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Role of anticardiolipin antibodies in bad obstetric history detected by ELISA test in a tertiary care centre

Therese Mary Dhason[1*], Euphrasia Latha Jairaj[1], Rajeswari Sankaralingam[1], Bhuvanesh Mahendren[1], Balaji Chilukuri[1], Sivakumar Vengudusamy[1], Mythili Seetharaman[1]

[1] Institute of Rheumatology, Rajiv Gandhi Government General Hospital, Madras Medical College, Tamil Nadu, India.

Abstract

Background: Antiphospholipid syndrome (APS) is one of the causes of recurrent abortions and intrauterine death among pregnant women. This study was undertaken to find out the association between bad obstetric history (BOH) and anticardiolipin (aCL) antibody.

Material and Methods: Blood samples were collected from women with BOH referred (recurrent abortion, intrauterine death, PIH) to a Tertiary Care Center in Chennai, Tamil Nadu. Blood samples from 100 pregnant women with BOH and 110 healthy pregnant women were collected and tested for the presence of aCL antibody by ELISA Method.

Results: Among the 100 patients tested 21 (21%) were aCL positive; IgG was positive in 16 (76.19%) patients and IgM was positive in 5(23.80%) patients. Anticordiolipin antibody (aCL) positivity in BOH was statistically significant (p .0048) when compared to healthy pregnant women. There was a positive correlation (p 0.005) between IgG and BOH. The antibody titer was high in 12 (57.14%) and moderate in 6 (28.57%). Among the controls 8(7.2%) were aCL positive.

Conclusion: Screening of patients with BOH for aCL by ELISA will reduce foetal morbidity and mortality as there is a significant association between high, moderate titers of aCL, (IgG) and BOH.

Key words: Antiphospholipid syndrome, Anticordiolipin Elisa, Bad Obstetric History.

Introduction

The systemic autoimmune disorder which causes recurrent vascular thrombosis and pregnancy losses is antiphospholipid syndrome (APS). The pathogenesis of APS is production of auto antibodies to phospholipid protein. The diagnosis is based on clinical and laboratory criteria. The diagnostic markerswhich clinch the diagnosis of APS are persistent rise in antiphospholipid antibodies and lupus anticoagulant positivity (1). Antiphospholipid syndrome may be either primary or secondary. When APS is present in patients without any underlying clinical illness it is primary. Secondary APS occurs in patientswith systemic lupus erythematosus (SLE) or any other underlying autoimmune disease.

The autoantibodies are present in 50% of patients with SLE and 1-5% of the general population. The antiphospholipid antibodies are found in serum in 1% of healthy persons and 3% of older age group (2). Though APS can involve any age group, the target group is young to middle aged adults.

Depending upon the site of vascular obstruction, various symptoms occur in patients with APS. Interference in the balance between procoagulant and anticoagulant factors and disruption of the clotting mechanism by the antiphospholipid antibodies (APLA) lead to leg ulcers, toe gangrene, myocardial infarction, purpura, stroke, recurrent miscarriage or preterm births.

***Corresponding Author:** Therese Mary Dhason, Senior Assistant Professor of Microbiology (Immunology) Institute of Rheumatology Rajiv Gandhi Govt. General Hospital Madras Medical College E.V.R.Periyar Road, Chennai-600003, TamilNadu India. **E-mail:** tmary1964@gmail.com **Received:** Feb 25, 2017 **Accepted:** Jul 07, 2017

Pregnant women with bad obstetric history are screened for Rh incompatibility, TORCH infections, structural abnormalities in uterus and also for APS in Tertiary Care Centers. The pregnant women with BOH referred to immunology laboratory from various maternity hospitals were screened for the presence of aCL antibodies in our study.

Antiphospholipid syndrome is detected either by a positive anticardiolipin antibody (aCL) or lupus anticoagulant test (3). Anticardiolipin ELISA is the test done to detect the antiphospholipid antibodies. The test results are reported by the specific aCL isotype (IgG, IgM, and IgA) and their levels in serum. The unit for measuring the levels is the phospholipid (PL) unit and is denoted as GPL (G phospholipid) MPL (M phospholipid) or APL (A phospholipid). According to the Second International Anticardiolipin Standardization Workshop < 20 PL is low positive, 20-80 PL is medium positive and > 80 PL unit is high positive (4). Though a moderate to high aCL IgG is the most specific diagnostic marker in APS, some patients are only IgM positive and in few patients IgA aCL is the sole marker of disease (5).

According to the Revised Sapporo classification, APS is diagnosed when one of the clinical and laboratory criteria are met (6).

The appropriate management of pregnant women with APS, increase the fetal survival and delivery of a healthy infant in more than 70% of them. Though the treatment protocol has not been formulated by randomized trials in bad obstetric history, a combined therapy with aspirin and heparin has been suggested to improve the fetal outcome (7).

Antiphospholipid antibodies (APLA) has been attributed in the pathogenesis of APS in pregnant women with BOH. Hence a study focused on anticardiolipin antibodies in bad obstetric history was undertaken at a Tertiary Care Center in Chennai so as to treat them effectively thereby preventing abortions and stillbirths.

Materials and methods

A cross sectional study was undertaken in a Tertiary Care Center at Chennai to find out the significance between anti cardiolipin antibodies and bad obstetric history. The study was conducted over a period of 1yr from January 2016 to December 2016. The study group included 100 pregnant women with BOH and 110 healthy pregnant women.

Blood samples were collected from 100 pregnant women with history of BOH and 110 pregnant women with normal course of pregnancy. Serum was separated and tested for the presence of aCL IgG and IgM using ELISA kits procured from Calbiotech (CA). All the samples and the kit reagents were brought to room temperature. Test samples were diluted with sample diluent in 1:21 dilution. In the first step, 100 µl of calibrators, controls (PC and NC) and diluted sera were added into the appropriate wells coated with bovine cardiolipin antigen. To the well 1A given as Blank 100 µl of sample diluent was dispensed. The microtiter plate was incubated at room temperature for 20mts. Wells were washed three times with 300µl of wash buffer. In the next step100µl of enzyme conjugate (IgG) was dispensed in all the wells and incubated for 20mts. Wells were again washed three times with 300µl of wash buffer. Then 100µl of TMB (Tetra methyl benzidine) substrate was dispensed and incubated for 10mts at room temperature. Finally100µl of stop solution was added to all the wells. Optical density (OD) of calibrators, controls (PC & NC) and samples was read within 15 minutes in an ELISA Reader at 450 nm wavelength. The same procedure was done in parallel with IgM enzyme conjugate.

The test was considered valid if the OD of the calibrator was greater than 0.250, negative control was less than 0.9 and positive control was greater than 1.2. Antibody index was converted to GPL units by multiplying antibody index value by 11 and MPL units by multiplying antibody index value by 17.

Inclusion criteria

History of recurrent abortions at or more than 10 weeks.

History of one or more premature births before 34 weeks of gestation due to placental insufficiency.

Exclusion criteria

Anemia complicating pregnancy, TORCH positive, other systemic diseases. TORCH positive. Other systemic diseases. Structural abnormalities of uterus.

Statistical analysis

The data were analyzed by SPSS package. Fishers 2x2 test was applied to analyze the significance of anticardiolipin antibodies in BOH. Chi-square and Fisher-exact two-tailed tests were used to find out the significance of isotypes IgG and IgM anticardiolipin antibodies in BOH.

Results

Out of the 100 pregnant women with BOH tested for aCL antibody, 21 (21%) were positive. Among the 110 controls 8 (7.2%) were aCL antibody positive.

Out of the 21 positive for aCL antibody 5 (23.81%) were IgM positive and 16 (76.19%) were IgG positive.

ELISA test for detection of anti-cardiolipin antibody is considered positive when the antibody index is above 1.1. The aCL antibody concentration was calculated based on the formula specified in the kit. The aCL positivity in pregnant women with BOH was compared with healthy controls (Table 1).

Table 1. aCL positivity in diseased versus controls**.**

Study group	n	ACL Positive n	ACL Negative n	p value
Diseased	100	21	79	**0.**0048*****
Healthy Controls	110	8	102	

*Significant.

Anticardiolipin anti body isotypes IgG, IgM and IgA play a key role in the pathogenesis of antiphospholipid syndrome. The IgG isotype appears to be more closely associated with clinical manifestations than either the IgM or IgA isotype. The IgG isotype positivity in pregnant women with BOH was statistically significant when compared with healthy controls (Table 2).

Table 2. aCL IgG in diseased versus controls.

		APLA		
		Present	**Absent**	**p value**
IgG	Positive	16	5	
	Negative	84	105	0.005*

*significant (Chi-square:7.636, Fisher exact: 0.009).

The aCL antibody IgM in pregnant women with BOH when compared with healthy pregnant women was not statistically significant (Table 3).

Moderate titers (20-80 GPL or MPL units) and high titers (> 80 GPL or MPL units) of anticardiolipin antibodies have definite association with antiphospholipid syndrome. The titer of IgG and IgM anticardiolipin antibody in pregnant women with BOH was categorized as high, moderate and low (Table 4).

A large proportion of patients had high and moderate titers and a few had low titer CL.

Table 3. aCL IgM positivity in diseased versus controls.

		APLA		
		Present	**Absent**	**p value**
IgM	Positive	5	3	0.39
	Negative	95	107	

*Not significant (Chi-square: 0.738, Fisher exact: 0.48).

Table 4. aCL IgG and IgM titers among diseased (n: 21).

Iso type	High (%)	Moderate (%)	Low (%)
IgG	10(47.62)	4(19.05)	2(9.52)
IgM	2(40)	2(40)	1(20)
Total	12(57.14)	6(28.57)	3(14.28)

Discussion

Primary APS is the commonest cause of acquired thrombophilia and leads to deep veins thrombosis either with or without pulmonary embolism in 15% - 20% of individuals, young strokes in one third and habitual abortions and still birth in 10% - 15% of pregnant women (8). APS secondary to SLE is implicated in thromboembolic disorders and bad obstetric history in a large proportion of patients. In SLE 30% to 40% of patients produce antiphospholipid antibodies and 10% to 15% present with the clinical manifestations of antiphospholipid syndrome (9).

In a small proportion of healthy individuals, antiphospholipid antibodies were found to be positive. Though infectious diseases like syphilis, acquired immunodeficiency syndrome and others produce antiphospholipid antibodies, they do not incite clinical APS. Similarly some drugs induce a PLs production but do not cause antiphospholipid syndrome. As per the earlier studies, the first episode of stroke, coronary artery disease and venous thrombosis depends upon the presence of antiphospholipid antibodies (10).

In pregnant women with APS, abortions occur usually after 10 weeks gestation and sometimes earlier than 10 weeks. Pregnancy losses in pre-embryonic or embryonic period is attributed to genetic and chromosomal defects in most of the cases. The course of pregnancy is uneventful until second trimester in some pregnant women with APS. After second trimester, intrauterine growth retardation occurs and leads to oligo hydramnios. Some patients land up with severe, early eclampsia or HELLP (Haemolysis, Elevated liver enzymes, Low platelets) syndrome.

The role of anti-cardiolipin antibodies in bad obstetric history has been studied on animal models like mice. The mice when injected with human polyclonal or monoclonal antibodies or with murine polyclonal antibodies showed fetal resorption and small sized offsprings. The most important hypothesis on pregnancy loss in antiphospholipid syndrome is displacement of Annexin V proteinsfrom trophoblast surface by antiphospholipids. Annexin proteins are strong anticoagulants and the displacement of these proteins make the trophoblast surfaces procoagulant. This leads to placental infarcts which in turn cause abortions or still births (11).The *in vitro* studies on the effects of antiphospholipid revealed impairment of trophoblastic invasion and production of human chorionic gonadotrophins thereby causing abortions, still births and also uteroplacental apoplexy (12) The predictive factors in the development of clinical syndrome are antibody level, isotype and many other unspecified properties of antiphospholipids. The risk of developing complications due to antiphospholipid antibodies in pregnancy is difficult to assert. But a previous bad obstetric history, history of thrombosis and triple positivity for lupus anticoagulant, anticardiolipin and anti-β2 glycoprotein antibodies are the high risk factors (13,14).

The anticardiolipin antibody assay by ELISA technique is a simple and highly sensitive method which helps in the diagnosis of APS. The microtiter plate wells are coated with the antigen cardiolipin or other phospholipids. In our study, cardiolipin coated plates were used. Earlier studies by Nesher et al., have shown that a small portion of patients with BOH were IgA aCL positive [(15)]. In our study IgA aCL was not done since we could not afford to procure the kit.

In the present study 21% of pregnant women with BOH were aCL antibodies positive. According to Stephenson, 20% of women with history of recurrent consecutive pregnancy losses were aCL positive (16). The finding is our study coincides with the above study.

The distribution of aCL IgG among pregnant women with BOH was high and statistically significant when compared to the isotype IgM in our study. Earlier studies by Levin et al have revealed IgG isotype was closely related to clinical feature of APS than IgM or IgA isotype (17). The present study is comparable with the above.

In our study aCL IgM was detected in 23.80% of pregnant women with BOH. Moderate and high titer CL were detected in 28.57% and 57.14% of pregnant women with BOH respectively. However, 14.28% with low titer CL presented with BOH. This is in contrary to the statement, a low titer CL of any isotype is less commonly associated with fetal losses (18).

Therapeutic intervention with anticoagulants heparin and low dose aspirin in aCL positive pregnant women with BOH improved the fetal outcome from 50% to 80% in the studies conducted by Rai and Kuttch (19).

Limitations of the study

As it is a Tertiary Care Centre, patients from all over the state and neighboring states are referred for APS screening. The resources are limited compared to turnover of patients. Hence ELISA test for aCL IgG and IgM could not be repeated after 12 weeks. Likewise aCL IgA was not done due to resources constraint.

Conclusion

One of the causes for recurrent pregnancy loss is Antiphospholipid syndrome. As there is a positive correlation between aCL IgG and BOH in our study, screening for APS by aCL ELISA can increase fetal survival by initiating early anticoagulant therapy when other causes for BOH are excluded.

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