

Study on Anticardiolipin Antibodies in Women With Recurrent Abortion in Duhok Province, Kurdistan Region, Iraq

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Abstract- Recurrent abortion is a worldwide issue. Anticardiolipin antibodies (ACA) are found to be among the most important factors related to recurrent spontaneous early pregnancy loss. This study aimed to investigate the prevalence of anticardiolipin IgM and IgG antibodies in women with recurrent abortion in Kurdistan Region of Iraq. The present study was conducted in Duhok and Zakho cities for the period from April 2014 to May 2019. A total of 1230 women aged between 18 to 46-year-old were included in this study. ELISA was used for the detection of anticardiolipin antibodies. Among the studied subjects, the prevalence of anticardiolipin antibodies was 74 (6.02%) for ACA IgM and 56 (4.6%) for ACA IgG. It was also observed that 16 (1.3%) subjects were positive for both ACA IgM and IgG antibodies. Additionally, the highest anticardiolipin antibody positivity rates were recorded in patients older than 30-year-old ($P<0.01$). The IgM ACA positivity was higher in Zakho city 45 (7.2%) when compared to Duhok city 29 (4.6%) ($P<0.04$). In conclusion, anticardiolipin antibodies can have a positive association among women with recurrent abortion. Therefore, it is suggested that women with recurrent abortion should be screened for anticardiolipin antibodies; this could increase fetal survival by initiating early anticoagulant therapy when other causes of abortion are excluded.

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Introduction

Recurrent abortion is a worldwide common problem influencing more than 500,000 women annually (1). Recurrent spontaneous abortions may occur due to immunological causes, hormonal, genetic, uterine factors, and infectious (2). Immunological factors are the most common causes of abortion, and amongst those, antiphospholipid antibody represents a major cause, especially in the first trimester of pregnancy (3).

Antibodies against cardiolipin belong to the group of antiphospholipid antibodies specific for negatively charged phospholipid. Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by the presence of antiphospholipid autoantibodies in association with a variety of health diseases including adverse pregnancy outcomes such as repeated abortion, fetal retardation placental insufficiency, and pregnancy-

induced hypertension (4). This syndrome is composed of a heterogeneous group of circulating antibodies against the different targets of antigens, most importantly, phospholipid containing structure or anionic phospholipids. The most frequently reported antiphospholipid antibody is the anticardiolipin antibody (5). The levels of anticardiolipin IgG and IgM antibodies are often high in people with abnormal blood clotting, autoimmune diseases such as systemic lupus erythematosus lupus (SLE) or recurrent miscarriage (6).

The exact mechanisms by which anticardiolipin antibodies to induce abortion are not yet fully established, but it appears to act on the placenta and its underlying decidual vessels. The most common causes of recurrent abortions could be genetic certain antiphospholipid antibodies, including ACA antibodies that interfere with fetal implantation, which might be associated with the pathological mechanism responsible for recurrent

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abortions (7).

Factors associated with recurrent abortions have been previously studied in our region (8,9). It was shown that infections were not major causes of abortion/stillbirth in the region. Therefore, this study aimed at evaluating the prevalence rate of anticardiolipin IgG and IgM among women who had a history of recurrent spontaneous abortion in Duhok Province, Kurdistan Region, Iraq.

Materials and Methods

Study design and sampling

This study was conducted in Duhok and Zakho cities, Kurdistan, Region, Iraq. Blood samples were taken from women with a history of abortion/stillbirth of unknown causes attending the Private Clinic and Laboratory in Duhok province. From April 2014 to May 2019, a total of 1230 participants aged between 18-46 were recruited and assessed to detect specific anticardiolipin antibodies.

Anticardiolipin antibodies assay

A sample of 5 ml of blood was collected aseptically from each patient without anticoagulants and centrifuged for 10 minutes at 3000 rpm. Anticardiolipin antibodies (IgG and IgM) were detected by enzyme-linked immunosorbent assay (ELISA) following the manufactures instructions (DIESSE Diagnostic Sense S.P.A, Monteriggioni, Italy). Briefly, the antigen was bound to the solid phase. The specific antibodies were then bound to the antigen through incubation with diluted human serum. After washing to remove non-specific proteins, incubation was performed with the conjugate, composed of antihuman immunoglobulins antibodies conjugated to horseradish peroxidase. The unbound conjugate was then removed, and the peroxidase substrate was added. The color which develops was proportional to the concentration of specific antibodies in the serum

sample. The result was considered negative if <12 GPL IU/mL, and positive if >18 GPL IU/mL.

Ethics consideration

The study proposal was approved by the ethics committee of the College of the Medicine/University of Zakho. Informed written consent was obtained from all the participants.

Statistical analysis

The result of this study was analyzed using the GraphPad Prism software package, version 8. The results were expressed as the mean±standard deviation or as simple percentages and ranges as appropriate. Comparisons were made using the *Chi*-Square and Fisher Exact Test. The results were considered significant if $P \leq 0.05$.

Results

During the study period, a total of 1230 women with a history of consecutive unexplained fetal loss/abortion were enrolled in this study (Table 1). Of these 1230 subjects, the mean age was 30.4±10.6-year-old. The prevalence of anticardiolipin antibodies among the studied subjects was 74 (6.02%) for ACA IgM (>18 MPL/ML) with a mean level of IgM anticardiolipin antibodies (26.14±17.03). On the other hand, the prevalence of anticardiolipin IgG antibodies (>18 GPL/ML) was 56 (4.6%) with a mean anticardiolipin IgG level (29.5±23.2) (Table 1). There was no significant difference between the number of patients with a high level of IgG and IgM ACA when analyzed using the Fisher exact test ($P=0.125$). It was also found that 16 (1.3%) was positive for both ACA IgG and IgG antibodies with a mean concentration (23.8±14.8) (Table 1).

Table 1. Prevalence and levels of Anticardiolipin (ACL) antibodies in women with a history of abortion from Kurdistan Region/Iraq

Anticardiolipin Antibodies (ACA)	Total Number	Positive (%) *Mean±SD	Negative (%) *Mean±SD	95% CI
ACA IgM	1230	74 (6.01) 26.14±17.03	1156 (93.9) 5.3±2.8	6.2-6.9
ACA IgG	1230	56 (4.6) 29.5±23.2	1174 (95.4) 4.4±2.3	5.1-5.9
Both ACA IgM and IgG	1230	16 (1.3%) 23.8±14.8	1214 (98.7) 5.8±6.5	5.7-6.4

*Mean: Levels of ACA antibodies, SD: Standard deviation of the mean
CI: Confidence Interval

We also found that the highest IgM ACA positivity 32 (6.8%) was in the age group of >30-year-old, and 42

(5.6%) for the age group of ≤ 30 ($P=0.39$) (Table 2). Additionally, the high positivity of IgG ACA 38 (8.1%) was identified in the age group of >30 years old with statistically significant differences ($P<0.01$) compared to other groups (Table 2). A high number of IgM ACA

positive cases were found in Zakho city 45 (7.2%) compared to Duhok city 29 (4.6%) (Fisher exact test, $P<0.04$). No significant differences were found between Zako city 30 (5%) and Duhok city 26 (4.1%) in terms of the IgG ACA antibody ($P=0.49$).

Table 2. Seropositivity of Anticardiolipin antibodies in women with a history of abortion from Kurdistan Region / Iraq

Variable	Total Number	Anticardiolipin Antibodies			*P	
		IgM Positive No. (%)	P	IgG Positive No. (%)		
Age group						
	≤ 30	756	42 (5.6)	0.39	18 (2.4)	0.01
	>30	474	32 (6.8)		38 (8.1)	
Region						
	Zakho	600	45 (7.2)	0.04	30 (5)	0.49
	Duhok	630	29 (4.6)		26 (4.1)	

*P were determined using Chi-Square (and Fisher Exact Test)
The results were considered positive if $P\leq 0.05$

Discussion

Recurrent abortion involves the loss of a pregnancy, usually within the first three months of conception in which many factors play a role such as genetic, hormonal disorders, maternal age, uterine factors, the infections, environmental and immunologic factors (3). Anticardiolipin antibodies are associated with placental vascular thrombosis, thrombocytopenia, intervillous fibrin deposition, and placental infarction, leading to the cause of fetal losses (10). Hence, the aim of the present study was to evaluate the prevalence of ACA IgG and IgM antibodies among women with a history of recurrent abortion in Kurdistan Region, Iraq.

Several studies showed that women with recurrent spontaneous abortion have a higher incidence of anticardiolipin antibodies (11,12). Another work reported that 20% of women with a history of recurrent consecutive pregnancy losses were CL positive (13). Furthermore, anticardiolipin antibodies positivity was previously reported in around 35% of patients with spontaneous abortion (14). In our study, ACA antibodies positivity was detected in 10.61% of women with a history of abortion. In another study from the Kurdistan region, it was shown that 31.7% of the women who experienced recurrent abortions of unknown causes attending the maternity hospital in Erbil City, Kurdistan region, showed positive anticardiolipin antibodies (15). In another study conducted in Iraq, anticardiolipin antibodies were detected in 17.6 % of women with recurrent spontaneous miscarriage; this rate is quite higher than the rate reported of the present study (16). In the current study, we found an overall prevalence rate of

anticardiolipin IgM and IgG antibodies of 6.01% and 4.6 %, respectively. Both ACA IgM and IgG antibodies positivity was detected in 1.3% of patients. Consistent with the current results, several previous studies showed that the prevalence rate of ACA IgG and IgM in the serum of women with a history of abortion was ranging from 5.1% -7.3%, respectively (17). In a study conducted in Sudan, the overall seropositivity of anticardiolipin antibodies among women with recurrent miscarriage was 6% (12). The results are also consistent with the previous report that showed that 7-25 % of recurrent pregnancy loss was due to positive anticardiolipin antibodies (18).

Variation in the frequencies of anticardiolipin antibodies in different reports may be due to a lack of the standardized assay for measuring the levels of antibodies, ethnic differences, and timing of fetal loss (19). Indeed, many different tests are available commercially, and reports from external quality control schemes show that these different assays make variable results. Most of these differences may also result from laboratory factors, including design of study assay, various pre-analytical variables, reference ranges, in addition to a very important factor, namely the selection of cut-off values for a positive result (20).

In the present study, there was no statistically significant difference in anticardiolipin IgM antibody levels between age groups. Significant differences were found in anticardiolipin IgG positivity among age groups ($P<0.01$). In our study, the highest rates of IgG and IgM anticardiolipin antibodies positivity were found in patients older than 30-year-old. The current result is similar to a recent study, which found that there were higher levels of antibody positivity in patients older than

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30-year-old (21). Additionally, in the present study, the ACA antibodies positivity was higher in Zakho city than that found in Duhok city. This discrepancy could be due to genetic, environmental factors, and pathological mechanisms. Further studies are needed to explore this.

To conclude, anticardiolipin antibodies could have a positive association among women with recurrent abortion. Additionally, the prevalence of anticardiolipin antibodies in the present study is higher in patients older than 30-year-old. It is therefore recommended that women with a previous history of unexplained fetal losses be screened for anticardiolipin antibodies. Further studies are needed to investigate the relationship between anticardiolipin positivity and pregnancy outcomes recruiting samples from all over Iraq.

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