



## **Antiphospholipid Antibodies and Acquired Thrombophilia: A Biological Marker for Recurrent Miscarriage**

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### **Authors' contributions**

*All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.*

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### **ABSTRACT**

**Background:** Repeated miscarriage can cause tissue injury can lead to the formation of antibodies to the phospholipids. Recurrent miscarriage (RM) is considering the one of the most common cause of sterility. Which has received more attention in recent years as a result of an increase in the number of reproductive-aged women.

**Materials and Methods:** Plasma samples were tested for antiphospholipid antibodies using ELISA, and platelet count using Sysmex (KX21) Hematology analyzer and Activated Partial Thromboplastin Time using semi-automated machine (STAGO PT31039352 (for coagulation).

**Results:** The prevalence of Anti phospholipid antibodies (APL) was 30.5% in Sudanese patients with recurrent miscarriage, the prevalence of (Anti phospholipid Antibodies-IgM and IgG) was found to be 23.6% in patients with recurrent miscarriage compared to (Anti phospholipid Antibodies-IgG) was found to be 11.1% ((P value $\leq$ 0.001), low platelets count (<50 $\times$ 10<sup>9</sup>/l) observed in 10 (13.5%),

as well as prolongation of activated partial thromboplastin time (APTT) among studied group were detected among 19 (26.1%).

**Conclusion:** Higher prevalence of antiphospholipids antibodies, and acquired thrombophilia was detected among Sudanese women with recurrent abortion; The findings are concerning because they link an increased risk of thrombosis and a hypercoagulable state lead to recurrent miscarriage in pregnant women.

*Keywords: Antiphospholipid antibodies; pregnancy; gestational age; thrombocytopenia.*

## 1. INTRODUCTION

Anti-phospholipid antibody syndrome (commonly called anti-phospholipids syndrome or (APS) is an autoimmune disease present mostly in young women. Anti-phospholipids are anti body syndrome is an autoimmune disease can cause frequent clotting in arteries and veins and /or miscarriages [1]. The clotting results from the presence of proteins in the blood called anti-phospholipids auto antibodies, which interfere with coagulation leading to increased clot formation. Antiphospholipids antibodies are present in 15-20% of all cases of deep vein thrombosis (blood clots) and in one-third of new strokes occurring in people under the age of 50. It is a major cause of recurrent miscarriages and pregnancy complications when no other causes are found [1,2]. Once the disease is diagnosed, adequate therapy in most cases can prevent the recurrence of the symptoms. Antiphospholipid antibodies can be present in the blood stream for long time but thrombotic events result only occasionally, which increase the risk for blood clotting, but thrombosis usually occurs when other conditions that favor clotting are present, such as prolonged inactivity (e.g. being restricted to bed), surgery or pregnancy. Additional risk factors for thrombosis are hypertension, obesity, smoking, atherosclerosis (hardening of the arteries), and use of estrogens (birth control pills) [3,4].

(APL) affects women five times more commonly than men. It's typically diagnosed between ages 30-40 years. While up to 40% of patients with systemic lupus erythematosus (SLE) will test positive for antiphospholipids autoantibody, only half will develop thrombosis and /or experience miscarriage [5,6]. Like most autoimmune disorders, (APS) has a genetic component, although there is not a direct transmission from parent to offspring [7]. Most people who have anti phospholipids syndrome have no symptoms, although though the disorder can cause blood clots and other health problem in some people. For women, recurrent miscarriage may be the only symptoms of the disorder. [3] refer properly

A definite diagnosis of antiphospholipid syndrome (APS) requires the presence of at least one clinical criterion and one laboratory criterion [8]. The diagnosis is made by testing the blood of patients with thrombosis and/or recurrent miscarriages for antiphospholipids antibodies (APL) [9]. During pregnancy women with (APS) may be at higher risk of developing blood clots and miscarriage. In antiphospholipid syndrome, pregnancies are thought to be lost because blood clots form in the placenta and starve the baby of nutrition. Some women may have trouble getting pregnant, while others may experience repeated miscarriages. Blood clots that develop in the placenta can cause fetal growth problems, fetal distress, preterm birth, or pregnancy loss [10].

Researchers have found that having antiphospholipid syndrome can increase women's chance of recurrent miscarriage. The reason for these is unclear; Some researchers believe that antiphospholipid syndrome causes tiny blood clots to block blood supply to the placenta. Other believes that having antiphospholipid antibody syndrome may interfere with fertilized eggs that prevent ability to implant in the lining of the uterus [11]. Moreover, they believed that the antibodies damage or affect the inner lining of the blood vessels, which causes clotting. Others believe that the immune system makes antibodies in response to blood clots damaging the blood vessels<sup>(31)</sup>. Maternal age and previous number of miscarriages are two independent risk factors for a further miscarriage. Fortunately, there is only a fraction of a percent that increase the risk of miscarriage in a subsequent pregnancy following one or even two previous miscarriages. Advanced maternal age adversely affects ovarian function, giving rise to a decline in the number of good quality eggs which often don't develop and hence the risk of miscarriage, also being underweight may also increase the risk [12,13]. Antiphospholipid syndrome is only proven thrombophilia that is associated with adverse pregnancy outcome [14], there were few published studies have been done in recurrent miscarriage in Sudanese

women, so current study was aimed to determine the frequency of acquired thrombophilia and to find the relationship with platelet, coagulation factors and antiphospholipid antibody.

## 2. MATERIALS AND METHODS

### 2.1 Study Design and Population

This was Descriptive, cross-sectional study done on 100 subjects; 72 women with recurrent miscarriage and 28 healthy pregnant women with no history of obstetric complications at first trimester (was involved as control subjects), whom attended four hospitals in Khartoum state (Khartoum Teaching Hospital –Khartoum Bahry Teaching Hospital Alrajaa –Janeen); the study was conducted over period of 4 months.

### 2.2 Sample Size

The sample size is calculated according to the known formula, which is used to reach a certain desired margin of error in the results. The sample size in this study is calculated for each category to give maximum error (0.01) with probability of ( $\alpha = 0.05$ ), prevalence 50%. As Follows:

$$n = \frac{z^2 \cdot p \cdot q}{d^2} = \frac{(1.96)^2 \times (0.05) \times (0.95)}{(0.05)^2} = 72 \quad \text{but the collected number is 100}$$

$z$  = the value in normal curve corresponding to level of confidence

$$95\% = 1.96$$

$p$  = probability prevalence in the community is 10% or 0.1

$$q = (1-p) = 1 - 0.1 = 0.9$$

$$d = \text{margin of error} = 0.05$$

### 2.3 Sample Collection

Blood sample was collected in to three containers, 2.5 ml in potassium EDTA container, 2.5ml in Trisodium citrate container and 2 ml in plain container, and allowed to clot at room temperature and this was provided adequate serum and the ratio of anticoagulant to blood 1:9, the amount of the blood in trisodium citrate container 2.5 ml, centrifuged in 3000r/min for 15 min for separation of platelet plasma (PPP).

### 2.4 Measurement of Platelet Count

EDTA blood sample were analyzed using Sysmex KX21 Hematology analyzer for performing platelet counts.

### 2.5 Method of Activated Partial Thromboplastin Time (APTT)

The reagent was brought to room temperature (RT) (rephrase) To the test tube add 50ul of test plasma and 50ul from BioCelin reagent (R1) then incubated for 3 min at 37C. Then add 50ul Calcium Chloride ( $\text{CaCl}_2$ ) following incubation and record the time. Repeat for duplicate test using same test plasma. Found the average from duplicate test values. The semi-automated machine (STAGO PT31039352) was calibrated, and sample normal control (NC) and pathologic control (PC) tests were conducted at the beginning of each patch.

### 2.6 Method of Antiphospholipid Antibodies

Samples were tested for antiphospholipid antibodies using ELISA, 2.5 ml of whole blood was taken and centrifuged to produce serum. The serum was freeze at -20 (Freeze and thawed once only). Then allow the sample to reach room temperature (RT). Dilution to the samples 1:100, in micro titer pipette add 100ul from diluted sample, pathogenic controls (PC), Normal control (NC) and cover plate with adhesive strip. Incubate for 1hour at Room Temperature (RT). Wash 3 times using wash solution 300ul. Add 100 ul conjugate (con) and cover plate with adhesive strip. Incubate for 30 min at room temperature (RT). Wash 3 times using 300ul wash solution. Add 100 ul substrate and incubate for 10 min at (RT). Add 100ul stop solution and read the absorbance at 450 nm within 10 min.

### 2.7 Data Processing

All data was entered and analyzed using statistical analysis software (SPSS) (statistical package for social sciences) frequencies, confidence regression were used for correlation, and descriptive statistic also used. The difference would consider significant when be value is less or equal 0.05.

### 2.8 Tool for Data Collection

Laboratory samples, clinical records and constructed questionnaire were used for collection of clinical and baseline data.

## 3. RESULTS

During the study period a total of 100 subjects enrolled 72 pregnant women in first trimester with

recurrent miscarriage and 28 healthy pregnant women in first trimester with no history of obstetric complication was admitted to the Khartoum state hospitals (Khartoum teaching hospital, Bahry teaching Hospital, AL raja, Janeen), for assessment of acquired thrombophilia in women with recurrent miscarriages. Antiphospholipid antibodies were detected in (30.5%) of women with recurrent miscarriages.

The frequency of antiphospholipid antibody syndrome (IgM) was significantly elevated (19.4%) in pregnant women with recurrent miscarriage compared to antiphospholipid antibody (IgG) was (11.1%) (P value≤0.001, and 0.005) respectively; which indicate the risk of acute (recent) infection was much higher than chronic (old) infection. The thrombocytopenia among case group, were found to have low platelets count (<50×10<sup>9</sup>/l) observed in 10

(13.5%), as well as prolongation of activated partial thromboplastin time (APTT) among studied group were detected among 19 (26.1%), which were too higher compared to control group, all data was summarized in Table 1. Table 2 illustrates the frequency of maternal age and number of recurrent miscarriages in pregnant women.

Table 3 illustrates the frequency of Recurrent Miscarriage and Antiphospholipid antibodies among pregnant women in different hospitals in Khartoum state 2020. Higher frequency of miscarriage detected in Al raga hospital 40.2%, however elevated prevalence of APL was revealed among patients attending Bahri hospital. A statistically significant differences between APL (IgM) and thrombocytopenia and prolonged APTT (P value 0.003), data displayed in Table 4.

**Table 1. Frequency of Antiphospholipid antibodies, platelets, and APTT among cases and control**

Parameters	Case n=72 (%)	Control n=29 (%)	P value
Antiphospholipid antibodies			
IgM anti-cardiolipin	19.4%	0	0.001
IgG anti-cardiolipin	11.1%	0	0.005
Total	72 (30.5%)	-	
Thrombocytopenia (<50×10 <sup>9</sup> /l)	10 (13.5%)	1 (3.4%)	0.001
APTT	19 (26.1%)	2 (6.7%)	0.001

**Table 2. Prevalence of maternal age and number of recurrent miscarriage among pregnant women**

Age	Miscarriage No			
	2	3	4	6
≤ 20 years old	-	-	-	-
20-25 years old	21.7	47.8	8.7	-
26-30 years old	21.2	39.4	15.2	3
31-35 years old	20.7	17.2	20.7	-
36-40 years old	30.8	46.2	7.7	-
Total	22.2%	35.4%	14.1%	1%

**Table 3. Frequency of recurrent miscarriage and antiphospholipid antibodies among pregnant women in different hospitals in Khartoum state 2020**

Hospital	Recurrent Miscarriage%	APL(Positive) %
Bahri	30.5 %	13.9 %
Al-Rga	40.2 %	9.7 %
Khartoum	26.3 %	6.9 %
Jeneen	-	-
Total	97%	30.5%

**Table 4. Association between antiphospholipid syndrome, platelet count and APTT among pregnant women with recurrent miscarriage**

Antiphospholipid antibodies	Platelets n=10 (%)	APTT n=19 (%)	P Value
IgM	8 (80%)	16 (84.2%)	0.003
IgG	2 (20%)	3 (15.8%)	0.076
<b>Total</b>	10 (100%)	19 (100%)	-

#### 4. DISCUSSION

Recurrent miscarriage (RM) is considered one of the most common causes of sterility. It had drawn a greater attention in the last decades because of it's a greater increasing among reproductive-aged women. Antiphospholipid syndrome is only proven thrombophilia that is associated with adverse pregnancy outcome, there were few published studies have been done in recurrent miscarriage in Sudanese women hence current study was aimed to determine and evaluate the frequency of acquired thrombophilia and to find the relationship with platelet, coagulation factors and antiphospholipid antibodies.

Present study was revealed high prevalence of positive antiphospholipid antibodies among study participants (30.5%), and none of the controls were positive. Our finding was much higher than that reported by Rajewski M et al who noted that prevalence of APL antibodies in 12.4% of ladies with recurrent pregnancy loss [13]. Same result concluded by Högdén A et al. [14], who stated that patients diagnosed antiphospholipid syndrome, particularly those with maternal antiphospholipid syndrome and triple antibody positive, are much more likely to have a negative pregnancy outcome, even if they are on antithrombotic medication. In high-risk patients, more frequent prenatal checks can improve outcomes even further.

Moreover, anti-cardiolipin IgM was detected in 19.4% of participants, while IgG was revealed in (11.1%) and 23.6% of subjects has both IgG, and IgM, Spegiorin LC et al. [15] documented that increased level of IgM ACA levels was observed among 41.1% of cases, and 17.6 percent had high IgG ACA levels, and 38.2 percent had both IgM and IgG ACA levels. The number of patients with high IgG and IgM immunoglobulin levels had no statistically significant difference. Our finding was similar to that globally reported in previous studies and literature which noted that the prevalence of anticardiolipin antibodies range between 15%-59% [16,17,18]. The fluctuations of prevalence of antibodies may attributed to difference in sample size, methodology used,

and status of participants and encounter co morbidities. Elevated titer of anticardiolipin antibodies revealed in present study is alarming, and should be taken as a reminder that hypercoagulability conditions should be investigated during pregnancy, particularly in relation to antiphospholipid antibodies, same conclusion reported by Di Prima FA, et al. [19].

Thrombocytopenia was reported among study participants with antiphospholipid antibodies as mild, and a sever thrombocytopenia when platelets count was ( $<50 \times 10^9/l$ ), which revealed in 10 (13.5%) patients. Our finding was come in contact with Di Prima FA et al. [15], who conclude that antiphospholipid antibodies cause an overproduction of tissue factor and thromboxane A2 by activating endothelial cells, monocytes, and platelets. Complement activation could play a key function in pathogenesis. These variables, when combined with the normal alterations in the hemostatic system that occur during pregnancy, result in hypercoagulability. This causes thrombosis, which is thought to be the cause of many of the pregnancy issues linked with APS. Many of the pregnancy issues linked with APS are thought to be caused by thrombosis.

With regard prolongation of activated partial thromboplastin time (APTT), our result was significantly found to be 26.1% in 19 patients have had prolongation of (APTT) (P value 0.001), and only 2 (6.7%) of control., additionally statistically significant correlation between presence of APL antibodies and decreased count of platelets, and prolonged APTT. This is sin agreement to finding reported by et al. [20] who revealed APTT was prolonged in (8%) of the patients, although it was normal in all of the controls. Three of the eight patients (18.7%) were found to be Antiphospholipid antibodies positive. A prolonged activated partial thromboplastin time (APTT) could reflect a deficit in one or more elements, therapeutic anticoagulation, the presence of a nonspecific factor inhibitor, or lupus anticoagulant (LA). Hence the LA-sensitive APTT and standard APTT reagents, have lately been found to be

successful in antiphospholipid antibodies diagnosis [21].

Any women with recurrent miscarriage or recurrent pregnancy loss two or more losses before 20 week of gestation should be screened for antiphospholipid antibodies and other cause of recurrent miscarriage. Moreover, a full coagulation assay should be done routinely to any pregnant women with positive antiphospholipid antibodies. Additionally, titration of (IgG) Antibodies in pregnant women with antiphospholipid antibodies should be done periodically to determine the severity of the disease.

## 5. CONCLUSION

Higher prevalence of antiphospholipid antibodies, and acquired thrombophilia was detected among Sudanese women with recurrent abortion; which is alarming results associated increase risk of thrombosis, and hyper-coagulable state that could lead to recurrent miscarriage among pregnant women.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT AND ETHICAL APPROVAL

The study was adopted by the Faculty of Medical Laboratory Science, Alzaeim Alazhari University ethical board. The consent was also taken from all participants before collection of samples. Personal identification was eliminated and each participant is assigned a unique identification number. Collected data had been secured and used only for research purposes.

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## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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