



## Original Research Article

# The Prevalence of Anticardiolipin Antibodies in women with Bad Obstetric History

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## ABSTRACT

### Keywords

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Anticardiolipin (aCL) antibodies;  
enzyme immunassay.

Bad obstetric history (BOH) implies previous unfavorable fetal outcome in terms of two or more consecutive spontaneous abortions, early neonatal deaths, stillbirths, intrauterine fetal deaths, intrauterine growth retardation and congenital anomalies. The immune factors associated with pregnancy loss are classified as autoimmune and alloimmune factors. The autoimmune factors include the synthesis of autoantibodies (anti-phospholipid antibodies, anti-nuclear antibodies, anti-thyroid antibodies). The main types of antiphospholipid antibodies are Lupus Anticoagulant (LA) and Anticardiolipin (aCL) antibodies (IgG & IgM). To measure the level of anticardiolipin antibodies in the sera of women with bad obstetric history. The study was conducted from October 2009 till June 2011 including 117 patients who attended private clinic & laboratory. Patients included in the study were those with history of two or more recurrent miscarriages, intrauterine fetal death & stillbirth. Fetal losses due to diabetes or congenital anomalies were excluded. Serum levels of anticardiolipin IgM & IgG antibodies were measured using AESKULISA phospholipid-Screen-GM (Germany) which is a solid phase enzyme immunassay for the separate qualitative & quantitative detection of IgG and /or IgM antibodies in human sera. From the total of 117 patients with BOH fourteen (11.96%) had positive anticardiolipin antibodies in their sera. When comparing between the different groups of patient who were classified according to the type of fetal loss the highest number & percentage of positive anticardiolipin antibody were found in those with three & more recurrent miscarriages (4 cases, 57.14%). When comparing the IgM & IgG levels in different patients groups, the highest levels were found in those with three & more recurrent miscarriages. Anticardiolipin antibodies can have a positive association in women with bad obstetric history especially those with three & more recurrent miscarriages. The level of IgM & IgG is highest in this group however these antibodies should be looked for in other patients with BOH

## Introduction

Bad obstetric history (BOH) implies previous unfavorable fetal outcome in terms of two or more consecutive spontaneous abortions, early neonatal

deaths, stillbirths, intrauterine fetal deaths, intrauterine growth retardation and congenital anomalies. For any given pregnancy, the reported risk of pregnancy

loss is 15% and the likelihood of consecutive three losses, which is the classic definition of bad obstetric history (BOH), would be 0.34%. However, one to two percent of couples experience three or more consecutive losses. Hence, medical help is sought in order to identify the causal factor as well as the strategy to alleviate the problem (Meka et al., 2006).

### **Causes of Bad Obstetric History**

Maternal age and previous number of miscarriages are two independent risk factors for a further miscarriage. Advanced maternal age adversely affects ovarian function, giving rise to a decline in the number of good quality oocytes. Other causes of BOH may be genetic, abnormal maternal immune response, abnormal hormonal response, maternal infection and anatomical.

### **Immune Causes**

The immune factors associated with pregnancy loss are classified as autoimmune and alloimmune factors. The autoimmune factors include the synthesis of autoantibodies (anti-phospholipid antibodies, anti nuclear antibodies & anti thyroid antibodies) (Rajangam et al., 2007; Bloomenthal et al., 2002; Sutandar et al., 2007).

The viscosity of blood slightly increases during pregnancy, but in some women the blood is found to clot more easily due to the presence of certain antibodies called antiphospholipid antibodies. These blood clots in the placental blood vessels may decrease the blood flow to the baby resulting in miscarriage.

Antiphospholipid antibodies are present in 15% of women with recurrent miscarriage. The main types of antiphospholipid

antibodies are Lupus Anticoagulant (LA) and Anticardiolipin (aCL) antibodies (IgG & IgM). The association between phospholipid antibodies and recurrent miscarriage is referred to as Antiphospholipid Syndrome (APS). Anti- $\beta$ 2-glycoprotein-I antibody, although associated with IUFD, has been shown to have little association with recurrent miscarriages (Higashino et al., 1998).

To diagnose APS it is mandatory that the patient should have two positive tests at least six weeks apart for either lupus anticoagulant or anticardiolipin (aCL) antibodies of IgG and/or IgM class present in medium or high titre.

According to Eng (1994) and others, it was Pangborn who in 1941, following Wasserman's test for syphilis in 1903, identified an acidic phospholipid (PL) as the apparent target antigen of the test, specifically, cardiolipin (CL). CL is named for the bovine heart muscle from which it was obtained, heart being rich in mitochondria, a main source of CL. In 1952, Conley and Hartmann first described the lupus anticoagulant (LA), later interpreted as a consequence of aPL, in association with a hemorrhagic diathesis. However, this and other early clinical observations were later overshadowed by frequent findings of thrombosis associated with positive anti-CL (aCL) test, leading to recognition of the aPL syndrome (APS) in the 1980s by Harris et al and by Hughes et al, originally called anticardiolipin (aCL) syndrome, now sometimes Hughes' syndrome (Harris et al., 1983).

Although diagnostic criteria vary somewhat depending on sources, APS is generally defined by a repeatedly positive test for one or more aPL in conjunction with thrombosis or recurring pregnancy

loss (Rand et al., 2001; Levine et al., 2002). It is often accompanied by thrombocytopenia, episodic neurological disturbances, and/or numerous other clinical manifestations (Brey et al., 2002). APS may be secondary to other underlying conditions, notably systemic lupus erythematosus (SLE); otherwise, in the absence of other disorders is known as primary APS (PAPS). In its most life-threatening form, it is known as catastrophic APS (CAPS). In patients with CAPS occlusion of small blood vessels leads to multi-organ failure. Many reviews of APS with focus on clinical manifestations and management, laboratory methodologies, and hypotheses to account for the association between aPL and thrombosis exist (Giannakopoulos et al., 2007).

### **What Are aPL and How Are They Measured?**

Originally, aPL were defined as antibodies reacting to cardiolipin (CL) but no widely accepted definition of aPL any longer exists. They are measured by two distinct kinds of tests, solid-phase for particular aPL, and coagulation-based for LA. The former is usually an enzyme-linked immunosorption assay (ELISA), consisting in outline of adding a sample of patient serum or plasma to a plastic well coated with some particular PL or mixture of PLs, with or without a specific protein cofactor, then measuring how much patient immunoglobulin (Ig) is captured by adding an anti-human IgG, IgM, or IgA conjugated with an enzyme that generates a colored product. Despite its simplicity, this procedure is subject to many subtle variations which can grossly affect results. In contrast, LA are detected by the prolonged time required for coagulation of the patient's plasma relative to normal

plasma in a test designed to be sensitive to PL. Most commonly, the dilute Russell viper venom time (dRVVT) is used. It is widely believed that the prolongation is caused by an aPL occupying sites on the PL which are required for binding the coagulation factors, thereby prolonging the time (Roubey, 1996).

The present study deals with measure the prevalence of anticardiolipin antibodies in women with bad obstetric history.

### **Materials and Methods**

The study was conducted from October 2009 till June 2011 including 117 patients who attended private clinic & laboratory. Patients included in the study had bad obstetric history i.e. those with history of two or more recurrent miscarriages, intrauterine fetal death &/or stillbirth. Exclusion criteria were history of diabetes mellitus, thyroid disease or fetal loss due to congenital anomalies or infections.

After taking full history & examination, patients were sent for a number of investigations to rule out those with possible underlying cause like TORCH, PCOS, diabetes & thyroid disease.

Serum levels of anticardiolipin IgM & IgG antibodies were measured using AESKULISA phospholipid-Screen-GM (Germany) which is a solid phase enzyme immunassay for the separate qualitative & quantitative detection of IgG and /or IgM antibodies in human sera. Diluted serum samples were incubated in microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to antigen. The unbound fraction is washed off in the following step. Afterwards, anti-human immunoglobulins

conjugated to horseradish peroxidase are incubated & react with the antigen-antibody complex. The unbound conjugate is washed off. Addition of TMB-substrate generates an enzymatic colorimetric reaction, which is stopped by diluted acid. The rate of color formation is a function of the amount of conjugate bound to the antigen-antibody complex. Results were expressed in U/mL with <12 U/mL regarded as normal range, 12-18 U/mL equivocal range & > 18 U/mL as positive results.

Those patients with positive anticardiolipin antibody tests were sent for another assessment of anticardiolipin level six weeks later to exclude any accidental elevation in these antibodies in the index patient. The mean of the two measurements were taken as the final reading & percentage calculation was carried out.

## **Results and Discussion**

The patients were grouped according to their age & type of fetal loss. Table.1 shows that the largest groups of patients were those who are 21-30 years old & the smallest groups were those above forty. Two & three recurrent miscarriages was the commonest type of fetal loss (71 patients).

The type of fetal loss was classified in a more detailed way to include five groups: patients with two 1<sup>st</sup> trimester miscarriages, those with 3& more 1<sup>st</sup> trimester miscarriages (recurrent miscarriage), those with IUD, those with miscarriage & IUD & those with 1<sup>st</sup> & 2<sup>nd</sup> trimester miscarriage. The anticardiolipin level was assessed in all patient groups. Table (2) shows the no. & percentage of positive anticardiolipin antibody cases in different groups. Of the total 117 patients with

BOH, 11.96% had positive anticardiolipin antibody test & the highest percentage was in those with recurrent miscarriage.

For patients with positive anticardiolipin antibody test IgM &/or IgG, another assessment done six weeks later & the mean of the two readings was calculated. Table (3) shows quantitative comparison of IgM antibodies in different patients groups. Positive cases of IgM were found in all patients groups with the highest level was found in those with recurrent 1<sup>st</sup> trimester miscarriage while IgG antibody was equivocal in all cases except in those with recurrent 1<sup>st</sup> trimester miscarriage.

The prevalence of the antiphospholipid antibodies in certain high risk groups like bad obstetric history is higher than previously realized. In our study we used anticardiolipin antibody (IgM & IgG) test as one of these antibodies to determine its prevalence in women with different kinds of fetal wastage. The percentage of anticardiolipin antibody positive patients was approximate that found in other studies of patients with BOH. Saha SP et.al found that the prevalence of antiphospholipid antibody ranged between 10-46.87% in women with BOH compared with 8.49% in women with history of normal uncomplicated pregnancies (Saha et al., 2009). Another study done by Mishra et al., (2007) showed that anticardiolipin antibody test was positive in 28.3% of patient with recurrent miscarriage. Singh & Sidhu studied different factors implicated in the causation of BOH & found that the prevalence of antiphospholipid antibody in test group was 10.13% (Col et al., 2010). Velayuthaprabhu and Archunan (2005) studied 155 patients with recurrent miscarriage & found that 40% of them were positive for anticardiolipin antibody.

**Table.1** Patients groups according to age & type of fetal loss

Age group(yr)	Total No.	2&3 Recurrent miscarriages	IUD	IUD & miscarriage
16-20	20	11	3	6
21-30	61	39	9	13
31-40	25	16	4	5
>40	11	5	6	0
Total	117	71	22	24

**Table.2** Number & percentage of anticardiolipin positive cases among different patient groups

Patients groups	Number	No. of anticardiolipin positive cases	Percentage
two 1st trimester miscarriage	25	3	12%
3& more 1st trimester miscarriage	7	4	57.14%
IUD	22	3	13.63%
Miscarriage & IUD	24	2	8.33%
1st & 2nd trimester miscarriage	39	2	5.12%
Total	117	14	11.96%

**Table.3** Mean serum anticardilipin IgM levels in different patients groups

Patients groups	Mean IgM level $\pm$ SD U/ml	Confidence interval 95%
two 1st trimester miscarriage	32 $\pm$ 3	24.55 - 39.45
3& more 1st trimester miscarriage	56.75 $\pm$ 25.9	15 - 97.97
IUD	26 $\pm$ 4	16.6 - 35.94
Miscarriage & IUD	31 $\pm$ 9.89	-57.94 - 119.94
1st & 2nd trimester miscarriage	49.5 $\pm$ 6.36	-7.68 - 106.68

The remaining negative samples were tested for aPS (antiphosphatidylserine antibody, another type of antiphospholipid antibodies), in which 18 (19%) patients were positive for aPS (Velayuthaprabhu and Archunan, 2005). Sonal Vora et al., (2008) studied four hundred and thirty women with unexplained fetal loss & found that the overall prevalence of IgG and/or IgM antibodies for cardiolipin was 27.9% .

The variation in the prevalence of these antibodies in different studies may be due to the different types of fetal wastage included, different types of antiphospholipid studied & the different localities in which studies were performed. In our study we found that anticardiolipin IgM antibody was higher than the IgG antibody in all patients groups & the highest level of anticardiolipin antibody was found in those with three or more recurrent miscarriages. Mackworth-young et,al found in their study on a group of patients with clinical features of APS that anticardiolipin antibodies IgM & IgG were equally prevalent (18 versus 20) (Mackworth-Young et al., 1989) , so we need further study regarding any significant difference in the dominance of IgM or IgG in an index patient.

Anticardiolipin antibodies can have a positive association in women with bad obstetric history especially those with three & more recurrent miscarriages.

The level of IgM & IgG is highest in this group however these antibodies should be looked for in other patients with BOH.

### **Recommendation**

Further studies are needed to test for the exact role of anticardiolipin antibodies in

the pathogenesis of recurrent miscarriage. Antiphospholipid antibody test should be included in the investigations of patients with bad obstetric history.

### **References**

- Bloomenthal D et al.2002. The effect of factor V Leiden carriage on maternal and fetal health. *CMAJ*. 167(1):48-54.
- Brey RL, Levine SR, Stallworth CL. 2002. Neurologic manifestations in the antiphospholipid syndrome. In *The Antiphospholipid Syndrome II: Autoimmune Thrombosis* Edited by: Asherson RA, Cervera R, Piette JC, Shoenfeld Y. New York: Elsevier.155-168.
- Col G Singh, Maj K Sidhu.2010. Bad Obstetric History : A Prospective Study. *MJAFI*. 66 : 117-120.
- Eng, A., 1994. Cutaneous expression of antiphospholipid syndromes. *Sem Thomb Haemost* 1994, 20:71-78.
- Giannakopoulos B, Passam F, Rahgozar S and Krilis SA: 2007. Current concepts on the pathogenesis of the antiphospholipid syndrome. *Blood* .109:422-430.
- Harris EN, Charavi AE, Boey ML, Patel BM, Mackworth-Young CG, Loizou S and Hughes GRV. 1983. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet*. 2:1211-1214.
- Higashino M et al., 1998. Anti-cardiolipin antibody and anti-cardiolipin beta-2-glycoprotein I antibody in patients with recurrent fetal miscarriage. *J Perinat Med*. 26(5): 384-9.
- Levine JS, Branch DW and Rauch J. 2002. The antiphospholipid syndrome (Review). *N Engl J Med*. 346:752-763.
- Mackworth-Young C G, Loizou S, Walport M J. 1989. Primary

- antiphospholipid syndrome: features of patients with raised anticardiolipin antibodies and no other disorder. *Ann Rheum Dis* 1989;48:362-367 .
- Meka A et al., 2006. Recurrent Spontaneous Abortions: An Overview of Genetic and Non-Genetic Backgrounds. *Int J Hum Genet*: 6(2):109-117.
- Mishra NM, Sapna Gupta1, Gupta MK. Significance of antiphospholipid antibodies in patients with bad obstetric history. *Indian Journal of Medical Science*. 2007 Volume:61 Issue:12 Page:663-664.
- Rajangam, S et al., 2007. Karyotyping and counseling in bad obstetric history and infertility. *Iranian J Rep Med*. 5(1):7-12.
- Rand JH, Macik B Ga and Konkle BA. 2001. Thrombophilia: What's a practitioner to do? (Diagnosis and treatment of the antiphospholipid syndrome). Edited by: Schechter GP, Broudy VC, Williams ME, Bajus JL. American Society of Hematology Education Program Book. Chapel Hill, NC. 322-324.
- Roubey, R.A.S. 1996. Immunology of the antiphospholipid antibody syndrome. *Arthritis Rheum*. 39:1444-1454.
- Saha, S.P; Bhattacharjee N; Ganguli RP; Sil S; Patra KK; Sengupta M and Barui G. 2009. Prevalence and significance of antiphospholipid antibodies in selected at-risk obstetrics cases: a comparative prospective study. *J Obstet Gynaecol*. 29(7):614-8.
- Sonal Vora, Shrimati Shetty, Vinita salvi, Purnima Satoskar. A comprehensive screening analysis of antiphospholipid antibodies in Indian women with fetal loss. *European Journal of Obstetrics & Gynecology and Reproductive Biology* Volume 137, Issue 2, April 2008, Pages 136-140.
- Sutandar M et al., 2007. Hypothyroidism in Pregnancy. *Obstet Gynaecol Can*. 29(4):354-356.
- Velayuthaprabhu S, and Archunan G. 2005. Evaluation of anticardiolipin antibodies and antiphosphatidylserine antibodies in women with recurrent abortion. 59.8: 347-352.