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RESEARCH ARTICLE

Factor XIII Val34Leu polymorphism and risk of recurrent pregnancy loss in Iranian population: a case control study

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BACKGROUND: Recurrent pregnancy loss (RPL) is a heterogeneous condition and thrombophilias have been considered as a probable cause.
OBJECTIVE: The aim of this study was to investigate the prevalence of the coagulation factor XIII Val34Leu polymorphism among women with unexplained RPL.
METHODS: A total of 140 women with a history of unexplained RPL and 100 age-matched healthy fertile women were recruited. The presence of FXIII Val34Leu polymorphism among the cases and controls was investigated using PCR-RFLP method.
RESULTS: Genotype analyses of the subjects revealed that the patients had a significantly higher prevalence of V/L and L/L than the controls ($P < 0.05$): 33.5% vs. 15%, and 9.2% vs. 2%, respectively.
CONCLUSION: These results indicate a significant association between FXIII Val34Leu polymorphism and unexplained RPL in the Iranian patient.

Keywords factor XIII, Iranian population, recurrent pregnancy loss, Val34Leu polymorphism

Introduction

Recurrent pregnancy loss (RPL), a common health problem, is described as consisting of three or more consecutive abortions before 20 weeks of gestation, and it occurs in 1%–2% of women of childbearing age (Brenner, 2002; López Ramírez et al., 2006). To date, a number of etiologies have been identified for RPL, including chromosomal abnormalities, anatomical problems of the uterus, endocrinological aberrations and autoimmune disorders (Brenner, 2002), hypothyroidism (Khalid et al., 2013), as well as environmental factors such as exposure to ethylene oxide (Calderwood and Thanoon, 2009); however, more than half of all cases remain unexplained (Kovalevsky et al., 2004). In such cases where there is no identifiable cause, both inherited and acquired thrombophilias have been demonstrated (Regan and Rai, 2002; Dossenbach-Glaninger et al., 2003). For instance, hyperhomocysteinemia (Nelen et al., 2000), plasminogen activator inhibitor (PAI-1) polymorphisms (Dossenbach-Glaninger et al., 2003), factor V Leiden and prothrombin G20210A gene mutations are introduced as causes of RPL (Finan et al., 2002). However, it is now suggested that the strength of the correlation between inherited thrombophilia and RPL is not very strong and inherited thrombophilia testing should not be a part of the routine workup for pregnancy loss except in the context of scientific studies (de Jong et al., 2011).

There are common morphological findings in spontaneous abortion tissue including intravenous blood clots and increased intervillous space fibrin representing a dysfunction of hemostasis which makes the coagulation factor XIII

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(FXIII) a considerable factor in the pathogenesis of RPL (Dossenbach-Glaninger et al., 2003). Plasma FXIII is a tetrameric structure (A2B2) arranged by two active A (FXIII-A) and two carrier/protective B subunits (FXIII-B). Although, several polymorphisms in the FXIII A-subunit gene have been found (Mikkola et al., 1994; Suzuki et al., 1996; Francis, 2002; Diz-Kucukkaya et al., 2004), V34L is the foremost functional polymorphism affecting FXIII activation (de Lange et al., 2006). This polymorphism is a G to T substitution at position 34 in exon 2, three amino acids away from the thrombin cleavage site that encodes the Val-Leu change. The release of activation peptide is accelerated by this point mutation; therefore, fibrin cross-linking, especially in the presence of homozygous form occur at a higher rate. As a result, the fibrin lattice is affected (Hayano et al., 1982; Balogh et al., 2000; Francis, 2002; Hancer et al., 2006). In other words, fibrin stability and its susceptibility to fibrinolysis is reduced (Dossenbach-Glaninger et al., 2003); however, its function is dependent on the fibrinogen concentration. On the one hand, the permeability of the fibrin lattice is enhanced at high fibrinogen concentrations while at low concentrations of fibrinogen, the permeability is diminished (Muszbek et al., 2008). Accordingly, it has been suggested that this polymorphism plays a protective role against both arterial (Elbaz et al., 2000; Rallidis et al., 2008; Bagoly et al., 2012) and venous thrombosis (Catto et al., 1999; Bagoly et al., 2012; Yioti et al., 2013).

Furthermore, FXIII-A has been observed in the cells of the uterus, placenta and in the cells of implantation tissue (López Ramírez et al., 2006). Thus, it has been suggested that the FXIII Val34Leu polymorphism is associated with RPL (Goodman et al., 2006); nonetheless, others have reported no significant association (Barbosa et al., 2004; López Ramírez et al., 2006; Madjunkova et al., 2012). In view of the fact that this association has not been convincingly established and because of the relatively high prevalence of FXIII val34Leu polymorphism in the Iranian population (Sajadi et al., 2008; Bagheri et al., 2011; Poursadegh Zonouzi et al., 2013), the relationship between unexplained recurrent pregnancy loss and the FXIII Val34Leu polymorphism was investigated.

Materials and methods

Study design

This case–control study was conducted to determine the association between RPL and the FXIII Val34Leu polymorphism. For this purpose, 140 cases of women with a history of three or more consecutive pregnancy losses before 20 weeks of gestation and 100 age-matched healthy women of reproductive age who had delivered at least two successful live births and had no history of pregnancy loss were enrolled by simple random.

All contributing women were informed about the study and written consent was obtained. Physical examinations and routine laboratory tests were carried out for all participants. According to the obtained medical histories, the cases or controls had no experiences of coagulation disorders. No certain causes were figured out for RPL; thereby, the cases were classified as unexplained recurrent pregnancy losses. Such a selection may lead to an increase in the prevalence of thrombophilia. Since none of the patients and controls were investigated for the polymorphisms of coagulation factors; thus, FXIII Val34Leu polymorphism was investigated as a probable cause.

Genetic analysis

Genomic DNA was extracted from anti-coagulated whole blood using a Roche kit (Mannheim, Germany, cat no: 11858874001). The stock solutions of super mix and master mix were prepared. To do so, 200 μL 10×buffer, 200 μL $MgCl_2$ (25 mmol/L), 200 μL betaine (5 mol) and 200 μL ddH_2O made the stoke of super mix and 5 μmol forward and reverse primer and 200 μL of each dNTP (2mmol/L) made the stoke of master mix. To obtain the PCR reaction mix, 8 μL of super mix, 6 μL of master mix, 6 μL of the patient's DNA and 0.3 μL of Taq DNA polymerase were added to each micro tube. All micro tubes were vortexed for 3 s at 1000 r/min. FXIII Val34Leu polymorphism was analyzed using the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method. The following oligonucleotide primers were used in PCR amplification:

Forward: 5′-ACTTCCAGGACCGCCTTTGGAGGC-3′

Reverse: 5′-GTTGACGCCCCGGGGCACCG-3′

The reverse primer contained a mismatch (G instead of A) at the 3′ end to create a *Cfo*I restriction site in the normal sequence (GCGC), which was lost when the Leu allele (GCTC) was present (Balogh et al., 2000). The PCR procedure encompassed 1 cycle of initial denaturation in 95°C for 5 min, followed by 40 cycles of denaturation (95°C for 1 min), annealing (55°C for 1 min), extension (72°C for 1 min) and a final extension at 72°C for 5 min. Following the amplification reaction, 10 μL of PCR product and 2 μL of loading dye were applied to 1.5% agarose gel stained by ethidium bromide. A positive control, a negative control, and a 50 bp size marker were also loaded onto the gel. DNA bands were then identified through use of a UV trans-illuminator. The presence of a 114 bp band confirmed the correctness of the DNA extraction protocol and the absence of Taq polymerase inhibitor. The PCR products were digested by RFLP using the *Cfo*I restriction endonuclease. The digested products were observable in the 4% agarose gel stained with ethidium bromide.

Statical analysis

Allele frequency and genotype distribution were compared

between the case and control groups by the χ^2 test (SPSS software version 16). A $p < 0.05$ was considered statistically significant.

Results

After digestion with *Cfo*I, in wild type samples, the digested fragment was 94 bp; in hemozygotes, no digestion was observed and the PCR products remained 114 bp long while in heterozygotes both 114-bp and 94-bp fragments were detectable in the 4% agarose gel stained with ethidium bromide (Fig. 1). Out of 140 patients and the 100 healthy individuals, FXIII Val34Leu polymorphism was detected in 60 and 19, respectively. According to the genotype, a significantly higher homozygosity (9.3% vs. 2%; OR = 5.01, $p = 0.04$) as well as heterozygosity (33.6% vs. 15%; OR = 2.86, $p < 0.001$) was observed for the patients than the controls. The wild type prevalence was 57.1% in the case group while it was 83% in the control group, which was significantly different. The leucine allele frequencies in cases and controls were 26.1% and 9.5%, respectively. Table 1 summarizes the results.

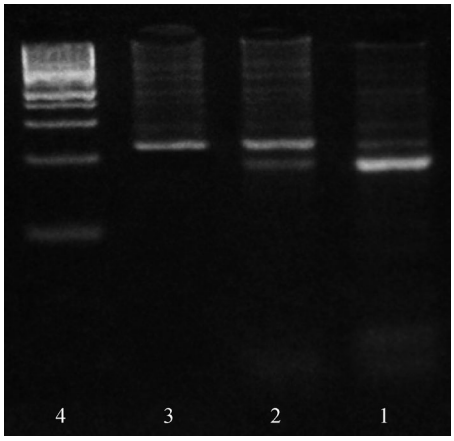

Figure 1 Molecular genetic analysis of FXIII Val34Leu polymorphism. Lane 1: Complete digestion (94 bp) in the wild-type (V/V) sample. Lane 2: Both the intact product (114 bp) and the digested fragment (94 bp) in the heterozygous (V/L) type. Lane 3: No restriction site for *Cfo*I in the homozygous (L/L) type (114 bp). Lane 4: 50 bp size marker.

Discussion

FXIII plays a major role in maintaining pregnancy (Muszbek et al., 2008). The extent to which Val34Leu polymorphism in the subunit A of coagulation factor XIII, however, is associated with an increased risk for RPL is still debatable. The findings of this study support the positive association between FXIII Val34Leu polymorphism and RPL reported by Goodman et al. (2006).On the contrary, other studies showed no correlation between them (Barbosa et al., 2004;; Coulam et al., 2006; López Ramírez et al., 2006; Aarabi et al., 2011; Bagheri et al., 2011; Ozdemir et al., 2012; Poursadegh Zonouzi et al., 2013;). In the presence of the Leu allele, some structural changes occur in the fibrin meshwork including the finer fibrin strands with decreased space between them, which in turn leads to impaired fibrinolysis. As a consequence, pregnancy loss may be observed due to inadequate trophoblast invasion and the disturbed deposition of fibrin in the early placental circulation (Dossenbach-Glaninger et al., 2003). In another view, the placenta, which is crucial for a healthy pregnancy, consists of specialized cells such as trophoblast, endoderm, and extra embryonic mesoderm. Coagulation factor XIII Val34Leu variant and PAI-I are known to be involved in the control of the placenta's functions (Bagheri et al., 2011). Some other studies have found that the co-inheritance of FXIII val34Leu alongside other thrombophilic polymorphism increases the chance of pregnancy loss (Dossenbach-Glaninger et al., 2003; Goodman et al., 2006). Other studies among Iranian populations, such as Jeddi-Tehrani et al. (2010) reported that FXIII A614T and FXIII C1694T increase the risk of RPL in Iranian women; however, such an effect was not suggested for the FXIII G103T polymorphism. Likewise, no significant association was found by Baghery et al. (2011) between this single nucleotide polymorphism and recurrent spontaneous abortion among Iranian Azeri women. In details, FXIII Leu34Leu genotype was not observed in patients and controls while FXIII Val/Leu and Val/Val genotype frequencies were 19 (35.19%) and 31 (64.81%) in patients and 12 (26.09%) and 34 (73.91%) in controls, respectively. In Zonouzi et al. (2013) study, the frequencies of mutant allele in case and control group were reported 18.53% and 13%, respectively.

The frequency of the homozygous form in the cases and

Table 1 FXIII Val34Leu polymorphism genotypes and allelic frequencies of patients and controls

Variables	Cases ($n = 140$)	Controls ($n = 100$)	OR	95% confidence interval of an odds ratio	p value
Age	27.8±3.1	28.3±2.17	-	-	0.28
Smokers	0	0	-	-	-
FXIII Val34Leu polymorphism No. (percent)					
Wild-type	80(57.1%)	83 (83%)	0.27	0.14-0.50	$p < 0.001$
Heterozygous	47(33.6%)	15(15%)	2.86	1.49-5.49	$p < 0.001$
Homozygous	13(9.3%)	2(2%)	5.01	1.07-20.21	$p = 0.04$
Val allele frequency	73.9%	90.5%	0.3	0.14-0.65	$p = 0.02$
Leu allele frequency	26.1%	9.5%	3.23	1.52-6.86	$p = 0.02$

control subjects of the present study was similar to that of Dossenbach-Glaninger et al. (9.2% and 2% vs. 8% and 2%, respectively) while the frequency of the heterozygous form and the wild type were different (Dossenbach-Glaninger et al., 2003). The criteria for patient selection would be the reason for the discrepancies between the results of this study and those of others. In this regard, the cases and controls were not ethnically matched which was a negative aspect of this study, since ethnic diversity has been shown for this polymorphism (Ariëns et al., 2000). For instance, the Leu allele frequency has been reported as being 25% for Brazilian (Franco et al., 1999), 2.9% for Spanish (Goodman et al., 2006), and 40% for Pima Indians (McCormack et al., 1998). Interestingly, absence or very low frequency has been reported for the Japanese population (Kangsadalampai and Board, 1998).

The results indicate that FXIII Val34Leu polymorphism may be a risk factor for occurring RPL. Thus, it could be used as a marker for determining high-risk individuals; however, further studies are needed to evaluate its true helpfulness. Moreover, the combination effect of this mutation with other thrombotic polymorphisms such as PAI-I 4G/5G, prothrombin G20210A and factor V Leiden should be explored.

In conclusion, the data point out that heterozygosity and, to a slightly larger extent, homozygosity for FXIII Val34Leu polymorphism might be considered as risk factors for recurrent pregnancy loss.

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Compliance with ethics guidelines

There is no conflict of interest.

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