

## ORIGINAL ARTICLE

# Estimation of Interleukin-10 and Soluble HLA-G in Aborted Women Having Herpes Simplex Virus-2 Infection

Lezan Medhat Mohammed<sup>1</sup>, Farhan Abood Risan<sup>2</sup>, Nazar Sh. Mohammed<sup>3</sup>

<sup>1</sup> Northern Technical University, College of Health and Medical Technology/Medical Laboratory Technique Department, 36001, Kirkuk, Iraq.

<sup>2</sup> Middle Technical University, Medical Technical Institute, Al-Mansour, 10011 Baghdad, Iraq.

<sup>3</sup> Middle Technical University, Medical Laboratory Technique Department, College of Health and Medical Technology, 10011 Baghdad, Iraq.

## ABSTRACT

**Introduction:** Spontaneous abortion (miscarriage) is one of extreme generic gestation complications. Yonder numerous causes leading to miscarriage like reproductive anatomical anomalies, genetic agents and virus infections in which viral infections, the infection with herpes simplex virus (HSV-2) is utmost common virus the occurs in the worldwide distribution. **Aims:** The objective of the study is to research the seroprevalence of Herpes simplex virus-2 in aborted women and detect the rate of interleukin-10 and HLA-G in the serum of aborted women compared with healthy pregnant women. **Methods:** One hundred and twenty which include 60 aborted women and 60 healthy pregnant women as a control group, the age of both groups are (15-43 years range). The blood samples are taken from both groups; the serum was separated and tested by ELISA to the detection of HSV-2 IgM, interleukin-10 & HLA-G. **Results:** Among aborted women most HSV-2 IgM antibody occur in age groups 21-30 & more than 30 years with rate 26(100.0%)&28(100.0%). There was a significant relation between IgM and cases in all ages ( $P=0.005$ ). There was a significant relation with  $P$ value=0.005 between IL-10 and cases and between sHLA-G and cases. **Conclusion:** IL-10 was increased in aborted women carrier with HSV-2 but the soluble HLA-G molecules are lowered in aborted women having HSV-2 infection.

**Keywords:** Spontaneous abortion, Herpes simplex virus-2 and enzyme linked immunosorbent assay

## Corresponding Author:

Lezan Medhat Mohammed, Ph.D.

Email: Lezan83lezan@gmail.com

Tel: +964750227531

## INTRODUCTION

Spontaneous abortion (miscarriage) is one of the extreme problems among women that may happens during early gestation (1) and is defined as termination of pregnancy prior the twenty weeks of gestation or the dismissal of an embryo weighing 500g or less (2). Genetic and uterine abnormalities, endocrine and immunological dysfunctions, environmental agents including infectious agents are extreme essential reasons of spontaneous abortion (3). There are different microorganisms infecting the mothers as TORCH agents including toxoplasma gondii, rubella virus, cytomegalovirus and the herpes simplex virus (4). Human herpes simplex virus infecting the placenta and causing disorders to fetal growth and spontaneous abortion (5). HSV-2 is a member of *Herpesviridae* family;

is a DNA virus that cause primary infection and have the ability to establish lifelong latency in sensory neural ganglia (6) and are able to cross the placenta and extends to the fetus, as a result of this reaches it affects the gestation via prompting the miscarriage of fetal growth disorders (5). Normal Pregnancy illustrate fetomaternal balance between the growing fetus and maternal immune system so any extrinsic stimulant as infection can alters maternal immune response (7). Th2-type response has been associated with normal pregnancy but Th1-type response has been related with pregnancy loss. Anti-inflammatory cytokines as Interleukin-10 is a critical cytokine for protects a normal pregnancy (8) and maintaining immune tolerance so the dysregulation of IL-10 associated with adverse pregnancy complications such as miscarriage, fetal growth restriction (9). Another molecules that act as immune-modulatory molecules is human leukocyte antigen (HLA-G) due to their role in maintaining immune tolerance at the fetomaternal interface, enhancing graft tolerance and decreasing immune responses (10). At the maternal-fetal interface, the HLA-G molecules are specifically expressed by

fetus-derived cells such as extravillous cytotrophoblast cells or chorionic endothelial cells (11) so any deficiency in HLA-G expression is associated with miscarriage (12).

**MATERIALS AND METHODS**

ELISA kit for the detection of Herpes simplex virus 2 IgM : the wells of the plate coated with specific antigens .Then the wells were washed and then a horseradish peroxidase (HRP) labelled conjugate was added , then washed to remove unbound conjugate then adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product & then stop solution added. Result in the production a yellow color. Absorbance at 450/620 nm is read using an ELISA reader (bioactive diagnostic, Germany)

**Interpretation of results:** The Cut-off is the mean absorbance value of the Cut-off Control determinations

$$\text{Sample (mean) absorbance value} \times 10 = \frac{[\text{Bioactive Units} = \text{U}]}{\text{Cut-off}}$$

- Cut-off= 0.287**
- Positive > 11 U**
- Equivocal 9 – 11 U**
- Negative < 9 U**

**Sandwich ELISA kit for the detection of human soluble human leukocyte antigen class-G:**

The antibody (capture) coated the wells & conjugated biotinanted antibody utilized as detection antibody. The serum samples , standard, detection antibody was added to the appropriate wells , incubates, washed, and then HRP-Streptavidin added and then washed with washing solution. The substrate TMB was added, and then the stop (acidic acid) solution added. read the absorbance by ELISA reader (mybiosource, USA).

Interpretation of results: using graph paper, a standard curve blotted by relative absorbance of standard solutions at 450nm( Yaxis )vs the respective standard solution concentration(X axis) & then the concentration of samples was interpolated from the curve.

**Sandwich ELISA kit for the detection of Interleukin-10:**

the wells coated with anti-interleukin 10 & biotinanted conjugated anti interleukin10 antibody was utilized as detection antibody. the serum samples , standards and detection antibody was added to the appropriate wells, then washed with wash solution. And then HRP-Streptavidin was added and washed with wash buffer then the TMB substrates was added then adding acidic stop solution. Finally, the absorbance was read at 450nm by ELISA reader and then the concentration of IL-10 can be calculated (mybiosource, USA).

**Interpretation of results:** using graph paper, a standard curve blotted by relative absorbance of each standard solutions at 450nm( Yaxis )vs the respective standard solution concentration(X axis) & then the concentration of samples was interpolated from the curve.

**RESULT**

Table (I) showed the incidence of Herpes Simplex virus-2 IgM antibody in studied groups according to age groups. among aborted women most HSV-2 IgM antibody occur in age groups 21-30 & more than 30 years with rate 26(100.0%)&28(100.0%) respectively. There was a significant relation between IgM and cases in all ages (P=0.005).

**Table I : Distribution of HSV-2 IgM in studied groups according to age.**

Age Years	HSV-2 IgM	Cases			
		Abortion		Normal	
		Count	%	Count	%
<=20	-ve	0	0.0%	16	100.0%
	+ve	6	100.0%	0	0.0%
21-30	-ve	0	0.0%	24	100.0%
	+ve	26	100.0%	0	0.0%
31+	-ve	0	0.0%	20	100.0%
	+ve	28	100.0%	0	0.0%

Table (II) reviewed the prevalence of interleukin-10 in aborted women and control groups. There was a significant relation between IL-10 and cases with P<sub>value</sub>=0.005

**Table II : Distribution of IL-10 in studied groups.**

IL-10	Cases				P value
	Aborted women		Non - aborted women		
	Count	%	Count	%	
<b>Negative</b>	13	21.7%	20	33.3%	0.005
<b>Positive</b>	47	78.3%	40	66.7%	

The pattern of distribution of HLA-G in studied groups is noticeable in table (III). There was a significant relation between HLA-G and cases with p<sub>value</sub> =0.005

**Table III : prevalence of HLA-G in studied groups.**

HLA-G	Cases			
	Aborted women carrier with HSV-2		Normal pregnant	
	Count	%	Count	%
Decreased	55	91.6%	0	0.0%
Normal	4	6.7%	36	60.0%
Increased	1	1.7%	24	40.0%

## DISCUSSION

HSV infection poses a serious threat to the reproductive health of the population. Primary infection or relapse during pregnancy is so serious for the foetus and also can lead to spontaneous miscarriage or developmental malformations (13).The trophoblast cells express entry mediators of herpes simplex virus and causing failure in placental invasion and spontaneous abortion (5). In the current study, the prevalence of HSV-2 IgM in aborted women are 60(100.0%) with  $p_{value}$  (P=0.005 ).

According to various studies conducted in India that nearly compatible with current results that showed the prevalence of HSV antibodies varies from 3.6% to 61.3% (14) and other study reviewed the seropositivity of herpes simplex virus- IgM were 31.06% (15). Another result also nearly similar to results of current study which records the HSV IgM in 73.9% of patients(16) but other study reported that IgM infection was 59.2% for HSV(17).

In the current study the HSV-2 IgM seropositivity was higher in ages (21-30)and more than 30 years with a rate of 26(100.0%)and 28(100.0%) respectively and the association was significant (P=0.005) . Other study showed the elevated HSV-2 positive seen in age group (30-39) years with a rate 7(18.9 %) with highly significant (P<0.001)(18). IL-10 actively suppresses the maternal immune system to avoid rejection of the fetal allograft (9). In current study, there is elevated level of IL-10 among aborted women carrier with HSV-2IgM with a significant relation between IL-10 and cases, these result nearly compatible with results of another study which showed increasing levels of interleukin10(IL-10 ) was observed in aborted women than pregnant control with great significance (19).Other study reported normal pregnant was characterized by transiently decreased pro-, and raised anti-inflammatory expression (20).The results of another study showed moderate increase in the anti-inflammatory cytokines such as IL-10 in the pregnant women with threats of miscarriage than the apparently healthy pregnant women (21)and other study may be agreed with these result that demonstrated the estimated levels of serum IL-10 for recurrent pregnancy loss women were which was significantly lower (22). The current study reviewed the 55(91.6%) aborted women carriers with HSV-2 have decreased HLA-G and 4(6.7%) aborted women carriers with HSV-2 have normal HLA-G but among healthy pregnant there

were normal or increased levels of HLA-G with rates 36(60.0%) or 24(40.0%) respectively.

These results was nearly compatible with study found that the sHLA-G level increased in normal pregnancy , HLA-G expression in maternal-fetal tolerance is important in protection of fetal semi-allograft against lysis by maternal NK cells (23) and decreased levels of HLA-G seen in women with history of two or more abortion(recurrent abortion) (24). Other study may be agreed with current study that found the presence of HLA-G in normal pregnant women may be involved in immune regulation so higher levels of these molecules were seen in their serum(25) and other showed the detection of decreased HLA-G levels in the urine of women correlated with pregnancy disorders as spontaneous abortion (26).HSV infection cause damage of HLA-G and cell death, these alters in trophoblast action is clarify why HSV-2 have been associated with spontaneous pregnancy loss (27).

## CONCLUSION

IL-10 was increased in aborted women carrier with HSV-2 but the soluble HLA-G molecules are lowered in aborted women having HSV-2 infection.

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