

Incidence of Anticardiolipin Antibodies Level in Patients with Recurrent Abortion

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Abstract

Background: Antiphospholipid syndrome is a major reproductive complication in women, that is characterized by recurrent abortion, thrombosis and thrombocytopenia in association with anticardiolipin antibodies .

Aims: To estimate the incidence of anticardiolipin (IgM & IgG) antibodies in pregnant women with the history of recurrent spontaneous abortion .

Design of study Case and Control : Serological estimation by Enzyme Linked Immunosorbent assay (ELISA) was done for the following tests:

1. Estimation of ACL IgM & IgG in (59) pregnant women.
2. Estimation of ACL IgM & IgG in (20) non pregnant women.

Methods & Materials: Fifty nine women with recurrent pregnancy loss in first and second trimester were selected and (20) non-pregnant women as control group. Anticardiolipin (IgM & IgG) antibodies were estimated in the sera by the enzyme linked immnvsorbent assay method (ELISA)

Results : The level of positive ACL IgM was 1.95 ± 0.8 , negative (0.62 ± 0.3 and control group 0.41 ± 0.21 , while the level of positive ACL IgG was 2.2 ± 1.05 , negative 0.54 ± 0.28 and control 0.39 ± 0.19 pg/mL.

The results showed 2 of 59 IgM positive, while 7 of 59 IgG positive.

Conclusion: Anticardiolipin antibodies in pregnant women are found to be the important factor for recurrent abortion especially in the first trimester of pregnancy.

Key words: Anticardiolipin, Antiphosphlipid Antibodies, Recurrent Abortion

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Introduction

Anticardiolipin syndrome is a major reproductive complication in women, which is characterized by recurrent fetal loss, thrombosis and thrombocytopenia in association with anticardiolipin antibodies (ACL) [1].

Pregnancy and related disorders in women of reproductive age group is common. Recurrent abortion is quite critical in which many factors play a crucial role including anticardiolipin , antiphosphatidyl serine antibodies and antinuclear antibodies. [2, 3]

The major underlying systems like anatomical, physiological and endocrine pathology are under investigation as well as management. Some studies hare been reported certain ACL interferes in very early pregnancy, that is at the stage of fetal implantation. This miscarriage or implantation failure may be related to pathological mechanism causing recurrent abortion, which is commonly diagnosed as infertility. [4]



There are many causes of early pregnancy loss, spontaneous abortion and intrauterine growth retardation. Although some of these losses are caused by an abnormal chromosome (genetic), others cannot be explained. It has been hypothesized that antibodies produced erroneously against the body's own tissues are involved in reproductive failure and fetal loss. [5]

Although, higher number of childhood onset lupus patients also had antiphospholipid syndrome, the antiphospholipid is widely abortion [6] There is clear documentation that ACL are involves in recurrent abortion irrespective of the patients whether having autoimmune disease or not [4] . Most of the women with pregnancy losses were negative for ACL and positive for other ACL [7] .

This study that aimed to estimate the anticardiolipin IgM & IgG antibodies in the sera of pregnant women with the history of recurrent spontaneous abortion.

Design of Study Case and Control

Subjects

A prospective study was done on the (59) pregnant women and (20) non-pregnant group as control. Their ages were ranged from (18-45) years and the period of samples collection was from (August 2010-December -2010).

Specimens

Three mL of blood samples were collected from pregnant and non-pregnant women, the whole blood samples were centrifuged for (5) minutes at (3000) rpm. The sera were kept in freezer until performed the tests.

Methods

Procedure of anticardiolipin IgM & IgG antibodies were detected by Enzyme linked immunosorbent assay (ELISA) as follows:

1. One hundred mL of each patient diluted serum pipetted in each well.
2. One hundred mL of each, negative, positive and cut off were pipetted in each well.
3. All the samples were incubated in incubator at (37) °C for 30 min.
4. The samples were washed with washing buffer (five times).
5. One hundred mL of conjugate were pipetted into each well.
6. All samples were incubated at (37) °C for 15 min.
7. All samples were washed (five times) by washing solution.
8. One hundred mL of TMB substrate were pipetted in each well.
9. All samples were incubated at (37) °C for 15 min.
10. One hundred mL of stop solution were pipetted into each well.
11. The absorbance of each sample was measured at 450nm (within 30min.).

Determination of Anticardiolipin Antibodies

According to the optical density of the cut-off control and the patient samples. Compare patients OD with the OD cut-off control.

All samples which are more than cut-off are considered positive, and the OD patient less than cut-off is negative .

OD patient > OD
cut-off = positive

OD patient < OD
cut-off = negative

All data were done by SPSS program (version- 10) and Excel application.

Statistical Analysis: All the statistical analyses were done by using Pentium- 4



computer through the SPSS program (version- 10) and Excel application

- The comparison of significant (P-value) in any test were:

S= Significant difference (P< 0.05)

HS= highly Significance (P<0.01)

NS= Non Significance (P>0.05)

Results

Table (1) expressed the occurrence of ACL IgM antibodies in pregnant and non-pregnant women. There are (2) of (59)

of pregnant women associated with antibody, while (57) of (59) were negative. No significant differences from negative and positive compared with control group (p > 0.05)

Table (2) Shows that are (7) of (59) of pregnant women have a positive ACL – IgG antibodies, while (52) were negative. Significant differences from positive, negative compared with control group (p < 0,05)

Table 1: Distribution of pregnant and non-pregnant women according to anticardiolipin- Ab (IgM)

	Studied groups		Total	Compassion of significance	
	Pregnant	Non-pregnant		P- value	Sig.
Anticardiolipin Ab (IgM)	Positive 2	-	2	0.7	N. S
	Negative 57	20	77	0.61	N. S
Total	59	20	79	0.58	N. S

Table 2 : Distribution of pregnant and non-pregnant women according to anticardiolipin- Ab (IgG)

	Studied groups		Total	Compassion of significance	
	Pregnant	Non-pregnant		P- value	Sig.
Anticardiolipin Ab (IgG)	Positive 2	-	7	0.3	S
	Negative 57	20	72	0.41	S
Total	59	20	79	0.23	S

Table (3) Show the mean concentrations in the sera of positive (1.95 ± 0.81), negative (0.61 ± 0.32) and control group 0.41 ± 0.21 Pg/mL. No significant difference from level of positive/negative compared with control group (P > 0.05)

Table (4) Show the mean concentrations in the sera of positive (2.2 ± 1.05), negative (0.54 ± 0.28) and in control group (0.39 ± 0.19) Pg/mL Significant differences from positive /negative compered with control group (p < 0.05)

Table 3: Distribution of positive / negative and control group according to ACL IgM conc. (Pg/mL)

ACL IgM Ab	No.	Mean	Std. Dev.	Comparison of significance	
				p-value	Sig.
Control	20	0.41	± 0.21	-	-
Negative	57	0.62	± 0.32	0.59	N. S
Positive2	2	1.95	± 0.81	0.91	N. S
Total	79	Negative vs. positive		0.61	N. S

Table (4): Distribution of positive / negative and control group according to ACL IgG conc. (Pg/mL) for studies groups.

ACL IgG Ab	No.	Mean	Std. Dev.	Comparison of significance	
				p-value	Sig.
Control	20	0.39	± 0.19	-	-
Negative	52	0.54	± 0.28	0.63	S
Positive2	7	2.2	± 1.05	0.03	S
Total	79	Negative vs. positive		0.03	S

Discussion

In present study, ELISA used to detect anticardiolipin (IgM & IgG) antibodies. Antiphospholipid syndrome is characterized in the presence of lupus anticoagulant as well as anticardiolipin antibodies, both cases were found in women with recurrent pregnancy loss [8, 9] This study noticed the (2) of (59) patients were recent infection (IgM) were positive, we have also observed (4) of (59) patients with old infection IgG positive. Many studies claimed that antiphospholipid syndrome might affect the outcome of pregnancy[10] .

There are two main adverse situations that are associated with antiphospholipid and pregnancy, first maternal venous thromboembolism, and second pregnancy complications associated with placental infraction, including miscarriage, preeclampsia and intrauterine death of premature birth [11] .

Our study is agree with Moerloss, 2008 [12] , that the presence of high titer of IgG anticardiolipin antibody is of greater clinical significance in identifying women at risk of pregnancy loss than IgM antibodies. AL-Abri *et al.*, (2000)[13] , they found that when both IgM & IgG anticardiolipin present, the rates of pregnancy losses was significant higher in first trimester than in the second trimester, while when only IgG alone, more abortion occurred in the second than the first trimester.

The causes of antiphospholipid antibodies production may related to the phospholipids molecules are ubiquitous in nature and are present in the inner surface of the cell (Inner or outer or intercellular organelles, and microorganisms including viral (HIV, EBV and CMV), bacterial (tuberculosis & Mycoplasma) Spirochaetal (syphilis) and parasitic (*plasmodium*), the disruption of cellular membrane may occur during cell damage and release and stimulate antiphospholipid antibodies [14] .

Conclusion

Anticardiolipin antibodies in pregnant women are found to be the important factor for recurrent abortion especially in the first trimester of pregnancy.

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