

Prevalence of factor V Leiden mutation and its relation with recurrent spontaneous pregnancy loss in a group of Syrian women

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ABSTRACT

Objective: The aim of our study was to investigate the prevalence of factor V Leiden and its relation with RPL in a group of Syrian women.

Materials and Methods: The study group included 35 women with a history of recurrent pregnancy loss (two or more abortions before 20th week of gestation) were referred to Orient hospital for obstetrics, gynecology and assisted reproduction, Damascus, Syria, for investigation between December 2005 and July 2006. All women with known causes of pregnancy loss after convenient investigations were excluded. The control group included 45 healthy women from the same ethnic background, who had at least one successful pregnancy, and none of them had a history of fetal loss or complicated pregnancy. FVL mutation was screened by Real-time PCR method.

Results: The results show that 10 women out of 35 with RPL and 4 women out of 45 controls had FVL mutation (28.6 versus 8.9 %, $P=0.022$, Odds ratio 4.1, 95% CI: 1.16-14.4). From the 25 women who were primary RPL, eight patients had the factor V Leiden (32 versus 8.9%, $P=0.014$, OR: 4.8, 95%CI: 1.2, 18.17). From the 10 women who were secondary RPL, two patients had the factor V Leiden (20 versus 8.9%, $P=0.30$, OR: 2.5, 95% CI: 0.4-16.4). All patients and controls carrying the factor V Leiden were heterozygote.

Conclusion: Our results revealed that the prevalence of FVL was significantly higher in women with RPL in comparison with controls, particularly in the subgroup with primary RPL, and there is an association between factor V Leiden mutation and recurrent pregnancy loss.

Key Words: Factor V Leiden mutation, recurrent pregnancy loss, Prevalence, Syrian women

Recurrent pregnancy loss (RPL) is a complex problem and a more frequent condition in obstetric practice. It affects about 5% of pregnant women when defined as two or more consecutive spontaneous of fetal losses (1).

Although some cases of RPL are due to known causes such as congenital uterine malformations, chromosomal abnormalities, systemic diseases and endocrine factors (2), up to 50% of all cases remain with unknown causes (3). Maternal thrombophilia such as activated protein C resistance may be a possible cause of RPL (2,3).

Activated protein C resistance (APC) is the most frequent thrombophilic defect associated with venous thrombosis (4). More than 95% of the APC resistance phenotype can be explained by the factor V Leiden (FVL) mutation. This defect is caused by a single point mutation (G-A) at nucleotide position 1691 in the factor V gene

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resulting in a replacement of Arg by Glu residue (5). The new form of factor V has the same procoagulant activity of the normal factor V, but it is resistant to APC degradation resulting in an increased thrombin generation and hypercoagulable state (6).

The FVL mutation is found in 4 -10 % in Caucasians and the risk of venous thrombosis increase seven times in heterozygote and eighty times in homozygote carriers compared to non-carriers (7).

Recently, several studies suggested that FVL mutation, through production of microthrombosis on placental bed vessels and placental infarctions which result in low placental perfusion, was strongly associated with RPL (8-10). Conversely, other studies failed to demonstrate any association between FVL mutation and RPL (11-13). In view of the high prevalence of FVL mutation among healthy population in countries of the Eastern Mediterranean, in Lebanon (14.4%), in Syria (13.6%) in Greece-Cyprus (13.4%), and Jordan (12.3%) (14), and the lack of data on the possible role of FVL mutation in RPL in Syria, our study aimed to determine the prevalence of factor V Leiden and its relation in a group of Syrian women with recurrent pregnancy loss.

MATERIALS AND METHODS

The study group included 35 women (mean \pm SD:29.6 \pm 6.3 years, range16-42 years) with a history of RPL (two or more abortions before 20th week of gestation, mean \pm SD:4 \pm 2.31, range 2-11) who referred to Orient hospital and assisted reproduction center, Damascus, Syria, for investigation between September 2005 and July 2006.

All women with known independent risk factor for pregnancy complication, such as uterine malformation, systemic disease (Diabetes mellitus, Lupus erythematosus), endocrine abnormality (Prolactin, Thyroid Stimulating Hormone, Follicular Stimulating Hormone and Luteal Hormone during the early follicular phase), and women who received induced abortion upon their request were excluded, in addition to the women with other thrombophilic defects, such as

antiphospholipid antibodies syndrome (Lupus anticoagulant, Anticardiolipin), or deficiency of activities of antithrombin III, protein C, and protein S.

On one hand, twenty-five patients were with primary RPL (they had never delivered a viable fetus), and 10 patients were with secondary RPL (they had delivered a viable fetus and then experienced recurrent pregnancy loss). On the other hand, seven patients had two miscarriages, 12 had three, and 16 had more than three.

The control group included 45 healthy women (mean \pm SD: 28.8 \pm 6.8 years, range 17-41 years) from the same ethnic background, who had at least one successful pregnancy, and none of them had a history of fetal loss or complicated pregnancy. No woman in this study had a personal or family history of thrombosis. The study was approved by the ethics committee of the hospital and written informed consent was obtained from each woman. Venous blood sample was collected from each woman into EDTA containing vacutainer tubes. The DNA was isolated from whole blood within 5 days after the sample collection using the Wizard[®] Genomic DNA purification kit (cat.# A1125, Promega, USA), as described by the manufacturer. The purified DNA was stored at -20°C until assay time. Aliquots of DNA were used for screening FVL mutation by Real-Time PCR using light Cycler[®] 1.2 Instrument and the Roche light-Cycler mutation detection kits for FVL (Roche Diagnostics GmbH, Germany) according to the method described and provided with the kit by the manufacturer.

Statistical analysis was performed on SPSS v.10 statistics software. Data were expressed as percentages. Chi-square test and student's t-test were used to assess inter-group significance. In addition, The Odds Ratio (OR) was used as a measure of the risk factor of RPL and 95% confidence intervals (CI) were calculated by standard methods. Statistical significance was set at $P < 0.05$.

RESULTS

Characteristics and outcome of previous pregnancies of the patients and controls are shown in

Table 1. Characteristics of patients and controls.

Characteristic	Patients (n=35)	Control(n=45)	P value
Age mean \pm SD (range)	29.6 \pm 6.3 (16-42)	28.8 \pm 6.8 (17-41)	0.56 ^a
Pregnancies			
Total number [*]	158	113	<0.001
No. of live births	15	113	
Mean \pm SD (range)	0.42 \pm 0.73 (0-2)08	2.5 \pm 1.08 (1-5)	
No. of fetal losses	143	-	
Mean \pm SD (range)	4 \pm 2.31 (2-11)		
Type of abortion			
Primary [†]	25		
Secondary [‡]	10		

^a Student's t-test (2-tailed)^b Pearson's χ^2 test^{*} Total number = live births + fetal losses[†] Primary = patient had never live births[‡] Secondary = patients had at least one live births

Table 1. Both patients and controls had a similar age. There was no significant difference between patients and controls in the pregnancy rate. The mean number of live births per women was significantly higher among controls (P < 0.001).

The results of screening FVL mutation in patients and controls are given in Table 2. The women with RPL had a significantly higher frequency of FVL mutation than did the control (P= 0.022).

Further, the higher prevalence of FVL mutation was found in women with primary RPL in comparison with controls (P= 0.014).

While, the higher prevalence of FVL mutation was found in women with secondary RPL in comparison with controls, but this prevalence did not reach significance in comparison with controls (P=0.30). All patients and controls carrying factor V Leiden mutation were heterozygote.

DISCUSSION

The fate of fetus is highly affected by the placental development and function, which, in turn depend upon the development of an adequate maternal-fetal circulation (15).

Table 2. Factor V Leiden (FVL) mutation in patients with recurrent pregnancy loss and in control

	Patients	Control	OR (CI) ^a	p-value ^b
RPL [*] (n=35)			4.1 (1.16-14.4)	0.022
Non carrier (G/G) [†]	25(71.4)	41 (91.1)		
Carrier (G/A)	10 (28.6)	4 (8.9)		
Primary [‡] RPL (n=25)			4.8 (1.2-18.17)	0.014
Non-carrier(G/G)	17 (68)	41 (91.1)		
Carrier(G/A)	8 (32)	4 (8.9)		
Secondary [§] RPL (n=10)			2.5 (0.4-16.4)	0.30
Non-carrier(G/G)	8 (80)	41(91.1)		
Carrier (G/A)	2 (20)	4(8.9)		

^a OR= odds ratio; CI= 95% confidence interval^b Pearson's χ^2 test.^{*} RPL=recurrent pregnancy loss[†] G/G: wild-type factor V gene; G/A= heterozygous factor V-Leiden mutation.[‡] Primary = patient had never live births[§] Secondary = patients had at least one live births

Values in brackets are percentages

A maternal thrombophilia caused by factor V Leiden mutation may result in production of microthrombosis on placental bed vessels and placental infarctions, which damage the maternal vessels supplying the placenta (the spiral arteries) leading to low placental perfusion and eventually in fetal death, or may interfere with initial formation of an adequate uteroplacental circulation.

Our results documented a clear association between FVL mutation and RPL (OR: 4.1). The prevalence of FVL was significantly higher in women with RPL in comparison with controls ($P=0.022$).

This result agrees with those reported by Mitraui N et al. (8), Finan RR et al (9), and Mahjoub et al. (10). Conversely, this result objected with those reported by Zahed et al. (11), Alfievic et al. (12), and Howard et al (13) who found no correlation between FVL mutation and RPL. Also, Pauer et al. (16) reported that the prevalence of FVL mutation was similar between patient and control group.

This conflict in the results reported by different studies may be due to different approaches in addressing the subject such as, number of abortions (11), to bias in patients selection (16), and ethnic heterogeneity among the patients (13).

It is of interest to note that the FVL mutation constitutes a major risk factor if Primary RPL is considered in comparison with control (OR:4.8), while the FVL mutation constitute a minor risk factor if secondary RPL is considered in comparison with control (OR: 2.5).

Also, the prevalence of FVL mutation was more prominent in women with primary RPL in comparison with control, and the deference was statistically significant ($P=0.014$). Our results agree with those reported by Wramsby et al (17) and Finan et al (9), who reported a high frequency of FVL mutation in women with primary RPL in comparison control. While the prevalence of FVL mutation was prominent in women with secondary RPL in comparison with control, but the deference was not statically significant ($P=0.3$). Consequently, if this result doesn't appear compatible with the hypothesis that FVL mutation may play in some cases of secondary RPL, this, perhaps, may be due to the small number of this subgroup.

According to our knowledge, this is the first report highlighted the role of FVL mutation in RPL in Syrian population. In conclusion, our data support the hypothesis that thrombophilia caused by FVL mutation may plays a role in the pathophysiology of primary recurrent abortion, and the investigation of FVL mutation should be routinely in women with recurrent pregnancy loss.

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