ORIGINAL RESEARCH

Prevalence of Antiphospholipid Syndrome in Recurrent Pregnancy Loss: A Case Control Study

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ABSTRACT

Background and Objectives: Antiphospholipid syndrome (APS) has been reported to be one of the most important causes of Recurrent Pregnancy Loss(RPL). In this study, we compared the prevalence of APS in women with and without history of RPL. **Methods**: A total of 30 women in reproductive age group (15-45) years with a history of three or more consecutive pregnancyloss upto 20 weeks of gestation were enrolled as cases and a total of 15 age- matched women with at least one successful pregnancy and no history of pregnancy loss were enrolled as controls. Women with history of still birth or intermittent pregnancy loss were excluded. Blood specimen from all the women were obtained and assessed for activated partial thromboplastin time (APTT), Dilute Russell's viper-venom test (DRVVT), Anticardiolipin, β-2 glycoprotein 1 and antiphospholipid antibody tests. Data was compared between cases and controls using Fisher exact test. **Results**: Mean age of cases and controls was 29.37±5.79 and 28.60±4.31 years respectively ($p>0.05$). Cases had an average of 3.33 pregnancy losses. Prevalence of APS was 36.7% in cases as compared to 0% in controls ($p=0.008$). None of the cases or controls had elevated APTT. Elevated DRVTT was the least common abnormality in cases (3.33%) whereas β-2 glycoprotein antibody was the most common abnormality (36.7%). At factorial level, except for β-2 glycoprotein antibody ($p=0.008$), none of the other APS factors showed a significant difference between cases and controls ($p>0.05$). **Conclusion**: APSinduced thrombosis could be one of the most important causes of RPL.

Key Words: Recurrent Pregnancy Loss, Antiphospholipid Syndrome, Anticardiolipin Antibody, B-2 Glycoprotein-1 Antibody, Antiphospholipid Antibody

INTRODUCTION

Recurrent pregnancy loss (RPL) is an obstetric condition defined as 3 consecutive pregnancy losses prior to 20 weeks from the last menstrual period. The incidence of RPL is reported to be 1 in 300 pregnancies; however, nearly 1 to 2% women experience it during their reproductive age.[1] In the recent years, the incidence of RPL has been shown to be rapidly increasing probably owing to environmental changes.[2] RPL can be caused by a spectrum of causes including uterine abnormalities, cervical conditions, chromosomal abnormalities, endocrine disorders, thrombophilia, ovarian factors, life style factors, immune factors, thyroid antibodies and antiphospholipid syndrome (APS).[3] Among all these causes, thrombophilia has been considered to be one of the most important causes of early pregnancy loss, however, most of the current evidence documents APS as the most important and difficult to manage causes of RPL.[4,5] APS has been documented to affect nearly 20-25% of RPL cases.[6,7] Without treatment, APS could contribute to 90% of RPL.[8] Thus, APS happens to be not only an important contributor to the total burden of RPL but also needs to be assessed and treated in order to improve outcomes in the subsequent pregnancies. Considering the significance of APS in RPL, the present study was carried out to assess prevalence of APS at factorial level in women with history of RPL and to compare it with a control population of women without history of RPL.

MATERIAL AND METHOD

This case-control study was carried out at Department of Pathology; King George's Medical University, Lucknow, UP, (India) and included a total of 45 women in reproductive age group (15-45 years). Of these a total of 30 women had a history of three or more consecutive pregnancy loss up to 20 weeks of gestation and comprised the Case group of the study

(Mean age 29.37±5.79 years) whereas a total of 15 age matched women with at least one successful pregnancy and no bad obstetric history were included as controls (Mean age 28.60±4.31 years). Those with history of still birth or intermittent pregnancy loss were excluded from the study. At enrolment patient's age, obstetric and medical history was obtained. All the patients were assessed for activated partial thromboplastin time (APTT), Dilute Russel's viper venom test (DRVVT), anticardiolipin antibody (aCL-ab) IgM & IgG, anti-β-2-glycoprotein-1 antibody (anti-β2-GP1) IgM & IgG and antiphospholipid antibody (APA) IgM & IgG.

Procedure

Venous blood samples (5 ml) were collected from the study participants under all aseptic conditions. One part of tri-sodium citrate (3.2%) and nine parts of freshly collected blood were mixed and centrifuged at 3000 rpm. Supernatant plasma was separated and subjected to further analysis within three hours of sample collection. APTT assessment was done as per method described by Dacie and Lewis.[9] APTT>34 seconds was considered as abnormal. Russel's viper venom test for lupus anticoagulant was done using LA Screen and LA Confirm assessment. The normal values for LA Screen were taken as 28-45 seconds. For correction of LA screen mixing studies were also done. For LA confirm the normal values were taken as 28-40 seconds. DRVTT ratio (R) was calculated by dividing the mean LA screen time with mean LA confirm time. The cut-off value for presence of Lupus anticoagulant was taken as >1.22. Assessment for aCL-ab IgM & IgG, anti-β2-GP1 IgM & IgG and APA IgM & IgG was done using ELISA. Following cut-off values were chosen for interpretation of a positive result:

	IgG	IgM
aCL-ab	≥10 GPL U/ml	≥7 MPL U/ml
anti-β2-GP1	>1.0 (Sample OD/Cut-off OD)	-
APA	≥10 GPL U/ml	≥10 MPL U/ml

Presence of any of these three parameters was considered as an indicator of Antiphospholipid Syndrome.

Data Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) 21.0 software. Fisher exact test was used for the purpose ofcomparison.

RESULTS

None of the cases as well as controls had abnormal APTT. DRVTT ratio above 1.22 was seen in 1 (3.33%) case as compared to none of the controls. Statistically, there was no significant difference between two groups with respect to elevated DRVTT (p=1.000). Anticardiolipin, Anti β-2 glycoprotein-1 and antiphospholipid antibody positivity (IgM/IgG) was seen in 2 (6.7%), 11 (36.7%) and 2 (6.7%) of cases as compared to none of the controls. Overall prevalence of APS was 36.7% in cases as compared to 0% in controls, thereby showing a statistically significant difference between two groups (p=0.008) (Table 1).

Table 1: Prevalence of Antiphospholipid Syndrome factors in Cases vsControls

SN	Factor	Cases (n=30)	Controls (n=15)	Statistical significance (Fisher Exact Test)
1.	APTT >34s	0	0	-
2.	DRVTT Ratio >1.22	1 (3.33%)	0	1.000
3.	Anticardiolipin antibodypositive	2 (6.7%)	0	0.545
4.	Anti β-2 glycoprotein-1antibody positive	11 (36.7%)	0	0.008
5.	Antiphospholipidantibody positive	2 (6.7%)	0	0.545
6.	Any positive	11 (36.7%)	0	0.008

DISCUSSION

Antiphospholipid Syndrome, sometimes also referred to as Hughessyndrome is a multisystemic autoimmune disorder that is associated with an increased risk of blood clotting.[10] It has a strong association with pregnancy complications like preeclampsia, thrombosis, autoimmune thrombocytopenia, fetal growth restriction, and fetal loss.[11] Its association with recurrent pregnancy loss/miscarriages has extensively been documented.[7,8,10,12] In the present study, we found its prevalence in women having a history of RPL to be 36.7%. At factorial levels, we found anti β-2 GP-1 antibody to be the most common abnormality (36.7%) whereas aCL and APA were abnormal in 2 (6.7%) cases each. Adelowo and Adetoro,[13] however, considered aCL and lupus anticoagulant to be diagnostic. In the present study, lupus anticoagulant (DRVTT) was confirmed in only 1 (3.3%) of cases. As such, Anti β-2 glycoprotein-1 antibody is now increasingly recognized as a laboratory criteria for diagnosis of APS.[14] Inclusion of Anti β-2 glycoprotein-1 helps to enhance the

sensitivity substantially. Compared to the present study, Verabally and Seedipally[15] who studied only anticardiolipin antibody found its positivity rate to be 16% in cases and only 2% in controls. However, Sheela *et al.* who included Lupus Anticoagulant (LA) - Anti Cardiolipin Antibody (ACA)- Anti β2 glycoprotein 1 (Anti-β2GP1Ab) for the purpose of identification of APS reported the prevalence of APS as 21.2%. Lee et al.[16] in their study similar to the present study also found Anti-β2GP1Ab to be positive in much higher proportion of RPL women (44%) as compared to anticardiolipin (11%). A high prevalence of APS in RPL women has also been documented by Velayuthaprabhu and Archunan, who found it to be positive in 40% using aCL IgG as the criteria. In the present study, we assessed both IgG and IgM for all the three markers and hence were able to find out a slightly higher prevalence of APS in the RPL women. The findings of the present study were encouraging from the point of view that they could help in formulating appropriate treatment strategies. It has also been reported that while APS could contribute to 90% of RPL cases when it remains untreated,[8] however, on treatment 70% of APS patients are expected to deliver successfully.[18,19] Hence, assessment for APS should mandatorily be done in case of RPL cases in order to rule out subsequent riskof pregnancy loss with proper intervention.

CONCLUSION

The present study showed that APS was prevalent in more than one- third cases of RPL, thus showing it as a possible etiological factor for RPL. Mandatory assessment for APS in women experiencing pregnancy loss is recommended to rule out the risk of RPL.

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