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Anu Nagpal
MBBS, MS, Department of Gynaecology and Obstetrics, MMIMSR, Mullana, Haryana, India

Prateek Girotra
MBBS, DNB, Department of Orthopaedics, Senior Resident, Hindurao Hospital and NDMC Medical College, Delhi, India

Varun Gautam
MBBS, DNB Orthopaedics, Senior Resident, Kalpana Chawla Medical College, Karnal, Haryana, India

Corresponding Author:
Anu Nagpal
MBBS, MS, Department of Gynaecology and Obstetrics, MMIMSR, Mullana, Haryana, India

Role of antiphospholipid antibodies in recurrent pregnancy loss

Anu Nagpal, Prateek Girotra and Varun Gautam

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Abstract
Antiphospholipid syndrome is associated with a hallmark of Obstetric complications including recurrent miscarriage, early delivery, oligohydramnios, prematurity, intrauterine growth restriction, fetal distress, fetal or neonatal thrombosis, pre-eclampsia/eclampsia, HELLP syndrome, arterial or venous thrombosis and placental insufficiency. Antiphospholipid antibodies promote activation of endothelial cells, monocytes and platelets, causing an overproduction of tissue factor and thromboxane A2. These factors lead to a hypercoagable state leading to various obstetric complications. The aim of this study was to evaluate the prevalence of anti-phospholipid antibodies in patients with RPL and to evaluate the relation of antibody positivity with other parameters.

Keywords: antiphospholipid, pregnancy loss

Introduction
Recurrent pregnancy loss is defined as 3 or more than 3 spontaneous miscarriages before 24 weeks, according to RCOG and ESHRE [1]. 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 [2, 3].
Anti-phospholipid syndrome (APS), anatomic uterine anomalies and chromosomal abnormalities in either partner are the only established causes of RPL. Other causes that have been suggested include inherited thrombophilias, endocrinopathies, infections and environmental exposures to as smoking or alcohol consumption.
Antiphospholipid Syndrome (APS) is an autoimmune thrombophilic condition that is marked by the presence in blood of antibodies that recognize and attack phospholipid-binding proteins, rather than phospholipid itself [4]. APS is a systemic autoimmune disorder characterised by the presence of anti-phospholipid antibodies (aPLA) in the serum directed against phospholipids or proteins associated with phospholipids. Clinical manifestations of APS include arterial thrombosis, venous thrombosis and/or obstetrical complications including RPL. Around 20% of women with RPL have autoimmune abnormalities with presence of aPLA being one of the most common autoimmune aetiologies for RPL [5].
The clinical features of APS are listed in table 1.
APS typically includes three types of antibodies:
1. Anticardiolipin antibodies.
2. Lupus anticoagulant.
3. Anti β2 Glycoprotein 1 antibodies.

aPLAs are thought to cause thrombotic events by various mechanisms. Some of the proposed mechanisms are [6]:
1. Binding and decreasing the function of antithrombin III.
2. Enhancing thromboxane release - leading to platelet aggregation.
3. Decreasing the activation of protein C needed to inactivate the clotting process.
4. Increasing oxidative stress.

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 was carried out in MMIMSR, Ambala. The aim of this study was to evaluate the prevalence of anti-phospholipid antibodies in patients with RPL and to evaluate the relation of antibody positivity with other parameters.

Aims and Objectives

Primary objectives

1. To evaluate the prevalence of anti-phospholipid antibodies in patients with RPL.
2. To evaluate the relation of antibody positivity with other parameters.

Secondary objective

To evaluate the clinical presentation of patients with RPL.

Material and Methods

60 patients coming to the Obstetric and Gynaecology department of MMIMSR, Mullana, Ambala from November 1st 2014 to June 30th 2016, with history of repeated pregnancy loss were recruited based on the below mentioned inclusion and exclusion criteria.

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 foetal 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 Anti-coagulant (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 > 15 GPL 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 v18.

Results

A total of 60 patients of recurrent miscarriage with two or more prior pregnancy losses were considered. Out of the 60 patients, 24 patients were excluded as per the exclusion criteria and 36 patients were included in the present study. The mean age of the study group was 24.63 years (Range: 20-28 years).
Three out of the 36 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 gestational age at 1st pregnancy loss was 12.81 weeks (Range: 9-18 weeks).
The mean gestational age at 2nd pregnancy loss was 13.24 weeks (Range: 7-19 weeks). The mean gestational age at 3rd pregnancy loss was 12.4 weeks (Range: 8-16 weeks). The overall mean gestational age at miscarriage for all pregnancies was 12.98 weeks (Range: 7-19 weeks). Karyotyping was done for four patients as a part of workup for RPL. Rest of the patients declined to undergo karyotyping due to lack of affordability or other reasons. USG pelvis was done for all the 36 patients and there was no evidence of uterine anomalies.
Seven patients (21.21%) were seen to have positive antiphospholipid antibody titres amongst the 36 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. ACA was positive in 5 patients with two previous mis-carriages. All these patients had positive IgGACA 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 year with the second miscarriage occurring in the second trimester for 4 patients and at 10 weeks POG for one patient.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 28years with a mean of 24 years. The age at first miscarriage for the four patients ranged from 20 to24 years with the first miscarriage seen to occur in the first trimester in two and the second trimester in the other two patients. 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. 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. 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 1stpregnancy 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 & gestational age at miscarriage during each of the previous pregnancies between individual and combined antibody positive and negative patients are given in the tables 1 and 2.

Table 1: Comparison of ACA positive and negative patients.

Parameter	Aca + Mean ± SD	Aca - 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

Table 2: Comparison of LA positive and negative patients.

Parameter	LA + Mean ± SD	LA - Mean ± SD	P value
Age at presentation	22 ± 2.42	26 ± 1.64	0.22
Age at 1st miscarriage	26 ± 1.84	21 ± 2.82	0.01
Pog at 1st miscarriage	13 ± 2.28	12 ± 2.64	0.50
Age at 2nd miscarriage	22.2 ± 2.42	24 ± 2.84	0.04
Pog at 2nd miscarriage	11 ± 1.84	12 ± 2.12	0.01

Discussion

Recurrent pregnancy loss is traditionally defined as a condition associated with three or more prior pregnancy losses occurring within 20-24 weeks of gestation. Due to the increase in its incidence and the minimal difference between the prognostic values between two and three miscarriages the recent consensus is to consider two or more previous pregnancy losses within 20 weeks as recurrent pregnancy loss. There are currently three established causes of RPL i.e. chromosomal abnormalities, uterine anatomical abnormalities and antiphospholipid antibody syndrome. While uterine abnormalities and chromosomal abnormalities can be diagnosed with USG pelvis and karyotyping the diagnosis of APS is not so simple with the disease having a heterogenous presentation.

The diagnosis of APS is made based on the modified Sapporo criteria with the presence of one of three major anti-phosholipid antibodies (aPLAs), which include anticardiolipin antibody, lupus anticoagulant and anti-beta 2 glycoprotein 1 antibody, along with clinical features mandatory for its diagnosis. 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 1 and a half year and patients with history suggestive of RPL. Overall 60 patients were recruited out of which 24 were excluded. Testing was done for the remaining 36 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 24.63 years. The mean age at 1st and 2nd prior miscarriages were 22.12 and 27.84 years respectively. A lower age at presentation has been notice din 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 [7].

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. IgG subtype was seen in all five ACA positive patients with none of the patients having IgM antibody positive. Three of four B2GP1 antibody positive patients had positive IgG 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 notstatistically 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 [8]. The present study reports a positive ACA titres in 15.15% which is comparable to the study by Yetman *et al*. [9]. 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*. [10] 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.

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 36 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 titres 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 strength of the study is that all the three antibodies have been assessed in the subjects with repetition of test in those positive titres 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 study. Further follow up 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.

References

1. Royal College of Obstetricians and Gynaecologists (RCOG). The investigation and treatment of couples with recurrent first-trimester and second-trimester miscarriage. Green-top guideline. London (UK): RCOG, 2011, 17.
2. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. Fertil Steril. 2013; 99(1):63.
3. Christiansen OB. Epidemiology of recurrent pregnancy loss. Recurrent pregnancy loss: causes, controversies and treatment. 1st ed. UK: Informa healthcare, 2007, 1-14.
4. Hughes GR. Thrombosis, abortion, cerebral disease and the lupus anticoagulant. BMJ. 1983; 287:1088-1089.
5. Ford H, Schust D. Recurrent pregnancy loss: etiology, diagnosis, and therapy. Rev Obstet Gynecol. 2009; 2(2):76-83.
6. Asherson RA. The catastrophic anti- phospholipid syndrome, 1998: a review of the clinical features, possible pathogenesis and treatment. Lupus. 1998; 7(Suppl 2):S55-

S62.
7. Pradhan T, Bhavthankar DP. An Observational Study of Causes of Recurrent Pregnancy Loss in Rural Population. Indian Journal of Basic and Applied Medical Research, 2014, 70-6.
8. 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; 26(5):407-10.
9. Yetman DL, Kutteh WH. Antiphospholipid antibody panels and recurrent pregnancy loss: Prevalence of anticardiolipin antibodies compared with other antiphospholipid antibodies. Fertil Steril. 1996; 66(4):540-6.
10. Sater M, Almawi W. Autoantibodies targeting phosphatidylserine, cardiolipin, β2-glycoprotein I, annexin V and prothrombin in patients with recurrent spontaneous miscarriage (148.18). The Journal of Immunology. 2011; 186(1 Supplement):148.18-8.