

Role of Autoimmunity in Recurrent Abortions

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Abstract:

Objective:- To evaluate the role of anticardiolipin antibodies in the etiopathogenesis of unexplained recurrent abortions.

Method:- A cohort of 28 female patient of recurrent spontaneous abortions which included 8 primary aborters and 20 secondary aborters and 20 normal fertile female controls were studied for IgG anticardiolipin antibodies by ELISA methods. Besides this, patients were also tested for Rheumatoid factor, antinuclear antibodies and LE cell phenomenon to know autoimmunity. Patients were also tested for VDRL tests by flocculation method.

Results:- IgG anticardiolipin antibody was positive in 25% cases; whereas all healthy controls were negative. Secondary aborters had an increased frequency of anticardiolipin antibody (35%) when compared with the primary aborters (12.5%). All positive cases were either weakly positive (7.1%) or moderately positive (17.9%). None of the cases were positive for LE cell phenomenon, anti nuclear antibody and VDRL test while rheumatoid factor was positive in 12.5% cases.

Conclusion:- Our study indicates that anticardiolipin antibodies are one of the potential causes of recurrent abortions and probably rheumatoid factor has a role in recurrent abortions. It is recommended these two biochemical parameters be tested in all cases of recurrent abortions.

Key Words: Anticardiolipin antibody, Rheumatoid factor, recurrent abortions.

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INTRODUCTION:

Antiphospholipid antibodies (apL) are a diverse family of autoantibodies reactive against negatively charged phospholipid protein complexes.

The study of apL antibody began in 1907 when Wassermann¹ introduced a diagnostic test for syphilis using a saline extract of liver from fetuses with congenital syphilis.

The clinically significant members include lupus anticoagulant (LA) that is known to prolong in vitro phospholipid dependent coagulation tests, and have been historically referred to as the lupus anti coagulant (LAC), reagenic antibodies causing biological false positive (BFP) venereal disease

laboratory test (VDRL) and anticardiolipin antibody (AcLAb).

The term antiphospholipid syndrome is used to describe the clinical manifestations of venous and arterial thrombosis, thrombocytopenia and recurrent fetal loss² in association with AcLAb or the LAC³. These autoimmune antibodies, which are associated with lupus anticoagulant and anticardiolipin antibodies, are immunoglobulin G (IgG) and immunoglobulin M (IgM).

AcLAb bind independently to the negatively charged phospholipid, a type of fat molecule that is a part of normal cell membrane (in which case, they are called authentic AcLAb) or they may require a cofactor, beta 2 glycoprotein - I (β 2GPI)⁴. Anticardiolipin antibody assay is more sensitive and specific for fetal loss in comparison to lupus anticoagulant⁵.

In pregnancy, the antibodies may react against the trophoblast resulting in sub placental clots and interfere with implantation and subsequently causing defective placentation. Necrotizing decidual vascular lesions are often seen in such placenta⁶. Thrombosis may occur in all the trimesters of pregnancy resulting in complications such as spontaneous abortions and intrauterine growth retardation (IUGR).

Recurrent abortion is defined as the occurrence of 3 or more conceptus losses before 20-22 weeks of gestation, with no intercalated pregnancy resulting in a live birth. In a considerable proportion of cases, the etiology of this disorder of abortion is not known, although several conditions such as defects of the luteal phase, genetic anomalies, anatomical defects of the uterus, systemic arterial hypertension, diabetes mellitus, and hypo- or hyperthyroidism have been proposed as possible causes. Using currently available methods, the cause such abortions is not detected in 40-50% of all cases .

Many studies have been conducted over the last few years in an attempt to establish an association between autoimmune phenomena and recurrent pregnancy wastage. Among the various antibodies studied, antiphospholipids seem to be the best markers of patients at risk for pregnancy wastage. Among them are the antibody responsible for the false positivity of serological tests for syphilis (BFP-STs),

the lupus anticoagulant and the anticardiolipin antibody plus others that have been less extensively studied. Their presence seems to be associated with clinical manifestations such as thromboembolism, thrombocytopenia, and neuropsychiatric disorders in addition to pregnancy wastage, which may form the so-called antiphospholipid antibody syndrome²⁶.

In the present study determinations of AcL of IgG type were conducted in patients who had recurrent abortions and compared with healthy controls to examine its clinical significance.

MATERIAL & METHODS:

A total of 28 pregnant patients at 6 to 8 week of gestation with 3 or more abortions were taken for the study along with 20 controls of same age and sex matched pregnant ladies of same gestations.

Depending upon the the obstetric history, patients were divided into two groups:

Group A: Primary aborters (with no previous child births)

Group B: Secondary aborters (with one or more previous live births)

In both cases and controls, patients giving history of factors known to affect the immune status of the individual such as clinical infection, liver disease, intestinal parasitic infestation, use of drugs such as steroids and chemotherapeutic agents were excluded from the study.

Details of history and physical examination findings were noted. In all patients, hemoglobin, blood group, fasting blood sugar, ultrasonography, TORCH test were done by standard methods.

Besides this LE cell phenomenon, RA factor, antinuclear antibody was done to see role of autoimmunity in these cases. VDRL was also done in all cases. LE cell phenomenon was done by method described in Dacie & Lewis (2001). VDRL was done by kit supplied by Span diagnostics, Surat. ANA was done by indirect fluorescent test, Sehgal 1992⁷. IgG anticardiolipin antibodies were tested by ELISA kit supplied by Omega, Agencies, Delhi The anticardiolipin tests were performed in accordance to the manufacturer's instructions.

Table 1: Levels of IgG aCl antibodies (GPL units.)

Interpretation	GPL units(IU/ml)
High positive	> 100
Moderate positive	20-100
Low positive	10-20
Negative	< 10

RESULTS:

Levels of IgG anticardiolipin antibody were evaluated in primary & secondary aborters, as well as in healthy controls (Table 1).

It was observed that in the healthy controls none of them were positive for AcLAb whereas patient groups were positive for AcLAb. About 17.85% of cases were moderately positive while 7.14% of women were

weakly positive in the group of aborters. None of the women in this study group showed high levels of aCL (Table 2). Besides, secondary aborters had a higher frequency of Acl when compared with primary aborters (35% vs 12.5%; Table 3).

Rheumatoid factor was positive in 12.5% women in aborters group while it was negative in all controls. All positive cases were positive for AcLAb also.

Table 2: Frequency of Anticardiolipin antibodies (aCL) in women with Recurrent abortions and Controls.

Groups	aCL levels (in GPL unit)							
	<10		10-20		20-100		>100	
	No.	%	No.	%	No.	%	No.	%
Aborters(n=28)	21	75	2	7.14	5	17.86	0	0
Controls(n=20)	20	100	0	0	0	0	0	0

About 17.86% cases were moderately positive (20- 100 GPL unit) and 7.14% case were weakly positive (10-20 GPL unit). None of the cases showed high positivity.

Table 3: Comparison of aCL frequency between Primary and Secondary aborters

Groups	aCL levels (in GPL unit)							
	<10		10-20		20-10		>100	
	No.	%	No.	%	No.	%	No.	%
Primary aborters(n=28)	7	87.5	1	12.5	0	0	0	0
Secondary Aborters(n=20)	13	65.0	2	10.0	5	25	0	0

When cases were analyzed according to type of aborters, it was found that secondary aborters had more increased frequency of Acl (35%) as compared to primary (12.5%). Severity of aborters was also high in secondary aborters.

Table 4: Frequency of Anticardiolipin antibodies in patients with Recurrent abortion and control subjects.¹³⁻²⁴

Author (year)	Cases(No. of AcL positive cases)	No. of total cases	Percentage of AcL positive cases	Controls (No.of AcL Positive cases)	No. of total cases	Percentage of AcL positive cases
1. Petri(1987)	5	44	11	1	40	2
2. Barbui T (1988)	4	49	8	0	141	0
3. Parazzini (1991)	11	99	11	4	157	3
4. Parke(1991)	6	81	7	0	88	0
5. O u t (1991)	8	102	8	2	102	2
6. Maclean MA(1994)	20	243	8.4	-	-	-
7. Yetman DL(1996)	150	866	17.3	-	-	-
8. Krysova(1999)	7	30	23.3	0	30	0
9. Chakrabarti's(1999)	12	50	24	-	-	-
10. Sheth JJ(2001)	47	178	11.79	-	-	-
I I. Present Study	7	28	25	0	20	-

Prevalence of AcL Ab in abortions in western literature varied from 7% to 23.3% while Indian studies showed a frequency between 11.8% to 24%.

DISCUSSION:

The exact etiology of recurrent abortion is not always explained. Anticardiolipin antibodies are associated with recurrent abortion and fetal wastage occurs in more than 90% of untreated patients with antiphospholipid syndrome and in those with autoimmune disease⁸.

Micro infarction of the placenta, possibly related to interference in prostaglandin metabolism, may be responsible for the fetal loss, but the role of antiphospholipid antibodies, including aCL, is not yet definitely ascertained⁹. In this study the aCL (IgG) levels were raised in total 25% cases. In 17.9% cases value was between 20-100IU/ml. None of the subjects in control were positive for aCL Ab ($p < 0.05$). None of the cases of aborters were strongly positive for aCL Ab.

Further analysis showed that secondary aborters had more prevalence (35%) than primary aborters (12.5%) indicating that aCL Ab develops as acquired disorder.

VDRL tests were negative in all the cases. Reason for this may be that aCL Ab may be in smaller amounts that could not be detected by ELISA method. As such in our study most of the cases (17.86%) were either moderately positive (20-100IU/ml) or weakly positive-7.14%, (10-20IU/ml). None of our cases were strongly positive for aCLAb.

Although LE cell phenomenon and ANA was absent in all the cases of abortions & control, rheumatoid factor was positive in 12.5% total cases.

Majority of positive cases were secondary aborters although clinically there is no evidence of rheumatoid arthritis. No appropriate data are available on the effects of rheumatoid factor on fertility.

A study conducted in England (McWugh et al 1989)¹⁰ on pregnancy outcome in various Rheumatic diseases revealed that aborters were highest in systemic sclerosis (44%) while in SLE (18%) and Rheumatoid arthritis (17%) its incidence was similar to control population (16%). Raised aCL IgG Ab were found in 25% cases of Rheumatoid arthritis. But investigations conducted by other workers (Fort et al)¹¹ reported that, aCLAb was detected in 38% cases of SLE, 28% cases of Psoriatic arthritis and 33% cases of Rheumatoid arthritis. In Rheumatoid arthritis there was co-relation with aCLAb and history of repeated aborters. Probably these cases in future may manifest as typical Rheumatoid arthritis because pregnancy has favourable effect on Rheumatoid arthritis.

Therefore, Anti-cardiolipin antibodies (aCL) are a

heterogeneous group of autoantibodies detected by enzyme-linked immunosorbent assay (ELISA) which occur in a variety of disorders and also in apparently healthy subjects both in systemic lupus erythematosus (SLE) and the primary anti-phospholipid antibody syndrome, the best-established clinical associations are with thrombosis, thrombocytopenia and spontaneous abortion. These correlations are strongest with anti cardiolipin antibodies of immunoglobulin G (IgG) class which are apparently specific for β_2 -glycoprotein I complexed to negatively charged phospholipid²⁷.

There is compelling evidence from two directions that aCL constitute a significant risk factor for adverse pregnancy outcomes; (i) passive administration²⁸ or induction by active immunization²⁹ in experimental animal models enhances the fetal resorption rate; (ii) several prospective studies of large obstetric populations comparing anti-cardiolipin antibody-positive and negative subjects have shown a significant association between presence of the antibody and fetal loss and other pregnancy complications^{8, 30, 31}.

Nevertheless, the strength of the association between aCL and recurrent miscarriage (RM) is less clear-cut and much more controversial. Although 35% of one series of RM patients were found to have these antibodies³² most studies of such patients report a prevalence of 2-10% for IgG antibodies provided a sensible definition of positivity is used. The largest series reported a value of 3.3%³³. The reported normal prevalence is also rather variable, and far too many studies have related patient data to reference values published elsewhere. Where patients and controls are appropriately compared, variable results and conclusions have been obtained, most reporting some degree of positive association³⁴ but others reporting no association at all³⁵.

Some studies have shown that in fetal deaths associated with antiphospholipid antibodies there is a certain degree of placental infarction. There may be a necrotizing decidual vasculopathy characterized by fibrinoid necrosis, thickening of the intima layer, acute atherosclerosis and intraluminal thrombosis of the spiral arterioles of the plate³⁶. A decrease in uteroplacental circulation is probably responsible for placental infarction and fetal death. It has been demonstrated that the serum of some patients with antiphospholipid antibodies has the ability to reduce prostacyclin (PGI_2) production by rat artery segments and human gravidic myometrium³⁷, and this deficiency is corrected by the addition of arachidonic acid. Since arachidonic acid is a product of endothelial phospholipids, it may be proposed that antibody binding to phospholipids may prevent

prostacyclin production. Since prostacyclin is the most potent physiological anti-platelet aggregation agent, this fact may lead to placental thrombosis. On this basis, decidual vasculopathy would simply be one more expression of the generalized thrombotic tendency of these patients. A decrease in prostacyclin production may be a common factor in the genesis of the decidual vasculopathy observed in several pathological conditions of pregnancy. Indeed, a decrease in prostacyclin production occurs in maternal and fetal vessels in specific hypertensive disease of pregnancy, in delayed intrauterine growth, in chronic hypertension and in diabetes mellitus³⁷. The antiphospholipid antibody syndrome may therefore be another condition leading to chronic placental insufficiency.

Although this is a controversial subject, there are several reports in the literature about patients with these anti-bodies who received treatment during pregnancy and who achieved a reasonable rate of live and viable fetuses. Low doses of acetylsalicylic acid

have been used in combination or not with prednisone or even heparin³⁸. The patients studied here have not yet become pregnant and therefore they have not yet received specific drug treatment.

The clinical significance of the different Acl isotopes is still under investigation. IgG- Acls are generally considered to have broader pathological sequelae¹². The isotopes occur with variable frequency & in individual patients each isotype may occur exclusively or in combination with another isotype.

CONCLUSION:

The present study suggests that a small but significant subset of patients with unexplained recurrent abortion has an immunological basis. Thus it is recommended that routine screening of anticardiolipin antibodies should be done in all cases of unexplained recurrent abortion, as it is a treatable condition.

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