

A STUDY OF ANTIPHOSPHOLIPID ANTIBODIES IN WOMEN WITH HISTORY OF MISCARRIAGES AT ZAKHO DISTRICTS, KURDISTAN REGION, IRAQ

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ABSTRACT

Background and aims: Antiphospholipid antibodies are associated with miscarriage and abortion, and this association has been reported in different countries. The aim of this study is to assess the prevalence of antiphospholipid antibodies IgM and IgG among pregnant women with previous history of miscarriage in Zakho City, Kurdistan region, Iraq.

Methods: This cross-sectional study was conducted in Zakho city among women who had history of miscarriages from 1st April 2019 to 31st May 2021. A total of 460 women age ranged between 16-46 years (Average age 29.73 ± 5.93 STDEV) were recruited in the study. Enzyme-linked immunosorbent assay (ELISA) was used to measure antiphospholipid IgM and IgG antibodies. The relationship between age and level of antiphospholipid antibodies was performed using the Chi-Square test (Fisher Exact Test).

Results: The overall rate of antiphospholipid antibodies was 31 (6.74%) for IgM, 16 (3.48%) for IgG and 9 (1.96%) was seropositive for both IgM and IgG antibodies. The study found that the highest IgM was 18 (11.25%) and IgG 10 (6.2%) seropositivity among age group >30 years old. There was a significant association between age groups and IgM seropositivity ($P=0.02$; $OR=0.4$; 95% CI 0.2-0.9) but statistically not significant between age groups and IgG seropositivity ($P=0.79$; $OR=0.9$; 95% CI 0.3-2). It was also found no significant differences between age groups and both IgM and IgG seropositivity ($P=0.28$; $OR=0.4$; 95% CI 0.1-1).

Conclusion: The prevalence of antiphospholipid antibodies among aborted women was relatively low compared to other study. There was a positively association between IgM seropositivity and age group. Therefore, it is essential to conduct APA screening for women who have recurrent pregnancy loss and investigate the therapeutic effects of heparin and aspirin; this approach might improve fetal survival by starting anticoagulant treatment early.

KEYWORDS: Miscarriage, antiphospholipid antibodies, IgM, IgG, Zakho city, Iraq

INTRODUCTION

Antiphospholipid syndrome (APS), an autoimmune condition was marked by the presence of antiphospholipid antibodies, may be identified via immunoassays or functional coagulation assays. Antiphospholipid antibodies (APA), which include anticardiolipin antibodies (aCL) and lupus anticoagulant, are a diverse collection of autoantibodies directed against negatively charged phospholipids (LAC) (Hanly, 2003). APA are frequently associated with blood clotting disorder including deep vein thrombosis, strokes, and heart attack and in pregnancy can lead to recurrent fetal loss, miscarriage, intrauterine growth restriction, stillbirth and pre-eclampsia (Mialdea et al., 2009).

These antibodies are considered to be etiologically important in the syndrome, although the precise pathogenic mechanisms are still being determined. Recurrent miscarriage, defined as three or more consecutive pregnancy losses before the 20th week of gestation, affects approximately 1-2% of women of reproductive age, and aPL are found in up to 15-20% of these cases (Ariela and Paolo, 2022).

According to evidence from a thorough study conducted in 2019, APS affects 1 in every 2000 people, making it an uncommon disease with a low frequency in the population (Duarte-García et al., 2019). APS can affect people of any age, from infants to the elderly, however the majority of patients are identified between the ages of 20 and 50 (Duarte-García et al., 2019). About 15% of recurrent miscarriages are caused by APS,

with 50% of those fetal losses happening in the first trimester (Di Prima et al., 2011). According to a study done in Iraq, APS is a significant factor in recurring mid-trimester abortions among women (Samarrai et al., 2012). However, the cause of APS-related miscarriages is still unknown; some experts think that blood clots detected in APS can stop the placenta's blood flow (Hughes and Khamashta, 1994).

The diagnosis of aPL is based on the detection of these antibodies in the blood using specific laboratory tests, such as the lupus anticoagulant, anticardiolipin antibodies, and anti-beta-2 glycoprotein I antibodies. However, the interpretation of these tests can be challenging, as aPL can be transiently present in some individuals and their clinical significance is not fully understood.

Although recurrent miscarriage has many causes such as infections, hormonal abnormalities, maternal age, incompetent cervix, exposure to radiation or toxic agents. Several risk factors associated with recurrent pregnancy loss and miscarriage have been extensively studied in our region (Naqid et al., 2020a, Naqid et al., 2020b, Naqid et al., 2019, Haydar and Naqid, 2022). It was determined that our community's abortion and stillbirth rates were not primarily caused by infections or anticardiolipin antibodies. Although the antiphospholipid syndrome continues to be the main reason for miscarriages, the exact frequency in our area is still unknown. Therefore, the aim in this study is to evaluate the prevalence of antiphospholipid antibodies in pregnant women with previous history of miscarriage in Zakho City, Kurdistan region, Iraq.

MATERIALS AND METHODS

Study design and period

This study was conducted as a cross-sectional and performed in Zakho city, Kurdistan Region, Iraq from 1st of April 2019 to 31st of May 2021. Overall, 460 subjects enrolled in this study and aged between 18-46 years old and assessed to detect specific antiphospholipid IgM and IgG antibodies. Blood samples were collected randomly from subjects with a previous history of unknown causes of abortion visited the outpatient's clinic centres. The inclusion criteria for the study included women who had a history

of having more than two recently abortions, were aged over 18 years, and agreed to participate in the study.

Blood collection and ELISA:

Five millilitres of blood were taken from subjects without anticoagulants using disposable syringe and centrifuged at 3500 rpm for 10 minutes to obtain serum. The enzyme-linked immunosorbent assay (ELISA) was used to assess phospholipid screen-GM test (aPL IgM and IgG antibodies) following the manufacturer's instructions (AESKU, Wendelsheim, Germany). Briefly, the serum was diluted to 1:100 by buffer reagent. Calibrators, controls and diluted serum samples (100µl) were added into the designed wells coated with specific antigen; the plate was incubated at 20 -35 °C for 30 minutes. After incubation, the plate was washed 3X with 300 µL washing buffer (1:50), 100µL of conjugate enzyme horseradish peroxidase (HRP) was added to each well. The plate was then incubated at 20 -35°C for 30min and subsequently washed 3 times with washing buffer (300 µL). Finally, 100µl of substrate TMB was added to each well, the plate was incubated at 20 -32°C for 30 minutes. Read absorbance of the plate at 450 nm after added stop solution with 100µL of hydrochloric acid. The result was then interpreted and considered positive if >18 U/mL and negative if <12 U/mL.

Data Analysis

The analysis of the study's findings was done with GraphPad Prism, Version 8. Simple frequency, percentages, and ranges were used as appropriate to express the data. The relationship between age and level of antiphospholipid antibodies was performed by the Chi-Square (Fisher Exact Test). The findings were considered significant if *P* value less than 0.05.

RESULTS

A total of 460 subjects with a previous history of miscarriage were recruited in the present study (Table 1). The average age of subjects was 29.73-Year-old (\pm 5.93 STDEV, range: 16-46). The overall prevalence of antiphospholipid antibodies was 31 (6.74%) for IgM antibody (>18 GPL IU/mL) and 16 (3.48%) for IgG antibody (>18 GPL IU/mL). Additionally, it was reported that only 9 (1.96%) was seropositive for both IgM and IgG antibodies (Table 1).

Table (1): Prevalence of Antiphospholipid Antibodies among women with History of Miscarriage

Antiphospholipid Antibodies	Total number	Positive (No.%)	Negative (No.%)
aPL IgM	460	31 (6.74)	429 (93.26)
aPL IgG	460	16 (3.48)	444 (96.52)
Both aPL IgM and IgG	460	9 (1.96)	451(98.04)

In the present study, it was also found that the highest IgM seropositivity 18 (11.25%) among age group >30 years old when compared to age≤30, 13 (4.33%) (Table 2). We found a significant correlation between IgM seropositivity and age groups and ($P=0.02$; OR=0.4; 95% CI 0.2-0.9) (Table 2). Additionally, the high seropositivity of IgG

antibody 10 (6.2%) was reported among age of >30 years old when compared to age ≤30, 6 (2%), but there was no significant correlation between age groups and IgG ($P=0.79$; OR=0.9; 95% CI 0.3-2) (Table 2). It was also reported, there was no correlation between age groups and both IgM and IgG seropositivity ($P=0.28$; OR=0.4; 95% CI 0.1-1) (Table 2).

Table (2): Seropositivity of Antiphospholipid Antibodies among Different Age Group of Women with a History of Miscarriage

Antiphospholipid Antibodies	Age Group (Year)		Statistical Analysis		
	> 30 (n= 160)	≤30 (n= 300)	OR	95% CI	*P value
	No. %	No. %			
aPL IgM	18 (11.25)	13 (4.33)	0.4	0.2-0.9	0.02
aPL IgG	10 (6.25)	6 (2)	0.9	0.3-2	0.79
Both aPL IgM and IgG	5 (3.13)	4 (1.33)	0.4	0.1-1	0.28

* P value is determined using Fishers exact test; CI; Confidence interval; OR; Odd Ratio, P value < 0.05 was considered significant.

DISCUSSION

Phospholipid molecules are the most abundant membrane lipids. The antibodies that are developed by the immune system are directed against proteins attached to these phospholipids molecules. These antiphospholipid antibodies come in different types which can lead to the development of blood clot in the veins or arteries, as well as repeated pregnancy losses (Rand, 2002, Ruiz-Irastorza et al., 2010).

The antiphospholipid antibody syndrome (APS) is an autoimmune condition which is commonly related with thrombosis and lead to several complications during pregnancy include preeclampsia, placental insufficiency, and fetal growth restriction and it is characterized by the presence of antiphospholipid antibodies (aPL) (Lockshin et al., 2000, Levine et al., 2002).

Anticardiolipin antibodies (aCL), Lupus anticoagulant (LA), and anti-beta-2-glycoprotein 1 antibodies are the most common types of aPL to be concerned during pregnancy (Xu et al., 2019). Thus, the current study aimed to estimate the prevalence of antiphospholipid antibodies among pregnant women with previous history of miscarriage/ abortion

In the present study, the rate of antiphospholipid antibodies among studied subjects with history of repeated abortion was 6.74% and 3.48% for IgM and IgG antibodies respectively. Furthermore, it has been found that only 1.96% was seropositive for both IgM and IgG antibodies. These results in agreement with the results of other studies and they have demonstrated that the rate of aPL was 1-5% among general population, while the rate of APS is between 4-5% per 100,000 individuals, present more prevalent among women

(Khamashta et al., 2016, Cervera, 2017). Another study reported that the prevalence of APAs among 1325 women with a previous history of miscarriage, 676 women who had repeated implantation failure, 789 women who had recurrent pregnancy loss and 205 fertile women as a control [19]. This study was found that more than one positive APA in 8% of women with unexplained infertility and 9% of women with recurrent implantation failure, compared to 1.5 % of fertile negative control women and 11% of positive control women suffering recurrent abortion (Sauer et al., 2010).

A study conducted in Iraq, reported that the prevalence of the antiphospholipid antibodies in Iraqi women were aged between 19-45 yrs with recurrent mid-trimester fetal loss (Al Samarrai et al., 2012) . This study had demonstrated that among two hundred enrolled women, only 68 patients (34%) demonstrated one or both of APA, 41(20.5%) had anticardiolipin antibodies, 15 (7.5%) had lupus anticoagulant and only 12 (6.0%) had both antibodies, therefore the overall prevalence of anticardiolipin antibodies were 53 cases, 35 of them with IgG aCL, while 15 with IgM aCL and only 3 with both types of aCLs and also they found a significant higher prevalence between APA with medical history of thrombosis while the association between APA and family history needs further observation and it would be very interesting if confirmed. Furthermore, they found that there was no significant correlation between age, APA and control group. Their findings are in agreement with our study as we demonstrated there were no significant correlation related to the age group and IgG ($P = 0.79$) and also with both IgM and IgG seropositivity ($P = 0.28$), however, we found a positive association between age groups and IgM seropositivity ($P=0.02$).

Many hypotheses are proposed to explain APAs mechanism; antiphospholipid antibodies stimulate the production of thromboxane A2 and tissue factor, which results in the induction of a procoagulant state by activating endothelial cells, platelets, and monocytes. Additionally, complement system activation could close the loop 7, causing thrombosis and fetal death was confirmed by a study showing that C3b and C4d fragments are deposited in the placenta of patients with antiphospholipid syndrome. Furthermore, antiphospholipid antibodies interaction with important proteins involved in the blood clotting regulation, such as proteins C and S, prothrombin, factor X, and plasmin can

interfere with the inactivation of coagulation factors and impede fibrinolysis. Interfering with annexin A5, a natural anticoagulant bind to exposed phosphatidylserine in trophoblasts syncytial formation, which may promote immediate impact on the structure of placenta, resulting formation of placental thrombosis and fetal death (Di Prima et al., 2011)

A study conducted on 83 Sudanese pregnant women with a history of recurrent abortion and miscarriage showed a 6% prevalence of anticardiolipin IgG antibodies positivity which is in the agreement with other studies who found that 19.2% of cases were positive anticardiolipin IgG antibodies (Abdulaziz et al., 2016). Furthermore, Sudanese group found that the highest prevalence of the recurrent miscarriage was 27.7% in women with age group 31-35 years which is in agreement with work done by (Dmitrovic et al., 2009) who demonstrated that 28% of recurrent miscarriage was found in women with age group between 31-35 years. On the other hand, they noticed that the first and second trimesters have the highest rate of recurrent pregnancy loss (45%) which is similar with other study (Clifford et al., 1997). In another work performed in Kurdistan region of Iraq (Naqid et al., 2020b), it was reported that the rate of anticardiolipin antibodies in two cities Dohuk and Zakho among 1230 women aged between (18-46 yrs.) with recurrent abortion was 6% for aCL IgM, and 4.6% for aCL IgG and for both aCL IgM and IgG seropositivity was 1.3%. This group also demonstrated that the highest positivity rate of aCL antibody were found among women older than 30 yrs.

In conclusion, the prevalence of antiphospholipid antibodies (APA) among women who experienced pregnancy loss in the present study was relatively low compared to other studies. Furthermore, a positive association was observed between IgM seropositivity and age group. Therefore, it is imperative to prioritize APA screening for women with recurrent pregnancy loss and to explore the therapeutic effects of heparin and aspirin. Such an approach may significantly enhance fetal survival by initiating early anticoagulant treatment.

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