

# Detection of Herpes Simplex -2 Virus in Women with Spontaneous Abortion in Al-Najaf City/Iraq

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## Abstract :

**Background:** HSV-2 is thought to be considered as the major sexual transmitted pathogen, which may cause severe complications in spontaneous abortion.

**Objective:** This study was carried out to detect the rate of HSV-2 infection in women with spontaneous abortion by HSV-2 Ags ELISA kit.

**Materials and methods:** Blood samples were collected from 200 pregnant women and 200 women with spontaneous abortion. Blood specimens (5 ml) were taken from aborted women and controls under aseptic conditions and left for 10 minutes for spontaneous clotting at room temperature before being centrifuged (6000 rpm) to separate the serum. Serum samples were kept frozen at  $-20^{\circ}\text{C}$  for determination of HSV-2 Abs.

**Results:** The results showed that, this virus was detected in 30 (15%) out of aborted women and 18 (9%) in pregnant women. The peak of infection level occurred in age group (20-29) years in both aborted and pregnant women. While in aborted women, the maximum positive HSV-2 cases were noticed at the second trimester.

**Conclusions:** Herpes Simplex -2 Virus may play a significant role in pregnancy loss. Its detection by sensitive ELISA techniques would permit instantaneous therapeutic intrusion to raise the eventuality of a felicitous pregnancy.

**Keywords :** Herpes Simplex type 2; spontaneous abortion; sexual transmitted diseases; latent infection; second trimester.

## INTRODUCTION

Abortion is the termination of pregnancy via eradicating a fetus or embryo from the uterus prior to becoming able to stay alive. Abortion is considered as a member of the main medical problems in society. Repeating of abortion more than twice results in a clinical situation known as Recurrent Pregnancy Loss (RPL)<sup>(1)</sup>. An abortion that occurs spontaneously is known as a miscarriage<sup>(2)</sup>. Various effectors associated with abortion such as Genetic and uterine anomalies, Endocrinopathy, immunological dysfunctions, infectious agents, environmental contaminants, psychogenetic elements and endometriosis<sup>(3)</sup>. Maternal infections are considered the main reason of pregnancy wastage in females with Bad Obstetric History (BOH)<sup>(4)</sup>.

*Herpes simplex virus* type 2 (HSV-2) is a worldwide-distributed virus, enveloped double strand DNA genome. It belongs to the family *Herpesviridae*<sup>(5)</sup>. HSV-2 spreads across epithelial mucosa, skin interruptions. Then, it transfers to nervous tissues, indeed lumbosacral ganglia to initiate a latent infection.

This virus has the ability to invade the genital tract. In general, this virus is one of the main viral sexually transmitted diseases (STD) causes globally<sup>(6,7)</sup>. High percentage of genital herpes infections are caused by HSV-2. Females during reproductive age become under high risk for exposure to occurrence of HSV-2 infection with a possibility of transmission to the embryo in pregnancies<sup>(8)</sup>. Prior to the 20<sup>th</sup> weeks of gestation, intrauterine transmission of HSV-2 leads to abortion, stillbirth and congenital abnormalities in live fetus<sup>(9)</sup>.

The aim of this study was to investigate the presence of HSV-2 infection in women with spontaneous abortion by HSV-2 Ags ELISA kit.

## SUBJECTS AND METHODS

This study was carried out during the period from 2/January/2017 to 31/August/2017, and included 200 women with spontaneous abortion (n=200), aborted by unknown causes (with age ranged between 16-45 years). The control group was 200 normal pregnancies, the age of all the tested groups ranged between 16-45 years.

Samples were tested in HSV-2 Ags ELISA kit (Bt-laboratory; China). After preparing reagents, standard solutions and samples according to the guideline information, all reagents were kept at room temperature before use because the assay was performed at the same temperature. The number of strips was determined then inserted in the frame. The unused strips should be stored at  $4^{\circ}\text{C}$  for up to one month. Blank wells were set without any solution. Fifty  $\mu\text{l}$  of both negative and positive control were added to negative control wells and positive control wells respectively. Then, the plate was incubated for a half hour after shielding with a sealer. All samples were diluted with sample diluent and mixed well and were covered with a plate sealer and incubated for 30 minutes at  $37^{\circ}\text{C}$ . The plate was washed and aspirated 5 times with wash buffer, overfilling wells with wash buffer. Then the plate was blotted on absorbent materials. Horseradish peroxidase (HRP) was added as  $50\mu\text{l}$  to all wells. Incubation of the plates for 30 minutes had been done after sealing it. The sealer was removed and the plate was washed as described above. Fifty  $\mu\text{l}$  of each substrate solution A and B were added to all the wells. This step was followed by covering and incubation for 10 minutes at  $37^{\circ}\text{C}$  in the dark. The stop solution was added as  $50\mu\text{l}$  to the wells of the plate, conversion of color from blue to yellow was occurred immediately. The O. D. of the plate's wells was determined immediately via microplate reader that set to 450 nm within 15 minutes after the addition of the stop solution had been done.

## STATISTICAL ANALYSIS

The statistical analysis of the presented study was performed by Statistical Package for the Social Sciences (SPSS) version 20. Variation of age was detected by ANOVA test.  $p$  value of  $\leq 0.05$  was measured to show statistical significance.

## RESULTS

The distribution of the tested groups according to the age in the presented study was as follows: aborted women were <20 years 21 (10.5%), 20-29 years 98 (49%), 30-39 years 73 (36.5%) and  $\geq 40$  years 8 (4%) (Fig 1). In pregnancies, <20 years 15 (7.5%), 20-29 years 120 (60%), 30-39 years 63 (31.5%) and  $\geq 40$  years 2 (1%) (Figure 2).

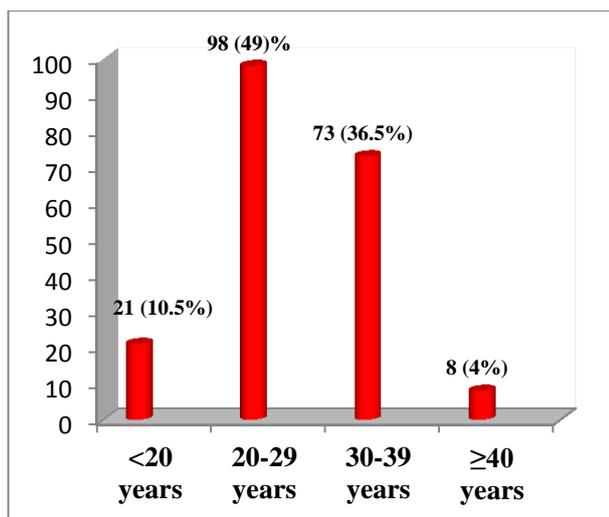


Figure 1: Distribution of aborted women according to age groups.

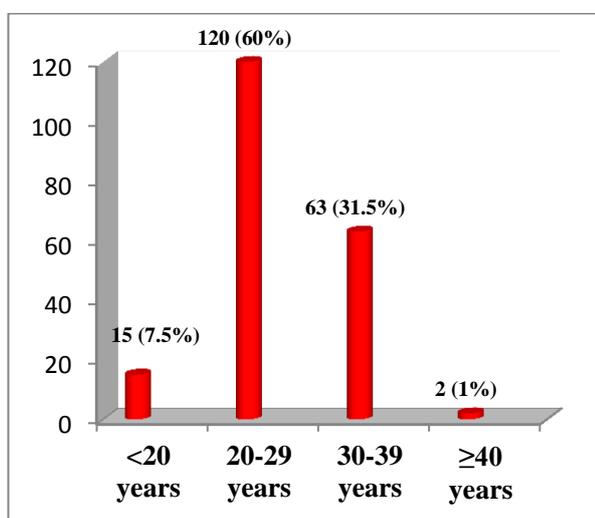


Figure 2: Distribution of pregnant women according to age groups.

The distribution of aborted and pregnant women according to the stages of pregnancy trimesters were as in Table 1.

Table 1: Distribution of aborted and pregnant women according to the stages of pregnancy trimesters.

Trimesters	Aborted	Pregnant	Total
1 <sup>st</sup> trimester	152 (38%)	118 (29.5%)	270 (67.5%)
2 <sup>nd</sup> trimester	48 (12%)	78 (19.5%)	126 (31.5%)
3 <sup>rd</sup> trimester	Nil (0%)	4 (1%)	4 (1%)
<b>Total</b>	<b>200 (50%)</b>	<b>200 (50%)</b>	<b>400 (100%)</b>

The positive sample to HSV-2 (Cutoff = 0.584) in aborted women were 30 sample (15%), while in pregnant women, the positive sample to HSV-2 were 18 samples (9%). In other hand, all the serum samples of non-pregnant women were negative to HSV-2. Therefore, the sum of HSV-2 positive cases in the presented study was 48 (8%) positive cases.

According to the age, the HSV-2 positive cases in aborted women were as following: Zero positive cases (0%) at <20 years, 17 positive cases (56.7%) at 20-29 years, 10 positive cases (33.3%) at 30-39 years and 3 positive cases (10%) at ≥40

years. The association between age and HSV-2 positive pregnant women was highly significant ( $P<0.05$ ).The positive sample to HSV-2 in pregnant women were 18 samples (9%); Zero positive cases (0%) at <20 years, 11 positive cases (61.1%) at 20-29 years, 6 positive cases (33.3%) at 30-39 years and 1 positive case (5.6%) at ≥40 years. The association between age and HSV-2 positive pregnant women was highly significant ( $P<0.05$ ).Finally, the distribution of HSV-2 between the tested groups according to the age in the presented study was listed in Table 2.

Table 2: Distribution of HSV-2 positive samples between aborted and pregnant women according to age.

Ages Groups	<20 years	20-29 years	30-39 years	≥40 years	Total
Aborted women	Nil (0%)	17 (35.4%)	10 (20.8%)	3 (6.3%)	30(62.5%)
Pregnant women	Nil (0%)	11 (22.9%)	6 (12.5%)	1 (2.1%)	18 (37.5%)
<b>Total</b>	<b>0 (0%)</b>	<b>28 (58.3%)</b>	<b>16 (33.3%)</b>	<b>4 (8.4%)</b>	<b>48 (100%)</b>

According to the pregnancy trimesters in the presented study, HSV-2 positive samples in aborted women were 8 positive samples (16.7%) at 1<sup>st</sup> trimester, 22 positive samples (45.8%) at 2<sup>nd</sup> trimester while there was absence of any HSV-2 detection at 3<sup>rd</sup> trimester. In pregnant women, the results were 14 positive samples (29.2%) at 1<sup>st</sup> trimester, 4 positive samples (8.3%) at 2<sup>nd</sup> trimester. At 3<sup>rd</sup> trimester, the results were similar to that of the aborted women (Table 3).

Table 3: Detection of HSV-2 according to the pregnancy trimesters.

Trimester	Abortion	Pregnant	Total
1 <sup>st</sup> trimester	8 (16.7%)	14 (29.2%)	22 (45.8%)
2 <sup>nd</sup> trimester	22 (45.8%)	4 (8.3%)	26 (54.2%)
3 <sup>rd</sup> trimester	Nil	Nil	0 (0%)
<b>Total</b>	<b>30 (62.5%)</b>	<b>18(37.5%)</b>	<b>48(100%)</b>

The distribution of aborted women in the presented study according to their frequency of abortion was as in Figure 3. Positive samples to HSV-2 in aborted women were distributed according to the frequency of the abortion as in Table 4.

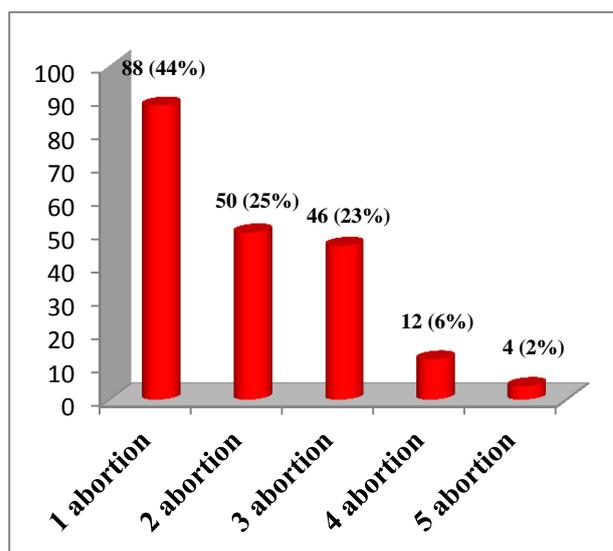


Figure 3: Distribution of aborted women according to the frequency of the abortion.

**Table 4:** Distribution of HSV-2 positive samples in aborted women according to frequency of the abortion.

	Frequencies of the abortion					Total
	1 abortion	2 abortion	3 abortion	4 abortion	5 abortion	
Aborted women	88 (44%)	50 (25%)	46 (23%)	12 (6%)	4 (2%)	200 (100%)
HSV-2	5 (5.7%)	4 (8%)	16 (34.8%)	4 (33.33%)	1 (25%)	30 (15%)

The results of HSV-2 distribution in aborted women with or without BOH in the presented study were as the following: The aborted women without BOH ( $\leq 2$  abortions) were 138 (69%) while those with BOH ( $\geq 3$  abortions) were 62 (31%). Most of the HSV-2 positive samples in aborted women with BOH were 21 (70%) with significant differences ( $P \leq 0.05$ ) with those without BOH 9 (30%). Positive samples to HSV-2 in pregnant women with BOH and in those without BOH were 13 (72.2%) and 5 (27.8%) respectively.

#### DISCUSSION

The rate of HSV-2 infection in aborted women in the current study was 30 positive samples (15%), this result lower than that which recorded in Baghdad 18.9 %<sup>(10)</sup>; Babylon 37.1%<sup>(11)</sup>; Athens 43.2%<sup>(12)</sup>; India 30.1%<sup>(13)</sup>, and higher than England 6.8%<sup>(14)</sup>.

The results of the pregnant women were 18 (9%), which lowered than these in Babylon 82.7%<sup>(15)</sup>; Diyala 73.9%<sup>(16)</sup>; Kirkuk 16.26%<sup>(17)</sup>; Tikrit 24.2%<sup>(18)</sup> and Syria 52%<sup>(19)</sup>. While the result was higher than that reported in Baghdad 8.26 %<sup>(20)</sup>, Dubai and Tehran 6.5%<sup>(21)</sup>. The use of ELISA kit in scientific researches has many advantages such as rapid; simple in procedure; high degree of accuracy; can test both antibodies or antigens; high sensitivity and more cost effective<sup>(22)</sup>.

The age of most positive cases in aborted and pregnant women located in 20-29 years (58.3%), then followed by 30-39 years (33.3%) and finally  $\geq 40$  years (8.3%). These result agreed with those of Aljumaili *et al.*<sup>(18)</sup>; Mawlood *et al.*<sup>(23)</sup>; Tuma *et al.*<sup>(20)</sup>; Hasan *et al.*<sup>(24)</sup>; Biswas *et al.*<sup>(25)</sup> and Al-Saeed *et al.*<sup>(26)</sup>, they established highest incidence of HSV-2 at the same age period. On other hand, results of the current study differed from these of Cusini and Ghislanzoni<sup>(27)</sup>; Mohammad and Salman<sup>(17)</sup> and Amar *et al.*<sup>(28)</sup>, they fixed most of positive cases at  $\geq 40$  years of age. Results also disagreed with that of Hassan *et al.*<sup>(10)</sup> he established that pregnant women at the age between 30-39 years become more susceptible to infection than other periods of age.

There are many reasons that may explain the high incidence of HSV-2 in women at age between 20 to 29 years, first: this age period considered the typical reproductive age of the women<sup>(8)</sup>. Second, women at this age are more susceptible to chronic infection such as HSV-2<sup>(29)</sup>. Finally, occurrence of primary or recurrent HSV-2 infection reaches to the peak at this age period<sup>(30)</sup>.

Most of positive samples of HSV-2 from aborted women were noticed at the second trimester followed by lower cases at the first trimester. These results agreed with Salman<sup>(31)</sup> when reported that the maximum rate of viral abortion occurs at the second and third trimester more highly than first trimester, and differed from results of Kapranos and Kotronias<sup>(12)</sup> and Hasan *et al.*<sup>(24)</sup> who established high rate of viral detection in serum samples aborted women at the first trimester. Baud *et al.*<sup>(32)</sup> established the infection as a causative agent of 15% and 66% of early and late abortion respectively. In addition, elimination of gestation due to infection with virus or inflammation of the placenta mostly occurs at second trimester<sup>(33)</sup>. Results of pregnant women showed high positive samples in the first trimester, which similar to that of Money and Steben<sup>(34)</sup>, while differed from Mohammad and Salman<sup>(17)</sup> who established maximum rate of HSV-2 infection at second trimester.

Both aborted and pregnant women with BOH in current study showed high level of HSV-2 infection than others without it. In the presented study, High percentage of aborted women with HSV-2 positive samples have BOH, this agreed with Nigro *et al.*<sup>(35)</sup>, who suggested that the recurrent infections of the genital tract with HSV-2 lead to loss of pregnancy.

Pregnant women with BOH had a rising in HSV-2 positive samples than pregnancies without it that agreed with Aljumaili *et al.*<sup>(18)</sup>, while differed from Hassan *et al.*<sup>(36)</sup> who report no significant difference between pregnant women with BOH and others with normal pregnancies.

#### CONCLUSIONS

Herpes simplex virus 2 was detected from aborted and pregnant women in noticeable level. Detection of HSV-2 in aborted women was higher than pregnant, this improve probability of this virus as one of main viral agents that cause abortion.

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