

Original Article

Factor V Leiden mutation and acquired activated protein C resistance in Indian women with recurrent fetal loss

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ABSTRACT

Objectives: To study the prevalence and association of factor V Leiden (FVL) mutation and acquired APC resistance (APCR) in women with recurrent fetal loss (RFL).

Patients and Methods: Fifty women with two or more RFLs and 50 age-matched controls with no history of fetal loss and at least one live birth were included in the study. Complete blood counts and screening tests for coagulation (PT, APTT), APCR, and FVL (PCR) were done in all women.

Results: Age of the patients ranged from 20–42 years with a mean \pm SD of 27.4 ± 4.8 years. Prolonged PT and APTT were observed in 2% and 8% cases, respectively. None of the controls had prolonged PT/APTT. APCR was observed in 8% cases and 2% controls. The prevalence of APCR was higher in women with first-trimester fetal loss (24.2%) as compared to women with the second trimester (13.3%) fetal loss. FVL was not observed in any of the cases or controls.

Conclusion: This study indicates that FVL mutation is not associated with RFL in the Indian population while APCR is observed in Indian women with RFL.

Key words: Activated protein C resistance; factor V Leiden; recurrent fetal loss; thrombophilia.

Introduction

Recurrent fetal loss (RFL) is defined as two or more consecutive pregnancy losses. It is a serious problem, affecting 1–5% of women in the reproductive age group with both psychological and social impact.^[1,2] Several etiological factors can cause RFL like chromosomal abnormalities, uterine anatomic malformations, endocrine dysfunction, infections, immunological, and environmental factors. However, in almost 50% of cases, the etiology remains unknown.^[3]

Thrombophilia or an increased tendency to develop venous or arterial thrombosis may be inherited or acquired. Activated protein C resistance (APCR) is the most frequent cause of venous thrombosis.^[4] In 90% of cases, APCR results

from a point mutation at nucleotide position 1691 in the factor V (FV) gene resulting in a replacement of arginine by glutamine and is referred to as FV Leiden (FVL). In the absence of FVL mutation, acquired factors can also cause APCR.^[4]

It is now established that thrombophilia increases the risk of first and second-trimester pregnancy loss by causing thrombosis of the placental bed.^[5-7] A meta-analysis revealed that FVL was associated with both early and late recurrent

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fetal loss.^[8] The reported prevalence of thrombophilia in women with RFL is highly variable.^[9,10]

With a high prevalence of APCR in Caucasians, most studies on APCR and RFL have been done in this population. There have been few studies on APCR in Asian women with RFL. The association is virtually unknown in Indians. The identification of these women and their treatment will increase the chance of a subsequent successful pregnancy.^[9] The present study assessed the frequency of FVL and acquired APCR in Indian women with RFL.

Materials and Methods

This case-control study was conducted on 50 women with ≥ 2 RFL. Fifty age-matched controls with no history of fetal loss and at least one live birth were also included. Patients with genital tract malformation, cervical incompetence, leiomyoma, chronic systemic disease, ABO and Rh incompatibility, diabetes mellitus, thyroid dysfunction, and the polycystic ovarian syndrome were excluded from the study. The study received clearance from the institutional ethics committee for human research. All patients gave informed consent before their inclusion in the study. After a detailed history and examination, the following investigations were done in all patients and controls: complete blood counts (automated hematology analyzer LH 500), prothrombin time (PT), activated partial thromboplastin time (APTT), and acquired APCR. Commercially available kits were used for estimation of PT (Thromborel S, Dade Behring USA), APTT (Dade Actin FS, Dade Behring USA), and APCR (clot based kit, Hemosil, Instrumentation Laboratory). FVL mutation was identified by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique after the extraction of DNA using the phenol-chloroform extraction method.

APCR was performed on semi-automated coagulometer START 4. APC sensitivity ratio (APC-SR) was calculated as the ratio of APTT with the addition of APC and APTT without added APC (APTT + APC/APTT-APC). APC-SR less than 1.92 was considered as positive, 1.92–1.99 borderline and more than 2 as negative.

Statistical analysis

Data were analyzed using SPSS software 23. The prevalence of APCR and FVL mutation was expressed as a percentage. A *P* value < 0.05 was considered significant.

Results

There was no significant difference in the age of patients (27.4 ± 4.8 y) and controls (28.3 ± 4.3 y). Two,

three, and >3 pregnancy losses occurred in 32%, 36%, and 32% women, respectively [Figure 1]. The majority (98%) of pregnancy losses occurred in the first/second trimester.

Hematological parameters

The hematological parameters of patients and controls are shown in Table 1. Hemoglobin was significantly (*P* < 0.01) lower in patients as compared to controls. Anemia was detected in 11 (22%) patients and 4 (8%) controls, being mild in severity.^[11]

Screening tests of hemostasis

PT was significantly (*P* = 0.02) higher in patients (12.5 ± 1.1 s) as compared to controls (12.0 ± 0.9 s). There was no significant difference in the APTT of patients (28.8 ± 3.1 s) and controls (28.1 ± 1.4 s). PT and APTT were prolonged in 1 (2%) and 4 (8%) patients respectively, being normal in all controls.

Acquired APCR and FVL mutation

This was measured using a clot-based assay and a sensitivity ratio was calculated. APC-SR < 1.92 was indicative of APCR. Ten (20%) women with RFL and 4 (8%) controls showed acquired APCR. FVL mutation was not identified in any patient/control.

APCR and time of fetal losses

Of the 10 women with APCR; 6 (60%) had 2 abortions, 3 (30%) had 3 abortions, and 1 (10%) had 5 abortions. The majority of the abortions occurred in the first trimester. [Figure 2]

Discussion

The role of maternal thrombophilias as an etiological factor of RFL has been evaluated in various studies. APCR has been proposed as a cause of placental thrombosis leading to fetal death.^[12] This study evaluated FVL mutation and APCR in women with RFL.

Prolonged PT and APTT were observed in 2% and 8% patients, respectively, being normal in all controls. The results in the present study are similar to those observed by Creagh *et al.*

Table 1: Complete blood counts of patients and controls

Parameter	Patients	Controls
Hb (g/dL)	11.4 ± 1.3*	12.1 ± 1.1*
RBC count ($\times 10^{12}/L$)	4.31 ± 0.49	35.4 ± 3.1
MCV (fL)	82.8 ± 8.3	82.6 ± 7.9
MCH (pg)	26.8 ± 3.2	27.4 ± 3.0
MCHC (g/dL)	32.2 ± 1.4	32.9 ± 1.2
TLC ($\times 10^9/L$)	7.6 ± 2.2**	6.2 ± 1.5**
Platelet count ($\times 10^9/L$)	249.0 ± 83.2	264.4 ± 76.7

P* < 0.01, *P* < 0.001

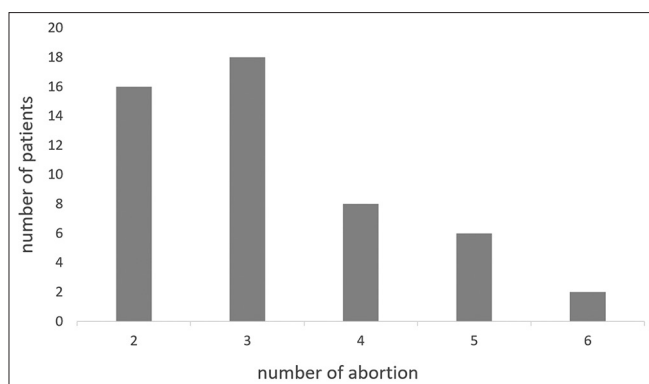


Figure 1: Number of abortion in patients

who reported mild prolongation of PT in 2 of 66 women with sustained fetal loss.^[13] There was no significant difference in the APTT of patients and controls. In contrast, a previous study done in our institution, observed significantly higher APTT in patients with RFL as compared to controls.^[14] However, Abraitis *et al.* did not find any significant difference in the APTT of patients with RFL and controls.^[15]

APCR was observed in 8% of women with RFL and 2% controls. Similar results have been reported by other authors.^[16-18] In a study on 1111 Caucasian women with RFL and 150 controls, APCR was observed in 8.8% cases and 3.3% controls.^[17] Al Allawi observed APCR in 9.7% of women with RFL and 1% controls.^[18] In contrast, Oner *et al.* observed a higher prevalence (14%) of APCR in women with RFL.^[19] In a previous study on 30 Indian women with RFL and 30 controls, acquired APCR was more frequent in the former (16.6%) as compared to the latter (3.3%). However, the difference was not statistically significant.^[16]

In this study, APCR was seen more often in women with first trimester (24.2%) as compared to those with the second trimester (13.3%) fetal losses. There are controversial data on the association of APCR and time of fetal loss. Abraitis *et al.* observed that APCR was associated with first-trimester RFL.^[15] In contrast, other authors have reported a greater association of APCR with second-trimester abortions.^[18,20]

Even though FVL is the most common cause of APCR, the present study did not observe FVL in any patient or control. The low frequency of FVL observed in this study agrees with other studies.^[17,21,22] Townson *et al.* did not identify FVL in any of the women with RFL.^[22] In contrast, Brenner *et al.* observed FVL in 19 (48%) patients with at least 3 successive first trimester or 2 successive second-trimester abortions.^[23]

The results of this study indicate that FVL is not associated with RFL in Indian women. This may be due to the low

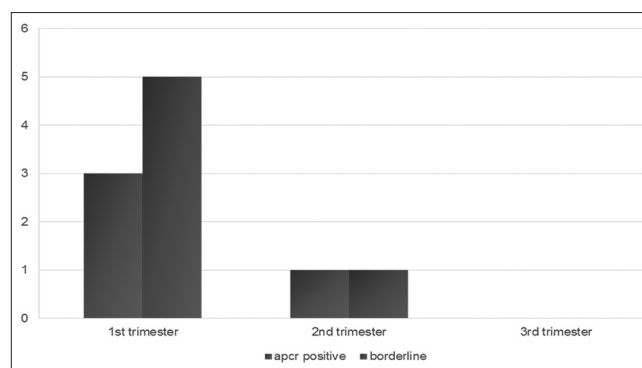


Figure 2: Time of abortion in APCR positive cases

prevalence of FVL in India. In an occasional study from India, heterozygosity for FVL was observed in 1/32 (3.1%) subjects from Gujarat and 1/29 (3.4%) Sikhs. Homozygosity was not identified in any of the subjects.^[24]

In this study, FVL was not identified in any of the women with acquired APCR. Acquired APCR is reported to be associated with a high concentration of FVIII, antiphospholipid antibodies, pregnancy, and the use of oral contraceptives.^[25] None of the women were on oral contraceptives. However, lupus anticoagulant, FVIII concentration, and other antiphospholipid antibodies were not measured in these women.

Conclusion

This study indicates that the frequency of FVL mutation in Indian women with RFL is low. The absence of FVL in the study group may be due to the low prevalence of FVL in India as also the lack of association of FVL with RFL. However, APCR was observed in women with RFL and was more common in women with first-trimester pregnancy loss. Screening for APCR is recommended in women with RFL. This study is limited by the small sample size. Further studies on a larger number of subjects with a long period of follow-up are required in our population to confirm these findings.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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