese and of various meat products. To be specific,*Penicillium* molds are found in Blue cheese. *Penicillium camemberti* and *Penicillium roqueforti* are the molds on Camembert, Brie, Roquefort, and many other cheeses. *Penicillium nalgiovense* is used to improve the taste of sausages and hams, and to prevent colonization by other molds and bacteria.

In addition to their importance in the food industry, species of *Penicillium* and *Aspergillus* serve in the production of a number of biotechnologically produced enzymes and other macromolecules, such as gluconic, citric, and tartaric acids, as well as several pectinases, lipase, amylases, cellulases, and proteases. Some *Penicillium* species have shown potential for use in bioremediation because of their ability to break down a variety of xenobiotic compounds.

The genus includes a wide variety of species molds that are the source molds of major antibiotics. Penicillin, a drug produced by *P. chrysogenum (formerly*

P. notatum), was accidentally discovered by Alexander Fleming in 1929, and found to inhibit the growth of Gram-positive bacteria

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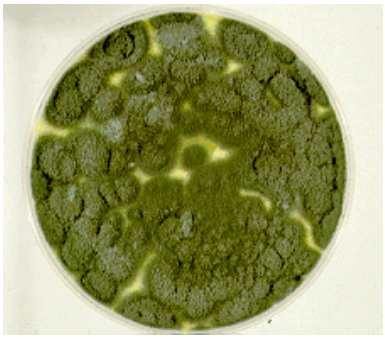


Culture of *Penicillium* sp.

Penicillium marneffei:-

Penicillium species are usually regarded as unimportant in terms of causing human disease. ***Penicillium marneffei***, discovered in 1956, is different. This is the only known thermally dimorphic species of *Penicillium*, and it can cause a lethal systemic infection (penicilliosis)with feverand anaemia similar to disseminated cryptococcosis.

Epidemiology:-

There is a high incidence of penicilliosis in AIDS patients in SE Asia; 10% of patients in Hong Kong get penicillosis as an AIDS-related illness. Cases of *P. marneffei* human infections (penicillosis) have also been reported in HIV-positive patients in Australia, Europe, Japan, the UK and the U.S.. All the patients, except one, had visited Southeast Asia previously.

Discovered in bamboo rats (*Rhizomys*) in Vietnam,it is associated with these rats and the tropical Southeast Asia area. *Penicillium marneffei* is endemic in Burma (Myanmar), Cambodia,Southern China, Indonesia, Laos, Malaysia, Thailad a nd Vietnam.

Although both the immunocompetent and the immunocompromised can be infected, it is extremely rare to find systemic infections in HIV-negative patients.

The incidence of *P. marneffei* is increasing as HIV spreads throughout Asia. An increase in global travel and migration means it will be of increased importance as an infection in AIDS sufferers.

Penicillium marneffei has been found in bamboo rat faeces, liver, lungs and spleen. It has been suggested that these animals are a reservoir for the fungus. It is not clear

whether the rats are affected by *P. marneffei* or are merely asymptomatic carriers of the disease.

Clinical Presentation:-

Patients commonly present with symptoms and signs of infection of the reticuloendothelial system, including generalized lymphadenopathy, hepatomegaly, and splenomegaly. The respiratory system is commonly involved as well; cough, fever, dyspnea, and chest pain may be present, reflecting the probable inhalational route of acquisition. Approximately one-third of patients may also exhibit gastrointestinal symptoms, such as diarrhea.

Laboratory diagnosis:-

The fact that *Penicillium marneffei* is thermally dimorphic is a relevant clue when trying to identify it. However, it should be kept in mind that other human-pathogenic fungi are thermally dimorphic as well. Cultures should be done from bone marrow, skin, blood and sputum samples.

Plating samples out onto two Sabouraud agar plates, then incubating one at 30 °C and the other at 37 °C, should result in two different morphologies. A mold-form will grow at 30 °C, and a yeast-form at 37 °C.

Mycelial colonies will be visible on the 30 °C plate after two days. Growth is initially fluffy and white and eventually turns green and granular after sporulation has occurred. A soluble red pigment is produced, which diffuses into the agar, causing the reverse side of the plate to appear red or pink. The periphery of the mold may appear orange-coloured, and radial sulcate folds will develop.

Under the microscope, the mold phase will look like a typical *Penicillium*, with hyaline, septate and branched hyphae; the conidiophores are located both laterally and terminally. Each conidiophore gives rise to three to five phialides, where chains of lemon-shaped conidia are formed.

On the 37 °C plate, the colonies grow as yeasts. These colonies can be cerebriform, convoluted, or smooth. There is a decreased production in pigment, the colonies appearing cream/light-tan/light-pink in colour. Microscopically, sausage-shaped cells are mixed with hyphae-like structures. As the culture ages, segments begin to form. The cells divide by binary fission, rather than budding. The cells are not yeast cells, but rather arthroconidia. Culturing isn't the only method of diagnosis. A skin scraping can be prepared, and stained with Wright's stain. Many intracellular and extracellular yeast cells with crosswalls are suggestive of *P. marneffei* infection. Smears from bone marrow aspirates may also be taken; this is regarded as the most sensitive method. These samples can be stained with the Giemsa stain. Histological examination can also be done on skin, bone marrow or lymph nodes.

The patient's history also is a diagnostic help. If they have traveled to Southeast Asia and are HIV-positive, then there is an increased risk of them having penicilliosis.

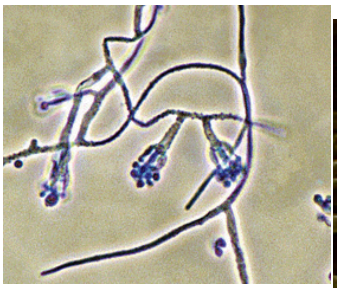
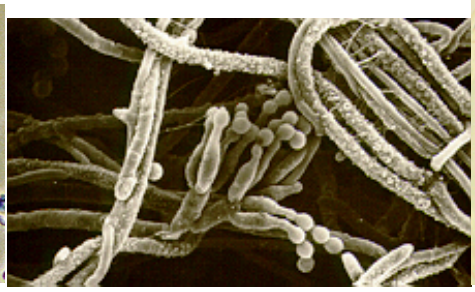
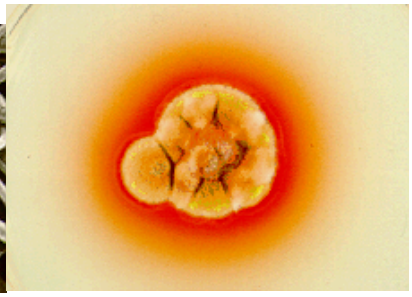
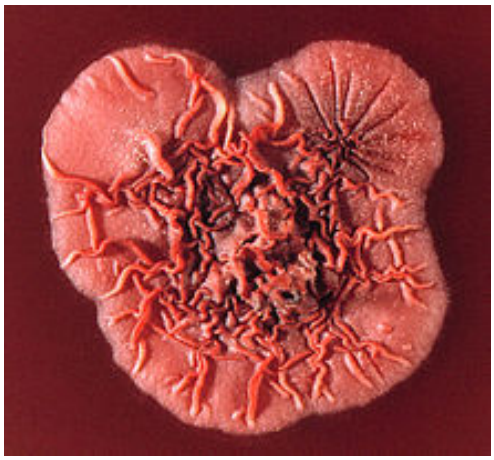
Antigen testing of urine and serum, and PCR amplification of specific nucleotide sequences have been tried, with high sensitivity and specificity. Rapid identification of penicilliosis is sought, as prompt treatment is critical. Treatment should be provided as soon as penicilliosis is suspected.

Treatment:-

2 weeks of amphotericin B, then 10 weeks of oral itraconazole.

Genomics Sexual reproduction

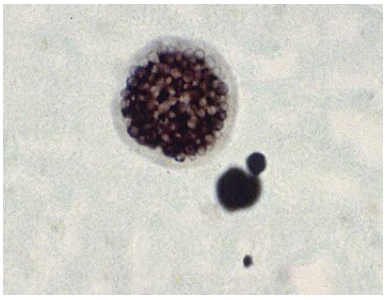
P. marneffei had been assumed to reproduce exclusively by asexual means based on the highly clonal population structure of this species. However, studies by Henk et al.[6](2012) revealed that the genes required for meiosis are present in *P. marneffei*. In addition, they obtained evidence for mating and genetic recombination in this species. Henk et al.[6] concluded that *P. marneffei* is sexually reproducing, but recombination in natural populations is most likely to occur across spatially and genetically limited distances resulting in a highly clonal population structure. It appears that sex can be maintained in this species even though very little genetic variability is produced.


Lecture 8: Systemic mycosis :

Coccidioides immitis (causing coccidioidomycosis):-

C. immitis is a dimorphic saprophytic fungus that grows as a mycelium in the soil and produces a spherule form in the host organism. It resides in the soil in certain parts of the southwestern United States, most notably in California and Arizona. It is also commonly found in northern Mexico, and parts of Central and South America. *C. immitis* is dormant during long dry spells, then develops as a mold with long filaments that break off into airborne spores when it rains. The spores, known as arthroconidia, are swept into the air by disruption of the soil, such as during construction, farming, or an earthquake. Windstorms may also cause epidemics far from endemic areas. In December 1977 a windstorm in an endemic area around Arvin, CA led to several hundred cases, including deaths, in non-endemic areas hundreds of miles away.

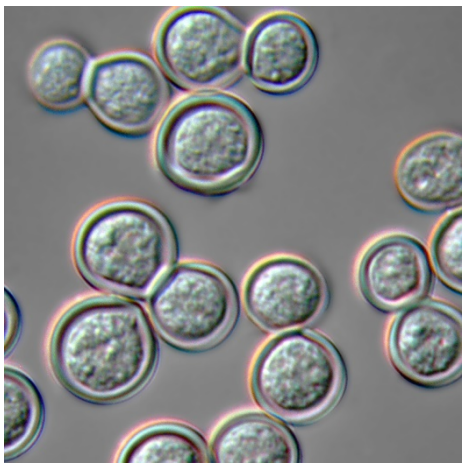


A Coccidioides immitis spherule containing endospores.

Blastomyces dermatitidis (causing blastomycosis):-

Blastomyces dermatitidis is the causal agent of blastomycosis, an invasive and often serious fungal infection found occasionally in humans and other animals in regions where the fungus is endemic. The causal organism is a fungus living in soil and wet, decaying wood, often in an area close to a waterway such as a lake, river or stream. Indoor growth may also occur, for example, in accumulated debris in damp sheds or shacks. The fungus is endemic to parts of eastern North America, particularly boreal northern Ontario, southeastern Manitoba, Quebec south of the St. Lawrence River, parts of the U.S. Appalachian mountains and interconnected eastern mountain chains, the west bank of Lake Michigan, the state of Wisconsin, and the entire Mississippi Valley including the valleys of some major tributaries such as the Ohio River. In addition, it occurs rarely in Africa both north and south of the Sahara Desert, as well as in the Arabian Peninsula and the Indian subcontinent. Though it has never been directly observed growing in nature, it is thought to grow there as a cottony white mold, similar to the growth seen in artificial culture at 25 °C (77 °F). In an infected human or animal, however, it converts in growth form and becomes a large-celled budding yeast. Blastomycosis is generally readily

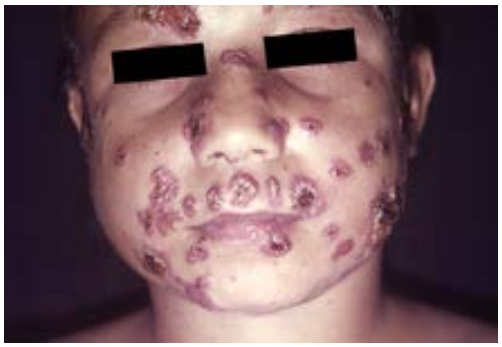
treatable with systemic antifungal drugs once it is correctly diagnosed; however, delayed diagnosis is very common except in highly endemic areas.



Paracoccidioides brasiliensis (causing paracoccidiodomycosis):-

P. brasiliensis is a thermally dimorphic fungus distributed in Brazil and South America. The habitat of the infectious agent is not known, but appears to be aquatic. In biopsies, the fungus appears as a polygemulating yeast with a pilot's wheel-like appearance.

Paracoccidioidomycosis is a systemic mycosis caused by the dimorphic fungus *Paracoccidioides brasiliensis*. Strong evidence indicates this fungus infects the host through the respiratory tract. It frequently involves mucous membranes, lymph nodes, bone, and lungs. Unlike other systemic mycoses, it can cause disease in immunocompetent hosts, although immunosuppression increases the aggressiveness of the fungus. Also uniquely, it rarely causes disease in fertile-age women, probably due to a protective effect of estradiol.



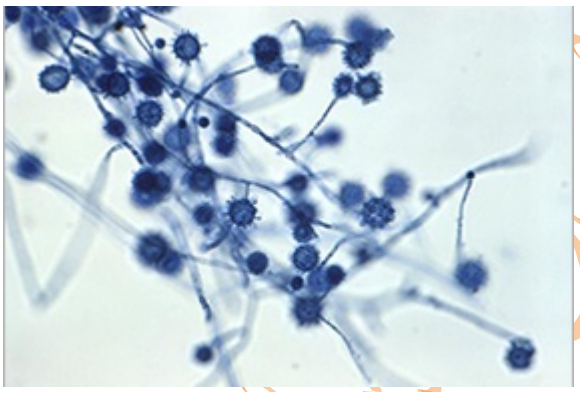
Lecture 9: Histoplasmosis:

Histoplasmosis:-

is an infection caused by breathing in spores of a fungus often found in bird and bat droppings. Histoplasmosis is most commonly transmitted when these spores become airborne, often during cleanup or demolition projects.

Soil contaminated by bird or bat droppings also can transmit histoplasmosis, so farmers and landscapers are at a higher risk of contracting the disease.

People can get histoplasmosis after breathing in the microscopic fungal spores from the air. Although most people who breathe in the spores don't get sick, those who do may have a fever, cough, and fatigue. Many people who get histoplasmosis will get better on their own without medication, but in some people, such as those who have weakened immune systems, the infection can become severe.



Symptoms of histoplasmosis include:

- Fever
- Cough
- Fatigue (extreme tiredness)
- Chills
- Headache
- Chest pain
- Body aches

Symptoms of histoplasmosis may appear between 3 and 17 days after a person breathes in the fungal spores. Histoplasmosis is diagnosed by:

- Biopsy of the lung, skin, liver, or bone marrow
- Blood or urine tests to detect histoplasmosis proteins or antibodies
- Cultures of the blood, urine, or sputum (this test provides the clearest diagnosis of histoplasmosis, but results can take 6 weeks)

Histoplasma capsulatum:-

Histoplasma capsulatum is found in soil, often associated with decaying bat guano or bird droppings. Disruption of soil from excavation or construction can release infectious elements that are inhaled and settle into the lung.

People can get histoplasmosis after breathing in the microscopic fungal spores from the air. Although most people who breathe in the spores don't get sick, those who do may have a fever, cough, and fatigue. Many people who get histoplasmosis will get better on their own without medication, but in some people, such as those who have weakened immune systems, the infection can become severe.

A dimorphic fungus species of worldwide distribution that causes histoplasmosis in hu mans and other mammals; its

LABORATORY DIAGNOSIS

Serologic tests for antibodies form the basis for diagnosis in most patients with mild infections, while cultures, stains, and tests for antigens are more useful in those with more severe disease. Biopsy of the involved organ for histopathology and culture may be required in some patients in whom test for antibodies in serum and CSF, test for antigens in urine, serum and other body fluids, and cytological analysis are negative or in severely ill patients in whom an immediate diagnosis is judged to be necessary to begin antifungal therapy before antigen results can be obtained.

Serologic Tests

Antibodies to *H. capsulatum* measured by immunodiffusion or complement fixation develop in most patients. H. precipitin bands can be demonstrated in less than 25% of patients and clear during the first 6 months following exposure . M bands occur in over three-quarters of cases and persist for years in some patients. Complement fixation titers of 1:8 or more are found in most patients with histoplasmosis while titers of 1:32 or higher are more suggestive of active infection..

Culture

Cultures are most useful in patients with disseminated or chronic pulmonary histoplasmosis. Culture is a particularly reliable diagnostic method for patients with disseminated histoplasmosis and HIV/AIDS. The sensitivity is only l0 to 15% in patients with other forms of histoplasmosis . In disseminated histoplasmosis, the highest yield is from bone marrow or blood, positive in over 75% of cases .

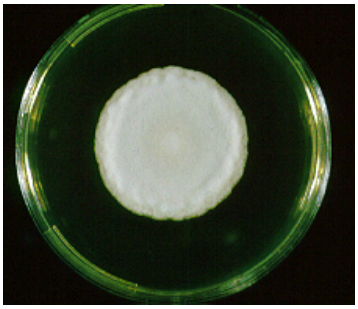
Organisms can be found in sputum or bronchoscopy specimens in 60 to 85% of cases of cavitary histoplasmosis . Due to their time consuming nature, fungal cultures cannot be relied up for a rapid diagnosis of histoplasmosis especially in patients with severe disease where timely initiation of antifungal therapy might be lifesaving.

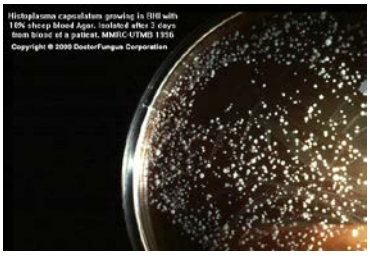
Antigen Detection

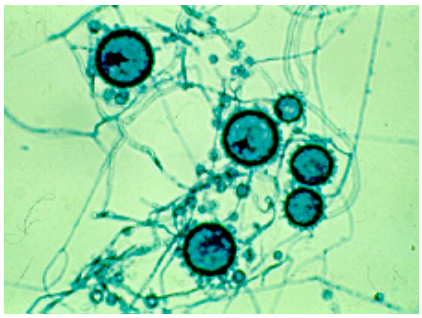
Sensitive methods for rapid diagnosis of histoplasmosis in patients with severe manifestations are essential to allow prompt initiation of therapy. Fungal stain is rapid but insensitive. Detection of antigen offers a valuable approach to the rapid diagnosis, especially in patients with the "epidemic" form of acute pulmonary, which follows within a week or two of a heavy exposure and is characterized by diffuse infiltrates and for disseminated histoplasmosis .

Fungal Stains

Silver stain of tissue sections or Wright stain of peripheral blood smears permits rapid diagnosis but with a lower sensitivity than culture or antigen detection. Fungal stains of tissues are positive in about half of cases of disseminated histoplasmosis . *Candida glabrata, Cryptococcus neoformans, Blastomyces dermatitidis, Penicillium marneffei, Pneumocystis carinii, Toxoplasma gondii, Leishmania* and staining artifacts may be misidentified as *H. capsulatum.*







Lecture 10:Antifungal

An **antifungal medication** is a pharmaceutical fungicide used to treat and prevent mycoses such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others.

Classes:-

Polyene antifungals:-

A polyene is a molecule with multiple conjugated double bonds. A polyene antifungal is a macrocyclic polyene with a heavily hydroxylated region on the ring opposite the conjugated system. This makes polyene antifungals amphiphilic. The polyene antimycotics bind with sterols in the fungal cell membrane, principally ergosterol. This

changes the transition temperature of the cell membrane, thereby placing the membrane in a less fluid, more crystalline state. (In ordinary circumstances membrane sterols increase the packing of the phospholipid bilayer making the plasma membrane more dense.) As a result, the cell's contents including monovalent ions (K^+, Na^+, H^+, and Cl^-), small organic molecules leak and this is regarded one of the primary ways cell dies. Animal cells contain cholesterol instead of ergosterol and so they are much less susceptible. However, at therapeutic doses, some amphotericin B may bind to animal membrane cholesterol, increasing the risk of human toxicity. Amphotericin B is nephrotoxic when given intravenously. As a polyene's hydrophobic chain is shortened, its sterol binding activity is increased. Therefore, further reduction of the hydrophobic chain may result in it binding to cholesterol, making it toxic to animals.

- Amphotericin B
- Candicidin
- Filipin – 35 carbons, binds to cholesterol (toxic)
- Hamycin
- Natamycin – 33 carbons, binds well to ergosterol
- Nystatin
- Rimocidin

Imidazole, triazole, and thiazole antifungals:-

Azole antifungal drugs (except for abafungin) inhibit the enzyme lanosterol 14 α-demethylase; the enzyme necessary to convert lanosterol to ergosterol. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth.

Imidazoles:-

- Bifonazole
- Butoconazole
- Clotrimazole
- Econazole
- Fenticonazole
- Isoconazole
- Ketoconazole
- Luliconazole
- Miconazole
- Omoconazole
- Oxiconazole
- Sertaconazole
- Sulconazole
- Tioconazole

Triazoles:-

- Albaconazole
- Efinaconazole
- Epoxiconazole
- Fluconazole
- Isavuconazole
- Itraconazole
- Posaconazole
- Propiconazole
- Ravuconazole
- Terconazole
- Voriconazole

Thiazoles:-

- Abafungin

Allylamines:-

Allylamines inhibit squalene epoxidase, another enzyme required for ergosterol synthesis. Examples include Amorolfin, Butenafine, Naftifine, and Terbinafine.

Echinocandins:-

Echinocandins may be used for systemic fungal infections in immunocompromised patients, they inhibit the synthesis of glucan in the cell wall via the enzyme Beta (1-3) glucan synthase:

- Anidulafungin
- Caspofungin
- Micafungin