neutral phospholipids such as phosphatidyl choline[2]. Some reports have indicated that patients can be identified who have antibodies that react with phosphatidyl ethanolamine, a zwitterionic phospholipid, and these patients have the same clinical manifestations as patients with lupus anticoagulant.

Lupus anticoagulant and anticardiolipin antibody assays have been standardised by international workshops and the clinical associations of these two antibodies are clearly established[5]. Importantly, the prevalence of lupus anticoagulant and anticardiolipin antibodies is well known and has been shown to be approximately 2-5% in the general obstetric population[6,7]. It is important to note that although lupus anticoagulant and anticardiolipin antibodies are grouped together under the common name aPL these two antibodies are not the same and many patients have one antibody but not the other[7].

aPL are associated with a variety of clinical manifestations of thrombosis including stroke, transient ischaemic attecks, pulmonary embolism and deep vein thrombosis[8]. The antibodies are also associated with obstetric complications including, still birth, recurrent miscarriage, intrauterine growth restriction and preeclampsia. There is now also evidence that aPL are associated with recurrent IVF implantation failure[10,11].

The incidence of aPL in women with recurrent miscarriage or still birth is estimated to range between 20 and 40%[6,7]. However, not all women with aPL have pregnancy complications. For example, in one large study of 933 pregnant women nine women had anticardiolipin antibodies and of these four had normal pregnancies[7]. In the same study, five of 11 women with lupus anticoagulants had normal pregnancies[7]. It is not possible to distinguish definitively between those women with aPL who will have pregnancy complications and those who will not but as is discussed later aPL are now known to bind to phospholipid-binding proteins and work is ongoing to determine whether assays using these proteins (particularly β_2 glycoprotein I or prothrombin) are more predictive of adverse pregnancy outcome than traditional aPL assays but results to date are inconsistent[11.12].

The term antiphospholipid antibody syndrome (aPLS) was coined to describe the condition of patients with aPLS and thrombosis or pregnancy loss[13]. The definition of this syndrome has been updated recently as an international consensus statement[5].

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 & arterial thrombosis, thrombocytopenia and recurrent fetal loss[14] in association with AcLAb or the LAC15. 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 membrance (in which ease, they are called authentic AcLAb) or they may require a cofactor, beta 2 glycopeotein - I (β2GPI)[16]. Anticardiolipin antibody assay is more sensitive and specific for fetal loss in comparison to lupus anticoagulant[17].

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 seen in the placenta[18]. Thrombosis may occur in all the trimester of pregnancy resulting in complications such as spontaneous abortions and intrauterine growth retardation (IUGR).

The aim of this investigation was to look for prevalence of AcL of IgG type antibodies in patients with recurrent abortions and to compare them with healthy controls.

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 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 effect 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, ultarsonography, TORCH test were done by standard methods.

Beside 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 described previously

by Selgal[19].
IgG anticardiolipin antibodies were tested by ELISA kit supplied by Omega agencies, Delhi. Instructions of original manufacturers were followed for anticardiolipin tests.

Table 1. Levels of IgG AcL antibodies were reported as GPL units.

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

Results:

Level of IgG anticardiolipin antibody were evaluated in primary & secondary aborters, as well as in healthy controls.
In healthy controls none of them were positive for AcLAb whereas patient groups were positive for AcLAb.
In healthy controls none of them were positive for AcLab whereas patient groups were positive for AcLAb.
Rheumatoid factor was positive in 12.5% women in aborters group while it was negative in all controls. All positive for AcLAb also.

Table 2. Frequency of Anticardiolipin antibodies (AcL) in females 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		20-100		>100	
	No.	%	No.	%	No.	%	No.	%
Primary aborters(n 8)	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.

DISCUSSION

The exact etioogy of recurrent abortion remains unknown at present.

A number of immune responses are stimulated in pregnant women, in whom an allogeneically competent fetus has been successfully transplanted, and the aberration of immunity in pregnant women might cause adverse pregnancy outcome including recurrent abortions. It has attracted increasing attention that autoimmune mechanisms, one of the pathological immune mechanisms, are involved in the pathophysiology of adverse pregnancy outcome based on the findings that such adverse pregnancies are common events in patients with autoimmune disease, such as systemic lupus erythematosus[44,45].

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 disease[20].

Recurrent pregnancy loss, three or more consecutive (primary or secondary) pregnancy losses with no more than one pregnancy extending into the third trimester, is the cause of childlessness in 2-5% of couples trying to conceive[21,22]. Some of the common factors incurring risk for recurrent miscarriage include anatomic defects of uterus, chromosmal aberrations, endocrine factors, and sub-clinical infections and immunological disturbances. Even after conventional investigations 40-60% of cases remain idiopathic. Accumulating evidence suggests that there are immunological mechanisms responsible for repeated abortions, with otherwise unknown cause[23].

Over the past decade increasing attention has been drawn to the association of antibodies having an apparent specificity for negatively charged phospholipids with thromboembolic episodes such as arterial and venous thrombosis, foetal loss and thrombocytopaenia, widely recognised as the antiphospholipid syndrome (APS)[6,24]. Antiphospholipid syndrome accounted for 5-20% of women with recurrent pregnancy loss.

Microinfarction 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 ascertained[25]. In this study the AcL (IgG) levels were raised in total 25% case . In 17.86% cases value was between 20-100IU/ml. None of the subjects in control were positive for AcLAb ($p<0.05$). 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 maybe 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 in 7.14% of case (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 and control, rheumatoid factor was positive in 12.5% total cases.

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

Studies conducted in England (McWugh etal 1989)[26] 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 study conducted by some other workers[27] reported that, AcLAb was detected in 38% cases of SLE 28% cases of Psoriatic arthritis and 33% cases of rheumatoid arthritis. In rhuematoid 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.

The distinguishing feature of LAC associated pregnancy loss is the high incidence of early foetal deaths. An adequate explanation for this association is still lacking and the most striking clinical implication is its association with systemic and placental vascular thrombosis with decidual vasculopathy leading to placental infarction[28].

Based presumably, on reports of infarcted placentae from women with lupus anticoagulant and the evidence that aPL cause systemic thrombosis[2, 29-31] received wisdom would have it that aPL cause fetal death and other obstetric complications by inducing thrombosis in the uteroplacental circulation, particularly the spiral arteries. Thrombosis of the spiral artery then leads to infarction of the placenta and subsequent fetal demise. Although this mechanism could possibly explain how aPL cause late fetal deaths there are a number of difficulties with this mechanism. It now appears that aPL causes first trimester miscarriages, indeed the most recent classification of aPLS includes three consecutive spontaneous abortions prior to 10 weeks gestation[5]. There is now substaintial evidence that during the first weeks of pregnancy the spiral arterties are occluded by trophoblast plugs and that significant blood flow into the intervillous space does not occur until the end of the first trimester of pregnancy[32]. Thus, intuitively it seems unlikely that aPL could cause in particular, early miscarriages (or disrupt IVF implantation) by inducing thrombosis in these vessels. Additionally, the available histological evidence is not supportive of a thrombotic mechanism for aPL inducing fetal demise. Out et al[33] in a survey of 17 placenta from women with aPL found no evidence

of thrombosis in 18% of cases. Another study of placenta from women with aPL demonstrated no evidence of thrombosis in any of the 11 placenta studied from untreated pregnancies ending prior to the 18th week of gestation[34]. The same study showed only three of five (60%) placenta from untreated pregnancies ending between 18 and 22 weeks of gestation had signs of thrombosis[34]. If thrombosis is the mechanism by which aPL cause fetal demise it would be expected that all affected placenta would have signs of significant thrombosis/infarction and this is clearly not the case. Thus, while it is possible that thrombosis could contribute to the pathogenesis of aPL-mediated fetal death (particularly in late fetal losses) it is unlikely that thrombosis is the primary cause of fetal demise or other aPL-mediated pregnancy complications. The search is now on to identify the mechanism by which aPL disrupt pregnancy but any proposed mechanism must be able to account for the first trimester losses that these antibodies cause.

The clinical significance of the different AcL isotypes is still under investigation. IgG - AcIs are generally considered to have broader pathological sequelae[12]. The isotypes occur with variable frequency and in individual patients each isotype may occur exclusively or in combination with another isotype.

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

Author (year)	Case (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. Out (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	-	-	-
11. 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 11.79% to 24% (Table 3)

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 treatable condition.

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