The Relationship between some Immunological Criteria and Women with Recurrent Miscarriages

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Abstract

The aim of this study is to determine the relationship between certain immunological parameters and recurrent miscarriages (RM) at first trimester among women patients with a past history of RM that associate with antiphospholipid syndrome (APS), as well as, know the positive prevalence of autoantibodies(IgM) in patients compared with healthy women . The study enrolled 88 women including, 55 aborted women in the first trimester of gestation with a history of RM, and 33 were selected as a control group , who attend the emergency and consulting divisions in maternity and pediatrics hospital at Babylon province, where their ages ranged between eighteen to thirty-five year. Sera from all participants were tested to assessment of some immunological parameters related to this study. It was observed that a high positive prevalence of IgM anti β2-glycoprotein I(aβ2GPI) which reached 50.0% in comparison with 21.6% and 18.2% to an index value of IgM for antiphospholipid(aPL) and anticardiolipin (aCL) respectively. The odds ratio (OR) for IgM aβ2GPI was 50.08 % ,whereas it was 6.93 % & 5.29 % for aPL and aCL IgM respectively, at a level of significant difference (P< 0.05). Also, the results showed a non-significant difference (P = 0.070) in the mean concentration of anti-thrombin III (AT III) in the sera of aborted women (58.60 ± 13.87 ng/ml), compared with healthy women (53.39 ± 11.09 ng/ml) , in addition the levels of CD56/ NCAM1 and IL-17A were higher in aborted women (129.15±11.60 pg/ml; 52.65±4.83 pg/ml) compared with a healthy subjects (92.42±8.32 pg/ml; 23.09±4.90 pg/ml). The ROC (Receiver Operating Characteristic) curve was employed to evaluate the prevalence of IgM for the parameters of APS, where was good to excellent in the degree of accuracy for all IgM in relying on the area under the curve (AUC). On the other hand, the sensitivity of aCL IgM and aPL IgM were 89.09% (95% CI 77.8-95.9) and 87.27% (95% CI 75.5-94.7) respectively , whereas the sensitivity of aβ2GPI IgM was 74.55% (95% CI 61.0-85.3), so it was the lowest among them.

***Keywords**: Recurrent miscarriages ; antiphospholipid syndrome; aCL; aβ2GPI; aPL.*

Introduction

According to the European Society of Human Reproduction and Embryology (ESHRE), recurrent miscarriages (RM) or recurrent pregnancy loss (RPL) was defined as the wastage of two or more gestations before the twenty-four weeks of pregnancy (**ESHRE, 2017**), where, happen clinically in 2-5% of the women at reproductive aged and there are many factors that implicated in RM like genetic, endocrinologist, anatomic, immunologic factors, and others (**Kutteh, 2014**). Antiphospholipid syndrome (APS) is associated with many gestation complications such as abortion, fetal death, pre-eclampsia, intrauterine growth retardation, thrombosis, and premature birth, as well as most of RM in the presence of antiphospholipid autoantibodies (aPL) which have a harmful influence on trophoblastic layer of the placenta, where those antibodies directly attack cardiolipin, β2GPI, and the other types of phospholipids (**Sanmarco, 2009; Ziakas, et al., 2010**).

However, the conclusion by **Opatrny, and others (2006)** pointed to the magnitude of a relationship between aPL and RM differs according to the kind of aPL, where certain studies showed an association between autoantibodies (aβ2GPI and aCL) and women patients with history of RM (**Alijotas-Reig, et al., 2010**; **Chauleur, et al., 2010**) .

Antithrombin is a glycoprotein that playing an essential role in regulating coagulation, works on inhibition activated coagulation factors, IIa, IXa, Xa, XIa, and XIIa, as well as act as anti-inflammatory within vascular endothelial (**Maclean and Tait, 2007**). Therefore, the acquired or inherited thrombophilia working on increasing thrombosis and may be reducing the placental perfusion, hence causing RM (**Raziel, et al., 2001** and **Mekaj, et al., 2015**).

There are two types of natural killer cells (NK cells), peripheral blood NK cells that form 90% in peripheral blood, expressing $CD56^{dim}$ $CD16^{+}$ on their surface and have cytotoxic activity; and the other type is uterine NK cells, represent 90% in uterine, expressing $CD56^{bright}$ $CD16^{-}$ on their surface and produce certain cytokines (IFN- γ, TNF- β, IL-10, and GM-CSF). Those cells have a function in female reproductive performance (**Sacks, 2014**), and may be associated with implantation failures, RM due to their cytotoxicity (**Kwak-Kim and Gilman-Sachs, 2008**).

Many reports suggested that Th17 cells are present in both normal pregnancy and recurrent miscarriages. In normal pregnancy, IL-17A cytokine produced by $CD4^{+}$ Th17 cells that are found in the peripheral blood and decidua (**Nakashima, et al., 2010; Ito, et al., 2010**).The ratio of $CD4^{+}$ Th17 cells in the decidua is higher than that in the peripheral blood in women during the first trimester of pregnancy (**Santner-Nanan, et al., 2009**). Furthermore, in vitro studies, human IL-17 can rise the invasive ability of JEG-3 cells (The trophoblastic-like human choriocarcinoma cell) and elevate progesterone secretion (**Pongcharoen, et al., 2006**; **Pongcharoenand and Supalap, 2009**), these evidences indicated that Th17 cells may be very important and beneficial in pregnancy fixation. Therefore , the aim of current study was to determine the relationship between certain immunological biomarkers and women with a history of recurrent miscarriages at first trimester of pregnancy .

Materials and Methods

1.Patients and control groups:

This work was done on fifty-five women suffering from recurrent miscarriages in the first trimester of pregnancy were selected from maternity and pediatric hospital / emergency division in the Hilla city, versus thirty-three women from consulting division as a control group, where their ages ranged between eighteen and thirty-five years.

2.Specimens of blood:

Blood was collected from all individuals via taking 5ml from a vein using disposable syringes, then was placed in a disposable tube at room temperature until clotting. The serum was separated by the centrifuge at 3000 rpm for fifteen minutes, after that the serum was carefully distributed to Eppendorf tubes and stored in a deep freeze until used (**Barbara and Anna, 2012**).

3.Immunological assays:

All parameters in existing study were evaluated by ELISA kits, IgM aCL and aPL were estimated according to the manual procedure of Aeskulisa-Company (German), while, IgM aβ2GPI, IL-17A, CD56/ NCAM1, and AT III were evaluated by using specialized kits provided by Cusabio- Company (China).

4.Statistical Analysis:

According to the version 24 SPSS (Statistical Package for Social Sciences), and MedCalc statistical software version (17.9.7) results were analyzed. Also data were expressed, mean, standard deviation (S.D), independent- samples T test, Chi square (x^2) test, Odds ratio, ROC curve, area under the ROC curve, sensitivity, and specificity.

Results

As mention in the tables (1) and (2), the index value of aCL IgM showed a significant increase in aborted women than that in control group ($x^2 = 5.215$, $P = 0.022$), also the OR was 5.29 (1.12-25.02) with a significant difference ($P = 0.0355$) in aborted women versus healthy women's group. Furthermore, the index value of aβ2GPI IgM revealed a significant increase ($x^2 = 40.776$, $P< 0.001$) in aborted women, whereas the OR was 50.08 (10.53-238.16) with a significant difference $P< 0.0001$. The aPL screen IgM index value was shown a significant increase in aborted women ($x^2 = 7.522$, $P = 0.006$), while the value of the OR was 6.93 (1.49-32.35), with a significant difference ($P = 0.0137$).

Table (1): The relationship between patients and control groups, according to the markers of APS .

Parameters		Aborted women	Control Group	Total	X2 Value	P. Value
aCL IgM index value	Positive count (%)	14(87.5%)	2(12.5%)	16(100.0%)	5.215	0.022
	% of Total	15.9%	2.3%	18.2%		
	Negative count (%)	41(56.9%)	31(43.1%)	72(100.0%)		
	% of Total	46.6%	35.2%	81.8%		
aβ2GPI IgM index value	Positive count (%)	42(95.5%)	2(4.5%)	44(100.0%)	40.776	< 0.001
	% of Total	47.7%	2.3%	50.0%		
	Negative count (%)	13(29.5%)	31(70.5%)	44(100.0%)		
	% of Total	14.8%	35.2%	50.0%		
aPL screen IgM index value	Positive count (%)	17(89.5%)	2(10.5%)	19(100.0%)	7.522	0.006
	% of Total	19.3%	2.3%	21.6%		
	Negative count (%)	38(55.1%)	31(44.9%)	69(100.0%)		
	% of Total	43.2%	35.2%	78.4%		

* Significant difference at P≤0.05

Table (2): The Odds ratio for biomarkers in patients with APS .

Parameters	OR (95% CI)	P. Value
aCL IgM index value	5.29 (1.12-25.02)	0.0355
aβ2GPI IgM index value	50.08 (10.53-238.16)	< 0.0001
aPL screen IgM index value	6.93 (1.49-32.35)	0.0137

CI: Confidence Interval

*P≤0.05

The data pointed out that there was non-significant difference (P = 0.070) in the concentration of AT III in the sera of aborted women (58.60 ± 13.87 ng/ml) when compared with control group (53.39 ± 11.09 ng/ml) as demonstrate in table (3).

The concentration of CD56/ NCAM1 in serum of aborted women was 129.15±11.60 pg/ml, while it was 92.42±8.32 pg/ml in non-aborted women . Furthermore, the mean concentrations of IL-17A in the sera of aborted women were reached 52.65±4.83 pg/ml, but it was 23.09±4.90 pg/ml in non-aborted women, where the data demonstrated that all aborted women have highly significant differences (P< 0.001) for both variables, as shown in Table (3).

Table (3): The concentrations of AT III, CD56/ NCAM1, and IL-17A in aborted women and control groups .

Parameters	Aborted women Mean ± S.D.	Control Group Mean ± S.D.	P. Value
AT III ng/ml	58.60 ± 13.87	53.39 ± 11.09	0.070
CD56/ NCAM1 pg/ml	129.15±11.60	92.42±8.32	< 0.001
IL-17A pg/ml	52.65±4.83	23.09±4.90	< 0.001

* Significant difference at P≤0.05

The results of this study showed the biomarker aCL IgM was more sensitive than aPL screen IgM, which reached [89.09 (77.8-95.9), AUC=0.81(0.71-0.88)] and [87.27(75.5-94.7), AUC = 0.92 (0.84-0.96)] respectively, but the specificity of the aCL IgM was lower than that of the aPL screen IgM which reached 63.64 (45.1-79.6) and 84.85 (68.1-94.9) consecutively. Generally, both of two variables have shown a higher sensitivity than aβ2GPI IgM , as shown in table (4) and figure (1).

Table (4): The sensitivity and specificity for biomarkers among patients with APS .

Parameters	AUC (95% CI)	P. Value	Sensitivity (95% CI)	Specificity (95% CI)
aCL IgM	0.81(0.71-0.88)	< 0.001	89.09 (77.8-95.9)	63.64 (45.1-79.6)
aβ2GPI IgM	0.88 (0.80-0.94)	< 0.001	74.55 (61.0-85.3)	93.94 (79.8-99.3)
aPL screen IgM	0.92(0.84-0.96)	< 0.001	87.27 (75.5-94.7)	84.85 (68.1-94.9)

CI: Confidence Interval

* Significant difference at P≤0.05

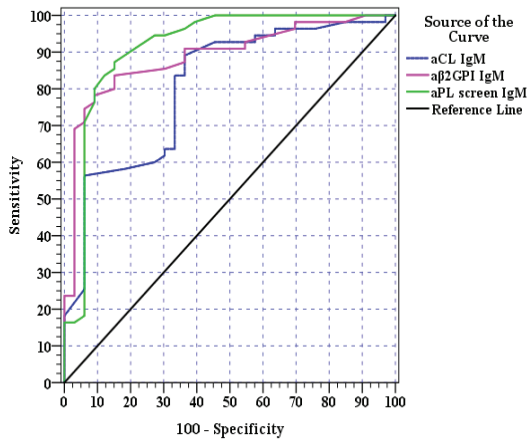

Figure (1): The sensitivity and specificity for IgM among three individuals of APS markers by ROC curve analysis.

Discussion

In this study there was a significant difference between aborted women and control groups , in which the total prevalence of aCL IgM was 18.2% (16/88) and 81.8% (71/88) in the aborted women and control groups respectively. The higher levels of these autoantibodies may be related to acute infection in patients. These results consisted with the data of **Spegiorin, et al., (2010)**, that found the prevalence of aCL IgM was 41.1%, where they pointed these antibodies are associated with a state of coagulability, and thus increased the risk of thromboembolic events. Furthermore, **Al Abri et al., (2000)** mention that 78% of all the miscarriages occur during the first trimester of pregnancy, where both aCL IgG and IgM are present, while in the presence of aCL IgM alone, more miscarriages will be occurring in the second trimester of pregnancy. Moreover, 3.39% (2/59) of aCL IgM was diagnosed in pregnant women at the first trimester of gestation has a past history of RM, in which the production of these antibodies may related to the phospholipids molecules that found in inner and outer part of the cell surface, also in the intracellular organelles, therefore any bacterial or viral infections lead to injury these membranes ,thus induced cells to release the cytokines and chemokines that triggers antibodies production (**Risan, 2011**). Also **Hessan(2016)** revealed that 13.34 % (4/30) of aCL IgM in aborted women with RM were more two times than aborted women without a history of RM. The aCL IgM was showing a significant difference in the OR (5.29 %), these results were in line with the study of **Hasan et al., (2010)**, on pregnant women with a past history of RM, where OR was 16.2% in comparison to the pregnant women without a past history of RM. Whereas, the OR was 2.8% and non- significant patients with a history of three or more consecutive miscarriages in the 1st trimester of pregnancy (**Žigon, et al. 2015)**.

aβ2GPI IgM antibodies were shown a total prevalence 50% (44/88) for both cases (positive &negative) with a significant difference ($p< 0.001$). In these cases, the acute infection may be contributed an increase in aβ2GPI IgM levels, hence leading to the emergence of these significant differences. This corresponds with the study of **Proietta, et al., (2014)** whom showed aβ2GPI IgM antibodies in 60 % of women with RM of the first trimester. Moreover, studies conducted by **Song et al., (2017)** demonstrated that the prevalence of aβ2GPI IgM was 91.66% (11/12) in women which suffering from RM, thus they concluded aβ2GPI IgM was the predominant form of the antibody in the patients with RM and APS. These results disagree with the study of **Zammiti, et al., (2006)**, where they pointed out that no significant difference between the positive sera of aβ2GPI IgM, which reached 62.5% and 37.5% in patients and control group respectively , this may be due to the heterogeneity of study samples. In current study the OR of aβ2GPI IgM was 50.08%, this came in line with study of **Zammiti, et al., (2006)** that recorded OR was 1.70%.

For screening the presence other forms of phospholipids such as phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylcholine, sphingomyelin as well as aCL, the aPL screen kit was used. The current study revealed that aPL IgM has a total positive prevalence in aborted women was 21.6%(19/88), while it reached 78.4%(69/88) in healthy women at significant difference ($P =0.006$). The acute infection may be elevating the index value of aPL IgM in all pathological cases. These results were consistent with the study of **Baleva, et al., (2014)** on 74 women with two or more RM at the first trimester of gestation, they noted that there was a significant increase in the prevalence of IgM phosphatidylethanolamine (aPE) in patients compared with control group ,in addition , they indicated that these antibodies may be involve in the pathogenesis of APS via inducing changes in the clotting system, and activating cells like monocytes and endothelial cells, in addition to platelets, hence the production of tissue factor or thromboxane which has prothrombotic properties. **Sugi, et al., (2004)** mention in their study the total positive prevalence of the aPE IgM was 12.4% in patient's women with recurrent miscarriages which was highly significant than that in healthy women, so they suggested the aPE may be a risk factor in patients with RM. Furthermore, the total positive prevalence of the phosphatidylserine (aPS) IgM was 27.8% (73/263), while aPE IgM was 11.8% (31/263) in women patients with RPL (**Umehara and Tanaka, 2012**).

Anti-thrombin III (AT III) deficiency is a rare occurrence where it was found in about three percent of patients with thrombosis. Fact, women which

having decreases of AT III more at risk of thrombosis from the beginning of pregnancy (**Kupferminc, 2003**; **Pabinger, et al., 2005**). The current study illustrated that there was non-significant difference (P = 0.070) in mean concentrations of AT III between aborted women and control group . These data was consistent with the majority of other studies like **Rey et al.,(2003**), which demonstrate the deficiency in AT III were not significantly associated with pregnancy loss, also **Lee, et al., (2016)** shown the ratio was 1.1 (2/178), among Korean women with RM suffering from deficiency of AT III without significant differences, whereas it was within the normal range (80-120%) with ratio 104% in Caucasian's women with RM (**D'Uva, et al.,2005**). Furthermore, **Ogasawara, et al., (2001)** found that non-significant differences in AT III among 536 patients with a history of two or more miscarriages during the first trimester of gestation, although the prevalence of AT III was 1.1% (1/88) among women with RM, but without significant difference (**Couto, et al.,2005**).

Certain studies were based on calculating the absolute number of NK cells by flow cytometry, while the current study depended on estimate the level of CD56 (pg/ml) in sera of aborted women and healthy individuals, which revealed statistically significant differences that may be as a result of the complex mechanisms in the production of cytokines by the cells of innate immune system, especially IL-12, by macrophages and dendritic cells which led to induce killer cells to express CD56/ NCAM1 in abortive women. Overall, in a study of **Kwak, et al., (2000)**, on 33 women with RM, they were demonstrated that 33.3% of them have high levels of CD56+ NK cells compared to 83.3% in women with normal CD56+ NK cell levels, so treatment of those women by intravenous immunoglobulin G after post-conception improves reproductive outcome. In addition, **Moraru, et al., (2012)**, during their study on Spanish's women with a past history of RM, 64 of them were notified an increase in $CD56^+$ NK cells. Moreover, Both $CD56^+$ and $CD16^+$ $CD56^+$ NK cells were significantly elevate in patients with more than two RM compared with healthy individuals (**Gao and Wang, 2015**; **Ghafourian, et al., 2015**).

The current study revealed a significant increase in the concentration of IL-17A, in women's with a past history of RM compared with healthy individuals. These results were compatible with the results of **Darmochwal-Kolarz, et al., (2017)**, that shown a significant increase in the levels of IL-17A in thirty-four of patients compared with thirty-five of healthy women which reached 3.9 pg/ml and 2.4 pg/ml respectively. Also, **Martínez-García, et al., (2011)** illustrated that there was a significant increase in the level of this cytokine during the 1st trimester of conception from 14.61 pg/ml to 21.4 pg/ml in the 2nd trimester, and up to 31.78 pg/ml in the 3rd trimester of gestation in healthy pregnant women. Therefore, in their conclusions the level of IL-17 was similar in their range to that present at all healthy women, but its average began to increase may be as a result of the implantation process, fetal development, inflammation or as a part of cytokines action. Also, **Li, et al., (2017)** shown a significant increase in the concentrations of IL-17 (320.85 ±63.15 pg/ml) and IL-23 in women with RM versus 251.69± 51.72 pg/ml in healthy women.

The ROC curve was used to evaluate the performance of IgM for parameters of anti-phospholipid syndrome. **Pericleous, et al., (2016)** showed the sensitivity of aCL IgM was 26.1%, which is less than the sensitivity of aβ2GPI IgM that reached 33.3% [AUC 0.74, CI 95% (0.68–0.80); AUC 0.80, CI 95% (0.75–0.85) respectively]. **Heikal , et al., (2015)** mention the sensitivity of both aCL IgM and aβ2GPI IgM in one hundred and sixty patients with APS was 0.0% [AUC 0.595, CI 95% (0.417–0.773); AUC 0.653, CI 95% (0.451–0.854) successively,. As well as, the study of **Perches, et al., (2009)**, on women has recurrent miscarriages and APS showed the sensitivity of the aCL IgM antibodies was 92% [AUC 0.466, CL 95% (0.332-0.6)].

Conclusions

Anti-β2GPI IgM in patients has a total positive prevalence and OR more than other autoantibodies, meantime, the IgM aCL was more sensitively others, furthermore, women patients with RM shown an association with APS.

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Conflict of Interest: None to declare.

Ethical Clearance: "All experimental protocols were approved under the College of Science and all experiments were carried out in accordance with approved guidelines".

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