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EVALUATION OF ANTICARDIOLIPIN ANTIBODIES AND ANTIPHOSPHATIDYLSERINE ANTIBODIES IN WOMEN WITH RECURRENT ABORTION

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ABSTRACT

BACKGROUND: Antiphospholipid syndrome (APS) is a major reproductive complication in women, which is characterized by recurrent fetal loss, thrombosis, and thrombocytopenia in association with anticardiolipin antibodies (aCL). AIMS: To analyze the prevalence of aCL and antiphosphatidylserine antibodies (aPS) in relation to pregnancy failures in women with the history of recurrent spontaneous abortion. SETTINGS AND DESIGN: A sequential study of 155 patients, who had three or more recurrent spontaneous abortions, was carried out. METHODS AND MATERIALS: Women with unexplained recurrent pregnancy loss in first trimester were selected for this study. Anticardiolipin antibodies IgG and aPS IgG were detected in the serum by the enzyme linked immunosorbent assay method. STATISTICAL ANALYSIS: Percentage calculation was carried out. Two-tailed t-test was performed to know the significance of aCL and aPS total population. RESULT: The levels of aCL IgG and aPS IgG were detected as 40% (62) and 19% (18), respectively in women with history of recurrent abortion. CONCLUSION: Anticardiolipin antibody is found to be the most important factor for recurrent abortion. In addition, women with negative aCL are having positive for another antiphospholipid antibodies like aPS, which may involve in recurrent abortion.

KEY WORDS: anticardiolipin; antiphospholipid antibodies; antiphospholipid syndrome; recurrent pregnancy loss

Pregnancy and related disorders in women of reproductive age group is common. Recurrent abortion is quite critical in which many factors play a crucial role including anticardiolipin antibody (aCL) and antiphosphatidylserine

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Correspondence G. Archunan Department of Animal Science Bharathidasan University Tiruchirappalli - 620 024 India. E-mail: garchu56@yahoo.co.in antibody (aPS). The major underlying systems like anatomical, physiological, and endocrine pathology are under investigation as well as management.

Patients with unexplained recurrent abortion often demonstrate many factors, autoantibodies in blood as antiphospholipid antibodies (aPL), and antinuclear antibodies (ANA).^[1] Mc Intry^[2] has reported certain aPL interferes in very early pregnancy, that is, at the stage of fetal implantation by impeding normal reproductive event. These miscarriages or implantation failure may be related to pathological mechanism causing recurrent abortion, which is commonly diagnosed as infertility.^[3] There is clear documentation that aCL are involved in fetal wastages and recurrent abortion irrespective of the patient whether having auto immune disease or not. Anticardiolipin antibody detection may be the most sensitive method in prevention of fetal wastage.^{[4],[5]} There is a controversy arising whether aCL are associated with recurrent abortion, because most of the women with pregnancy losses were negative for aCL and positive for other aPL.^[6]

After a widespread global study screening of patients with antiphospholipid syndrome (APS) for aCL was done, astonishingly it was found to have other antibodies such as antiphosphatidylinositol, antiphosphatidylglycerol, and aPS.^[7] There is difference of opinion whether these antibodies play a crucial role or not. Individual aPL have their own part in pregnancy loss. Furthermore aPL induce thrombocytopenia thrombotic episodes, recurrent pregnancy loss, or a combination of the above.^[8] Although, higher number of childhood onset lupus patients also had APS, the aPL is widely accepted as a risk factor for recurrent abortion.^[9] However, the significance of aCL and aPS is unclear in patients with recurrent abortion. To determine if any women with negative aCL were positive for other aPL such as aPS levels in women with recurrent pregnancy loss were estimated by conventional enzyme linked immunosorbent assay (ELISA).

This study is an attempt to address the prevalence and correlation of aCL and aPS in

women with recurrent spontaneous abortion by the conventional ELISA method.

MATERIALS AND METHODS

One hundred and fifty-five women with history of three or more consecutive unexplained recurrent abortion in the first trimester were followed up sequentially in certain Government hospitals of Tamil Nadu, accounted for this study over a period of 2 years. The selected patients belonged to the age group of 25-31 and they were thoroughly investigated for all baseline blood parameters, and other infectious diseases including metabolic diseases. All other pathologies were ruled out except aPL. Those patients who showed negative for the above said tests were selected for the next step of aPL screening test. Serum is the recommended sample for the evaluation of aCL and aPS. Therefore, for the present investigation blood was collected from the women who had experienced an abortion. The blood sample was then allowed to clot for 30 min-1 h and serum was collected from the sample. The serum was again centrifuged at 4000 rpm at 10°C in cooling centrifuge and then the purified serum sample was used for further evaluation. Serum samples were evaluated to see the presence of aCL IgG. Samples, which showed negative for aCL, were further processed to find out the presence of other aPL such as aPS. In order to fulfill the Sapporo criteria, both the autoantibodies were tested on two occasions with 6 weeks apart to distinguish antibody response. Antiphosphatidylserine antibody and aCL estimation were done by the ELISA technique as described by Harris et al.[10] In brief, individual 96 well micro titer plates were

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coated with 30 ml of cardiolipin at a concentration of (45 mg/ml) or phosphatidylserine (50 mg/ml) and blocked with 200 ml of blocking buffer containing 10% fetal calf serum for 2 h. To this plate, 50 ml of test samples in 1:150 dilutions with PBS were added and allowed to react. After washing, equal amount of affinity purified horseradish peroxidase enzyme linked secondary antibody (antibodies to IgG) was added to respective wells and allowed to react for 3 h at 4°C. Subsequently, the plates were washed and enzyme substrate (H₂O₂) was added along with evolving chromogen (3,3¢ 5,5¢ tetramethyl benzidine). The reaction was allowed at 56°C until the blue color end point was obtained. After the completion of reaction, the stop solution was added to stop the reaction and optical density read in ELISA reader at 405 nm. The result, which was calculation against concentration, was interpreted in GPL units <10 negative, 10-19 border line, 20-80 positive, and >80 high positive.[11]

The two tailed *t*-test was performed to find out significance variance of positive aCL and aPS among the total test population by using SPSS statistical software,11th Version.

Among the 155 patients with unexplained

negative for aPS. The negative aCL samples were evaluated for aPS IgG antibody; in this category, 93 women (i.e., samples) were studied. A correlation was observed between the aCL and aPS in patients with recurrent abortion. As demonstrated in Table 1, those women who exhibited (40%; 62 of 155) recurrent pregnancy loss were positive for aCL but the remaining patients were negative to these antibodies. The remaining negative samples were tested for aPS, in which 18 (19%) patients showed positive for aPS. The APS was found in approximately 51.6% of women with recurrent abortion. Therefore, a total of 80 patients were reported to have APS and 75 patients did not have APS in women with recurrent abortion. However, these 75 patients also showed recurrent abortion but the etiology was not known. The samples of women who had borderline, positive and high positive values of both aCL and aPS are given in the Table 1. The individuals 32, 24, and 6 had borderline, positive, and high positive values of aCL, respectively. By contrast, the patients viz 6, 11, and 01 showed borderline, positive, and high positive values of aPS, respectively. Borderline, positive, and high positive values were considered as significant factors to eradicate crucial circumstances in recurrent abortion. The risk ratio of pregnancy

recurrent spontaneous abortion 62 were

positive for aCL IgG antibody and showed

Table 1: Analysis of positive (aCL and aPS) cases and interpretation with different GPL units

Diagnosis	Number of positive patients among different GPL units			Proportion and percentage of
	Borderline (10–19)	Positive (20–80)	High positive (>80)	positive patients
aCL positive *	32	24	06	62/155 (40.00)†
aPS positive *	06	11	01	18/93 (19.00)

* Values are significant at P < 0.05

RESULT

Legend: aCL, anticardiolipin antibodies; aPS, antiphosphatidylserine antibodies

failure is likely to be 51.6% in those women having positive aCL and aPS. The positive aCL (t = 2.00 for 60 d.f.) and aPS (t = 2.16 for 17 d.f.) among three different GPL units were significant at P < 0.05 level.

DISCUSSION

In the present study, ELISA used to detect aPL is a common and popular tool for detecting aPL. Generally, solid phase Immunoassay was performed on cardiolipin-coated plate usually in the presence of bovine or fetal calf serum which consists of b2 glycoprotein I. This b2 glycoprotein I give antigenic epitope to aCL and this protein has 89% homology between bovine and human in amino acid level.^[12]

The present study showed that 40% of patients with recurrent abortion had a positive result for ACL. The results are consistent with the previous reports that 8-42% of recurrent pregnancy loss is due to positive aCL.[13]-[15] Additionally, our results indicated that 19% of women with recurrent pregnancy loss were positive for aPS. The presence of aCL has been noted in the sera of women who had a recurrent abortion for which no cause has been found.^{[16],[17]} There is an association between the aCL and recurrent pregnancy loss. Previous studies have subsequently confirmed the adverse effect of aCL on pregnancy with the experimental mouse model. The experimental induction of APS causes the increased resorption rate and at the same time decreased placental and embryos weight in pregnant mice.^[18] It is interesting to note that in the present study aCL negative patients also had recurrent abortion, in which 18 of 93 women revealed the presence of aPS. The IgG aPL of each

phospholipid were more prevalent and were found frequently in women with recurrent pregnancy loss and IgG aCL is reported to be more strongly associated with clinical events than IgM aCL.[19],[20] Therefore, 80 women were considered to have APS at the end of investigation about the presence of positive aCL and aPS, whereas 75 of 155 women did not have the APS. Controversy exists in relation to the unexplained abortion and APS. Antiphospholipid syndrome is a clinical disorder with at least one specific clinical and one laboratory abnormality must be present either aCL and/or lupus anticoagulant.^[21] The fetal outcome of pregnancy in patients with recurrent pregnancy loss associated with APS was virtually identical and guite satisfactory.[22] In some cases, the women with recurrent pregnancy loss did not have positive aCL. The absence of aCL and aPS in patients with recurrent abortion is comparable. In the previous study, women with recurrent pregnancy loss and positive aCL were compared with women with recurrent pregnancy loss and negative aCL.[6] Antiphospholipid syndrome is also characterized in the presence of lupus anticoagulant. Although, lupus anticoagulant and aCL are grouped together under the common name aPL, these two antibodies are not the same and many patients have one antibody but do not have the other.[23] Earlier studies stated that the aCL-type B (without LA) were found in women with recurrent pregnancy loss^[24]. Further more, positive LA test is much more strongly associated with the clinical manifestation of thrombosis than the recurrent pregnancy loss in patients with APS.^[21] Although it is now accepted that the screening test should be sensitive to lupus anticoagulant,

the basis for this sensitivity is unclear.^[24] Hence, clinical and laboratory classification criteria of Sapporo conference have again become controversial.^[21]

The present results also showed an increase in the incidence of aCL in the group with unexplained pregnancy loss, which is in agreement with other findings.[25]-[27] It is also quite common the women who had recurrent abortion in the case of aCL and aPS remains negative. The study carried out by Kutteh et al.[28] in the group of 125 women who had pregnancy loss in aCL-negative vs -positive patients. We have also observed 18 of 98 patients with recurrent pregnancy loss showed positive for aPS, whereas showed negative for aCL. This result is in agreement with previous investigations that a range of 10.1-17.3% of recurrent pregnancy loss sample was positive for other aPL. Many studies claimed that aPS might affect the outcome of pregnancy. Antiphosphatidylserine antibody has been closely associated with implantation failure, which indicates that aPS is particularly pathogenic to trophoblast.^[29] Hence, this study further provides an awareness to evaluate other antibodies like antiphosphotidylinositol, antiphosphatidylethanolamine and aPS, which could be a factor for recurrent miscarriages. Earlier studies showed a strong association between recurrent pregnancy losses and other aPL.[30] It is hoped to establish the clinical significance of APS in women with recurrent pregnancy loss because the identification of antibodies to phospholipids other than cardiolipin is most important. This study emphasizes the correlation and the significance of aPL such as aCL and aPS in the occurrence of recurrent abortion.

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