



## Detection of Lupus Anticoagulant Antibodies Among Sudanese Women With Habitual Abortion

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### ABSTRACT

This study is a cross- sectional study, conducted in Khartoum state during the period from December 2013 to July 2015 to determine lupus anticoagulant among Sudanese women with habitual abortion. Onehundred and Forty patients with three or more habitual abortion were enrolled in this study and sixty healthy pregnant women without history of abortion were selected as control group. The age was ranging between 17 -45 years. Patients and control group were screened for the presence of LA by using coagulometer (CoatronM2), ACA by using enzyme linked immunosorbent assay (ELISA). The results were analyzed by using the program Statistical Packages of Social Sciences version 21 (SPSS program) and express as means. The results showed that the means of LA, Fibrinogen level and ACA for patients were  $(61.4 \pm 21.6)$ ,  $(265.3 \pm 118.9)$  and  $(242.5 \pm 124.3)$ , respectively, compared to the meanof LA, Fibrinogen level and ACA forcontrol group were  $(36.4 \pm 4.6)$ ,  $(255.1 \pm 68.5)$  and  $(40.4 \pm 4.5)$  respectively. Thirty seven of the patients (26.4 %) had lupus anticoagulant while the geographical distribution of the One hundred and Forty patientswere: 23 of them (16.4 %) from South Kurdfan. The study concluded that the presence of LA was significantly associated with habitual abortion.

**KEYWORDS** :*Recurrent pregnancy loss, Lupus Anticoagulant, Anticardiolipin Antibodies*

### المستخلص

هذه الدراسة الوصفية أجريت في ولاية الخرطوم في الفترة من ديسمبر 2013 الي يوليو 2015م لتحديد مضاد التجلط لوبص في النساء السودانيات المصابات بإجهاض متكرر ، تم اختيار مائة وأربعون مريضة مصابة بالإجهاض المتكرر ( ثلاث أو أكثر ) . كما تم أخذ ستون عينة من نساء حوامل لا يعانين من مشاكل لها علاقة بفقدان الحمل كمجموعة ضبط تراوحت أعمارهن من 17 الي 45 سنة .تم اختيار كل النساء في الدراسة لمضاد التجلط لوبص باستخدام جهاز قياس التجلط ، (Coatron M2) مضاد الكارديوليبيين باستخدام تقنية ELISA وتم تحليل النتائج بواسطة برنامج الحزم الاحصائية للعلوم الاجتماعية إصدار 21 ، تم حساب المتوسط وكانت النتائج كالآتي : متوسط مضاد التجلط لوبص ، الفبرينوجين ومضاد كارديوليبيين  $(21.6 \pm 61.4)$  ،  $(118.9 \pm 265.3)$  ،  $(124.3 \pm 242.5)$  على التوالي للمرضى ، وبينما كان متوسط مضاد التجلط لوبص ، الفبرينوجين ومضاد كارديوليبيين في مجموعة الضبط  $(36.4 \pm 4.6)$  ،  $(255.1 \pm 68.5)$  ،

(4.5±40.4) على التوالي. وقد أظهرت الدراسة أن 37 من المريضات (26.4%) مصابات بهذا المضاد كما أظهرت الدراسة أن معظم المرضى من ولاية جنوب كردفان 23 (16.4%) خلصت هذه الدراسة إلي أن وجود مضاد التجلط لوبص له علاقة بالإجهاض المتكرر.

## INTRODUCTION

Recurrent pregnancy loss (RPL) is the loss of 3 or more spontaneous consecutive pregnancies <sup>(1)</sup>. There are many causes, such as genetic, anatomic, hormonal, medical and immunologic causes <sup>(2)</sup>. If properly screened the cause will be found in almost all women <sup>(1)</sup>. The most common singular defect in women with recurrent miscarriage syndrome is a hemostatic defect and if thorough-antiphospholipid evaluation is performed. [The most common of these is found to be antiphospholipid syndrome <sup>(1)</sup>. Antiphospholipid antibodies are acquired autoantibodies against a phospholipid that have been associated with slow progressive thrombosis and infarction in the placenta <sup>(3)</sup>. Lupus anticoagulants and anticardiolipin antibodies belong to a heterogeneous group of antibodies directed against negatively charged phospholipids. Although antiphospholipid antibodies are frequently found in otherwise healthy persons, they have been associated with thrombosis, thrombocytopenia, and fetal loss <sup>(4)</sup> as well as a variety of other clinical condition<sup>(5)</sup>. Extensive placental infarction in patients with spontaneous abortion or fetal loss and antiphospholipid antibodies has been reported in some studies <sup>(6)</sup> but not in others <sup>(7)</sup>. An underlying pathophysiologic mechanism is still being sought for fetal loss and thrombosis. A number of therapeutic approaches have been proposed to prevent pregnancy loss, <sup>(8)</sup> but their efficacy has not yet been evaluated in randomized clinical trials. Most studies suggesting an association between pregnancy loss and lupus anticoagulant or anticardiolipin antibodies involve a case series of

patients. Thus, antiphospholipid antibodies have not been convincingly shown to be a risk factor for pregnancy loss.<sup>(9)</sup>

## MATERIALS and METHODS

This study is a case-control study, conducted in Khartoum state during the period from 2013 to 2014 to determine lupus anticoagulant among Sudanese women with habitual abortion. 140 patients with three or more habitual abortion and 60 healthy pregnant women without history of abortion were selected as control group. The age was ranging between 17 -45 years. Patients and control groups were screened for the presence of LA by using coagulometer (CoatronM2), ACA by using enzyme linked immunosorbent assay (ELISA). 4.5 ml of whole blood was collected from all patients and control group in 0.5 ml (3.8 % ) Tri-sodium citrate as anticoagulant and proceed for plasma separation using centrifuge at 4000 RPM for 5 minutes. The obtained plasma was separated from the red blood cell using a plastic pipette, was placed it in a plastic test tube, was frozen at -70°C., and was stored until performing the tests. Lupus anticoagulant and fibrinogen were performed using coagulometer (CoatronM2), ACA was detected by an ELISA method. The collected data was analyzed to obtain the mean, standard deviation. Frequencies and the probability (p- value) between patients and control using Statistical package of Social Sciences ( SPSS version 21) computer program .

## RESULTS

The result of the study showed that the most affected age of patient in range between (28-33) years comprising (27.9%) of the study group, another

affected age group was between (40-45) years comprising (5.0%) of the study group, see table (1). In addition, the most affected population was from South Kurfan, were 23 (16.4%) were affected. As for Central Darfur only 1(0.7%) was affected, see table (2). The mean of LA was significantly increased in patients with habitual abortion compared with control group (p, value 0.00 ). Also the mean of ACA was significantly increased in women

habitual abortion compared with control group (p, value 0.00 ). The mean of fibrinogen level was insignificantly increased in habitual abortion women compared with control group (p, value 0.449), see table (3). There was significant correlation between the presence of lupus anticoagulants (LA) and the age (p= 0.03 ) in women with habitual abortion. It is seen more in age group between 34-39 years, see table (4).

Table 1: Distribution of Study population's according to age group

Characterize Ages/years	N(%)	
	Number of Patient and %	Control and %
16-21	19 (14)%	17(28)%
22-27	37 (26)%	16(27)%
28-33	39(28)%	20(33) %
34-39	38(27)%	5(8)%
40-45	7(5)%	2(3)%
Total	140 100	60 100

Table 2: Sudanese States distribution of patients and controls

Sudan States	Patients	%	Control	%
White Nile	12	( 9) %	3	(5) %
Khartoum	13	(9) %	5	(8) %
Al Qadarif	5	(4) %	3	(5) %
Northern	17	(12) %	5	(8) %
River Nile	3	(2) %	4	(7) %
Sennar	8	(6) %	5	(8) %
North Darfur	8	(6) %	6	(10) %
West Darfur	6	(4) %	2	(3) %
South Darfur	2	(1) %	0	(0) %
East Darfur	4	(3) %	0	(0) %
South Kurfan	23	(16) %	4	(7) %
North Kurfan	16	(11) %	6	(10) %
West Kurfan	2	(1) %	0	(0) %
Central Darfur	1	(7) %	0	(0) %
Kassala	5	(4) %	2	(3) %
Blue Nile	0	(0) %	3	(5) %
Red Sea	0	(0) %	3	(5) %
Al jazirah	10	(7) %	9	(15) %
Total	140	100	60	100

Table 3: The mean of LA, ACA and Fibrinogen levels in case compared to control group.

Parameters	Mean ± SD		P.value
	Patients	Control	
Lupus Antibodies(LA)	61.4 ± 21.6	36.422 ± 4.6	0.00
AntiCardiolipin Antibodies (ACA)	242.5 ± 124.3	40.4 ± 4.51	0.00
Fibrinogen	265.28 ± 118.9	255.1 ± 68.5	0.449

P. value < 0.05 is significant

Table 4: Distribution of LA, ACA and fibrinogen levels according to age groups

Age groups/years	Mean ± SD		
	Lupus antibodies screening/seconds	Anticardiolipin antibodies U/ml	Fibrinogen mg/ 100mL
16 – 21	60.3±10.3	226.8±108.9	260.2±130.9
22 – 27	57.0±15.0	241.3±121.4	278.9±109.0
28 – 33	61.4±26.4	259.9±138.9	275.4±134.6
34 – 39	66.2±25.6	239.5±121.1	236.9±96.4
40 – 45	61.5±20.2	210.9±131.5	304.9±156.3

## DISCUSSION

The mean of LA was significantly increased in women with habitual abortion compared with control group ( $p < 0.05$ ). The mean of ACA was significantly increased in habitual abortion compared with control group ( $p < 0.05$ ). In the present study there was insignificant difference between the mean level of fibrinogen in study group of patients and control group ( $p > 0.05$ ). The study is consistent with the study of Tagreet and Tagwawhich shows statistically significant difference of lupus anticoagulant in recurrent miscarriage which compared between Sudan and other countries. The present study showed that 26.4 % of patients with habitual abortion had a positive result for LA, while 34.3 % had positive for ACA. These findings are consistent with studies, which has been done in Iraq, India and Nigeria. In Iraq study the frequencies of LA and ACA in sera of women with recurrent miscarriage were (LA 13.5 %, ACA 26.5 %) carried out by <sup>(12)</sup>. In Indian the frequencies of ACA in sera of women with recurrent miscarriage was 40% carried out by <sup>(13)</sup>. Another report by <sup>(14)</sup>

from Nigeria shown that the frequencies of LA were 4.8 % . The prevalence of ACA in patients with habitual abortion , as determined in this study is 34.3% and this is lower than the rate reported by <sup>(13)</sup> on the other hand, a much higher frequency of 40.3 % was reported from India. Also the results of the current study were confirmed by <sup>(10)</sup>, the study showed that 4% of women with recurrent had LA, other result of study in Sudan done by <sup>(11)</sup>, which agreed with our result. The variations noted in the rates of LA and ACA is related to patient selection, various pre-analytical variable, differing sensitivities of tests, isotypes tests and the population studies<sup>(16)</sup>. 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 <sup>(17-18)</sup>.

## CONCLUSION

In conclusion, the presence of LA and ACA in sera of women with recurrent abortion were significantly associated with recurrent abortion and the age. It is there for advisable to routinely use the test (dRVVT) when screening for LA.

LA is a significant cause of recurrent fetal loss in Sudanese women. The dRVVT is the best screening test for LA. The detection of LA in the healthy pregnant Sudanese women without history of abortion is absent according to our study.

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