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Original Research Article

The study of antiphospholipid antibodies in recurrent pregnancy loss

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ABSTRACT

Background: Around 15% of clinically recognised pregnancies in women less than 35 years old result in spontaneous miscarriage. However recurrent pregnancy loss (RPL) is encountered in 5% of couples with two or more losses and in around 1–2% of couples with three or more losses. In view of the increasing burden of recurrent pregnancy loss in the society and in view of Anti-phospholipid syndrome being one of the undisputed treatable cause for recurrent pregnancy loss, this study aims to evaluate the significance of the antibody profiles of APS in relation to RPL in OBG dept of VIMS and RC.

Methods: Patients coming to the Obstetric and Gynaecology department of Vydehi Institute of Medical Sciences and Research Centre, Bangalore, from December 1st 2014 to June 30th 2016. A detailed history of patients was taken based on set questionnaires. Detailed general and gynaecological examination findings were taken. Routine blood investigations were sent along with investigations for aPLAs which included: - Lupus Anticoagulant (LA) - Anti Cardiolipin Antibody (ACA) - Anti β2 glycoprotein 1 (Anti-β2GP1Ab). Final results are statistically evaluated.

Results: A total of 56 patients of recurrent miscarriage with two or more prior pregnancy losses were considered. Out of the 56 patients, 23 patients were excluded as per the exclusion criteria and 33 patients were included in the present study. The mean age of the study group was 24.63 years (Range: 20-32 years). Overall, seven patients (21.21%) were seen to have positive antiphospholipid antibody titers amongst the 33 patients, with repeat testing done after 12 weeks to confirm the positivity. Five patients (15.15%) were positive for ACA antibody. Four (12.12%) patients were positive for LA and B2GP1 each. Two patients (6.06%) were positive for both LA and ACA antibodies. Two patients (6.06%) were positive for LA and B2GP1 antibodies and two other (6.06%) patients were positive for ACA and Anti-β2GP1Ab. There was a statistically significant association noticed between ACA positivity and POG at 1st pregnancy loss. However, the overall association of APLA positivity and POG at pregnancy loss was not statistically significant.

Conclusions: There was a significant difference of POG at first pregnancy loss in ACA positive patients as compared to the ACA negative patients. However, when all the APLA positive patients were considered the difference was not statistically significant.

Keywords: APLA (Anti phospholipid antibody), ACA (Anti cardiolipin antibody), Lupus anticoagulant (LA), POG (Period of gestation)

INTRODUCTION

Around 15% of clinically recognised pregnancies in women less than 35 years old result in spontaneous miscarriage.[1] However recurrent pregnancy loss (RPL) is encountered in 5% of couples with two or more losses and in around 1-2% of couples with three or more losses.[2] Different definitions are being employed all over the

world for recurrent pregnancy loss, leading to the lack of a proper consensus. The Royal College of Obstetricians and Gynecologists (RCOG) and the European Society of Human Reproduction and Embryology (ESHRE) define recurrent miscarriage as three or more consecutive losses before 24 weeks gestation.[3] Due to an increasing number of childless couples, the improved availability of diagnostic tests, and most importantly the minimal difference in the prognostic value between two and three losses, the American Society for Reproductive Medicine (ASRM) updated the definition of RPL to two or more clinical pregnancy losses, before 20 weeks period of gestation, documented by either ultrasonography or approved in a histopathologic examination.[3] This confusion has led to discrepancies in the incidence and prevalence of RPL.

Many entities have been proposed to be causative of RPL including parental chromosomal aberrations, uterine anomalies, endocrine abnormalities, autoimmune disorders, and thrombophilias. Apart from these, many cases do not have an obvious cause and are termed as Unexplained RPL. Thrombophilias can be because of hereditary thrombophilias (HT) or acquired thrombophilias (AT). The most common HT is due to Factor V Leiden, prothrombin gene mutation, protein C deficiency, protein S deficiency and antithrombin III deficiency accounting for other causes.[4] Acquired thrombophilias are mostly attributed to anti-phospholipid syndrome (APS) which is encountered in 5-20% of patients with RPL. There is an increasing burden of recurrent pregnancy loss in the society and AntiPhospholipid Syndrome being one of the undisputed treatable cause for recurrent pregnancy loss. This study aims to evaluate the significance of the antibody profiles of APS in relation to RPL in obstetrics and gynaecology department of Vydehi Institute of Medical Sciences and Research Centre.

Primary objective was to evaluate the prevalence of anti-phospholipid antibodies in patients with RPL and evaluate the relation of antibody positivity with other parameters. Secondary objective was to evaluate the clinical presentation of patients with RPL.

METHODS

Patients coming to the Obstetrics and Gynecology department of Vydehi Institute of Medical Sciences and Research Centre, Bangalore, from December 1st 2014 to June 30th 2016, with history of repeated pregnancy loss were recruited based on the below mentioned.

Inclusion criteria

History of two or more previous spontaneous pregnancy losses

- With ultrasound confirmed pregnancy with Intrauterine gestation sac
- Less than 20 weeks
- With or without fetal cardiac activity.

Exclusion criteria

- Previous medical termination of pregnancy
- Previous Ectopic Pregnancy
- Previous pregnancy losses of more than 20 weeks gestation
- Trauma induced previous pregnancy loss.

A detailed history of patients was taken based on set questionnaires. Detailed general and gynaecological examination findings were taken. Routine blood investigations were sent along with investigations for APLAs which included: - lupus anticoagulant (LA), Anti cardiolipin antibody (ACA), Anti β2 glycoprotein 1 (Anti-β2GP1Ab). If any of the above-mentioned tests for antiphospholipid antibodies came positive for a patient, a repeat of that particular test was done after 12 weeks, since the diagnosis of APS requires a test to be positive on two or more occasions at least 12 weeks apart. Lupus Anticoagulant was measured using dilute Russell viper venom test (DRVVT) using the principle of electromechanical clot detection. Normal values are between 32-42 seconds with higher values suggestive of antibody positivity. Serum ACA levels were tested by Enzyme immune assay method. Values >15GPL for IgG antibody subtype and >12.5 MPL for IgM antibody subtype were taken as positive. Serum anti-β2GP1Ab levels were tested by Enzyme immune assay method. Values >20 SGU for IgG type and >20 SMU for IgM type antibody were considered to be positive. Statistical analyses were done using SPSS version 18. Prevalence data were noted as percentages or proportions. Categorical variables were compared using Fisher's exact test while continuous variables were compared using Student's t test. P value less than 0.05 was taken to be significant. Consent was taken from all the participants on consent forms written in the language comfortable to them.

RESULTS

A total of 56 patients of recurrent miscarriage with two or more prior pregnancy losses were considered.

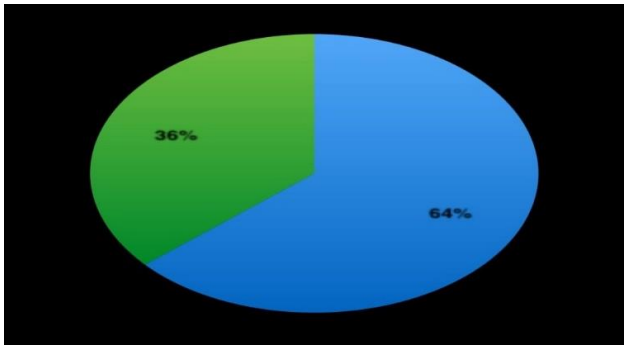

Figure 1: Age distribution.

Out of the 56 patients, 23 patients were excluded as per the exclusion criteria and 33 patients were included in the present study. The mean age of the study group was 24.63 years (Range: 20-32 years). The break-up of the age is given in Figure 1.

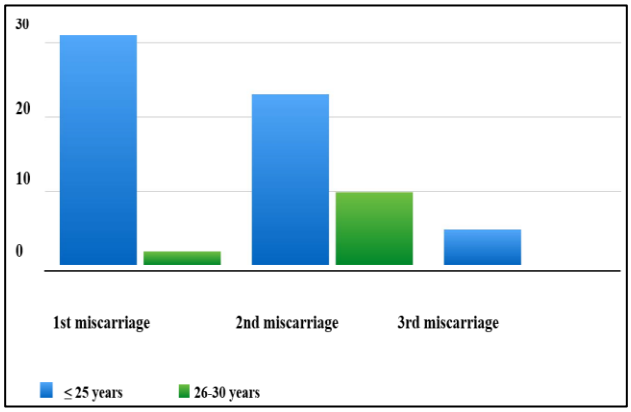

Figure 2: Patient age at previous miscarriages.

Three out of the 33 patients had a previous live born pregnancy while the rest of the patients were all nulliparous. Twenty-eight patients had two previous miscarriages while five patients had three previous miscarriages. The mean age at first miscarriage was 22.12 years (range: 18-26 years) and the mean age at second miscarriage was 27.81 years. (Range: 22-32 years). All the patients with at least three miscarriages had all the miscarriages before 25 years of age. The distribution of age at miscarriage is given in Figure 2.

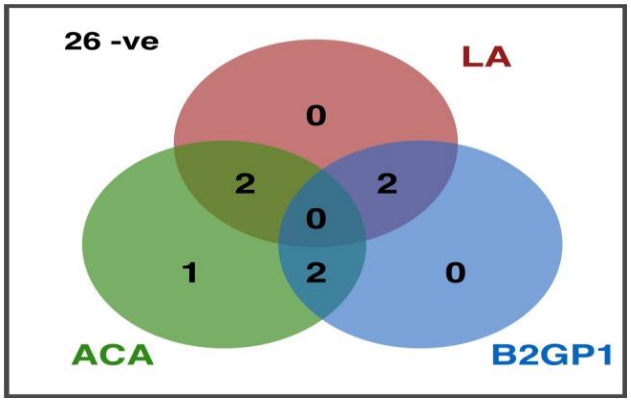

Figure 3: Venn Diagram showing APLA positivity.

Overall, seven patients (21.21%) were seen to have positive antiphospholipid antibody titers amongst the 33 patients, with repeat testing done after 12 weeks to confirm the positivity. Five patients (15.15%) were positive for ACA antibody. Four (12.12%) patients were positive for LA and B2GP1 each. Two patients (6.06%) were positive for both LA and ACA antibodies. Two patients (6.06%) were positive for LA and B2GP1 antibodies and two other (6.06%) patients were positive for ACA and Anti-β2GP1Ab. No patient was positive for all three antibodies. One patient had only ACA positivity.

A Venn diagram representing the antibody positivity is given in Figure 3. ACA was positive in 5 patients with two previous miscarriages. All these patients had positive IgG-ACA antibodies with IgM-ACA antibodies being within normal limits.

The age at presentation of the five patients ranged from 23 to 28 years with a mean of 25.6 years. The age at first miscarriage for the five patients ranged from 21 to 24 years with the first miscarriage seen to occur in the second trimester for all the five patients. The age at second miscarriage ranged from 22 to 27 years with the second miscarriage occurring in the second trimester for 4 patients and at 10 weeks period of gestation (POG) for one patient. Figure 3 venn diagram showing APLA positivity.

Table 1: Comparison of ACA positive and negative patients.

Parameter	ACA +ve (Mean±SD)	ACA -ve (Mean±SD)	P value
Age at presentation	25.6±1.82	24.46±2.35	0.31
Age at 1st miscarriage	22.8±1.64	22±2.65	0.52
POG at 1st miscarriage	15.4±1.52	12.36±2.44	0.01
Age at 2nd Miscarriage	24.8±1.79	23.36±2.54	0.23
POG at 2nd miscarriage	13.4±2.19	13.21±2.92	0.89

LA was positive in 4 patients with three patients having two previous miscarriages and one patient having 3 previous miscarriages. The age at presentation of the four patients ranged from 22 to 28 years with a mean of 24 years. The age at first miscarriage for the four patients ranged from 20 to 24 years with the first miscarriage seen to occur in the first trimester in two and the second trimester in the other two patients.

Table 2: Comparison of LA positive and negative patients.

Parameter	LA +ve (Mean±SD)	LA -ve (Mean±SD)	P value
Age at presentation	24±2.71	24.72±2.27	0.56
Age at 1st miscarriage	21.25±1.89	22.24±2.8	0.47
POG at 1st miscarriage	12.55±2.99	12.90±2.84	0.64
Age at 2nd Miscarriage	23±2.71	23.66±2.48	0.62
POG at 2nd miscarriage	13.25±3.77	13.24±2.72	0.99

The age at second miscarriage ranged from 21 to 27 years with the second miscarriage occurring in the first

trimester for 2 patients and in the second trimester for the other two patients. The patient with the third pregnancy loss had the miscarriage at the age of 22 years during the 12th week of gestation. Anti-β2GP1Ab was positive in 4 patients with three patients having two previous miscarriages and one patient having 3 previous miscarriages. Three of the four had IgG Anti-β2GP1Ab positivity while one patient had positive IgM Anti-β2GP1Ab positivity.

Table 3: Comparison of Anti-β2GP1Ab positive and negative patients.

Parameter	B2GP1 +ve (Mean±SD)	B2GP1 -ve (Mean±SD)	P value
Age at presentation	24.25±2.05	24.59±2.35	0.72
Age at 1st miscarriage	21.25±1.89	22.24±2.60	0.47
POG at 1st miscarriage	13±3.65	12.79±2.46	0.88
Age at 2nd Miscarriage	23.25±2.06	23.62±2.56	0.78
POG at 2nd miscarriage	13.75±2.87	13.17±2.83	0.70

The age at presentation of the four patients ranged from 22 to 26 years with a mean of 24.25 years. The age at first miscarriage for the four patients ranged from 20 to 24 years with the first miscarriage seen to occur in the first trimester in two and the second trimester in the other two patients.

Table 4: Comparison of aPLA positive and negative patients.

Parameter	aPLA +ve (Mean±SD)	aPLA -ve (Mean±SD)	P value
Age at presentation	24.71±2.14	24.62±2.37	0.92
Age at 1st miscarriage	22±1.91	22.15±2.69	0.88
POG at 1st miscarriage	13.86±2.97	12.54±2.42	0.23
Age at 2nd Miscarriage	23.86±2.19	23.50±2.58	0.74
POG at 2nd miscarriage	13.43±2.70	13.19±2.67	0.84

The age at second miscarriage ranged from 21 to 25 years with the second miscarriage occurring in the first trimester for 1 patient and in the second trimester for the other three patients. The patient with the third pregnancy loss had the miscarriage at the age of 22 years during the 12th week of gestation. There was a statistically significant association noticed between ACA positivity and POG at 1st pregnancy loss. However, the overall association of APLA positivity and POG at pregnancy loss was not statistically significant. The comparative analyses of parameters such as age at presentation and age and gestational age at miscarriage during each of the previous pregnancies between individual and combined antibody positive and negative patients are given in Tables 1-4.

DISCUSSION

The present study aims at evaluating the presence of APLAs in a cohort of women having 2 or more previous pregnancy losses without any obvious cause of RPL. The study was carried out over a period of one and a half year and patients with history suggestive of RPL were recruited from the outpatient department of VIMS and RC. Overall 56 patients were recruited out of which 23 were excluded. Testing was done for the remaining 33 patients for the evaluation of ACA, LA and Anti-β2GP1Abs.

Five of the patients had more than two miscarriages. Three of the patients had a previous live born baby. The mean age of presentation of the patients was 24 years.[5] The mean age at 1st and 2nd prior miscarriages were 22.12 and 27.84 years respectively. A lower age at presentation has been noticed in the present study in comparison with previous studies wherein the mean age at presentation was in the early 30s. Most of these studies are from the western literature. A younger age at presentation however has been reported in previous studies from India which can be explained by the comparatively earlier age at marriage and conception in Indian women as compared to women in the west.[6]

The antibody positivity rate seen in the present study was 15.15% for ACA, 12.12% for LA and 12.12% for Anti-β2GP1Ab respectively. None of the patients had triple positivity while six patients had two of the three antibodies positive. Ig G subtype was seen in all five ACA positive patients with none of the patients having Ig M antibody positive. Three of four B2GP1 antibody positive patients had positive Ig G type Antibody with the remaining patient having IgM positive antibody and LA positivity.

The overall mean gestational age at miscarriage was 12.98 weeks. There was a significant difference of POG at first pregnancy loss in ACA positive patients as compared to the ACA negative patients. However, when all the APLA positive patients were considered the difference was not statistically significant. In similar studies done previously the prevalence of RPL has been seen to be slightly increased in the second trimester in APLA positive patients.

The overall prevalence of 21.21% for APLA is comparable to previous studies done from India and worldwide with rates reported to be ranging from 15-50%.

The present study reports a positive ACA titer in 15.15% which is comparable to the study by Yetman et al.[7] which

was the largest study done on APLAs in RPL patients. In that study, positive ACA were detected in 17.3% of patients with RPL with 10.1% who were negative for anticardiolipin antibodies having positive levels of another antiphospholipid antibody. Sater et al in a more recent study of 277 patients, reported higher prevalence rates of ACA with 10.1% for IgM ACA and 36.5% for IgG ACA taking patients more than or equal to 3 miscarriages.[8]

In previous studies from India by Ghosh et al, a comparatively higher prevalence of ACA has been reported in a much larger study group.[9] Another study done by Indu et al. reported a much lower prevalence of 10% for ACA which is comparable to the present study. While the first two studies included women with 3 or more pregnancy losses the latter study included women with 2 or more RPL. Similar studies from Pakistan and Babylon have reported ACA prevalence of 11.96% and 16.19% respectively which are comparable to prevalence rate of the present study.[10,11]

The present study has a rate of 12.12% for Anti-β2GP1Ab positivity with one patient positive for IgM type and the other three for IgG type which was similar in comparison to the study by Stern et al. who in 1998 had previously reported a higher prevalence of Anti-β2GP1 Ab in patients with RPL than ACA with prevalence rates of 6.2 % for IgG Anti-β2GP1Ab and 15.5 % for IgM Anti- β2GP1Ab.[12]

In a study by Kumar et al including 150 patients 3 or more spontaneous pregnancy losses, LA was positive in 10.28% and β2GP1 antibodies seen in 40.24%. The present study has similar prevalence of LA but comparatively fewer number of patients were positive for Anti-β2GP1 Ab.[13] None of the positive patients had any other history suggestive of thrombotic events in the past. Features of SLE were looked for and none of the patients had any other additional features suggestive of SLE. The absence of other thrombotic events can be attributed to the younger age of the patients and regular follow-up of these patients will have to be done in view of increased morbidity and mortality due to thrombotic events in the feature in patients with obstetric APS.

CONCLUSION

There are few studies which assess the prevalence of APLAs in patients with RPL. The present study was carried out in a single centre and included 33 patients with RPL. APLA were evaluated in all the patients with testing being done for ACA, LA and anti β2GP1Ab. Patients who had positive antibody titers were reevaluated after 12 weeks.

There was a significant difference of POG at first pregnancy loss in ACA positive patients as compared to the ACA negative patients. However, when all the APLA positive patients were considered the difference was not statistically significant.

The overall prevalence of APLA in the study group was seen to be 21.21%. Most of the patients had positivity for ACA (15.15%) with 4 patients (12.12%) showing positivity for LA and anti β2GP1 antibody each. All the patients with initial positive antibody titers had positive titers even after 12 weeks. None of the patients had triple positivity.

There was a statistically significant association noticed between ACA positivity and POG at 1st pregnancy loss. However, the overall association of APLA positivity and POG at pregnancy loss was not statistically significant.

The mean TSH of the study group was 1.96mIU/ml. One patient had subclinical hypothyroidism with a TSH of 5.6 mIU/ml and normal T3, T4 levels. She was not started on treatment as she was TPO antibody negative and did not have any goiter.

The strength of the study is that all the three antibodies have been assessed in the subjects with repetition of test in those positive titers as per the diagnostic criterion. The relatively small sample size and the absence of karyotype testing for all patients are some of the limitations of the present 48 study. Further follow up of the APLA positive patients will have to be done to asses for other signs of systemic thrombosis in the future. In conclusion APS is an important cause of recurrent miscarriages and testing for aPLAs should be routinely done for all patients with RPL.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril. 2012;98(5):1103-11.
2. Jauniaux E, Farquharson RG, Christiansen OB, Exalto N. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. Hum Reprod. 2006;21:2216-22.
3. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. Fertil Steril. 2013;99(1):63-67.
4. De Stefano V, Chiusolo P, Paciaroni K, Leone G. Epidemiology of factor V Leiden: clinical implications. Semin Thromb Hemost. 1998;24(4):367-79.
5. Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps et al. Antiphospholipid syndrome: Clinical and immunologic manifestations

and patterns of disease expression in a cohort of 1,000 patients. Arthritis Rheum. 2002;46:1019-27.
6. Pradhan T, Bhavthankar DP. An observational study of causes of recurrent pregnancy loss in rural Population. Indian J Basic Appl Med Res. 2014;4(1):70-6.
7. Yetman DL, Kutteh WH. Antiphospholipid antibody panels and recurrent pregnancy loss: prevalence of anticardiolipin antibodies compared with other antiphospholipid antibodies. Fertil Steril. 1996 Oct;66(4):540-6.
8. Sater M, Almawi W. Autoantibodies targeting phosphatidylserine, cardiolipin, β2-glycoprotein I, annexin V and prothrombin in patients with recurrent spontaneous miscarriage (148.18). J Immunol. 2011 Apr 1;186(1 Supplement):148.
9. Ghosh A, Ghosh M, Bhattacharya SM. Anti-phospholipid antibodies as a cause of recurrent pregnancy loss: a study in Calcutta, India. Journal of Obstetrics and Gynaecology. 2009 Jul;26(5):407-10.
10. Al-Hilli NM, Al-Mosawi HM. The prevalence of anticardiolipin antibodies in women with bad obstetric history. Int J Curr Microbiol App Sci. 2014;3(2):547-3.
11. Nizamani GS, Memon RA, Kazi N, Khoharo HK, Kazi S. Anticardiolipin antibodies in women having recurrent spontaneous abortions. Pak J Physiol. 2015;11(3):38-9.
12. Stern C, Chamley L, Hale L, Kloss M, Speirs A, Baker HWG. Antibodies to β2 glycoprotein I are associated with in vitro fertilization implantation failure as well as recurrent miscarriage: results of a prevalence study. Fertil Steril. 1998 Nov;70(5):938-44.
13. Ames PR, Nourooz-Zadeh J, Tomma- sino C, Alves J, Brancaccio V, Anggard EE. Oxidative stress in primary antiphospho- lipid syndrome. Thromb Haemost. 1998;79:447-9.

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