



Original Research Article

Phospholipids as a Predisposing Factor of Recurrent Miscarriage in Sudanese Women

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ABSTRACT

Study Design and Objective: This was a case control study performed to determine the haemostatic abnormalities and frequency of the antiphospholipid syndrome in Sudanese women with history of recurrent miscarriage.

Material and Methods: During the period between January and June 2011, a total of 100 Sudanese women with recurrent miscarriage as case group and 100 non complicated pregnant women as control group were interviewed through structured questionnaire to assess the risk factors for recurrent miscarriage, then screened for presence of antiphospholipid antibodies and anticardiolipin antibodies by using Enzyme linked Immuno-Sorbent Assay. Platelets were counted and coagulation profile was measured for all patients and controls. All tests were analyzed using Statistical Package of Social Science Version 17.

Results: Frequency for both antiphospholipid antibodies and anticardiolipin antibodies was 20%, thrombocytopenia was 8%, lupus anticoagulants was 20% and 5% had prolonged prothrombin time (PT). There was a significant correlation between Age and the above results, except PT which had not influenced by age.

Conclusion: The frequencies obtained in this study were in agreement to the frequencies for these parameters in other populations.

Key Words: phospholipids, recurrent miscarriage.

INTRODUCTION

Recurrent miscarriage (RM) is defined as three or more consecutive pregnancy losses. [1] It affects about 5-15% of all pregnancies worldwide. [2] It has been attributed to a large number of etiological factors. [3] Presence of lupus anticoagulants (LA), anticardiolipin antibodies (ACA), and antiphospholipid antibodies (APA) can

increase the incidence of recurrent miscarriage. [4] The antiphospholipid syndrome (APS) has emerged as the most important treatable cause of RM. [5] Several studies had reported an increased prevalence of the natural inhibitors of coagulation such as antithrombin III in women with recurrent miscarry. [6] Pregnancy complications are still challenge for Gynecologists as

knowledge of pathogenesis is still limited. The determination of predisposing factors for the development of recurrent miscarriage is useful to determine the incidence of abortion in women. Therefore, this study aimed to determine and evaluate LA, APA, ACA, and haemostatic abnormalities in Sudanese women with recurrent miscarriage, and to estimate the frequencies of primary and secondary antiphospholipid syndrome.

MATERIAL AND METHODS

A number of 100 women with recurrent miscarriage were included in this case control study. Six ml of venous blood was collected in a syringe from each woman. Two ml of the blood sample were added to a tube containing trisodium citrate for analyzing the coagulation profile including prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin clotting time (TCT). To count platelets, another two ml of the blood were added to a container with EDTA. From the remaining blood sample, plasma was collected after centrifugation and stored at -20°C to be used for measuring APA, ANA, and ACA using ELISA method on the basis of the manufacture's guidelines.

Platelets counts were done using Sysmex KX-21 N (TOA Medical Electronics Company). The machine automatically dilutes an EDTA venous anticoagulated blood sample, lyses, counts, and gives a printout result of absolute numbers of platelets per liter. Reference value of the platelets count $150-400 \times 10^9$ c/l was considered. [7] Reference value applied for prothrombin time (PT) was 11-16 seconds, [8] for APTT was 30-40 seconds, [7] and for TCT was 15-19 seconds. [9]

Anti-phospholipid antibodies (IgM/IgG) and Anti-Cardiolipin (IgG) were considered negative if less than 12 MPL or GPL U/ml,

borderline if 12-18 MPL or GPL U/ml, and positive if more than 18 MPL or GPL U/ml. All positive APA cases were repeated after 6 weeks for confirmation.

RESULTS

Only 20% of the study cases were positive for APA compared with the control group (0%). APA/IgM was positive in the first test and negative when repeated after 6 weeks, while APA/IgG was negative in the first test and positive after 6 weeks. (Figure No 1).

There was a significant correlation between the presence of APA (IgM/IgG) and the age of the patients ($P=0.03$). APA (IgM/IgG) was more frequent in the age group (35-39) years (9 cases). (Table No 1).

ACA was positive in 20% of cases and in 0% of control group. There was a significant correlation between the presence of ACA (IgG) and the age of patients ($P=0.04$). ACA (IgG) was more frequent in the age group (35-39) years (12 cases). (Table No 2)

All cases APA-positive were also ACA-positive. (Table No 3).

Only 8% of cases showed decreased levels of platelet counts (thrombocytopenia). There was a significant correlation between the presence of thrombocytopenia and recurrent miscarriage (p value=0.001).

The presence of lupus anticoagulants (LA) was screened by prolonged APTT that not corrected by addition of normal plasma. Prolonged APTT and LA were positive in 20% of study cases. There was a significant correlation between the presence of LA and recurrent miscarriage (p value=0.018).

The prothrombin time (PT) was prolonged in only 5% of women with RM. The prolonged PT results were not corrected by addition of normal plasma indicating the presence of antiphospholipid antibodies. There was no significant correlation

between the presence of prolonged PT and recurrent miscarriage (p value=0.5).

All patients and controls showed normal TT. (Figure No 2). There was no significant correlation between TT and recurrent miscarriage ($P= 0.5$).

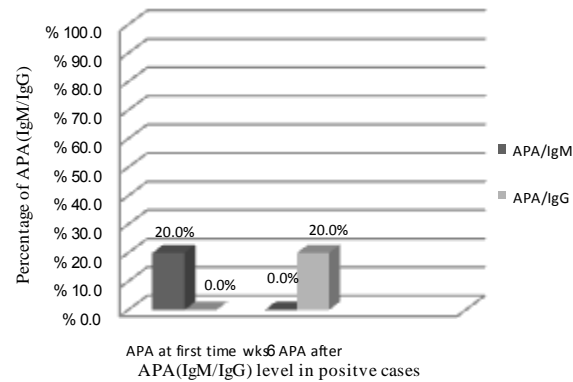


Figure (1): Frequency of APA (IgM/IgG) in study cases.

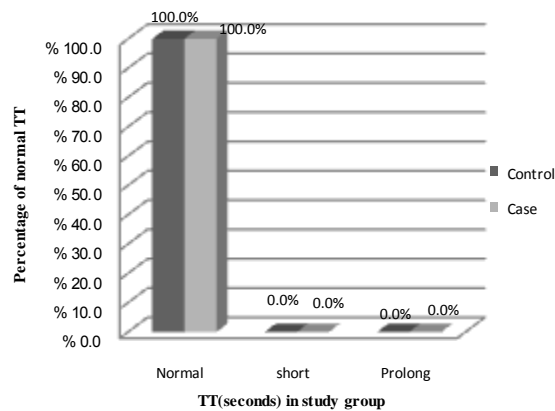


Figure (2): Frequency of Thrombin time (TT) in cases and controls.

Table (1): Frequency of APA (IgM/IgG) according to age in cases:

Age(years)	APA(IgM/IgG)	
	No of Positive case	No of Negative cases
20-24	0	20
25-29	4	4
30-34	7	29
35-39	9	22
40 and above	0	5
Total	20	80

($P = 0.03$)

Table (2): Frequency of (ACA/IgG) according to age in cases:

Age(years)	ACA(IgG)	
	No of Positive cases	No of Negative cases
20-24	0	10
25-29	4	14
30-34	4	20
35-39	12	31
40 and above	0	5
Total	20	80

($P = 0.04$)

Table (3): Correlation between APA and ACA antibodies

APA/ACA	ACA		Total
	+ve no	-ve no	
APA	15	5	20
Total	20	80	100

($P=0.01$)

DISCUSSION

This study showed significant correlation of antiphospholipid antibody (APA), anticardiolipin antibodies (ACA), thrombocytopenia, and lupus anticoagulants (LA) with recurrent miscarriage. The frequencies of APA, ACA, and LA were 20% of cases; that is in agreement with a study of 500 Sudanese women with recurrent miscarriage that revealed the frequencies of APA was 20.2% and ACA was 19.3%. [10] Another study of 52 Sudanese women with RM reported that the frequencies for both APA and ACA were 22.7% and LA was 21%. [11] In an Indian study of 235 women with recurrent miscarriage, frequencies of APA and LA were 25% and frequency of ACA was 23%. [12] In another study from Iran included 138 women with RM, the frequencies of APA, ACA, and LA) were 19.4%, 21.6% and 18.6% respectively. [13] Vaidyanathan *et al.*, from Oman found the frequencies of APA and ACA were 23% and LA was 18.2%. [14]

Munther *et al.* from the Lupus Unit in London showed the frequencies of APA and ACA were 25% and 18.2% respectively among British women with recurrent fetal loss. [15]

In the current study, the sensitivity of both APA (IgM/IgG) and ACA (IgG) in RM patients was 73% and specificity was 93%, indicating that testing for APA and ACA in RM patients was more specific (the percentage of healthy people who were correctly identified as not having RM) and less sensitive (the percentage of sick women who were correctly identified as having the RM). These results are similar to those obtained from the study of El.Hassan, [11] which reported the specificity of both APA and ACA was 94% and sensitivity was 71%.

In the current study, about 12% of women with recurrent miscarriage were positive for antinuclear antibodies (ANA), anti-double strand DNA antibodies (Anti-dsDNA), and also antiphospholipid antibodies (Secondary Antiphospholipid Syndrome), while about 8% of women were positive only for APA (Primary Antiphospholipid Syndrome). These results are similar to a Spanish study that reported frequencies of ANA was 25.2% and Anti dsDNA was 18% in women with recurrent miscarriages. [16] A study in Colombian women reported that the first trimester of pregnancy was found to be associated with high frequency of ANA and Anti dsDNA, and about 10-33% of recurrent miscarriages were due to positive ANA and Anti dsDNA. [17]

Clotting aspects are an important etiological factor in recurrent miscarriages; therefore, the role of platelets had been explored in this study. Frequency of thrombocytopenia in cases of this study was 8%. In a Russian study, reported frequency of thrombocytopenia in 540 women with recurrent miscarriage and antiphospholipid syndrome was 2-10%. [18] Another study in French women with recurrent miscarriage showed that the frequency of thrombocytopenia was 11%. [19]

Prothrombin time (PT) in women with recurrent miscarriage in this study was

not affected significantly ($P=0.5$). Only 5% had prolonged PT; addition of normal plasma failed to correct the prolonged PT due presence of antiphospholipid antibodies in the sera obtained from these women. In a study performed in women with recurrent miscarriage in Greece, prothrombin time was prolonged in 7% of patients due to the presence of antiphospholipid antibodies. [20] Another report in 145 French women with recurrent miscarriage showed that PT was prolonged in 7% of patients due to the presence of antiphospholipid antibodies and lupus Anticoagulants. [21]

Thrombin time (TT) was normal in women with recurrent miscarriage in this study. This is similar to the normal results of TT reported in 287 Canadian women with recurrent miscarriage. [22]

In the current study, the correlation between the presence of Antiphospholipid antibodies (APA), anticardiolipin antibodies (ACA), lupus anticoagulants (LA), antinuclear antibodies (ANA), anti-double strand DNA (Anti dsDNA), Thrombocytopenia and Age was significant. These findings were matched to the results obtained by an American study which reported that the presence of APA, ACA, LA, Anti dsDNA and thrombocytopenia was increased with age in women with recurrent miscarriage. [23]

CONCLUSION

It is concluded that the frequencies obtained from this study are in agreement with the frequencies for the same parameters in other populations.

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