

# Anticardiolipin antibody in women with recurrent spontaneous miscarriage

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## ABSTRACT

**Objectives:** To determine the association between the presence of anticardiolipin antibody (ACA) and history of recurrent spontaneous miscarriage. Also, to evaluate the association between raised ACA and activated partial thromboplastin time (APTT).

**Methods:** This is a case-control study which was carried out in Basrah Hospital for Maternity and Children, Basrah, Iraq during January to September 2004 on 91 women with recurrent spontaneous miscarriage, matched with 109 women with no history of pregnancy wastage. Sera were collected from these 200 women and analyzed for ACA by enzyme linked immunosorbent assay (ELISA).

**Results:** In women with pregnancies that ended with a loss,

17.6% were positive of ACA, compared with none among the control group. Women with 4 or more miscarriages had almost higher percentages of ACA (26.3% and 22.2%) than women with only 3 miscarriages (13%) but with no statistical significance. Prolonged APTT was detected among 18.8% of patients having positive ACA while only 1.6% of patients who were negative for this antibody.

**Conclusions:** A significant association was observed between recurrent spontaneous miscarriage and the presence of ACA. Also, there was a significant relationship between positive ACA and prolonged APTT.

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Anticardiolipin antibodies (ACA) are one the family of auto-antibodies directed against phospholipids,<sup>1</sup> may bind independently to the negatively charged phospholipid (in this case, they are called "authentic" (ACASs) or may require a cofactor, beta 2 glycoprotein-1 (B2GPI).<sup>2</sup> Anticardiolipin antibody syndrome is usually an acquired condition. There is no data to suggest that this is an inherited condition, and therefore it cannot be transmitted from mother to child. It is not a condition that may be transmitted from person to person through contact such as an infection.<sup>3</sup>

In pregnancy, the ACA may react against the trophoblast resulting in sub-placental clots and interfere with further placentation. Necrotizing

residual vascular lesions are seen in the placenta.<sup>4</sup> Thrombosis may occur in all trimesters of pregnancy resulting in complications such as spontaneous miscarriage and intrauterine growth retardation. Thus, ACA associated with an increased risk of platelets aggregation, venous and arterial thrombosis, recurrent fetal loss and thrombocytopenia.<sup>4</sup> In addition, pregnancy loss may be due to genetic, anatomic, endocrine, infectious or immunological factors.<sup>5</sup> Thus, this study was designed to determine the association between the presence of ACA and the history of recurrent spontaneous miscarriage. Also, to evaluate the association between raised ACA and activated partial thromboplastin time (APTT).

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**Methods.** This case-control study was carried out on 200 pregnant women who attended the outpatient gynecological clinics of Basrah Hospital for Maternity and Children, Al-Basrah General Hospital, Basrah, Iraq and those who attended a private clinic during the period January to September 2004. The subjects were randomly selected and arranged into 2 different groups, the first one (the study group) was consisted of 91 women with a history of 3 or more spontaneous consecutive first or second trimester or both miscarriages. The second group (control group), was of 109 women with one or more live birth and with no history of any spontaneous miscarriage, stillbirth, gestational hypertension, or low birth weight. Their ages ranged from 15-45 years. Elapsed time from the last delivery or miscarriage to blood sampling varied from 6 months to 2 years to assess the IgG antibody. Also, excluded in the study were women with a history of low birth weight, gestational hypertension, diabetes mellitus, women receiving aspirin, heparin or oral contraceptives.

**Blood samples.** Each woman was informed regarding the test and their consents were taken. Blood samples (4 ml each) were obtained from the patients at least after 6 months from the last miscarriage or normal delivery. The blood sample was divided into 2 groups, 0.9 ml of the blood collected into a tube containing trisodium citrate (3.2%) an aqueous solution W/V and mixed well for APTT examination. The rest of the blood was centrifuged for 10 minutes at 1200 rpm and the serum was kept at -20° C until needed.

**Laboratory diagnosis.** The APTT procedure measure the time required to generate thrombin and fibrin polymer via intrinsic pathway.<sup>6</sup> Six control blood samples were tested and their mean time was 33 seconds, with a normal range of 25-37 seconds, while any reading below this range was considered as a hypercoagulable state, within the normal range value and prolonged if the value was above the range. Anticardiolipin antibody assay was performed according to the manufacturer's instructions of the commercial enzyme linked immunosorbent assay (ELISA) kit of Aeskulisa Cardiolipin-GM, Aesku. Lab Diagnostika, Germany.<sup>7</sup>

**Statistical analysis.** Chi-square and Fisher's Exact tests were used for statistical analysis. Student's t-test was used for comparing the means and the differences between the 2 proportions. A test of Standard Normal Deviate was used to assess the significance of difference between 2 proportions. The significance level was set at  $p < 0.05$ .

**Results.** The mean age of the study group (29.7 ± 6.6 years) and that of the control group (28.3 ±

5.8 years) were comparable with no statistical differences between them ( $t=1.9, p > 0.05$ ). Out of the 91 patients with recurrent spontaneous miscarriage, 16 (17.6%) patients had positive ACA (IgG) test, while all of the control group were negative to ACA test (SND=25.4,  $p < 0.05$ ). The high percentage of positive ACA test results in the second trimester is due to the small number of women suffering from their second trimester pregnancy loss, statistically however, the difference was not significant (Fisher's Exact test=0.502,  $p > 0.05$ ) (Table 1). There was no significant effect of age on the pregnancy outcome in women with recurrent spontaneous miscarriages of immune etiology ( $X^2=2.084, p > 0.05$ ). Women with 4 or more miscarriages had almost higher percentages of ACA than women with only 3 miscarriages but with no statistical significance ( $X^2=2.06, p > 0.05$ ) (Table 2). Out of 16 patients who were positive to ACA test, 18.8% had prolonged APTT, while only (1.6%) of the whole negative (184) women to ACA test had prolonged APTT, provided that only one women of the control group had prolonged APTT. The results were statistically highly significant (Fisher's Exact test=8.21,  $p < 0.05$ ) (Table 3).

**Discussion.** The data shows that the level of ACA activity was higher among those with recurrent spontaneous miscarriage as compared with none of the controls, indicating a significant association between ACA and recurrent pregnancy losses. Similar findings were found in studies carried out in West Indies Mona, Kingston Jamaica in 2004.<sup>8</sup> It was found that 53 (38.4%) out of 138 Jamaican women who had recurrent spontaneous miscarriages were positive for IgG to cardiolipin.<sup>8</sup> A study in Jordan in 2001, found that in a group of 26 women defined as habitual aborters, 19.23% had positive ACA test results as compared with none of the control group.<sup>9</sup> Also found in northern Italy a raised ACA in 19% of women with miscarriage history, compared to 3% in

**Table 1 -** The anticardiolipin antibody (IgG) test results in relation to the timing of pregnancy.

Timing of pregnancy	No. of ACA (IgG) (%)		Total
	Positive	Negative	
1st trimester	10 (19.6)	41 (80.4)	51 (100)
2nd trimester	1 (33.3)	2 (66.7)	3 (100)
1st and 2nd	5 (13.5)	32 (86.5)	37 (100)
<b>Total</b>	<b>16 (17.6)</b>	<b>75 (82.4)</b>	<b>91 (100)</b>

Fisher's Exact test = 0.502,  $p > 0.05$   
ACA - anticardiolipin antibody, IgG - immunoglobulin G

**Table 2** - The relation between ACA (IgG) and frequency of previous miscarriage.

No. of previous miscarriage	No. of ACA test results (%)		Total	
	Positive	Negative		
3	7 (13.0)	47 (87.0)	54	(100)
4	5 (26.3)	14 (73.3)	19	(100)
≥5	4 (22.2)	14 (77.8)	18	(100)
<b>Total</b>	<b>16 (17.6)</b>	<b>75 (82.4)</b>	<b>91</b>	<b>(100)</b>

$X^2=2.06, df=2, p>0.05$   
ACA - anticardiolipin antibody

**Table 3** - The association between active partial thromboplastin time test results and the results of anticardiolipin antibody in both groups.

APTT test results	ACA test results		Total	
	Positive	Negative		
Normal	13 (81.2)	181 (98.4)	194	(97)
Prolonged	3 (18.8)	3 (1.6)	6	(3)
<b>Total</b>	<b>16 (100)</b>	<b>184 (100)</b>	<b>200</b>	<b>(100)</b>

Fisher's Exact test = 0.502,  $p>0.05$   
APTT - active partial thromboplastin time,  
ACA - anticardiolipin antibody

the control group.<sup>10</sup> In a work carried out on a Brazilian population, found that ACA positive occurred in 12.5% out of 88 women with recurrent spontaneous miscarriage while among another 88 fertile women, it was detected in 1.1%.<sup>11</sup> In a prospective study on 96 women with repeated spontaneous miscarriage in Senegal, 21.1% of them have ACA.<sup>12</sup>

The present study revealed that there was no significant relationship between ACA test results and timing of miscarriage (whether occur in the first or second trimester). Similarly, Al-Abri et al<sup>13</sup> found that the presence of ACA led to same frequency of unsuccessful pregnancies in the first and second trimester. In contrast, a study carried out in Switzerland, found that most of the pregnancy wastages occurred after the first trimester in ACA-positive patients and during the first trimester in ACA-negative patients.<sup>14</sup>

In agreement with this study, workers in USA showed that the prevalence of auto antibodies to cardiolipin was not different in relation to the various ages of women with recurrent spontaneous miscarriage.<sup>15</sup> The current study revealed a significant relation between positive ACA and prolonged APTT. Non specific inhibitors, such as lupus antibodies and ACA that bind to chemicals called phospholipids found on the surface of platelets. Since phospholipids assist in the clotting process, and since the APTT test reagents (chemicals used to run the tests) contain phospholipids, such antibodies may prolong the APTT.<sup>6</sup> Furthermore, APTT is sensitive to decreased concentrations of several coagulation Factors I, II, VIII, IX, X, XI and XII. Factor V acts as a co-factor to accelerate clot formation, called Factor V accelerin (Va). The inactivation of Factor Va is fundamental in controlling the clotting process and prevents expanding of the clot. In normal individuals, Factor Va generation and inactivation is regulated by activated protein C.<sup>16</sup> The ACA impair the normal functioning of protein C thus, delay the inactivation of Factor Va, leading to a

prolongation of the clotting process. Other proteins that are important in regulating coagulation, such as prothrombin, proteins C and S and annexin V may also be targeted by antiphospholipid antibodies.<sup>17</sup>

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