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

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الخلاصة:
ان اسباب الاسقاطات المتكررة والولادات الميتة كثيرة من بينها مضادات الكارديوليبين لدى المرأة الحامل. لذا كان الهدف من هذه الدراسة قياس مستوى مضادات الكارديوليبين في دم المريضات اللائي لديهن تاريخ ولادي سيء. اشتملت الدراسة 117 امرأة ممن لديهن ولادة ميتة او اسقاط منسي او اسقاطات متكررة مع استبعاد من لديهن اسباب واضحة لفقدان الجنين كأن تكون مصابة بداء السكري او ان يكون الجنين مشوه خلقيا.وبعد اخذ عينات الدم و قياس مستوى المضادات واعادة التحليل بعد مضي ستة اسابيع من التحليل الاول للتأكد من النتيجة، تبين ان مضادات الكارديوليبين كانت موجودة عند 11.9% من المريضات تحت الدراسة واعلى النسب وجدت عند النساء اللائي لديهن اسقاطات متكررة مما يدل على وجود علاقة للمضادات بالتاريخ الولادي السيء و ان هذا الاختبار يجب ان يؤخذ بنظر الاعتبار عند البحث عن اسباب هذه الحالة. .

**Abstract:**

**Background**: 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).

**Objective:** To measure the level of anticardiolipin antibodies in the sera of women with bad obstetric history.

**Patients & method:** the study was conducted from October 2009 till June 2011 including 117 patients who attended private clinic & laboratory. Patients included in the sudy 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.

**Results:** 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.

**Conclusion**: Anticardiolipin antibodies can have a positive association in women with bad obstetric history especially those with three & more recurrent miscarrigages. 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[1].

**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)[2,3,4].

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-β2-glycoprotein-I antibody, although associated with IUFD, has been shown to have little association with recurrent miscarriages[5].

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[6] 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[7].

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[8,9]. It is often accompanied by thrombocytopenia, episodic neurological disturbances, and/or numerous other clinical manifestations[10]. 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.[11]

**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.[12]

**Aim of study:** to measure the prevelance of anticardiolipin antibodies in women with bad obstetric history.

**Patients & Methods:**

The study was conducted from October 2009 till June 2011 including 117 patients who attended private clinic & laboratory. Patients included in the sudy 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 generate 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 the these antibodies in the index patient. The mean of the two measurements were taken as the final reading & percentage calculation was carried out.

**Results:**

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

Table(1) Patients groups according to age & type of fetal loss

| Age group(yr) | Total No. | 2&3 Recurrent miscarriges | 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 |

The type of fetal loss was classified in a more detailed way to include five groups: patients with two 1st trimester miscarriges, those with 3& more 1st trimester miscarriages ( recurrent miscarrige), those with IUD, those with miscarriage & IUD & those with 1st & 2nd 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 miscarrige.

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

| Patients groups | Number | No. of anticardiolipin positive cases | Percentage |
|---|---|---|---|
| two 1st timester 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% |

For patients with positive anticardiolipin antibody test IgM &/or IgG, another assessment done six weaks later & the mean of the two readings was calculated.

Table (3) shows quantitative comparison of IgM antibodies in diffrent patients groups. Positive cases of IgM were found in all patients groups with the highest level was found in those with recurrent 1st trimester miscarriage while IgG antibody was equivocal in all cases except in those with recurrent 1st trimester miscarriage.

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

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

**Discussion:**

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 prevelance 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 prevelance of antiphospholipid antibody ranged between 10-46.87% in women with BOH compared with 8.49% in women with history of normal uncomplicated pregnancies[13]. Another study done by Mishra et,al showed that anticardiolipin antibody test was positive in 28.3% of patient with recurrent miscarriage[14]. Singh & Sidhu studied different factors implicated in the causation of BOH & found that the prevalence of antiphospholipid antibody in test group was 10.13%[15]. S Velayuthaprabhu & G Archunan studied 155 patients with recurrent miscarriage & found that 40% of them were positive for anticardiolipin antibody. The remaining negative samples were tested for aPS (antiphosphatidylserine antibody, another type of antiphospholipid antibodies) , in which 18 (19%) patients were positive for aPS[16]. Sonal et,al 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%[17] .

The variation in the prevelance 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 prevelant (18 versus 20)[18] , so we need further study regarding any significant difference in the dominance of IgM or IgG in an index patient.

**Conclusion**:

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

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