

Antiphospholipid Antibodies in Iraqi Women with Recurrent Mid-Trimester Abortions

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ABSTRACT

Purpose: Antiphospholipid antibodies are often associated with recurrent pregnancy loss, and although many studies have addressed this association in Western countries, such studies are not so frequent from developing countries. The current study aims to determine the frequency of Antiphospholipid antibodies (Anticardiolipin antibodies and Lupus anticoagulant) among Iraqi women with recurrent mid-trimester abortions and to evaluate various tests used for their detection.

Materials and Methods: Two hundred women with recurrent mid-trimester abortions were randomly enrolled from a main referral center in Baghdad-Iraq. The enrollees had their IgG and IgM anticardiolipin antibodies assayed by ELISA, and Lupus anticoagulant by a combination of the following screening tests: Activated Partial Thromboplastine Time (APTT), and Partial Thromboplastine Time-LA (PTT-LA), Kaolin Clotting Time (KCT) and confirmation was made by Hexagonal phospholipid neutralization test.

Results: The women were aged between 19 and 45 years (median 30 years). Fifty three (26.5%) had one or both anticardiolipin antibodies present, while 27 (13.5%) were positive for lupus anticoagulant. The KCT and KCT index appeared to be the most sensitive tests, while the KCT index and APTT were the most specific for Lupus anticoagulant. Patients with antiphospholipid antibodies had higher rates of history of thrombosis, thrombocytopenia and family history of recurrent abortion ($P = 0.0009$, 0.0056 and 0.0003 respectively).

Conclusions: Antiphospholipid antibodies constitute an important cause of recurrent mid-trimester abortion in Iraqi women, with frequencies intermediate between Western and Indian reports. While thrombocytopenia and thrombosis are well documented associations of antiphospholipid antibodies, the significant association with family history of recurrent fetal loss is intriguing and requires further scrutiny.

Key words: Anticardiolipin antibodies, antiphospholipid antibodies, Iraq, lupus anticoagulant, recurrent abortion

INTRODUCTION

Antiphospholipid antibodies (APA) comprises a heterogeneous group of autoantibodies directed against negatively charged phospholipids and include lupus anticoagulant (LAC) and anticardiolipin antibodies (aCL).^[1] The importance of these

antibodies stems from their established association with thrombosis, thrombocytopenia and recurrent fetal loss.^[2] The first well documented association between antiphospholipid antibodies and recurrent fetal loss was reported in 1975 by Nilsson and coworkers.^[3] Thereafter, many reports documented the latter association and studied the prevalence, clinical and laboratory associations of the antiphospholipid antibodies in the general obstetric population and in those with recurrent fetal loss in western countries.^[4] However, such reports from developing and Asian countries were not as frequent, and more studies from the latter countries are important, particularly because interethnic differences (which maybe due to genetic and/or environmental factors) have been noted both

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in the frequency and the clinical complications of these antibodies.^[5]

The aim of this study, which is the first to be reported from Iraq, is to determine the prevalence of the antiphospholipid antibodies in a group of Iraqi women referred for the evaluation of recurrent mid-trimester abortion and to evaluate the various tests used for their detection.

MATERIALS AND METHODS

A total of 200 women with a history of spontaneous recurrent (3 or more) mid-trimester abortions, referred to the Central Health Laboratory-Baghdad were randomly enrolled. All the latter patients had a detailed clinical history taken and had their clinical records re-evaluated. The women were questioned particularly regarding the number and timing of their abortions, family history of recurrent abortions, history of thrombotic episodes, history of still births or intrauterine deaths. The inclusion criteria necessitated that the women were not pregnant, and were not on any hormonal therapy, contraceptive pills or anticoagulants. Furthermore, those with uterine abnormalities and diabetes mellitus were excluded.

Twenty healthy non-pregnant adult women, who had at least one successful pregnancy, had no miscarriage and were not on medications, were taken as a normal control group, their ages ranging from 18 to 45 years (mean 29.05 + 8.13 years).

Ten ml of venous blood were obtained from each patient and control by clean venipuncture, and divided between EDTA tubes (for blood counts), tubes containing 0.13M sodium citrate (9:1) (for coagulation studies) and plain tubes (for serological studies).

EDTA anticoagulated blood was used to perform full blood counts using MS9 electronic counter (MeletSchloesing-France). For coagulation studies, platelets poor plasma was prepared by double centrifugation at 3000 rpm for 20 minutes of citrated blood. The coagulation tests performed included: Prothrombin time using Calcium Thromboplastin (BioMerieux-France), activated partial thromboplastine time (APTT) by Cephalite (BioMerieux-France) and PTT-LA (an LAC sensitive APTT test) (Diagnostica Stago-France) according to the manufacturer's instructions, while kaolin clotting time (KCT) was performed by a method modified from Exner *et al* 1978.^[6] All APTTs or PTT-LAs that were significantly prolonged (> 5 seconds above the upper

limit) were screened for the presence of an anticoagulant by performing an APTT on a 50:50 mixture of patient and normal pooled plasma. While a KCT index was performed for samples with prolonged KCT using the formula:^[7]

$$\text{KCT index} = \frac{\text{KCT of (80\% normal plasma + 20\% patient plasma)}}{\text{KCT of 100\% normal plasma}}$$

In cases where there was failure of correction of prolonged APTT and/or PTT-LA or the KCT index was >1.2, a hexagonal phospholipid neutralization procedure (HPNP) using Staclot-LA Kit (Diagnostica-Stago, France) was performed. A Lupus Anticoagulant was considered present when one or more of the screening procedures were prolonged with failure of correction by normal plasma, confirmed by the Hexagonal phospholipid neutralization procedure.^[8]

Sera were used for quantitative determination of Anticardiolipin antibodies (aCL) of IgG and IgM types using an ELISA method (Sanofi Diagnostica Pasteur, France). The mean aCL IgG and IgM of the 20 healthy controls was determined, and for the purposes of this study the cut off points for positive aCL antibodies were taken as the mean + 3 SD and was 13.72 GPL units for IgG and 14.2 MPL units for IgM. Further tests included Anti-dsDNA (by ELISA using Melisa Cambridge Life Science, England), Antinuclear Factor (ANF) (by fluorescent technique using Sanofi Diagnostica Pasteur, France), and VDRL using Biokit RPR (Biokits Barcelona, Spain). All above tests were performed according to the manufacturers' instructions.

The study was approved by the appropriate ethical committee and informed consent was obtained from all enrollees. Statistical evaluation utilized the Chi-squared (with or without Yates correction as appropriate) and Mann Whitney U test as applicable. $P < 0.05$ was considered significant.

RESULTS

The age of the patients ranged between 19-45 years with a median of 30 years, and had a median of 3 mid-trimester abortions, with a range of 3-14. Among the enrolled patients, 68 cases (34%) demonstrated one or both of antiphospholipid antibodies (APA) (aCL and/or LAC), 41 (20.5%) had aCL and 15 (7.5%) had LAC only, while 12 (6.0%) had both antibodies. Therefore the overall frequency of aCL was 53/200 (26.5%) and that of LAC 27/200 (13.5%). The main hematological and clinical parameters in those with or without APA are outlined in Table 1.

Among the 27 patients with HPNP confirmed Lupus anticoagulant, the APTT was prolonged in 16 (59.2%), PTT-LA in 22/27 (81.4%) while a prolonged KCT and a KCT index of >1.2 was documented in all the 27 cases. Based on the above findings the sensitivity, specificity and the predictive values of the different coagulation tests for LAC were calculated [Table 2]. As demonstrated in the latter table the KCT and KCT index had the highest sensitivity and the KCT index and the APTT had highest specificity for LAC.

Anticardiolipin antibodies were positive in 53 cases, including 35 with IgG aCL, 15 with IgM aCL and 3 with both types of aCLs. On the other hand, VDRL was positive in 28 of the 53 aCL positive cases.

Table 1: Some hematological and clinical parameters in APA positive and negative women with recurrent fetal loss

Parameter	APA positive (no. 68)	APA negative (no. 132)
	Mean±SD	
APTT (sec)	40.6±5.9	36.4±3.3
KCT (sec)	124.8±35.1	97.9±16.9
PTT-LA (sec)	43.7±9.0	36.4±2.8
Platelets count (×10 ⁹ /L)	205±58.2	246±71.7
Hemoglobin (g/L)	120±14	125±12
Leucocyte count (×10 ⁹ /L)	6.2±2.0	6.6±2.0
Age (years)	31.4±6.6	29.8±5.4
	Number (%)	
Positive Anti dsDNA	14 (20.6)	1 (0.76)
Positive ANF	21 (30.9)	8 (6.1)
Positive history of thrombosis	12 (17.6)	5 (3.8)
Positive history of stillbirth	4 (5.9)	6 (4.5)
Positive history of Intra-uterine death	5 (7.4)	5 (3.8)
Positive family history of RFL	13 (19.1)	5 (3.8)
Positive history of congenital abnormalities	1 (1.5)	4 (3.0)

RFL: Recurrent fetal loss, APA: Antiphospholipid antibodies, APTT: Activated Partial Thromboplastine, KCT: Kaolin Clotting Time PTT-LA - Partial Thromboplastine Time-LA, ANF: Antinuclear Factor

Furthermore, patients with antiphospholipid antibodies had significantly higher prevalence of anti-dsDNA antibodies and ANF (20.6% and 30.9% respectively) compared to APA negative group (0.76% and 6.1% respectively) ($P < 0.0001$ for both). Five of the enrolled patients fulfilled the diagnostic criteria for the diagnosis of systemic lupus erythematosus.^[9]

Platelets counts were reduced ($<150 \times 10^9/L$) in 10 of the 68 patients (14.7%) with APA, compared to 4 out of the 132 patients (3.0%) who were negative for APA, a finding which was significant ($P = 0.0056$).

Clinically there was no significant difference in age between the APA positive and negative groups ($P = 0.153$), but those with APA were found to have significantly higher prevalence of positive medical history of thrombosis and family history of recurrent abortions (17.6% and 19.1% respectively) than APA negative group (3.8% each) ($P = 0.0009$ and 0.0003 respectively). Patients with a positive history of thrombosis had 5.3 times the risk of having APA compared to those with negative history, similarly those with positive family history of recurrent abortions were found to have 5.8 times the risk of having APA compared to those with negative history. No significant differences were found in the prevalence of a positive history of still births, intrauterine death and congenital abnormalities between APA positive and negative groups respectively ($P = 0.7, 0.3$ and 0.66 respectively).

DISCUSSION

Antiphospholipid antibodies are often but not always associated with adverse obstetric outcomes, including first trimester miscarriage, mid-trimester and later fetal loss, intrauterine death and stillbirth.^[4,10] However, the risk of

Table 2: The performance characteristics of different screening tests in the detection of Lupus anticoagulant

LA screening test	Positive	Negative	Total	Sensitivity	Specificity	PPV ^{††}	NPV [‡]
APTT							
Prolonged (>45 sec)	16	2*	18	59.3	98.8	88.9	94.0
Normal	11	171	182				
PTT-LA							
Prolonged (>46 sec)	22	5**	27	81.5	97.1	81.5	97.1
Normal	5	168	173				
KCT							
Prolonged (>120 sec)	27	10*	37	100	94.2	73	100
Normal	0	163	163				
KCT Index							
Positive (>1.2)	27	3 [†]	30	100	98.3	90	100
Negative	0	170	170				

*Only one case was positive for aCL, **Two cases were positive for aCL, †None were positive for aCL, ††Positive predictive value, ‡Negative predictive value

pregnancy loss is greatest during the mid-trimester.^[1] The exact mechanism of action of APA in relevance to fetal loss is still uncertain, but it appears to act on the placenta and its underlying decidual vessels. Decidual vasculopathy, thrombosis, extensive infarction and necrosis in the placenta of women with APA, was documented in many studies and linked to fetal death.^[11] Other studies have suggested that APA have inhibitory effects on the growth and differentiation of the trophoblast, or suggested an inflammatory basis.^[12]

The prevalence of Lupus Anticoagulant in association with recurrent fetal loss, as determined in the current study was 13.5% which is comparable to the rate of 10.28% reported by Kumar and coworkers from India^[13] and is within the range reported by previous studies from Western countries of 3-14%.^[14-16] The variations in the prevalence of LAC in recurrent fetal loss is due to several factors, including the use of variable types and numbers of tests with variable sensitivities and methodologies, differences in the numbers of cases included and the criteria of patient selection.^[10,17] One of the most important of the latter factors is related to the number and types of the tests employed for detection of LAC. In the current study a combination of screening (PTT, PTT-LA and KCT), correction (for PTT, PTT-LA and KCT index) and confirmatory tests (HPNP) were used. Such a combination of screening, correction and confirmatory tests has been recommended by all major laboratory accreditation authorities.^[8]

The results of the current study reveal that the KCT and the KCT index are the most sensitive screening tests, with the latter being also the most specific with the highest predictive values [Table 2]. The high sensitivity of the KCT and its superiority over APTT and other LAC screening procedures has been documented by several previous studies, including that of Lesperance and coworkers,^[18] who reported that the KCT is at least four times more sensitive than APTT or dilute Russell Vipers Venom's time. The high sensitivity and specificity of the KCT and the KCT index as employed in the current study, in addition to their relative low cost and procedural simplicity, offsets their disadvantages namely: Being unyielding to automation and needing strictly platelet poor plasma. This would make such tests quite useful screening test for LAC especially in developing countries.

The prevalence of Anticardiolipin antibodies (aCL) in recurrent fetal loss, as determined in this study is 26.5%, and this is higher than the rates reported by many Western studies of 5-21%.^[11,14-16] On the other hand, a much higher frequency of 40.24% was reported from India.^[13]

The variations noted in the rates of aCL in those with recurrent fetal loss is related to various pre-analytical variables, differing sensitivities of tests, isotypes tested, the population studied, in addition to a very important factor, namely the choice of cut-off point for a positive result.^[19] The selection of the appropriate cutoff point requires setting a careful balance to ensure adequate sensitivity and specificity of the test, and thus in the current study we employed the compromise mean + 3SD of the normal control group as a cut-off point.

The significant association of APA with thrombocytopenia and thrombosis is quite expected, since other than fetal loss, these two features are the most important clinical associations of APA,^[2] but the association with family history of recurrent fetal loss is rather interesting, and may suggest a genetic predisposition to the development of APA, which if confirmed would be a very intriguing observation adding to the mystery of these already vague antibodies. Genetic susceptibility for the development of these antibodies has been the subject of several studies, although it was difficult to conclusively determine genetic risk factors for APA. The latter warrants further research including genome wide linkage studies and international collaboration to obtain better insight into this intriguing issue.^[5]

The importance of the detection of the above antibodies in women with recurrent fetal loss is paramount, since in contrast to women who are untreated, those who are managed properly for APA may have favorable pregnancy outcomes.^[20] The latter fact, in addition to the high frequency of these antibodies in those with recurrent adverse pregnancy outcomes, necessitates that better standardized and preferably cheaper tests for their detection should be provided in any major general obstetric practice, especially in developing countries.

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