IJHG 2(1): 65-67 (2002)
PRINT: ISSN 0972-3757 ONLINE: 2456-6360
DOI: 10.31901/24566330.2002/02.01.13

ß$_2$-Glycoprotein I Dependent Anticardiolipin Antibodies in Women Experiencing Recurrent Pregnancy Loss

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KEY WORDS Anticardiolipin; autoantibodies; ß$_2$-glycoprotein; phospholipids; predictive risk factor; recurrent pregnancy loss

ABSTRACT Enzyme linked immunosorbent assay (ELISA) for ß$_2$-Glycoprotein I dependent anticardiolipin antibodies (ß$_2$I aCL) was carried out in 82 women experiencing 3 or more recurrent pregnancy losses and 82 normal healthy controls. The mean aCL levels were 14.53 (µ/ml) ± 16.22 in women with repeated abortions and 7.26 (µ/ml) ± 3.65 in controls. The difference in the values between the two groups was found to be highly significant (P<0.001). We suggest the usefulness of screening ß$_2$I aCL as a routine marker in predicting the risk for future pregnancies.

INTRODUCTION

Women experiencing recurrent pregnancy loss have a higher prevalence of antiphospholipid autoantibodies in their blood (Ogasawara et al. 1999; Bick et al. 1999). Anticardiolipin antibodies (aCL) belong to a family of autoantibodies that react with negatively charged phospholipids. Recently the antigen for autoimmune aCL was identified as ß$_2$-Glycoprotein I (ß$_2$ I), a 50 KDa ß$_2$ globulin, which occurs in plasma at a level of 200 mg/ml (McNeil et al. 1990; Polz and Kostner 1978). ß$_2$ I enhances the cardiolipin binding with autoimmune aCL but inhibits the binding of aCL associated with syphilis (Maejima et al. 1997).

Anticardiolipin antibodies exist in the immunoglobulin classes IgG, IgM and/or IgA. Meagre studies on ß$_2$ I dependent aCL from abroad and no studies from India has necessiated to take up this study to establish the prevalence of aCL autoantibodies in Indian women and to find their role in the aetiology of immune pregnancy loss.

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MATERIALS AND METHODS

The present study was carried out at the Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad over a period of 5 years (January 1996- January 2001). A total of 150 women with a history of recurrent pregnancy loss were investigated. Detailed clinical histories and information pertaining to age, region, religion, habits, number of previous spontaneous abortions, number of live born children, pedigree, past medical histories were recorded in special case proformas. All the patients underwent chromosomal investigations, ultrasonography, hormonal screening tests and tests to rule out infections. Among 150 women, 82 had no conventional causes for abortions and were selected for aCL screening test. The age of the patients ranged from 18-35 years with a mean of 25.47 years and had previously experienced 3 or more consecutive abortions (Mean 3.42, range 3-8 abortions). 82 age matched healthy women who had experienced atleast one successful pregnancy and no previous history of abortions were taken as controls.

Among the study group the majority, 46 (56.09%) had experienced only early pregnancy losses (<13 weeks gestation), 23 (28.04%) had only experienced late abortions (>13 weeks gestation), and 13 (15.85%) had both early and late miscarriages. In all, five women (6.09%) had previously experienced a successful pregnancy, followed by recurrent abortions (secondary recurrent pregnancy loss). Serum samples obtained from patients and controls were stored at –20^{0}C until used.

ELISA kit for aCL screen with human ß$_2$ I as cofactor was obtained from ORGen Tec, GmbH, Germany. The test was performed as per manufacturer's instructions. The optical density (OD) was measured at 450nm. The amount of aCL was determined based on the standard curve produced using standard antibodies, provided with the kit. Differences between the two groups

were analysed for significance by using Z-test.

RESULTS AND DISCUSSION

The ß$_2$-glycoprotein I dependent aCL levels were estimated in serum samples of 82 women with RPL and in equal number of controls by ELISA kit method (Table 1). The amount of ß$_2$I aCL >10 units/ml with this kit was considered to be positive for the presence of the sum of IgG/IgM/IgA autoimmune antibodies and <10 µ/ml was considered to be negative. From the standard curve, the mean ± SD aCL concentration in the study group was found to be14.53 (µ/ml) ± 16.22 (range 0 to 90.4 µ/ml). This was much higher than in the control group with a mean ± SD of 7.26 (µ/ml) ± 3.65 (range 0 to 18 µ/ml). The difference in the mean values between the two groups was found to be highly significant (Z=3.96, P<0.001). The binding of the antibodies to the antigen was observed in 40.24% (n=33) of the cases compared to 6.09% (n=5) in controls.

Table 1: ß$_2$ -Glycoprotein I dependent anti-cardiolipin antibody levels (µ/ml) in women with recurrent pregnancy loss compared with control subjects

Groups	*Number of Cases*	*Anticardiolipin levels (µ/ml) Mean ± SD*	*Range*
Study Group	82	14.53 ± 16.22	0-90.4
Control Group	82	7.26 ± 3.65	0-18

***P<0.001, highly significant

According to previous reports the frequency of aCL in recurrent pregnancy loss ranged from 11% to 42% (Unander et al. 1987; Taylor et al. 1990; Rai et al. 1995). This wide distribution in part may be accounted for variations in assay protocols of various laboratories used to detect aCL (Peaceman et al. 1992). A new aspect relating to antigen specificity demonstrated the necessity of a co-factor, namely ß$_2$-glycoprotein I, for the detection of autoimmune aCL in ELISA test systems, to selectively eliminate aCL associated with syphilis. Present study on ß2-glycoprotein-I dependent aCL in women experiencing RPL is the first one to be reported from India.

Human ß$_2$I is a protein of 345 amino acids, with several interesting cell biological characteristics. *In vivo* ß$_2$I is associated with lipoproteins, anionic phospholipids, platelets and mitochondria. *In vitro* it has been found that ß$_2$I inhibits the prothrombinase activity of platelets and ADP-mediated platelet aggregation, suggesting a possible role of ß$_2$I in the regulation of blood coagulation (Mehdi et al. 1991; Chamley 1997).

In recent years several authors have found ß$_2$-glycoprotein-I dependent aCL as superior in predicting adverse pregnancies involving autoimmunity, than co-factor independent aCL (Aoki et al. 1995; Maejima et al. 1997; Aoki 2000). In the present study we found ß$_2$-glycoprotein-I dependent anticardiolipin antibodies in 40.24% (n=33/82) of the cases investigated. In controls low serum concentrations of aCL were found in 5 (6.09%) women. Yap et al. (1998) suggested that the significance of aCL in general population in the absence of clinical features is uncertain. Autoimmune aCL may induce pregnancy failure either by impairing embryonic implantation (Sthoeger et al. 1993) and/or binding directly to placenta (Katano et al. 1996). Hence it is clear that the presence of autoimmune aCL may induce pregnancy loss at any trimester. Data from the present study with increase in the mean activity of ß$_2$I dependent aCL (Table 1) in women with first and second trimester idiopathic pregnancy losses seems to be involved significantly in the pathogenesis of the pregnancy failure. Hence we suggest the usefulness of ß$_2$I aCL as a screening marker for predicting future pregnancies and to offer proper counselling.

ACKNOWLEDGEMENTS

Financial support extended by University Grants Commission, New Delhi is highly acknowledged.

REFERENCES

Aoki K, Dudkiewicz AB, Matsuura E, Novotny M, Kaberlein G, Gleicher N 1995. Clinical significance of ß$_2$-glycoprotein I dependent anticardiolipin antibodies in the reproductive autoimmune failure syndrome: correlation with conventional antiphospholipid antibody detection systems. *Am J Obstet Gynecol,* **172:** 926-931.

Aoki K 2000. Antiphospholipid antibody syndrome in adverse pregnancy. *Rinsho Byori*, **48:** 323-327.

Bick RL, Arun B, Frenkel EP 1999. Antiphospholipid thrombosis syndrome. *Haemostasis*, **29:** 100-110.

Chamley LW 1997. Antiphospholipid antibodies or not? The role of ß2- glycoprotein 1 in autoantibody mediated pregnancy loss. *J Reprod Immunol,* **36:** 123-142.

Katano K, Aoki K, Sasa H, Ogasawara M, Matsuura E, Yagami Y 1996. ß$_2$-glycoprotein-I dependent anticardiolipin antibodies as a predictor of adverse pregnancy outcomes in healthy pregnant women. *Hum Reprod,* **11:** 509-512.

Maejima M, Fujii T, Okai T, Kozuma S, Shibata Y, Taketani Y 1997. ß$_2$-glycoprotein-I dependent anticardiolipin antibody in early recurrent spontaneous abortion. *Hum Reprod*, **12:** 2140-2142.

Mc Neil HP, Simpson RJ, Chesterman CN, Krilis SA 1990. Antiphospholipid antibodies are directed against a complex antigen that includes a lipid binding inhibitor of coagulation: ß$_2$-glycoprotein-I (apolipoprotein H). *Proc Natl Acad Sci USA*, **87:** 4120-4124.

Mehdi H, Nunn M, Steel DM, Whitehead AS, Perez M, Walker L, Peeples ME 1991. Nucleotide sequence and expression of the human gene encoding apolipoprotein H (ß$_2$-glycoprotein-I). *Gene*, **108:** 293-298.

Ogasawara M, Aoki K, Katano K, Aoyama T, Ozaki Y, Suzumori K 1999. Prevalence of autoantibodies in patients with recurrent miscarriages. *Am J Reprod Immunol*, **41:** 86-90.

Peaceman AM , Silver RK , Mac Gregor SN, Socol ML 1992. Interlaboratory variation in antiphospholipid antibody testing. *Am J Obstet Gynecol*, **166:** 1780-1787.

Polz E, Kostner GM 1978. The binding of ß$_2$-glycoprotein-I to human serum lipoproteins: Distribution among density fractions. *FEBS Lett*, **102:** 183-186.

Rai RS, Regan L, Clifford K, Pickering W, Dave M, Mackie I, Mcnally T, Cohen H 1995. Antiphospholipid antibodies and ß$_2$-glycoprotein-I in 500 women with recurrent miscarriage: results of a comprehensive screening approach. *Hum Reprod*, **10:** 2001-2005.

Sthoeger ZM, Mozes E, Tartakovsky B 1993. Anticardiolipin antibodies induce pregnancy failure by impairing embryonic implantation. *Proc Natl Acad Sci USA*, **90:** 6464-6467.

Taylor M, Cauchi MN, Buchanan RR 1990. The lupus anticoagulant, anticardiolipin antibodies, and recurrent miscarriage. *Am J Reprod Immunol*, **23:** 33-36.

Unander AM, Norberg R, Hahn L, Arfors L 1987. Anticardiolipin antibodies and complement in ninety- nine women with habitual abortion. *Am J Obstet Gynecol*, **156:** 114-119.

Yap C, Yeoh SC, Viegas OAC 1998. Antiphospholipids and pregnancy- A review. *Sing Med J*, **39:** 331-334.