Original Article

Determination of antiphospholipid antibodies and Thrombophilia in women with recurrent miscarriage

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ABSTRACT

Background: Recurrent miscarriage is a critical problem in which many factors play a crucial role such as antiphospholipid antibodies (APA) and anticardiolipin antibodies (ACA). Recent studies pointed to a potential role of thrombophilias as a possible cause of recurrent miscarriage (RM).

Objectives: This study was conducted to determine the frequency of the primary and secondary antiphospholipid syndrome and the haemostatic abnormalities in women with history of RM.

Materials and Methods: This study was a case control study, conducted from Febraury-2008 to February -2011 in Khartoum State. A total of 100 women with three or more consecutive recurrent miscarriage without previous living babies as case group and 100 non complicated pregnant women were screened for the presence of antinuclear antibodies (ANA), anti-double stranded DNA (anti-dsDNA), antiphospholipid antibodies (APA IgM/IgG), and anticardiolipin antibodies (ACA/IgG) by using Enzyme Linked Immuno-Sorbent Assay (ELISA). Patients with history of three miscarriages with delivery of full-term or preterm babies in between or with medical termination of pregnancy were excluded from this study. Women positive for APA were asked for a repeat sample after 6 weeks to run confirmatory test. Patient and control groups were also screened for the count of platelets using Sysmex KXN-21, Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) and Thrombin Time (TT) using coagulometer Biobas 10.

Results: The frequencies for both APA and ACA were 20%. ANA and Anti-dsDNA were 12%. There was a significant correlation between age and the presence of APA (P=0.03), ACA (IgG) (*P*=0.04), ANA and Anti-dsDNA (*P*=0.013). Frequencies for, thrombocytopenia and lupus anticoagulant (LA) were 8% and 20% respectively. 5% had prolonged PT, whereas the remaining patient and control groups had normal results.

Conclusion: The data concluded that the frequencies of APA, ACA, ANA, Anti-dsDNA, in women with RM obtained in this study were in agreement to the frequencies for these parameters obtained in previous studies and their presence were significantly associated with recurrent miscarriage.

Recommendation: Frequencies of LA and thrombocytopenia were significantly associated with recurrent miscarriage. Prolongation in PT may be due to the presence of anti-prothrombin antibodies in serum of patients with recurrent miscarriage. The current study recommends measuring of APA, ACA, Anti-dsDNA-antibodies and assessment of platelets count, APTT and PT in all women with recurrent miscarriage and late pregnancy loss.

Keywords: APA, ACA, ANA, Anti-dsDNA, RM, LA, APTT, PT.

Recurrent miscarriage (RM) is defined as three or more consecutive pregnancy losses[1]. It affects about 5-15% of all pregnancies worldwide[2].

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It has been considered as primary RM when pregnancy has never been carried to viability[3] or secondary RM when a live birth has occurred at sometime[4].

RM has been attributed to a large number of etiological factors, in approximately two-third of cases the cause is known to be genetic error, anatomic abnormalities of the reproductive tract, hormonal abnormalities, infection or immunologic factors or systemic

disease whereas, idiopathic in one third of all cases[5].

Presence of lupus anticoagulant (LA) and anticardiolipin antibodies (ACA) increased incidence of RM. Also the presence of the antiphospholipid antibodies (APA) was shown to associate with recurrent miscarriage due to thrombosis of the uteroplacental vasculature and subsequent placental infarction[6]. Antiphospholipid antibodies (APA) are heterogeneous group of auto-antibodies directed against different antigens, predominantly anionic phospholipids or phospholipids-containing structures[7]. APA that associated with APS are anticardiolipin antibodies (ACA) or antibodies against other negatively-charged phospholipids such as phosphotidylserine, phosphatidyl inositol, phosphatidic acid and phosphatidyl glycerol[8]. Lupus anticoagulants (LA) which are immunoglobulins directed against plasma proteins such as prothrombin or annexin V that are bound to phospholipids. Anti-β_2-glycoprotein1(anti-(β_2GP1) are antibodies which recognize a plasma protein known as apolipoprotein H or beta-2 glycoprotein I and have higher specificity than ACA for thrombosis[9].

Some cases of recurrent first trimester miscarriage (RM) may have a thrombotic etiology. The evidence for this comes from histological studies reporting microthrombi to be a common finding in the placental vasculature of pregnancies amongst women with RM[10]. The mechanism of thrombosis in patients with antiphospholipid antibodies (APA) is still unknown, although several mechanisms have been proposed[11]. There are data suggesting that antiphospholipid antibodies induce thrombosis through any one or more of several mechanisms: (1) antiphospholipid antibody interference with endogenous anticoagulant mechanisms (disruption of the annexin A5 anticoagulant shield,[12] inhibition of protein C pathway, inhibition of antithrombin); (2) binding and activation of platelets; (3) increased thromboxane production by platelets; (4) interacting with endothelial cells and inducing expression of adhesion molecules and tissue factor, and thus a prothrombotic state occurs as a result of the reaction between APA with cellular antigens including platelet and endothelial cell membrane protein[13] and (5) decreased prostacyclin production by endothelial cells[14]. Determination of other problems related to APA in women with recurrent miscarriage such as thrombocytopenia as a risk factor for pregnancy complications has gained much attention in the scientific community. Clinical studies reported that thrombocytopenia was associated with 2%-10% of APS in women with RM and was presumably due to anti-platelet auto-antibodies directed against platelet-bound P2GPI[15, 16].

In Sudan, few studies reported the prevalence of antiphospholipid antibodies (APA) and anticardiolipin antibodies (ACA). Ahmed and others reported a prevalence of 20.2% for APA and 19.3 % for ACA in women with RM[3]. Another study in 52 Sudanese women with RM demonstrated a prevalence of 22.7%, 22.7% and 21% for APA, ACA and LA respectively[17].

Some cases of RM may have a non-thrombotic etiology. The evidence for this comes from studies reporting antinuclear antibodies (ANA) and anti-double stranded DNA among women with recurrent miscarriage[18]. These antibodies were also a common finding In Italian[19] and Colombian women[20] with recurrent miscarriageduring the first trimester of pregnancy.

The diagnosis of APS requires the presence of both clinical and laboratory criteria. It requires at least one of the clinical criteria plus the presence of either LA or ACA. The LA or ACA must be shown to persist for at least six weeks before the diagnosis of APS may be made[21].

The aim of the current study was to determine the frequencies of antiphospholipid antibodies (APA), Anticardiolipin antibodies (ACA) and lupus anticoagulants (LA), antinuclear antibody (ANA) and Anti-double strand DNA (Anti ds-DNA) in women with history of recurrent miscarriage. To elucidate the role of haemostatic abnormalities in inducing RM, the platelets count, Activated Partial

Thromboplastin Time (APTT), Prothrombin Time (PT) and Thrombin Time (TT) were assessed.

Materials and Methods

Study Design:

This was a case-control study, conducted in Khartoum state at Military Hospital and Fath Alrahman Albashir referral clinics during the period between February-2008 and February-2011. Cases were defined as patients who had three or more consecutive recurrent miscarriage and control group was consisting of pregnant females with non-complicated pregnant women.

Sampling:

Since the global reported-risk of recurrent miscarriage was 1%; by applying the classical equation to estimate the sample size $n= (Z\sigma^2/d)^2$

Z= was the value of specified level of significance, in this study the level of significance was 1% which gives a value of Z= 0.799

σ = was the standard deviation of the cases, the value of S.D was estimated to be 2.5.

d = was the difference between the case mean and control mean was estimated not to exceed 0.5. Hence, applying these values $n= (0.799\times2.5^2 / 0.5)^2 = 100$ women

Five ml of venous blood was collected from each woman with history of 3 or more consecutive miscarriage. Plasma was collected after centrifugation and stored at -20ºC for measuring of APA, ANA, ACA and anti- dsDNA using ELISA (Organic company-Germany).

Immunometric Enzyme Immunoassay for the quantitative determination of Anti-nuclear antibodies (ANAs):

This method collectively detects ANAs against purified antigens (SS-A/Ro, SS-B/La, RNP 70, Sm, RNP/Sm, Scl-70, centromere B and Jo-1). Briefly, samples were transferred into 96 well plates coated with human recombinant SS-A/Ro, SS-B/La, RNP 70, Sm, RNP/Sm, Scl-70, centromere B and Jo-1 antigens along with controls. The plates were incubated for 30 minutes at room temperature. Washing was undertaken in triplicate by using washing buffer (PBS, NaN3<0.1%). Enzyme conjugate (Horseradish peroxidase (HRP) conjugated anti-human IgG) was added to each well and then incubated for 15 min at RT. Reaction was catalyzed by adding TMB substrate solution to each well and the plate were incubated for 15 minute in dark at room temperature and then washed as before. The reaction was stopped by adding stop solution (1 M hydrochloric acid). Read O.D by ELISA reader at length of 450nm.

Immunometric Enzyme Immunoassay for the quantitative determination of IgG auto-antibodies to double-stranded DNA:

Serum levels of IgG auto-antibodies against human recombinant double-stranded DNA (ds-DNA) were measured by Immunometric Enzyme Immunoassay. Briefly, samples were transferred into 96 well plates coated with human recombinant double stranded DNA along with controls. The plates were incubated for 30 minutes at room temperature. Washing was undertaken in triplicate by using washing buffer (PBS, NaN3<0.1%). Hundred µl of enzyme conjugate (Horseradish peroxidase (HRP) conjugated anti-human IgG) was added to each well to detect the specific antibodies forming a conjugate/antibody/antigen complex. The plates were then incubated for 15 minutes at room temperature. An enzyme substrate (100µl of TMB substrate in each well) in the presence of bound conjugate hydrolyzes to form a blue color and incubated in dark. Then 100µl of stop solution (1 M hydrochloric acid) were added to each well of the modules. Plates were incubated for 5 minutes at room temperature in dark. The intensity of the yellow color is measured photometrically at 450 nm by read O.D using ELISA reader.

Immunometric Enzyme Immunoassay for the quantitative determination of anti-phospholipid antibodies (IgM/IgG):

A mixture of highly purified Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and human β2-Glycoprotein1 were bound to micro wells. Briefly, samples were transferred into 96 well

plates coated with a mixture of phospholipids along with controls. The plates were incubated for 30 minutes at room temperature. Washing was undertaken in triplicate by using washing buffer (PBS, NaN3<0.1%). Hundred µl of enzyme conjugate (Horseradish peroxidase (HRP) conjugated polyclonal rabbit anti-human IgG) was added to each well to detect the specific antibodies forming a conjugate/antibody/antigen complex. The plates were then incubated for 15 minutes at room temperature. An enzyme substrate (100µl of TMB substrate in each well) in the presence of bound conjugate hydrolyzes to form a blue color and incubated in dark. Then 100µl of stop solution (1 M hydrochloric acid) were added to each well of the modules and stopped the reaction forming a yellow end-product. The intensity of the yellow color is measured photometrically at 450 nm by read O.D using ELISA reader.

Immunometric Enzyme Immunoassay for the quantitative determination of Anti-Cardiolipin IgG:

IgG class auto-antibodies directed against Cardiolipin were measured by using Immunometric Enzyme. Briefly, samples were transferred into 96 well plates coated with bovine cardiolipin and saturated B2-glycoprotein-1 along with controls. The plates were incubated for 30 minutes at room temperature. Washing was undertaken in triplicate by using washing buffer (PBS, NaN3<0.1%). Enzyme conjugate (Horseradish peroxidase (HRP) conjugated polyclonal rabbit anti-human IgG) was added to each well and then incubated for 15 min at RT. Reaction was catalyzed by adding TMB substrate solution to each well and the plate were incubated for 15 minute in dark at room temperature and then washed as before. The reaction was stopped by adding stop solution (1 M hydrochloric acid). Read O.D by ELISA reader at length of 450nm.

Platelets count using automated blood cell counter sysmex KXN-21:

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 150-400×10^9 c/l was considered.

Prothrombin Time (PT) test:

To rule out the presence of inhibitors to prothrombin that may be found in conjunction with Lupus Anticoagulant (LA), plasma sample from each case was tested for Prothrombin time (PT). 100µl of control and patient citrated platelet poor plasma pipetted into a warmed cuvette. Then, 200µl of calcified thromboplastin were added, test was performed in duplicate. End point was observed and the mean of the double determination was plotted. Reference value for PT is in the region on 11-16 second.

Activated Partial Thromboplastin Time (APTT):

The test is based on activation of the intrinsic pathway by the addition of external cephalin phospholipid mixture in a test tube and measuring time to clot formation. The typical screening test for LA antibodies is the standard APTT that is prolonged and fails to correct when the patients plasma is mixed with normal plasma. Briefly, 100µl of control and patient citrated platelet poor plasma were added into a warmed cuvette, and then100µl of Cephalin – kaolin mixture was added and incubated for 10 minutes. Following that, 100µl of $cacl_2$ was addedand the end point was observed. The mean of the double determination was plotted. Reference value for APTT is 30 to 40 seconds.

APTT Mixing Experiments with normal plasma:

By mixing Patients plasma with normal pooled plasma, factor deficiencies can be differentiated from Inhibitors. An inhibitor, if present, can generally be characterized as "Lupus- Like".

Procedure of APTT with normal plasma: 50µl of patient citrated platelet plasma were added to 50µl of normal citrated platelet poor plasma and the same dilution for control plasma and follow the same procedure in APTT method. Correction by adding

frozen/thawed platelets concentrate strongly suggests presence of Lupus Anticoagulant (LA) antibodies.

PT mixing experiment with normal plasma:

Fifty µl of patient citrated platelet plasma were added to 50µl of normal citrated platelet poor plasma and the same dilution for control plasma and follow the same procedure in APTT method. If PT is prolonged in patients plasma with no correction this indicates the presence of inhibitors to prothrombin which may be found in conjunction with Lupus Anticoagulant (LA).

Thrombin Clotting Time (TT):

The test measures the clotting time of the plasma after adding thrombin, thrombin is affected by the reaction & concentration of fibrinogen & the presence of inhibitory substances. Briefly, 100µl of control and patient citrated platelet poor plasma were pipetted into a warmed cuvette. Then 200µl of Thrombin –bovine were added and the end point was observed and the mean of the double determination was plotted. Reference value for TT is at the range between 15 and 19 second.

Statistical analysis:

All tests were analyzed using Statistical Packages of Social Sciences Version 17 (SPSS program).

Ethical consideration:

An informed consent was obtained from each volunteer. The confidentiality of the patients was established by coding of the questionnaires and the data list by a different code from their files to insure the anonymity of respondents. All investigations were carried out for patients free of charge.

Results

Of the 100 women with recurrent miscarriage that were tested, 20% had detectable levels of antiphospholipid antibodies (APA) (IgM/IgG). The frequency of APA in controls group was significantly lower compared with that in women with recurrent miscarriage (*P*=0.02). All women with recurrent miscarriage were positive for APA (IgM/IgG) at day 0 (figure 1) and at day 42 (figure 2).

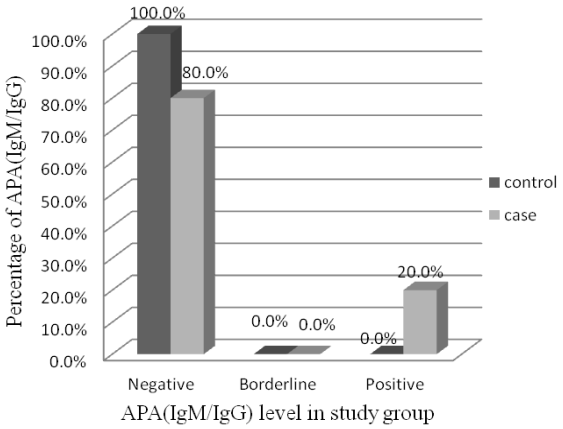

Figure 1: Frequency of APA (IgM/IgG) in cases and controls.

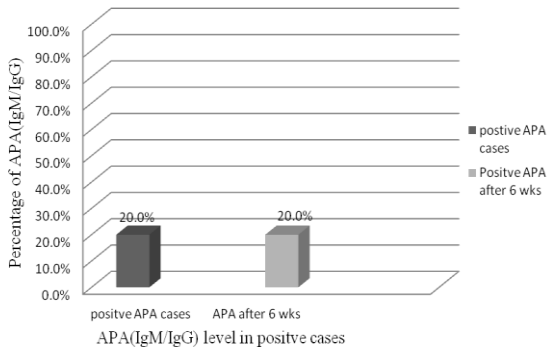

Figure 2: Frequency of APA (IgM/IgG) in cases with positive antiphospholipid antibodies at day 42.

Table 1: Frequency of anticardiolipin antibody ACA (IgG) in cases and controls

Case/control	ACA(IgG) Positive n (%)	Borderline n (%)	Negative n (%)	Total
Cases	20(20%)	0(0%)	80(80%)	100(100%)
Controls	0(0%)	0(0%)	100(100%)	100(100%)

*P = 0.02

Table 2: Frequency of antiphospholipid antibody APA (IgM/IgG) according to age in cases

	APA(IgM/IgG)	
Age (years)	+ve no (%)	-ve no (%)
20-24	0(0%)	20(20%)
25-29	4(4%)	4(4%)
30-34	7(7%)	29(29)
35-39	9(9%)	22(22%)
40 and above	0(0%)	5(5%)
Total	20(20%)	80(80%)

*P = 0.03

Table 3: Frequency of anticardiolipin antibodies (ACA/IgG) according to age in cases

	ACA(/IgG)	
Age(years)	+ve no (%)	-ve no (%)
20-24	0(0%)	10(10%)
25-29	4(4%)	14(14%)
30-34	4(4%)	20(20)
35-39	12(12%)	31(31%)
40 and above	0(0%)	5(5%)
Total	20(20%)	80(80%)

*P = 0.04

Table 4: Sensitivity and specificity to recurrent miscarriage of APA and ACA (IgG) antibodies

		ACA		
APA/ACA		+ve no (%)	-ve no (%)	Total
APA	+ve no (%)	15(75%)	5(25%)	20(100%)
	-ve no (%)	5(25%)	75(75%)	80(80%)
Total		20(20%)	80(80%	100(100%)

*P =0.01

Anticardiolipin antibodies were positive in 20% women with RM. The frequency of ACA in control group was significantly lower compared with that in women with recurrent miscarriage (P=0.02) (table 1). APA were more seen at the age group between 35-39 years. Similarly ACA (IgG) was a significantly correlated to the age (P=0.04) and more seen at the same age (table 3). Table 4 summarizes the ELISA true positives and true negatives samples. 15 women with recurrent miscarriage had

APA and ACA, whereas the remaining 5 women with RM had either APA or ACA. The sensitivity for both APA (IgM/IgG) and ACA (IgG) was 73% and specificity reaches 93%.

Anti-nuclear antibodies (ANA) and anti-double strand DNA antibodies (Anti-dsDNA-Ab) were measured in sera from cases and controls. 12% of patients had ANA and anti-dsDNA (figure 3) and were also positive for APA i.e., had secondary APS or Systemic lupus erythematosus (SLE) (Figure 4).

The frequency of ANA and Antids-DNA according to age was illustrated in table5.

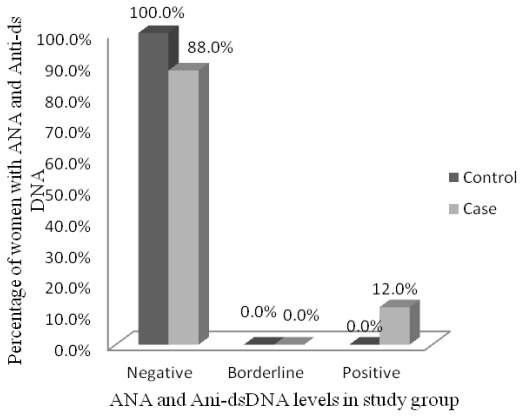

Figure 3: Frequency of antinuclear antibodies (ANA) and Anti-double stranded DNA (Anti-dsDNA) in cases and controls.

The correlation between ANA, anti-dsDNA and recurrent miscarriage among these women was significant (*p value*=0.01). None of controls had detectable levels of either antibodies. ANA and Antids-DNA were more prevalent at the age group between 35-39 years (*P*=0.013).

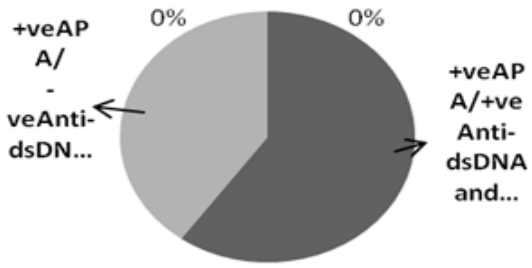

Figure 4: Frequency of the secondary and primary antiphospholipid syndrome incases

Table5: Frequency of ANA and Antids-DNA according to age in cases.

	ANA & Antids-DNA(IgG)	
Age(years)	+ve no (%)	-ve no (%)
20-24	0(0%)	10(10%)
25-29	0(0%)	16(16%)
30-34	3(3%)	23(23%)
35-39	9(9%)	34(34%)
≥ 40	0(0%)	5(5%)
Total	12(12%)	88(88%)

(*P* = 0.013)

Eight percent had thrombocytopenia whereas; the remaining patients (92%) and controls (100%) had normal platelets count. The correlation between the presence of thrombocytopenia and recurrent miscarriage was significant (*p*=0.001) (Table 6).

The presence of LA was screened by Activated partial thromboplastin time (APTT) and the prolonged results were not corrected by addition of normal plasma indicating the presence of LA.

Table 6: Frequency of the Platelets count in cases and controls

	Platelets count			
	Normaln (%)	Thrombocytopenian (%)	Thrombocytosisn (%)	Total
Cases	92(92%)	8(8%)	0(0%)	100(100%)
Controls	100(100%)	0(0%)	0(0%	100(100%)

**P* = 0.001

Table 7: Frequency of the lupus anticoagulants in cases and controls

	APTT			
	Normal No. (%)	Short No (%)	Prolong and no correctionwith normal plasma No (%)	Total
Cases	80(80%)	0(0%)	20(20%)	100(100%)
Controls	100(100%)	0(0%)	0(0%)	100(100%)

*P = 0.018

Table 8: Frequency of the Prothrombin time (PT) in cases and controls

	PT			
	Normal no (%)	Short no(%)	Prolong and no correction with normal plasma no (%)	Total
Cases	95(95%)	0(0%)	5(5%)	100(100%)
Controls	100(100%)	0(0%)	0(0%	100(100%)

*P = 0.5

This has been shown clearly by table 7 where 20% in women with RM had prolonged APTT. Results also revealed a significant correlation between the presence of LA and recurrent miscarriage (p=0.018). On the other hand, the prothrombin time (PT) was prolonged in 5% of women with RM as shown in Table 8, whereas the remaining patients (95%) and controls (100%) had normal PT. The prolonged PT results were not corrected by addition of normal plasma indicating that the presence of antiphospholipid antibodies interfered with PT.

Discussion

This study is the first one in Sudan to show a statistically significant correlation of antiphospholipid antibody (APA), anticardiolipin antibodies (ACA) and recurrent miscarriage. On the other hand, the data obtained in this study extends and confirms the data obtained in other studies in Sudan[17].

The present study shows that women with unsuccessful pregnancy had mounted APA and ACA. This was in line to the reported frequencies of APA and ACA in many studies from Iran, India and Oman. In Indian study the frequencies of antiphospholipid antibodies (APA) and lupus anticoagulant were 25%in 235 women with recurrent miscarriage[22]. In Iran the frequencies of antiphospholipid antibodies (APA), anticardiolipin antibodies (ACA) in sera from 138 women with RM were 19.4% and 21.6% respectively[23]. Another report by Vaidyanathan and othersfrom Oman had shown frequencies of 23% for both antiphospholipid antibodies (APA) and anticardiolipin antibodies (ACA)[24] with no significant difference in frequencies obtained in the current study and other studies in Sudan. The role of APA in reproductive failure appears to be more diverse. The adhesion properties of phospholipids play a major role in the physiology of reproduction; APA can interfere with the phospholipid adhesion which may result in reproductive failure. ACA are known to be associated with specific autoimmune diseases such as SLE and with overt thromboembolic phenomena, including recurrent pregnancy loss. The mechanism of action is thought to be an increase in the hypercoagulable state via inability to activate protein C, inhibition of prostacyclin, and endothelial wall and platelet membrane damage.

In the present study, the recurrent miscarriage was more associated with the secondary antiphospholipid syndrome rather than with primary antiphospholipid syndrome that, 12%

of women with recurrent miscarriage who were positive for antinuclear antibodies (ANA) and anti-double strand DNA antibodies (Anti-dsDNA), were also positive for antiphospholipid antibodies i.e., had secondary APS. 8% of women who were positive only for APA had primary APS. Presence ANA and Antids-DNA with similar frequencies was reported in Spanish women with recurrent miscarriages[18]. Another study reported that the first trimester of pregnancy was found to be associated with the increased positivity of ANA and Antids-DNA in Colombian women[13]. However, the frequencies of ANA and Anti-dsDNA in the current study were conflicting with a study in Italy that had shown a frequency of 51.5% for ANA and 39% and for antids-DNA in women with recurrent miscarriage and these conflicting results may be due to difference in the genetic makeup between the population of the current study and Italian women[19].

The current study is the first one in Sudan to show a statistically significant correlation of thrombocytopenia, lupus anticoagulants (LA) and recurrent miscarriage. On the other hand, the data obtained in this study extends and confirms the data obtained in other studies[17, 22, 23]. According to the results of the current study, 20% of women with recurrent miscarriage had prolonged APTT. That was in agreement with a previous study in Sudan by El.Hassan[17], with no significant difference between the frequencies of the two studies. Also the frequencies of LA in the current studies were in line to the reported frequencies of LA in studies conducted in India, Iran and Oman. One study from India in 235 women with recurrent miscarriage reported frequencies of 25% for lupus anticoagulant[22]. Similarly, the frequency of lupus anticoagulant (LA) in sera from 138 Iranian women with RM was 18.6%[23]. Another report by Vaidyanathan and others from Oman had shown a similar frequency for Lupus anticoagulant[24]. However, a conflicting report by Munther and others from the lupus unit in London showed a high frequency of LA of 65.7% in 500 British women with recurrent miscarriage[25]. A good explanation for different reports may be due to lack of presence of a standard assay for measuring the antibodies.

The role of platelets as an important etiology in recurrent miscarriages had been explored in this study. The thrombocytopenia was 8% in women with recurrent miscarriage. These results were in line to the reported frequency of the thrombocytopenia (2-10%) in 540 women Russian with recurrent miscarriage. Thrombocytopenia in these women was presumably due to anti platelet auto-antibodies directed against platelet-bound B2GPI[15]. Another study by Lin and others in French women with recurrent miscarriage had shown that the frequency of thrombocytopenia was 11%[16].

Only 5% of the current study population had prolonged PT. Addition of normal plasma failed to correct the prolong PT. That is most probably due to the presence of antiphospholipid antibodies in sera obtained from these women. This findings was in line with the obtained results by study performed in women with recurrent miscarriage in Greece, revealed that prothrombin time was prolonged in 7%[26]. Another report by Cauchi and othersin 145 French showed that only 7% of women with recurrent miscarriage had prolonged PT and that was due to the presence of antiphospholipid antibodies and lupus anticoagulant (LA)[27].

Conclusion:

The presence of antiphospholipid antibodies (APA), anticardiolipin antibodies (ACA) in sera of women with RM were significantly associated with recurrent miscarriage and the age. The frequency of secondary antiphospholipid syndrome (SLE) was higher than the frequency of primary APS among women with RM. The presence of lupus anticoagulant (LA) and thrombocytopenia in sera from women with RM were significantly associated with recurrent miscarriage and the age. Prolongation in PT may be due to the presence of anti-prothrombin antibodies in serum of these patients. The Activated Partial Thromboplastin Time (APTT) was more sensitive than Prothrombin Time (PT) in the presence of lupus anticoagulant. Accordingly,

the current study recommends assessment of antiphospholipid antibodies (APA), anticardiolipin antibodies (ACA), antinuclear antibodies (ANA), anti-double strand DNA antibodies (Anti-dsDNA) and LA in all women with recurrent miscarriage and late pregnancy loss.

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