

## Original Article

# Incidence of anticardiolipin antibodies and lupus anticoagulant factor among women experiencing unexplained recurrent abortion and intrauterine fetal death

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Received January 11, 2015; Accepted February 28, 2015; Epub March 1, 2015; Published March 15, 2015

**Abstract:** The aim of this research study was to estimate anticardiolipin (IgG & IgM) antibodies (aCL) and lupus anticoagulant (LA) factor in patients of recurrent unexplained pregnancy loss and intrauterine fetal deaths (IUFD). 82 women were selected for this study by virtue of having more than two consecutive unexplained pregnancy losses in their first trimester and were referred by the department of Obstetrics and Gynecology, King Saud Medical City Hospital, Riyadh, KSA. All patients had gone through a standardized investigation sequence. Lupus anticoagulants and Anticardiolipin antibodies (IgM and IgG) were detected in the serum by the enzyme linked immunosorbent assay method. To check the significance of aCL and LA, two-tailed t-test was done. Non parametric data was calculated either by Chi-Square test or Fischer exact test when relevant. Total 82 females grouped as 52 cases of recurrent ( $\geq 2$ ) mainly first and second trimester miscarriage and 30 cases of recurrent ( $\geq 2$ ) late intrauterine fetal death. Lupus anticoagulants was observed in twenty one (21) cases (25.6%) while anticardiolipin antibodies IgM and or IgG positive cases were estimated in forty four (44) cases (53.65%). The prevalence of APS in both studied group was thirty five (35) cases (42.68%). Antiphospholipid antibodies are calculated as the most important reason for recurrent abortion. The patients with unexplained recurrent pregnancy loss must be advised to go for a screening test for all this aPL antibodies.

**Keywords:** Anticardiolipin antibodies, antiphospholipid antibodies, intrauterine foetal death, lupus anticoagulant, recurrent pregnancy loss, unexplained infertility

## Introduction

Unexplained recurrent pregnancy loss is consistently detailed as more than two successive pregnancy losses. It found in about 1% of the general worldwide population focusing to have children. Autoimmunity performs an important role in recurrent pregnancy loss. It has been concluded that immunologic peculiarity may be a cause in many of such cases. The affected women have usually no other signs or symptoms referring an autoimmune disease.

Antiphospholipid syndrome is an extraneous condition, defined as the presence of thrombosis or pregnancy loss or maternal morbidity and perpetual circulating antiphospholipid antibodies (aPL) in plasma [1, 2]. Nowadays, antiphospholipid antibodies (aPL) are being noticed as the most frequently acquired risk factor for thrombophilia as they are responsible for clots in the placental blood vessels generating fetal growth retardation and as a curable cause for recurrent pregnancy loss [3]. APS can cause preeclampsia (18%), pregnancy-induced hypertension, foetal death (7%), retardation (31%), premature labor (43%), stillbirth, and ultimately sterility [4-7].

APS syndrome aggregate a heterogeneous group of circulating antibodies against anionic phospholipids with the most important ones are antiphosphatidyl choline, anticardiolipin antibodies (ACA), antiphosphatidyl serine, lupus anticoagulants and antinuclear antibodies [18, 19]. Inflated levels of immune markers of vari-

pholipid antibodies (aPL) are being noticed as the most frequently acquired risk factor for thrombophilia as they are responsible for clots in the placental blood vessels generating fetal growth retardation and as a curable cause for recurrent pregnancy loss [3]. APS can cause preeclampsia (18%), pregnancy-induced hypertension, foetal death (7%), retardation (31%), premature labor (43%), stillbirth, and ultimately sterility [4-7].

## Antiphospholipid antibodies (ACA and LA) in recurrent abortion and IUFD cases

**Table 1.** Clinical characteristics of the studied group (82 cases of recurrent miscarriage and IUFD)

N = 82		
1	Age (yrs) mean yrs $\pm$ SD	27.7 $\pm$ 5.3
2	Residency (n & %)	
	Rural	40 (48.7%)
	Urban	42 (51.2%)
3	Weight (kg) mean $\pm$ SD	67.5 $\pm$ 7.3
4	Height (cm) mean $\pm$ SD	164 $\pm$ 4.7
5	Body Mass Index (BMI), kg/m <sup>2</sup> mean $\pm$ SD	27 $\pm$ 3.5
6	Hb% gram (mean $\pm$ SD)	114 $\pm$ 0.95

ous types of antiphospholipid antibodies are thought to be concerned with an immune reaction to an implanting embryo on trophoblast intracellular fusion, hormone secretion and invasion may cause early pregnancy loss. Once placentation is settled, thrombogenic action points to decreased placental perfusion and subsequent infarction [5].

As for the pathogenesis of APS, there are several known interpretations relating to the pathogenic role of aPLs which shows displaced placental function. In pregnancy, placenta is the target organ of APS, where ischemic infarctions evolve due to intimal proliferation, fibrinoid necrosis and intraluminal thrombosis of the spiral arteries [5, 6, 8].

Arachidonic acid metabolites fluctuation establish in the placenta in presence of aPLs. In the endothelial cells, aPLs disrupt the production of prostacyclins which plays an important role by helping vasodilatation and obstructing platelet aggregation following in the aggregation of the procoagulant thromboxane A<sub>2</sub>. On the other way, after connecting with placental anticoagulant proteins, aPLs restrict the behavior of natural anticoagulants [6, 10]. In addition, in the first trimester of pregnancy, aPLs may cause ataxia of placentation and embryonal implantation by precisely inhibiting hormonal secretion and aggression of trophoblasts [6].

The aim of this work was to assess the prevalence of Anti phospholipids syndrome in the cases of unexplained recurrent miscarriage and correlation between the level and the type of the antibody if found positive.

### Material and methods

This research study was carried out in the Department of Obstetrics and Gynecology, King

Saud Medical City Hospital, Riyadh, KSA. Eighty two (82) women, age group of 22-35, were selected for this study after examining with all standard parameters' including STDs, hormonal disorders and metabolic disorders [9, 10]. In all cases full history and complete physical examinations including body weight, height and body mass index were measured. Subjects were excluded from this research study if having any uterine abnormalities, endocrinal irregularities, suffering from any disease like diabetes mellitus, thyroid dysfunction, STD or having hypertension, detected Rh- negative, or abnormal semen findings if found in their male partner.

### Lupus anticoagulant and anticardiolipin estimation

Lupus anticoagulant (LA) estimation was performed according to the guidelines of the International Society on Thrombosis and Haemostasis, ISTH [11]. Screening for LA was performed by the Kaolin Cephalin Clotting Time (KCCT) testing and by the Dilute Russell's Viper Venom Time (DRVVT) test.

In these assay the enzyme and the cofactor of the clotting cascade connect with the phospholipids and forms a template. This template interrupts with the timely interaction of the clotting factors resulting in a delayed clotting time which can be confirmed by another confirmatory test. For this test 9 ml of blood sample was collected in 1 ml of anticoagulant (0.109 M trisodium citrate). Centrifugation of blood samples was done for 15 minutes at 2,500 rpm. Plasma remains stable for 4 hours at 20  $\pm$  5°C.

Serum is the suggested sample for the identification of aCL. Therefore, 5 ml of blood sample was collected from each selected patient and serum was separated. This purified serum sample was used for the estimation of aCL (IgG and IgM) by the ELISA technique as described by Harris et al [12].

### Statistical analysis

Two tailed t-test was used to calculate significance variance of positive aCL. Non parametric data was calculated by using Chi-Square test or Fischer exact test. Statistical significance was confirmed when *P* value < 0.05.

## Antiphospholipid antibodies (ACA and LA) in recurrent abortion and IUFD cases

**Table 2.** Clinical characteristics' of the studied group (82 cases of RM and IUFD), classified in two groups

s. no	Character	Abortion N = 50	IUFD N = 32	P value	
a.	Age (yrs) (mean ± SD)	28.3 ± 5.4	29.2 ± 5.3	0.08	NS
b.	Weight (Kg) (mean ± SD)	66.3 ± 7.5	68.8 ± 7.2	0.16	NS
c.	Height (cm) (mean ± SD)	165 ± 5.3	162 ± 4.2	0.30	NS
d.	BMI (Kg/m <sup>2</sup> ) (mean ± SD)	26.9 ± 3.5	27.1 ± 3.6	0.03	NS
e.	Hb% (gm%) (mean ± SD)	11.3 ± 0.99	11.5 ± 0.89	0.15	NS

The correlation between the number of abortion and increasing maternal age, weight, BMI, and APA was observed. All of these factor show highly significant positive correlation ( $P = 0.016, 0.006$  and  $0.003$ ) (Table 5).

**Table 3.** Prevalence of antiphospholipid antibodies in studied group

N = 82	
Lupus anticoagulant positive (LA)	21 (25.6%)
ACA	
IgM positive only	19 (23.17%)
IgG positive only	25 (30.48%)
ACA	
IgM and IgG positive	18 (21.9%)
IgM or IgG positive	26 (31.7%)
Both are negative	57 (69.5%)
All negative (ACA IgM/IgG and LA)	47 (57.3%)
One of them positive	35 (42.68%)

The correlation between APLAs antibodies showed that LA positive cases were associated with ACA IgM and/or IgG positive cases were found in twenty one (21) cases (Table 6).

### Discussion

Antiphospholipid syndrome is a multiple system disease with the prevalent features of venous/arterial thrombosis, recurrent miscarriage and the existence of antiphospholipid antibodies. Many epidemiological studies focus on the presence of antiphospholipid syndrome (APS) as a cause of recurrent spontaneous abortion (RSA). Auto antibodies autoimmune conditions are frequent causes underlying unsuccessful attempts for having a child. In a significant number of cases these are not recognized as the affected women have no any sign or symptoms usually suggest that having an autoimmune disease. APS can also cause preeclampsia (18%), pregnancy-induced hypertension, foetal death (7%), retardation (31%), premature labor (43%), stillbirth, and eventually also sterility [4-7]. Various research studies also focus on antiphospholipid antibodies syndrome (APS) as a cause of recurrent spontaneous abortion (RSA). In different clinical studies, APS-related abortions occurred with an incidence of 15-90% [9]. APS was the underlying cause in 36% and 16% of abortions/foetal deaths occurring in early and late pregnancy respectively [11, 12]. The catastrophic APS (CAPS) is an unusual (< 1%) but a life-threatening variant of APS (results in widespread thrombotic microangiopathy and multiple organ failure) that appears infrequently during pregnancy, especially in those women with previous history of abortions and/or thrombosis. It is important to consider the possibility of the development of CAPS because it can lead to a high mortality rate for the mothers (46%), and for the babies (54%) [13].

### Results

A total of 82 women with a history of two or more consecutive unexplained recurrent fetal loss were selected for this study (Table 1). These included fifty (50) of recurrent ( $\geq 2$ ) mainly first or second trimester abortions and thirty two (32) cases of recurrent ( $\geq 2$ ) late intra uterine fetal death. Baseline characteristics are shown in Table 2.

The prevalence of antiphospholipid antibodies among the studied group showed that LA was present in 21 (25.6%) cases. Anticardiolipin antibodies IgM or IgG positive cases showed prevalence of 26 cases (31.7%). Patient can be diagnosed as suffering from antiphospholipid syndrome if they contain any one of these antibodies. The prevalence of APS was diagnosed in total of 35 (42.68%) cases (Table 3).

In the 21 (25.6%) positive cases of LA, 15 (30%) cases were of abortion group while 6 cases (18.75%) were of IUFD group ( $P = 0.45$ ). The prevalence of ACA (IgM) antibodies were detected in 12 (24%) cases of abortion group and 7 (21.8%) cases of IUFD group ( $P = 1.00$ ).

Regarding ACA (IgG) positive cases in abortion group was 17 (34%) while in IUFD group positive cases were 8 (25%) ( $P = 0.63$ ) (Table 4).

In the current research study, eighty two cases of unexplained pregnancy loss (recurrent abor-

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**Table 4.** Prevalence of positive APA (LA and ACA among studied group)

N = 82; Abortion = 50, IUFD = 32				
Markers	Abortion N = 50	IUFD N = 32	Total N = 82	P value
Lupus anticoagulant (LA)				
(n & %)	15 (30%)	6 (18.75%)	21 (25.6%)	0.45
(mean ± SD)	2.58 ± 0.42	2.93 ± 0.37	2.75 ± 0.39	0.42
Anticardiolipin antibodies (ACA)				
IgG positive	12 (24%)	7 (21.8%)	19 (23.1%)	1.00
(mean ± SD)	18.55 ± 4.62	28.6 ± 4.32	23.5 ± 4.47	1.09
IgM positive	17 (34%)	8 (25%)	25 (30.4%)	0.63
(mean ± SD)	29.3 ± 3.56	33.7 ± 3.81	31.5 ± 3.68	0.85
ACA Both (IgM and IgG) Positive	11 (22%)	7 (21.8%)	18 (21.9%)	1.00
ACA both IgM and IgG negative	32 (64%)	24 (75%)	56 (68.2%)	0.72

**Table 5.** Correlation between the no. of abortion and antiphospholipid antibodies level, age, weight and BMI

	Number of abortion (82)	
	Coefficient (R)	P value
APA	0.225	0.042
Age	0.264	0.016
Weight	0.298	0.006
BMI	0.318	0.003

tion and IUFD) were included. We found that the prevalence of APS among those cases was 42.68%. The results are consistent with the previous reports that 8-42% of recurrent pregnancy loss is due to positive aCL [14-16]. Additionally our results also indicate that 25.6% of women with recurrent pregnancy loss were positive for LA and this result is consistent with Velayuthaprabhu and Archunan, 2005 [17]. The presence of aCL has been noted in the sera of women who had a recurrent abortion for which no other cause was found [18, 19].

Most authorities agree that lupus anticoagulant (LA) and anticardiolipin (aCL), the two best characterized antiphospholipid antibodies are associated closely with recurrent pregnancy loss [20-23]. A research study confirms the higher frequency of antiphospholipid antibodies in women with recurrent pregnancy loss, shows that only LA and aCL level were elevated and clinically relevant. Using cut off at the 95th percentile, 16% women with recurrent miscarriage and 6% of fertile controls tested for IgG anticardiolipin antibodies [24]. These results are considerably higher than those reported by others in healthy adults [25, 26].

In our research study percentage of ACA is higher than lupus anticoagulant in both studied group. Lupus anticoagulant was positive in 30% of abortion and 18.75% of IUFD cases while ACA IgG positive cases were 34% in abortion group and 25% on IUFD group. Various research studies also have confirmed a strong correlation between anticardiolipin antibodies (IgM/IgG) and lupus anticoagulant. It was found that ACA were twice of lupus anticoagulant [27]. A research study found that ACA is five times more often than LA in patient with APS [28].

There is strong correlation between number of abortions and the level of LA, and with level of ACA in some clinical research studies [27, 28]. This is with agreement of our study (Negative predictive value with LA 84.2% and with ACA IgM 85%). In the current study we concluded that only correlation is between number of abortion and LA but not with either anticardiolipin antibodies IgM or IgG. There was a positive correlation between the number of abortion and the increasing maternal age, weight, BMI, and APA.

### Acknowledgements

The authors are very thankful to be supported by grant from the Research Centre of the "Centre for Female Scientific and Medical colleges", Deanship of scientific research, King Saud University, Riyadh to carry out the research.

### Disclosure of conflict of interest

None.

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**Table 6.** Association between different antiphospholipid antibodies among the positive cases of the studied group

s. no	Number N = 82	Number (N) and percentage (%)
1	Cases positive for both LA and ACA (IgM)	5 (6.09)
2	Cases positive for both LA and ACA (IgG)	11 (13.4)
3	Cases positive for all	5 (5.09)

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