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KEYWORDS :

Anticardiolipin antibodies in patients of Explained Vs Unexplained Abortions

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ABSTRACT

Background : *Abortion is termination of pregnancy by any means before the fetus is sufficiently developed to survive on its own. The growing recognition that in this group of patients there may be an immunologic basis aroused interest in research workers. The anticardiolipin antibodies (aCL) which are commonly associated with recurrent pregnancy loss are lgG and lgM. lgM antibody is the largest immunoglobulin and first to appear in an immune response. Women with phospholipid antibodies have a fetal loss of 90% when no specific treatment is given during pregnancy so this study was planned to find anticardiolipin antibody levels (IgM & IgG) in patients of Abortions. Material/Methods: A total of 125 pregnant subjects were included in the study which includes study group (75 subjects) and a control group (50 subjects). Their blood was tested to assess the levels of IgM & IgG aCL by enzyme-linked immunosorbent assay (ELISA).*

Result : *IgG anticardiolipin antibodies were raised in 16.66% of explained vs. 30.7% of unexplained abortion. IgM anticardiolipin antibodies were raised in 83.3% of the explained vs. 4.35% of unexplained abortions. Conclusion : Patients with increased IgM & IgG levels indicate a higher antibody burden with greater antigenic affinities explaining IgM & IgG mediated pregnancy wastage.*

Introduction

Abortion is termination of pregnancy by any means before the fetus is sufficiently developed to survive on its own. This corresponds to a gestational age of 20 weeks or a fetal weight of 500 gms. When abortion occurs spontaneously, the term miscarriage has been applied. The incidence of spontaneous abortion has commonly been quoted as 10% of all the pregnancies . A number of immunologic and non-immunologic factors have been implicated as probable causes of abortions. Current methods of detection do not find any recognized causes in 40-50% of the patients with recurrent abortions (Harris et al 1983) [1] .The growing recognition that in this group of patients there may be an immunologic basis aroused interest in research workers. The antiphospholipid antibodies are gaining importance as a potential cause of recurrent pregnancy loss and their presence in these is clearly documented.

The antiphospholipid antibodies were first detected by Wasserman in 1906 [2]. The term antiphospholipid antibodies include three types of antibodies i.e. anticardiolipin (aCL), lupus anticoagulant and the antibodies causing false positive test for syphilis. The antiphoshoplipid antibodies are a diverse family of autoantibodies which share a common reactivity with negatively charged phospholipids. Although all three are associated independently with fetal loss, aCL are seen to have a definite edge over the other two.

Cardiolipin is a doubly negatively charged phospholipid held together by a glycerol linkage. It reacts with a lipid part of bovine heart and hence the name cardiolipin. It is a part of the antigen used in the VDRL assay. It is due to this reason that antibody to cardiolipin brings about a biological false positive reaction in the VDRL test for syphilis. They play a crucial role in the coagulation abnormalities and are associated with scattered placental infarctions, arterial and venous thrombosis, mild thrombocytopenia and connective tissue disease [3] (Elias et al 1984). The presence of anticardiolipin antibodies with one or more of the above clinical manifestations is referred to as Antphospholipid syndrome (Silver et al 1994).[4]

The aCL which are commonly associated with recurrent pregnancy loss are lgG and lgM. These immunoglobulins are serum glycoproteins that are produced by plasma cells in response to antigens.

lgG, the most abundant of the antibodies (80% of all circulating immunoglobulin's), circulates in the body fluids and is the immunoglobulin which crosses the placenta and provides passive immunity to the fetus. Elevated lgG anticardiolipin antibodies have also been detected in patients with autoimmune disorders such as primary Sjogrens syndrome, mixed connective tissue disease, rheumatoid arthritis, idiopathic thrombocytopenic purpura, Bechet's syndrome, Myasthenia gravis and other undefined autoimmune disorders (Harris et al 1985)[5].

lgM antibody is the largest immunoglobulin and first to appear in an immune response. It constitutes approximately 10% of normal immunoglobulin's. High level of lgM anticardiolipin appear to be more distributed than lgG anticardiolipin antibodies and have been found in autoimmune, drug induced and infectious disorders including some patients with syphilis.

The antiphospholipid antibodies act via myriad of prothrombotic mechanisms to bring about thrombosis and infarction of the placental vasculature, thereby causing fetal compromise and death. The antibodies might affect platelet membranes leading to subtle and sometimes non-subtle changes in platelet function [6] (Hughes et al 1986). The antibodies might damage the endothelial cell membranes, leading to decreased prostacyclin release. Prostacyclin is a vasodilator which prevents platelet aggregation. Any decrease in the former leads to increased thrombosis [7] (Carreras et al 1981). Women with phospholipid antibodies have a fetal loss of 90% when no specific treatment is given during pregnancy.

MATERIALS AND METHODS

The study was conducted in the Department of Anatomy and the subjects for the study were obtained from the Department of Obstetrics and Gynaecology of Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital and Kalawati-Saran Children's Hospital. New Delhi. A total of 125 subjects were for the study. _

A total of 125 pregnant subjects were included in the study which includes study group (75 subjects) and a control group (50 subjects). Subjects in the Study group included patients with a history of two or more first trimester or second trimester spontaneous abortions who were otherwise healthy without any obvious medical or surgical cause for the pregnancy loss. Con-

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trol group included subjects in similar age groups but without any history of recurrent spontaneous abortions and with one or more live births.

Subjects in which some medical disorder or a congenital anomaly was responsible for the abortions, were excluded from the study. An informed content was obtained from the volunteers. A detailed history was obtained from the patients which included parity, detailed obstetric history including the time and reason (if known) for the previous spontaneous abortions. Also past history of any autoimmune diseases, drug intake (Chlorpromazine, procainamide etc.) or bleeding disorders were ruled out.

A detailed clinical & obstetric history was taken with reference to age, gravidity, parity ,date of last menstrual period (LMP). A detailed history including the time and reason for the previous spontaneous abortions was also taken. History of present pregnancy with regard to fever, drug intake, radiation exposure, blurring of vision, headache and pedal edema was taken. Any history of autoimmune disease or history of bleeding tendencies was ruled out.

Examination of levels of aCL (IqG & IgM) were done by ELISA technique. ELISA is a quantitative test based on the principle of enzyme immunoassay. It is the test of choice adopted in this study. ELISA technique is simple, has a longer shelf life and there is no material involved in it. The results of the test were interpreted as insignificantly positive or significantly positive. Prevalence of a positive aCL test was assessed in cases of recurrent abortions and was compared with subjects in the control group. Patients with history of recurrent abortions and a positive test for aCL were treated with Aspirin (80 mg/day) or heparin given subcutaneously twice daily in a starting dose of 10,000 IU/ day and adjusted so as to keep the Activated partial thromboplastin time (APTT) between 1.5-2.5 times the normal (50-80 sec).

Interpretation of IgG & IgM values as per the manufacturer's advice in the kit

	IgG (GPL-U/ml)	IgM (MPL-U/ml)
Negative	< 12	< 6
Equivocal	12-18	6-10
Positive	> 18	> 10

OBSERVATIONS

Observations :

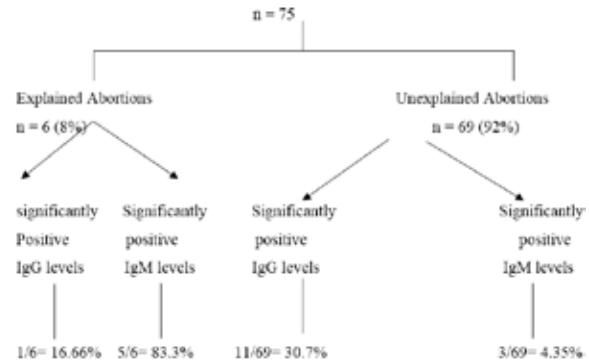
Mean age of study group = 23.57 ± 3.09 yrs. Mean age of control group = 22.88 ± 2.81 yrs. P > 0.05, not significant (ages of both groups were comparable). Mean gravidity in study group = 3.65 ± 0.78. Mean gravidity in control group = 2.72 ± 0.99.

Table 1 : Distribution of patients according to number of abortions.

Number of Abortions	Study Group n = 75 No. (%)	Control Group n = 50 No. (%)
0	0	50 (100)
1	0	0
2	46 (61.3)	0
3	25 (33.33)	0
4	2 (2.66)	0
5	2 (2.66)	0

Mean number of abortions in study group was 2.49 (Range 2-5).

Table 2. Significantly raised levels of anticardiolipin antibodies (IgG & IgM) in patients of explained vs unexplained abortions



IgG anticardiolipin antibodies were raised in 16.66% of explained vs 30.7% of unexplained abortion. IgM anticardiolipin antibodies were raised in 83.3% of the explained vs 4.35% of unexplained abortions.

DISCUSSION

The premise that antiphospholipid antibodies have pathogenetic relevance gathered wide spread acceptance because of the association of these antibodies with thrombosis in patients with systemic lupus erythematosus and in otherwise normal individuals. Several intermediary thrombotic mechanisms have been suggested as causative, perhaps the most rational suggestion concerning pathogenesis to date is that some antiphospholipids possess a critical property which promotes thrombosis upon interaction with platelet or endothelial membrane phospholipids.

This study was conducted in the Department of Anatomy in collaboration with the Department of Obstetrics and Gynecology of Lady Hardinge Medical College and associated Hospitals.

In the present study pregnant patients with a history of two or more spontaneous first or second trimester abortions were included and were compared with patients without any history of abortion or pregnancy loss and at least one previous live birth.

The mean age of the subjects in our study group was 23.57 ± 3.09 years and that in the control group was 22.88 + 2.81 years (p > .05, not significant). There was no significant correlation of recurrent abortions with any particular age group. Maximum number of patients (62.7%) in our trial were in the age group of 21-25 years whereas in a study conducted by Parazzini et al [8] (1991) maximum number of patients ie. 38 percent each were in the age groups 25-29 and 30-34 years. Similarly the mean age reported by Unander et al [9] (1987) was 31.0 ± 4.6 years which was much higher than that observed in our study. The lower age of patients observed in our study was probably due to early marriages in our country.

The mean gravidity of the patients in our study group was 3.65 ± 0.78 with range of 3-6 and that in the control group was 2.72 ± 0.99 ranging between 2 and 6. In the study group 49.3% and 40% of the patients respectively were third fourth gravida and none of the patients were below gravida three. In the control group however, maximum number of patients i.e 56% were gravida two. Mean gravidity of 3.65 ± 0.78 in our present study, nearly conforms with the gravidity of 3.9 as reported by Cowchock et al (1986)[10] despite the lower age up of patients in our study.

In the present study 85.33% of the cases were primary abortors which was twice as high compared to the findings reported by Cowchock et al [10] (1986), and Maclean et al [11] (1994) who in different researches reported primary abortions in 49% and 48% of the patients.

In the patients in our study group 61.3% had two spontaneous abortions whereas 38.7% of the patients had three or more mis-

carriages compared to the findings of Maclean et al [11] (1994) who reported 47% of the patients with two and 53% with three or more previous miscarriages.

Mean number of abortions in the study group was 2.49 with a range of 2 to 5 abortions as compared to a mean of 3.4 abortions (range 2-11) reported by Cowchock et al (1986) in a study on 61 patients of recurrent spontaneous abortions. However no correlation was observed between the number of abortions and the prevalence of anticardiolipin antibodies.

Prevalence of anticardiolipin antibodies in general obstetric population

In the study on control group of patients with one or more live births and no history of any previous abortion, anticardiolipin antibody positivity for IgG was 8% and for IgM was 4%, but all the patients in this group had insignificant levels of cardiolipin antibodies i.e. < 18 GPL units and <10 MPL units respectively, mean of 5.02 ± 3.01 GPL units for IgG and 2.57 ± 1.44 MPL units for IgM anticardiolipin antibody.

Prevalence of anticardiolipin antibodies in general obstetric population as reported by different studies

TABLE D-1

Studies	Prevalence
Lockwood et al (1989) [12]	2.2%
Parazzini et al (1991)	3%
Kumar et (1997) [13]	5%
Present study (2002)	0%

Prevalence of anticardiolipin antibodies in general obstetric population as reported in various studies ranged between 2.2-5% (Refer table D-1) whereas in the present study no case with significantly increased IgG or IgM anticardiolipin antibody was observed which is in concordance with the findings in a previous unpublished study conducted in our hospital in 1999 which reported 0% prevalence of significantly raised levels of aCL in general obstetric population. This also comforms to the report of Harris et al (1983)[14] that anticardiolipin antibodies are only rarely demonstrated in the healthy control subjects.

Prevalence of IqG anticardiolipin antibodies

The prevalence of significantly raised levels of IgG anticardiolipin antibody (>8 GPL units) in our study group of patients i.e. patients with two or more first or second trimester abortions was 16% with a mean level of 11.38 ± 6.44 GPL units. Our findings were comparable to those of Creagh et al (1991)[15] who reported prevalence of 17% and nearly thrice as high compared to the findings of Cowchock et al (1986) and Maclean et al (1994) who reported a prevalence of 6.5% and 6.6% respectively. Unander et al (1987) and Kumar et al (1997) reported a prevalence of 35.3% and 32.5% which was nearly twice that obtained in our study.

Prevalence of raised IqG anticardiolipin antibody as reported by different workers.

TABLE D-2

Studies	Prevalence
Cowchock et al (1986)	6.5%
Unander et al (1987)	35.3%
Parazzini et al (1991)	11%
Creagh et al (1991)	17%
Maclean et al (1994)	6.6%
Kumar et (1997)	32.5%
Present study (2002)	16%

After ruling out patients who had raised IgM anticardiolipin antibodies and explained cause of recurrent abortions i.e. toxoplasmosis, prevalence was 30.7%. This observation was well within the range i.e. 11% - 35.3% as reported in various other studies. In 30.7% of patients with two or more spontaneous abortions, we could not find any other factor but for raised IgG anticardiolipin antibody, to attribute to their recurrent abortion. Hence a strong association between raised IgG anticardiolipin antibody and recurrent abortion ($p < 0.001$, highly significant) has been confirmed by the present study.

Prevalence of IgM anticardiolipin antibodies

The prevalence of raised anticardiolipin antibodies (IgM) was found in 10.7% of the study group of patients with mean levels of 5.83 ± 4.47 MPL units. These findings were comparable to those of Unander et al (1987) and Parazzini et al (1991) who reported 7% and 12% prevalence of IgM anticardiolipin antibodies in cases of recurrent spontaneous abortions. Silver et al (1996)[16] in a study reported raised IgM anticardiolipin antibodies in 20.4% of the women who underwent clinically indicated testing for antiphospholipid antibodies. In our study it was observed to be prevalent in half the percentage compared to the findings of Silver et al (1996).

Prevalence of IgM anticardiolipin antibody as reported by different authors

TABLE D-3

Authors Name	Prevalence
Cowchock et al (1986)	9.8%
Unander et al (1987)	7%
Parazzini et al (1991)	12%
Maclean et al (1994)	1.6%
Sliver et al (1996)	20.4%
Present study (2002)	10.7%

Five of the eight patients ie. 62.5 percent with increased levels of IgM anticardiolipin antibodies in our study had some other infection also (such as toxoplasmosis, cytomegalovirus and tuberculosis) suggesting the polyclonal binding of IgM. This proves the findings reported by Lockwood et al (1989) that there is polyclonal binding of IgM.

In our study prevalence of increased IgM anticardiolipin antibody in patients with explained recurrent abortion was 83.3% (i.e. five out of six patients). In patients with unexplained recurrent abortion the prevalence of IgM cardiolipin antibody was 4.35% ie. (three of 69 patients) which is in comparison to the observation of 7% as reported by Unander et al (1987).

Patients with increased IgG tended to have higher absolute levels of immunoglobulin than increased IgM levels. A higher antibody burden, together greater antigenic affinities and transplacental antibody passage of IgG explains the difference in anticardiolipin antibody IgG and IgM mediated pregnancy wastage.

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