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The relationship between FXIII Val34Leu and PAI-1 4G/5G gene polymorphisms and recurrent miscarriage in women from Golestan province

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A B S T R A C T

The main purpose of this study was to work out the relationship between different polymorphisms of PAI (Plasminogen Activator Inhibitor type 1) 4G/5G and XIII Val34Leu genes in women with first-semester recurrent miscarriage (RM) syndrome. Recurrent miscarriage (RM) is an obstetric challenge. Polymorphisms of factor XIII (FXIII)and plasminogen activator inhibitor-1 (PAI-1) may cause an imbalance between coagulation and fibrinolysis that can end in RM. This case-control study enclosed 48 women with at least two or three abortions and 50 women with at least one pregnancy as a control. DNA molecule was extracted from peripheral blood samples by the phenol-chloroform methodology. Different variants of the two genes were amplified by Amplification Refractory Mutation System - Polymerase chain reaction (ARMS-PCR) method. Finally, we analyzed allele frequencies and genotypes, Odd Ratios, Chi-square, Fisher's and Students T-test for the data. During this study, it has been found that 50% of the case population have the normal genotype for the PAI-1 (rs1799762) gene, 31.25% had a heterozygous genotype and 18.75% had a mutant homozygote. The frequency of the mutated allele in the patient population compared to the controls have p-values <0.001 and it is statistically significant for this allele (OR = 0.068, p-values <0.001, CI = 0.014-0.322). In contrast, no significant results were observed for the factor XIII (rs5985) gene and this variant was considered in this population as an ineffective polymorphism on recurrent miscarriage syndrome (p-values = 0.238). Finally, it is suggested that other variants of the given gene should be examined in Golestan.

Introduction

Abortion is defined as the loss of pregnancy before the twenty-work [1]. Recurrent miscarriage (RM) refers to the loss of three or more consecutive pregnancies, and about 1-5% of couples suffer from this complication (Rai and Regan 2006). Various risk factors for RM have been identified to date, including genetic disorders, uterine pathology, endocrine disorders, autoimmune diseases, acquired and inherited thrombophilia, as well as environmental factors

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(Toth, Jeschke et al. 2010). Among these, Genetic abnormalities account for only 2-4% of RM [4].

Successful pregnancy has a prothrombotic phenotype that causes implantation and ultimately a successful hemostatic process during labor. As the pregnancy progresses, there is a significant increase in thrombin production (Szecsi, Jørgensen et al. 2010). Some studies in women with RM have shown that the presence of certain polymorphisms in the genes of coagulation factors and fibrinolysis can be a reason for miscarriage (Elmahgoub, Afify et al. 2014). Coagulation factors in which mutations cause miscarriage include: XIII V34L, β-fibrinogen, PAI-1, HPA1, MTHFR C677T, prothrombin, and V Leiden (Coulam, Jeyendran et al. 2006).

PAI-1 gene polymorphisms have been identified as a thrombophilic factor in different researches. Identifying its variants and examining them can help our knowledge in predicting RM. The 4G/5G variant involves a single-nucleotide deletion/addition mutation in the guanine position 675 of the promoter region. The mutation causes two different alleles, 4G and 5G, which alter the regulation of PAI-1 protein. People with the Wild-type (4G/4G) genotype have higher concentrations of protein. In addition, homozygous genotypes have the lowest concentration and heterozygous individuals have moderate levels of this protein. PAI-1 plays a vital role in the fibrinolysis process, and any change in concentration and activity may cause a thrombotic change in the uteroplacental unit (Dawson, Hamsten et al. 1991, Parveen, Tuteja et al. 2013).

Coagulation factor XIII which participates in the last stage of the coagulation cascade, is a plasma transglutaminase (Muszbek, Adany et al. 1996). Factor XIII helps to implant embryos during normal pregnancy and homozygous women with FXIII deficiency suffer recurrent miscarriages. Furthermore, The exact cause of pregnancy loss among women with FXIII deficiency is not known. FXIII deficiency could well lead periplacental bleeding and spontaneous loss of the fetus (Dardik, Loscalzo et al. 2006). The common G to T SNP in exon 2 of this gene converts the amino acid Valine to luecine at position 34. This change represents a potential risk factor for the RM (Bagheri, Rad et al. 2011).

Materials and methods

In this study, we considered 48 women with recurrent miscarriage syndrome with a mean and standard deviation of 32.06 ± 5.88 who experienced at least two miscarriages before the twentieth week. These individuals have no anatomical, hormonal, immunological, or cytogenetic problems. In contrast, for the control group, 50 women with a history of at least two successful pregnancies were examined as a control group for these polymorphisms. The average age and standard deviation for the control group are 29.35 ± 4.98. All blood samples (control and case) were collected in Gorgan Genome Laboratory. Blood samples (5 mL) from the control group to RM women were taken from EDTA-coated collection flasks and the DNA was extracted using the phenol-chloroform method. The study has been approved by the Research Ethics Committees of Iran (IR.USB.REC.1399.035).

3.1 Genotype analyzing

Genotype analyses of various SNPs associated with thrombophilia PAI-1 4G/5G and FXIIIa Val34Lue were performed based on the Amplification Refractory Mutation System of PCR (ARMS-PCR) amplified products. The sequence of the primers is indicated in Table 1. The volume of material in the PCR reaction has to be 20 microliters. Add 10 µl of Master-Mix Amplicon, 2 µl of DNA, 0.8 µl of Reverse and forward primers, and transfer 6.5 µl of distilled water to the Micro-tube to reach the desired volume. Initial denaturation at 96◦C for 2 minutes, followed by 10 cycles of denaturation at 96◦C for 15 seconds, annealing at 63◦C for one minute, and extension at 72◦C for 30 seconds. The latest extension took place at 72 C for 4.5 minutes. PCR products were subsequently confirmed using 1.5% agarose gel electrophoresis.

Table1. The sequence of primers and length of PCR products to study polymorphism PAI-1 and FXIII

Gene name	Sequence	Product length
Factor XIII F	TGG AGC TTC AGG GCG	352
Factor XIII FM	GTG GAG CTT CAG GGC T	
Factor XIII R	AAG AGG CCC TAG CTA ATC CAG C	
PAI-1 F	CAC TGC TCC ACAGAA TCT ATC GG	356
PAI-1 R PAI-1 RM	CTG ACT CCC CCA CGT GCTGAC TCC CCA CG	

3.2 Statistical Analysis

A statistical analysis of genotypes and allele frequency was conducted in the R (Version 4.0.3 2020-10-10) programming environment. The independent data analysis was performed by Student's T-test and Chi-square test was carried out for significant comparison among different genotypes. Genotypes less than five were examined by the Fisherz test to ensure P-value. odd ratio and 95% CI were performed with the help of Epitools package (Aragon, Fay et al. 2017). Also, ggpolt2 package was made for drawing a bar graph (Wickham 2011). The p-values <0.05 were regarded as statistically significant.

Results

Our results showed that the PAI-1 4G/5G allele and the FXIII Val34Lue allele were somewhat higher in the cases (Table 2).

PAI-1: The PAI-1 wild-type genotype frequencies for the case and control groups were 50% and 94% respectively (Table 2). The rate of PAI-1 heterozygous and PAI-1 homozygous were 31.25% and 4% in the case and 18.75% and 2% in the control groups (Figure 1). The statistical analysis found significant differences between the control and control groups in these polymorphisms (p <0.05), (Table 2). Using the "epitools" package to analysis the effect of polymorphisms on the risk of RM, it has been demonstrated that between the two polymorphisms, only PAI-1 (OR: 0.068, 95% CI for OR: 0.0143_0.3224, p-value<0.001) significantly increase the risk of RM (Table 3).

FXIIIa: Among the RM cases, five women (10.41%) were heterozygous for the FXIII Val34Lue polymorphism and three (6.25%) were homozygous, while among the controls, four (6.25%) were heterozygous and no one was recognized with homozygous genotype for this polymorphism (Table 2). The P-value for this polymorphism is not statistically significant (Table 3).

Concerning the risk of RM, we found that women with RM have a higher incidence of the heterozygous and homozygous PAI-1 4G/5G polymorphism than controls. This suggests that carriers of the heterozygous and homozygous PAI-1 4G/5G polymorphism present an increased risk of RM. Moreover, a clear relationship with the risk of RM has been established with the combined polymorphism FXIII Val34Lue and PAI-1 4G/5G.

Table 2. Frequency of studied polymorphisms for two genes FXIII Val34Leu and PAI-1 4G/5G

	Cases(n=48)	Controls(n=50)	P-value
Age (SD)	32.06 (5.88)	29.35 (4.98)	0.016*
Number of RM (SD)	3.56 (1.25)	---	---
PAI-1 4G/5G polymorphism			
Wild-type	24/48 (50%)	47/50 (94%)	0.0063*
Heterozygous	15/48 (31.25%)	2/50 (4%)	0.0027*
Homozygous	9/48 (18.75%)	1/50 (2%)	0.0066*
4G allele frequency	63 (65.62%)	96 (96%)	0.0088*
5G allele frequency	33 (34.38%)	4 (4%)	<0.001**
FXIII Val34Leu polymorphism			
Wild-type	40/48 (83.33%)	46/50 (92%)	0.5176
Heterozygous	5/48 (10.41)	4/50 (8%)	1
Homozygous	3/48 (6.25%)	0.0	1
Val allele frequency	85 (88.54%)	96 (96%)	0.4136
Leu allele frequency	11 (11.45%)	4 (4%)	0.0707
Combined FXIII Val34Leu and PAI-1 4G/5G polymorphisms	6/48	0	0.1431
Both heterozygous	3/48	0	0.0832
34Leu + 4G/5G	1/48	0	0.3137
Val34Leu + 4G/4G	1/48	0	0.3137
Both homozygous	1/48	0	0.3137

statistically significant (p <0.05)
*significant.
** highly significant.

Table 3. Odd Ratio related to recurrent miscarriage in PAI-1 and FXIII polymorphisms

Polymorphism	P-value	OR	95% CI for OR	
			Lower	Upper
PAI 4G/5G	<0.001*	0.068	0.0143	0.3224
FXIII Val34Leu	0.2385	0.6956	0.1747	2.7689

*statistically significant (p <0.05)

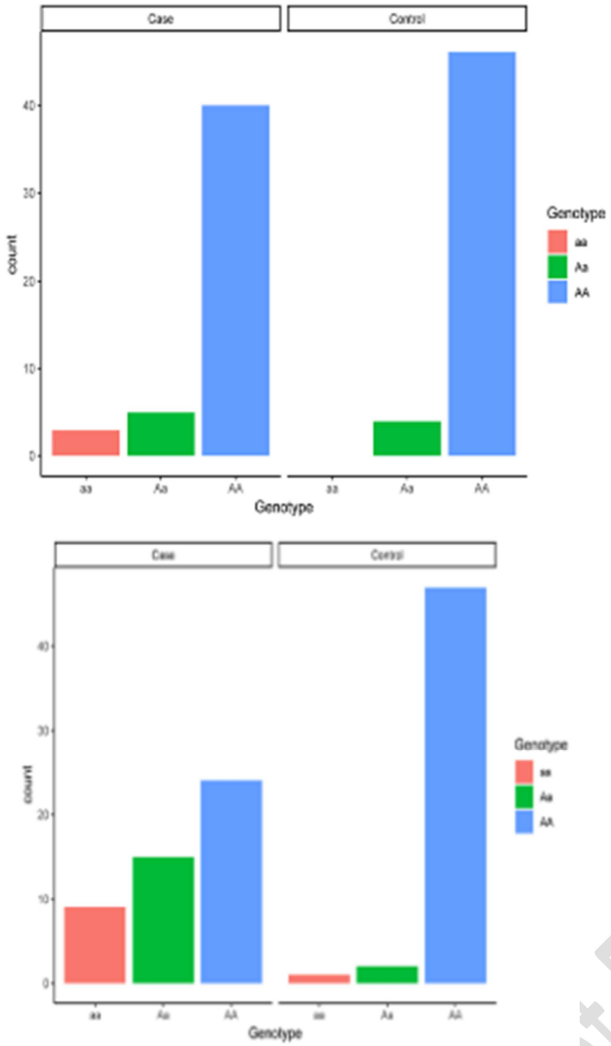

Figure 1. Bar-plot for the frequency of PAI-1 and FXIIIa genotypes

Discussion

Pregnancy is a physiological condition that is prone to coagulation changes and thrombosis. As a consequence, thrombophilia could be one of the important causes of recurrent miscarriage (RM) syndrome (Adler, Mahmutbegovic et al. 2018). PAI-1 gene polymorphisms have been identified as a thrombophilic factor in the given research. Also, identifying its different variants and examining them would help our knowledge in predicting RM. The 4G/5G variant involves a single-nucleotide insertion/deletion mutation in the guanine position 675 of the promoter region. PAI-1 plays a vital role in the fibrinolysis process, and any modification in concentration and activity will result in a thrombotic change in the uterine-placental unit (Dawson, Hamsten et al. 1991, Parveen, Tuteja et al. 2013).

FXIII deficiency is an inherited bleeding disorder characterized by severe manifestations of bleeding, delayed wound healing, and recurrent miscarriage in homozygous women. Women who are homozygous for FXIII deficiency cannot carry the fetus until the end of pregnancy unless they are treated with factor XIII concentrate during pregnancy. The minimum level of FXIIIA required for a normal pregnancy is unknown. However, only 0.5 to 2% of FXIIIA is required for normal homeostasis (Burrows, Ray et al. 2000).

In a case-control study by Elmahgouba et al., Heterozygous and homozygous FXIII Val34Leu polymorphisms and the frequency of Leu allele was higher in women with RM rather than controls. Also, this study showed that the prevalence of PAI-1 4G/5G homozygous and heterozygous polymorphisms were not significant among case and control individuals, although the frequency of 4G alleles was higher among the cases. Furthermore, their findings suggest that RM might be higher in women with FXIII Val34Leu and PAI-1 4G/5G combined polymorphisms than among women with single nucleotide polymorphisms (Elmahgoub, Afify et al. 2014).

The results of various studies that were carried out in different parts of the world do not report the same results and different reports were announced. In a study by Sallot et al., the PAI-1 was referred to as an important member of the fibrinolytic path, however no vital link was found between RM and the polymorphism of this sequence (Al Sallout and Sharif 2010).

A study by Torabi and colleagues on women with RM in northwestern Iran in 2014. The results of this study reported that there were no significant differences between the expected and observed frequencies of FV G1691A and A4070G polymorphisms, prothrombin G20210A ($p> 0.05$), while the expected frequency of 4G allele was greater than the observed frequency in the study population ($p <0.01$). The frequency of PAI-1 polymorphism (4G/5G) did not show a major distinction between the population of northwestern Iran and Italy or China ($P> 0.05$) (Bargahi, Farajzadeh et al. 2014).

In the present study, it is found that women with primary RM (women who have not had a successful pregnancy) are more likely to carry the 4G allele for the PAI-1 gene. As it is mentioned before another study by Torabi and et al. confirmed similar results to our study and confirmed the role of PAI-1 in the RM of Iranian women in 2012. However, in 2003, a study by Al Sallout et al. Found that in their study population, no significant differences were observed between the control and case groups in relation to this polymorphism. Statistical analyzes in this study have shown that recurrent miscarriage is much more common in women with PAI-1 mutation (RR = 0.106, OR = 0.068, 95% CI = 0.14 - 0.322, p-Value <0.001). In contrast, the FXIII gene variant examined in this population lacked a significant role. Examination of the data shows that the presence of mutations combining the two polymorphisms in individuals may increase the risk of miscarriage.

Differences in the results could be due to the ethnic diversity. Also, the frequency of alleles in different geographical conditions does not affect the results of the study. These results are confirmed in a study by Cavalli-Sforza et al. They showed that gene abundance in North Africa is mediated by communities near Egypt and southern Europe (Cavalli-Sforza, Cavalli-Sforza et al. 1994).

Interestingly, among these patients, it was recorded that patients with mutations in the PAI-1 promoter region loss the fetus in the second to third months of pregnancy and the fetus is lost due to heart problems. For the FXIII gene, fetal loss occurs at less than two months and between the seventh and twelfth weeks. Most of the relevant laboratory questionnaires in this area ignore this option, so recording such information would be very helpful in order to identify the factors.

Finally, it is suggested that the studied polymorphisms be sequenced in order to obtain a more definite result. Sequencing could help identifying other possible variants as well.

Conclusion

According to the results of this study, it can be said that PAI-1 4G/5G polymorphism in the study population is significantly associated with recurrent miscarriage syndrome. Variant rs5985 FXIII in this population has no significant result. It is suggested that other variants of this gene be studied in Golestan.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement

Informed consent was obtained from all women participants in the Genome laboratory of Gorgan.

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