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Seroprevalence of Anti-Phospholipid Igm and Igg Antibodies in Aborted Women Carrier with Herpes Simplex Virus Type 2

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Abstract
Background: The existence of anti-phospholipid antibodies produces a prothrombotic states and elevated risk of pregnancy complications.
Objective: the existing study aimed to detect the rate of anti-phospholipid IgM and IgG antibodies in aborted women carrier with Herpes simplex virus type 2 and compared with pregnant women.
Patients and methods: The current study done on 120 women who include 60 aborted women and 60 pregnant women as a control group, the age of both groups are ranged from 15-43 years. The blood samples are obtained from both groups and then centrifuged to obtain the serum that used for detection of HSV-2IgM and anti-phospholipid IgM and IgG antibodies.
Results: Within aborted women group, about 13(61.9%) and 30(76.9%) aborted women carrier with HSV-2 seropositivity have seropositive anti-phospholipid IgM antibody existent in ≤6 weeks and more than 6weeks respectively while in non-aborted group there was only 2(6.3%) positive anti-phospholipid IgM antibody. There was no significant relation between apl IgG and cases in all gestational age groups (P>0.05).
Conclusion: The most antiphospholipid IgM antibody seropositivity observed in aborted women's serum at gestational age more than six weeks. There was no significant relation between seropositivity of anti-phospholipid IgM antibody with number of abortion.
Keywords: Spontaneous abortion, Herpes simplex virus-2 and enzyme linked immunosorbent assay and anti-phospholipid antibodies.

Introduction

The spontaneous abortion meaning loss of pregnancy prior twenty weeks of gestational age(El-Hachem *et al.*,2017). Numerous reasons leading to pregnancy loss including factors that are involved in human reproduction, anatomic features, genetic factors, diabetes mellitus, systemic hypertension, hypo or hyperthyroidism(endocrine), environmental agents psychogenetic and immunological factors (Gao *et al.*, 2018). Different autoimmune illness as maternal causes include systemic disorders such as endocrinopathies and hypercoagulable states which are risky for the placenta (Stankiewicz , 2017) including antiphospholipid syndrome (Tektonidou *et al.*, 2019).

The infection with Herpes simplex virus (HSV) is one of the extreme critical causes for spontaneous abortion (Hassan *et al.* , 2017).Human Herpesviruses occurs worldwide, more than 90% of adults are infected by one or numerous HHVs (Bruno *et al.*, 2019). HSV-2 are members of alphaherpesviridae subfamily, posses a large linear double-stranded DNA genome (Serdaroglu

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and Kutlubay, 2017). The primary mechanism of antiphospholipids in the first trimester gestational period depends on a deleterious effect directly on trophoblastic cells, inhibition of secretion of human placental chorionic gonadotropin, and the expression of trophoblast cell adhesion molecules (a1, a5 integrins, E, VE-cadherins)(Tsikouras *et al.* , 2019). Human β_2-glycoprotein I (β_2GPI) is the major antigen recognized as a target for antiphospholipid antibodies , expressed by placental endothelial cells and placenta itself .β2GPI is expressed in human extravillous trophoblast cell membrane, thus making these cells a target for aPL (Bruno *et al.*, 2019).The syncytiotrophoblast produces human chorionic gonadotropin (hCG), Apl reduce the growth of the syncytiotrophoblast resulting in decreased production of hCG. aPL prevent the formation of new syncytiotrophoblast and increase cell death, leading to reduced transplacental transport (Hamulyak *et al.*, 2020). Thrombogenic action caused by HSV on the uteroplacental vessels leading to circulatory disturbance cannot be ruled out as a mechanism of spontaneous pregnancy loss, given the fact that HSV has the ability to cause thrombin production and endothelial damage (Kapranos and Kotronias,2009). Presence of antiphospholipid antibodies can lead to blood clots and/or pregnancy loss (miscarriage) in the first trimester and also after 10 weeks of pregnancy and placental insufficiency(Houghton and Moll, 2017).

Materials and methods:

From each subject; 5 mL of blood samples was collected by vein puncture using disposable syringes placed in plain test tube and left to clot at room temperature and then separated by centrifuge at 1500 round /minute (rpm) for 10 minutes to obtain the serum. ELISA used to detect Herpes simplex virus 2 IgM antibody(Bioactiva diagnostic /Germany) , the result interpreted as
Positive > 11 U: It means antibodies against the pathogen are present.
Equivocal 9 – 11 U: It means antibodies against the pathogen could not be detected clearly.
Negative < 9 U: it means: The sample contains no antibodies against the pathogen.
ELISA kit used for the detection of antiphospholipid IgM and IgG(Aeskulisa/ Germany), the result interpreted as
Normal Range : < 12 U/mL
Positive value: >18 U/mL.

Statistical Analysis

Statistical analyses were performed using SPSS Statistical Package for Social Sciences (version 20.0). Data are presented as number and percentage as applicable. Comparison between abortion and normal groups was done using Student's t-test. Relation between qualitative data was studied using Chi-square test. Correlation test was done to examine relation between markers. P value of <0.05 was considered statistically significant.

Results and Discussion

Table (1) illustrated the incidence of anti-phospholipid IgM antibody (aPL-IgM) in studied cases according to gestational age. Within aborted women group, about 13(61.9%) and 30(76.9%) aborted women carrier with HSV-2 seropositivity have seropositive anti-phospholipid IgM antibody existent in ≤6 weeks and more than 6weeks respectively while in non-aborted group there was only 2(6.3%) positive anti-phospholipid IgM antibody. There was a significant relation between aPL-IgM and cases in gestational age groups (P=0.005) .

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Table (1): Allocation of anti-phospholipid IgM antibody in studied cases based on gestational age.

Gestational age	aPL-IgM	Cases				P_{value}
		Aborted women		Pregnant women		
		Count	%	Count	%	
≤6 weeks	Negative	8	38.1%	28	100.0%	0.005 Significant
	Positive	13	61.9%	0	0.0%	
>6 weeks	Negative	9	23.1%	30	93.7%	
	Positive	30	76.9%	2	6.3%	

The prevalence of anti-phospholipid IgG antibody in aborted and non-aborted pregnant women was clarified in table (2). The positive anti-phospholipid IgG antibody in aborted and non-aborted group found in gestational age ≤6 weeks with a rate 1(4.8%) and 1(3.6%) respectively but at gestational age more than 6 weeks with a rate 1(2.6%) and 3(9.4%) respectively. There was no significant relation between apl IgG and cases in all gestational age groups (P>0.05).

Table (2): Prevalence of anti-phospholipid IgG antibody in studied cases based on gestational age.

Gestational age	aPL-IgG	Cases				P_{value}
		Aborted women		Pregnant women		
		Count	%	Count	%	
≤6 weeks	Negative	20	95.2%	27	96.4%	0.796 >0.05
	Positive	1	4.8%	1	3.6%	
>6 weeks	Negative	38	97.4%	29	90.6%	0.860 >0.05
	Positive	1	2.6%	3	9.4%	

Table (3) described the distribution of anti-phospholipid IgM antibody among examined groups. Twenty-six (70.3%) aborted women that have positive antiphospholipid IgM antibody have one abortion and 17(73.9%) have two or more abortion. Statistically, there was no significant relation between seropositivity of anti-phospholipid IgM antibody with number of abortion with p_{value} (0.184)

Table (3): Distribution of molecules aPL-IgM molecules according to number of abortion.

Apl-IgM IL-10		Number of abortion				Pvalue
		One		Two or more		
		Count	%	Count	%	
Apl-IgM	Negative	11	29.7%	6	26.1%	0.184
	Positive	26	70.3%	17	73.9%	>0.05

Viral agent was considered the infectious trigger for the induction of antiphospholipid antibodies (Mohammed, 2020). The present study may agree with another review that showed

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the antiphospholipid antibody was increased in recurrent aborted women (Heng *et al.,* 2015) and may be nearly compatible with study that showed the antiphospholipid antibody found in about 50% of cases of pregnancy loss beyond 10 weeks of gestation period and also the outcome of miscarriage most commonly seen in frequent miscarriages. Moreover, the recurrent miscarriage is linked to the elevation in the levels of phospholipids antibodies, in about 15% of women. The women showing positive result of phospholipids antibody have a rate of fetal loss up to 90%. Thus, this is another reason that recurrent miscarriages can put women at risk of abortion (Khangura *et al.*,2019). The existing result may agree with another study that found the antiphospholipid antibody IgM levels were decreased during pregnancy and their alteration were not associated with pregnancy outcomes (Yelnik *et al.,* 2016).Another study found there was no elevation in frequency of raise antiphospholipid antibody in women with recurrent losses (Abrahams *et al.,* 2017) and another study reported found no relationship between changes in aPL titers and adverse pregnancy outcomes, while others reported favorable pregnancy outcomes associated with declining titers of aPL (Yelnik *et al.,* 2016) and another study that clarified there no antiphospholipid IgM antibody positivity in control healthy group (Bhasker *et al.,* 2018). The current study may be disagreed with another study that found a significant relation between antiphospholipid IgM antibodies and recurrent abortion (Santosa *et al.*,2017).The present results may agree with others that showed the incidence of antiphospholipid antibody positivity may range from 0% to 11.4% in healthy pregnant women so the antiphospholipid antibody positivity was significantly associated with lower pregnancy rate and elevated abortion rate (Hong *et al.,* 2018). The current result may disagree with another results that showed the significant increase in level of antiphospholipid IgG antibody seropositivity in aborted women ($P < 0.001$) when compared with the control with rate about 60.2% in aborted women, compared with 39.8% in healthy women(Jabber *et al.*,2020). Other study showed the prevalence of aPL antibody was about 15% in recurrent aborted women(Diejomaoh , 2015).

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