Lupus Anticoagulants and Anticardiolipin Antibodies in Indian Women with Spontaneous, Recurrent Fetal Loss

Akanksha Rawat · Meera Sikka · Usha Rusia ·
Kiran Guleria

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Abstract Spontaneous and recurrent pregnancy loss are common complications of pregnancy resulting from varied causes including antiphospholipid syndrome (APS). Treatment of women with APS increases the chance of a subsequent successful pregnancy. The study aimed to find the prevalence of lupus anticoagulants (LA) and anticardiolipin antibodies (ACAs) in women with spontaneous/recurrent fetal loss and compare with women with normal obstetric history. Hundred women with spontaneous/recurrent fetal loss and 50 healthy pregnant controls were tested for LA by complete blood counts, Prothrombin time, Activated partial thromboplastin time (APTT), LA sensitive APTT and dilute Russell viper venom time (dRVVT) (screening and confirmatory) and ACAs (ELISA). LA was detected in 15 % patients using dRVVT confirmatory test and ACA in 5 %, all controls being negative. Twenty one % patients were detected by LA sensitive APTT (sensitivity 92.9 %, specificity 100 %) and 100 % with dRVVT screening test (sensitivity 98.8 %, specificity 100 %). We recommend that screening for antiphospholipid antibodies must be done in women with spontaneous/recurrent foetal

loss even in the absence of other clinical manifestations using a combination of tests.

Keywords Recurrent pregnancy loss · Lupus anticoagulant · dRVVT · APTT · Anticardiolipin antibodies

Introduction

Spontaneous pregnancy loss is a common complication of pregnancy with a reported rate of 12–15 % [1]. Recurrent pregnancy loss (RPL) (3 consecutive pregnancy losses) during the first trimester affects 1–3 % of pregnancies [2]. While most cases of spontaneous pregnancy loss result from chromosomal abnormalities, RPL has a diverse etiology [1, 3].

Antiphospholipid syndrome (APS), a systemic autoimmune disorder is responsible for about 10 % of cases of repeated miscarriage [4]. It is associated with several obstetric complications such as pre-eclampsia, retarded fetal growth and intrauterine fetal death [5]. Diagnosis of APS includes laboratory testing for antiphospholipid antibodies. Of the antiphospholipid antibodies, the most important are Lupus anticoagulant (LA) and anticardiolipin antibodies (ACAs) [6].

Identification of women positive for antiphospholipid antibodies is of importance as there are reports which have described successful pregnancies in women with these antibodies who have received treatment [7, 8]. If untreated, these patients have a miscarriage rate of 90 % in the subsequent pregnancy [9].

The present study aimed at evaluating the prevalence of lupus anticoagulants (LA) and ACAs in women with spontaneous/recurrent fetal loss who did not have clinical evidence of SLE and compare it with women who had

A. Rawat · M. Sikka (✉) · U. Rusia
Department of Pathology, University College of Medical
Sciences and GTB Hospital, Delhi 110095, India
e-mail: meerasikka55@gmail.com

A. Rawat
e-mail: rawat.akanksha@yahoo.com

U. Rusia
e-mail: sprusia@gmail.com

K. Guleria
Department of OBG, University College of Medical Sciences
and GTB Hospital, Delhi 110095, India
e-mail: kiranguleria@yahoo.co.in

uncomplicated pregnancy outcome in the past. A combination of tests was used for detection of LA including PT, APTT, LA sensitive APTT and dRVVT.

Materials and Methods

Subjects

One hundred women with spontaneous/recurrent foetal loss (mean $\pm$ SD age 25.3 ± 3.7 years) attending the Outpatient Department of Obstetrics and Gynaecology, UCMS and GTB Hospital, Delhi were enrolled in the study. A detailed history was elicited and clinical examination done on each patient. The patients had no obvious clinical evidence of SLE or any history of deep vein thrombosis, joint symptoms or dermal manifestations. Testing was done within 7 days of the abortion. Fifty pregnant women (mean $\pm$ SD age 23.7 ± 2.8 years) with no history of previous abortion/any other obstetric complaints or other disease state were included as controls.

The study received prior clearance from the Institutional Ethical Committee. All women provided a written informed consent before enrolment in the study.

Methods

Venous blood was collected in EDTA for complete blood counts, in citrate for coagulation studies and in a plain vial for separation of serum for ACA. After rendering the citrated sample platelet free, tests of coagulation including PT (Thromborel S, Dade Behring, USA), APTT (Dade Actin FS, Dade Behring, USA), LA sensitive APTT (Cephen LS, Hyphen BioMed, France) and dRVVT (LADS Detection System, TULIP diagnostics Pvt Ltd, India) screening test were done in all patients and controls using commercially available kits. In patients with prolonged APTT, mixing studies were done with normal plasma to demonstrate that prolongation was due to an inhibitor and not factor deficiency. In patients with abnormal screening tests, the presence of LA was confirmed by the dRVVT confirmatory test which was based on the platelet neutralisation principle (LADS Detection System, TULIP diagnostics Pvt Ltd, India). LA activity was further quantitated as moderate, high and very high as per manufactures instructions (ratio of mean screen time/mean confirm time; ratio 1.5–1.8 moderate activity, 1.8–2.4 high activity and more than 2.4 very high activity).

All women who were initially positive for LA underwent repeat testing at 12 weeks and LA was reported to be present when both results were positive.

ACA were estimated by ELISA (Varelixa cardiolipin IgG and IgM, Phadia Inc, Sweden).

Table 1 Number and time of occurrence of abortions in patients

No. of abortions	No. of patients (n = 100)	Time of occurrence of abortion		
		1st trimester	2nd trimester	3rd trimester
2	13	15	8	3
3	58	108	64	8
4	19	35	24	11
5	8	26	7	7
6	2	8	2	2
Total	100	192	105	31

Statistical Analysis

The data was analysed using unpaired student t test and Chi square test. Level of significance and level of confidence were 5 and 95 % respectively.

Results

Obstetric History

The patients presented with history of spontaneous first or second trimester or recurrent abortion. Majority (87 %) of the patients had more than two abortions (Table 1). Most (60 %) abortions occurred in the first trimester, 32 % in the second trimester while 8 % occurred in the third trimester.

A history of other obstetric complications was elicited in one or more previous pregnancies including prematurity (14 %), pre eclampsia and eclampsia (15 %), IUGR (12 %) and neonatal death (9 %). In some patients the complications co-existed, the commonest being prematurity with IUGR.

Complete Blood Counts

There was no statistically significant difference between the complete blood counts (Automated Hematology Analyser LH500) of patients and controls.

Tests of Coagulation

The range and mean $\pm$ SD of various tests of coagulation and number of patients with abnormal results is shown in Table 2.

Prothrombin time was not significantly different in patients and controls and was not prolonged in any patient or control.

APTT was significantly ($p < 0.001$) higher in patients as compared to controls. APTT was prolonged in 8 (8 %)

Table 2 Mean $\pm$ SD of parameters of coagulation and abnormal results in patients and control

Parameter (s)	Patients (n = 100)	Controls (n = 100)	Abnormal result	
			Patients (%)	Controls (%)
PT	12.3 $\pm$ 0.6*	12.3 $\pm$ 0.5*	0	0
APTT	28.6 $\pm$ 3.0**	27.0 $\pm$ 2.0**	8	0
LA sensitive APTT	37.0 $\pm$ 7.1***	33.9 $\pm$ 2.0***	21	0
dRVVT screening time	38.1 $\pm$ 7.0****	35.6 $\pm$ 4.5****	16	0
dRVVT Confirmatory time	–	–	15	0

* NS, ** $p < 0.001$,*** $p < 0.004$, **** $p < 0.02$ **Table 3** Sensitivity and specificity of tests of hemostasis in detection of LA

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
APTT	94.1	20	37.5	86.9
LA sensitive APTT	92.9	100	71.4	100
dRVVT screening test	98.8	100	93.7	100

PPV positive predictive value, NPV negative predictive value

patients and was normal in all controls. Three of these 8 (18.7 %) patients were confirmed to be LA positive.

LA Sensitive APTT

APTT using LA sensitive reagent was significantly ($p < 0.004$) higher in patients as compared to controls and was prolonged in 21 (21 %) patients being normal in the controls. Of these 21 patients only 7 (33.3 %) had a prolonged APTT. Fifteen of these 21 (71.4 %) patients were confirmed to be LA positive.

Mixing studies with normal plasma did not show correction of the prolonged APTT/LA sensitive APTT in all 22 patients establishing the presence of an inhibitor.

DRVVT screening time was significantly ($p < 0.02$) higher in patients as compared to controls and was prolonged in 16 (16 %) patients and normal in all controls. LA sensitive APTT was prolonged in all these patients.

DRVVT confirmatory test based on a platelet neutralisation procedure was done in patients with a prolonged dRVVT screening time/LA sensitive APTT and was positive in 15 women. The prevalence of LA in this study was 15 %.

Screening Tests of Hemostasis in LA Confirmed Cases

In the 15 LA confirmed cases APTT was prolonged in 3 (20 %) women, LA sensitive APTT and dRVVT screening test were prolonged in all (100 %) patients. The sensitivity, specificity, PPV and NPV of various tests of hemostasis in identification of LA is shown in Table 3.

Quantitation of LA was done in 15 women with positive dRVVT confirmatory test. Moderate, high and very high levels were seen in 9 (60 %), 5 (33.3 %) and 1 (6.6 %) patient respectively.

Anticardiolipin Antibodies

IgG and IgM were measured by ELISA in 78 patients and all controls. IgG ACA's were detected in 1 (1.3 %) patient and IgM antibodies in 3 (3.8 %) patients. None of the controls were positive for ACA's.

Repeat testing at 12 weeks was done in all women who were positive for LA and ACA and the test was positive in all patients.

Discussion

RPL is a common obstetric problem caused by various etiologies of which APS is an important cause [10]. Anti-phospholipid antibodies both lupus anticoagulant and ACL antibodies are secondary to autoimmune disorders mainly SLE [4]. They have been described even in the absence of any underlying disorder and are also associated with obstetric complications [5]. Fetal death and recurrent spontaneous abortions represent the obstetric criteria of APS [10, 11]. While treatment of these women reduces pregnancy loss, untreated women have a miscarriage rate of 90 % in subsequent pregnancies [8, 9].

The present study assessed the prevalence of Lupus anticoagulant and ACAs in women with recurrent/spontaneous abortions. LA's are considered more specific for APS and ELISA for ACA are more sensitive.

LA and ACA were identified in 15 and 5.1 % women with fetal loss respectively and were absent in all controls. This difference was statistically significant ($p < 0.003$).

The reported frequency of antiphospholipid antibodies in women with fetal loss is variable with values ranging from 3 to 48 % for LA and 8–50 % for ACA [12–17]. This wide variation is due to different laboratory tests used for diagnosis. Andreoli et al. [18] observed APA in 6 % women on analysis of 120 papers published in literature prior to year 2000. In contrast, in a study on 81 women with recurrent spontaneous abortion the prevalence of APA

was found to be 16 % [19]. In a study on 30 Indian women with unexplained fetal loss, LA was observed in 16.6 % patients [17]. Saha et al. [15] reported LA in 13.4 % patients with obstetric complications. Vora et al. [16] observed LA positivity in 8.1 % of 430 women with fetal loss. Screening for LA in 2,257 patients with RPL, revealed repeat positivity in 62 (2.7 %) only [20]. The authors concluded that a confirmed, repeat LA was infrequent even in a high risk setting.

In this study, other obstetric complications including prematurity, eclampsia, pre-eclampsia, IUGR and neonatal death were significantly ($p < 0.047$) higher in LA positive women as compared to LA negative patients. APS is known to be associated with several obstetric complications [10, 11]. Similar results have been reported earlier [17].

There is no gold standard test for identification of LA. As the antibodies are heterogeneous, no single test can identify all lupus anticoagulants. It is recommended that two tests with different assay principles be used to screen for LA of which dRVVT should be the first test and a sensitive APTT the second. If either of the test is positive, LA should be considered as positive [21, 22]. All positive test results should be re-confirmed after 12 weeks.

In this study a combination of tests such as PT, APTT, LA sensitive APTT and dRVVT were used for screening LA and the presence of inhibitor confirmed by mixing study. All positive results were confirmed by dRVVT confirmatory test and were repeated after 12 weeks.

PT was not prolonged in any patient. It has been reported that LA does not prolong PT due to a high concentration of phospholipid in the thromboplastin reagent [23]. APTT is a common screening test for LA though it is reported to be insufficiently sensitive [24]. In the present study 3 of 8 (18.7 %) patients with prolonged APTT were confirmed to be LA positive. The sensitivity and specificity of the test were 94.1 and 20 % respectively. In a study done in patients with SLE, APTT was prolonged in 9.5 % women who had a miscarriage, the sensitivity and specificity 20.8 and 100 % respectively [25]. The sensitivity of APTT to lupus anticoagulant is reported to vary depending on the reagent and the test system and depends on the nature and quantity of phospholipid used [26].

LA sensitive APTT was used in this study as a screening test as is recommended [23]. It was found to have a high sensitivity (92.9 %) and specificity (100 %). The test uses a reagent which is sensitive to LA because of the low phospholipid content. It identified 13 additional patients suspected of having LA which would have been missed by use of APTT alone. In our study 71.4 % patients with prolonged LA sensitive APTT were confirmed to be LA positive. In a study on 105 adults with various disorders, 44.8 % patients with prolonged LA sensitive APTT were

confirmed as LA positive [27]. LA sensitive APTT has not been used in most studies.

DRVVT screening time was prolonged in 16 patients, 15 of whom were confirmed as LA positive. The sensitivity and specificity of the test were 98.8 and 100 % respectively with a PPV and NPV of 93.7 and 100 % respectively. dRVVT confirmatory test showed the presence of LA in 15/16 women.

dRVVT is a sensitive and specific test for detection of LA. It is a frequently used test for identification of LA and assay systems which include a confirmatory test are often used as was also done in this study [27, 28]. Various commercially available dRVVT assays vary in phospholipid origin, concentration and source of Russel viper venom. This highlights the importance of using two tests with different assay principles in identification of LA's.

The prevalence of LA (15 %) observed in this study was in agreement with other studies [19, 29, 30].

ACAs are the most frequently measured antibodies and are detected by ELISA. In this study IgG and IgM antibodies were seen in 1 (1.3 %) and 3 (3.8 %) patients respectively. The prevalence is lower than that observed by other authors [15, 16]. The prevalence of LA was thrice that of ACA and this observation was in contrast to other studies [6, 30]. This variation is attributed to difference in sensitivity of various ELISA kits used, lack of standardization and difference in normal cut off levels (range 2 SD to 5 SD) for ACA. All four patients positive for ACA also showed the presence of LA.

Patients with LA are often asymptomatic. LA's are identified frequently in women with RPL and treatment of LA positive women raises the chance of a subsequent successful pregnancy. These women should be routinely screened for antiphospholipid antibodies even in the presence of otherwise good health. A combination of sensitive tests like LA sensitive APTT and specific test like dRVVT will help in the detection of these antibodies as was observed in this study.

Conflict of interest The authors declare no conflict of interest.

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