Prevalence of mutations in V Leiden and prothrombin genes in women with recurrent pregnancy loss: A retrospective study on Iranian Azeri women

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Abstract

Introduction: The thrombophilia is one of the most important cause of maternal thromboembolism, which is associated with recurrent pregnancy loss (RPL) Risk. The aim of present study was to investigate prevalence of prothrombin (FII, G20210A) and factor V Leiden (FVL, G1691A) genes mutation, as two important cause of thrombophilia, in Iranian Azeri women with RPL.

Materials and methods: The subjects in this retrospective study consisted of 100 women (20-40 years old) with RPL recruited from Iranian Azeri population in East Azerbaijan province, Tabriz in Iran. The genomic DNA was extracted from 5 ml peripheral blood samples using the proteinase K method. The Allele and genotype of FII (G20210A) and FVL (G1691A) mutations were assessed using restriction fragment length polymorphism (RFLP) polymerase chain reaction (PCR) method.

Results: Our results showed that the frequency of normal homozygous, heterozygous and mutation homozygous for the FII G20210A and FVL G1691A mutations were equally distributed among Iranian Azeri women with RPL. The genotype distribution in RPL patients was, 99% AA, 1% AG, and 0% GG in both of the mutations.

Conclusion: In general, our study showed that the prevalence of FVL (G1691A) and FII (G20210A) mutations is low in the Iranian Azeri women with RPL. However, these mutations can be the important reasons for RPL, and more studies with large sample size are required to determine the exact frequency of FVL (G1691A) and FII (G20210A) mutations in Iranian Azeri women with RPL.

Keywords: Mutation, Factor V Leiden, Prothrombin, Recurrent pregnancy loss

Introduction

Recurrent pregnancy loss (RPL) is a multifactorial condition with two or more successive abortions (1), which occurs in 1-5% of woman at reproductive age (2). The main causes of RPL are heterogeneous, such as chromosomal, hematological, genetic, anatomical, and endocrinological factors (3-5). Moreover, environmental factors and exposure to chemical compounds such as lead and ethylene oxide are associated with RPL (5). In some cases, RPL arises from immunologic problems and infections (6, 7).

Thrombophilia is a condition with increased potential of blood coagulation. Due to various physiological alterations

during pregnancy, women are at a higher risk of venous thrombophilia (8). The presence of an equivalence between coagulation system of mother pregnant women and fibrinolysis cause to inhibits fibrin deposition in the fetus vessels and the spaces between the tufted capillaries, thus stabilizing the blood coagulation (9). However, the thrombophilia in women during pregnancy cause to increase of venous thromboembolism risk and other vascular complications, such as abortion and preeclampsia (10).

The thrombophilia is a main cause of RPL in the first half of pregnancy, after chromosomal abnormalities (11). Moreover, pregnancy is a hypercoagulable condition, thus thromboembolism is an important cause of maternal mortality in antepartum and postpartum (12). The various mutations on prothrombin (FII, G20210A) and factor V Leiden (FVL, G1691A) were considered as important risk factors of thrombophilia, which can cause to possible RPL (13, 14).

Generally, the exact frequency of FII (G20210A) and FVL (G1691A) mutations in patients with RPL is still unclear. Therefore, it is necessary to investigate the prevalence of mutations in these genes. In this study, we investigated the prevalence of prothrombin (G20210A) and V Leiden (G1691A) genes mutation in Iranian Azeri women with RPL.

Materials and methods

Patients collection

This retrospective study was performed to determine the prevalence of prothrombin (G20210A) and V Leiden (G1691A) mutations with RPL. During the period from January 2018 to June 2019, 100 Iranian Azeri women with RPL, referred to clinical reproductive medicine centers in Tabriz, were investigated in this study. The women with known causes of RPL (semen anomalies, karyotype abnormalities, uterine malformations, etc.) were excluded from study. The studied subjects were women aged 20-40 years old with at least two consecutive miscarriages before 20 weeks of gestation. The structure of uterus and karyotypes of the patients were normal, and not identified any infection and other causes related to miscarriages. Therefore, the events were classified as unexplained pregnancy loss. The information's such as clinical characteristics, lifestyle and demographic were collected using interview and questionnaire from case and control groups. The collected information's included age (year), body mass index-BMI (kg/m^2), age at menarche (year), menopausal status, tobacco smoking, alcohol drinking, age at first delivery (year) and family history. In order to prevent the epidemiological bias, all selected women in this study were from East Azerbaijan province of Iran and matched for age and ethnic and were genetically unrelated. All studied women were signed a consent form and informed about this study according to the Declaration of Helsinki ethical standards (Ethical code: IR.TBZMED.REC.1397.257).

Extraction of DNA

Genomic DNA extraction was performed using the proteinase K method from 5 mL blood samples containing EDTA as anticoagulant. The quantity and quality of extracted DNA was investigated according to OD 260/280 ratio using a Nanodrop instrument and electrophoresis on 1% agarose gel, respectively.

Detection of mutations

The extracted DNA samples were amplified using polymerase chain reaction (PCR) method. The specific used primers presented in Table 1. The amplification was performed in 50 µl volume using 2 µl each praimers, 2 µl template DNA, 5 µl dNTP, 5 µL PCR buffer, 1.5 µl Mgcl2, 0.5 µl Taq DNA polymerase, and 32 µl distilled water. The PCR conditions encompassed as following: initial denaturation (1 cycle in 94°C for 5 minutes), denaturation (35

cycles in 94°C for 45 seconds), annealing (35 cycles in 58°C for 45 seconds), extension (35 cycles in 72°C for 45 seconds) and final extension (1 cycle in 72°C for 5 minutes). The amplified fragments were electrophoresed on 1.5% agarose gel. The restriction fragment length polymorphism (RFLP) using *Hind III* restriction enzyme was performed for genotype analyses. In FII G20210A mutation, the PCR product (506 bp) yields two fragments (99 bp and 407 bp) for the G allele, and yields three fragments (384 bp, 99 bp and 23 bp) for the A allele. In FVL G1691A mutation, the PCR product (241 bp) remains intact if G allele is present and yields two fragments (209 bp and 32 bp) for the A allele. After incubation in 37°C, genotypes determination was performed using electrophoresis on 3% agarose gel.

Table 1. Sequences and characteristics of primers used amplify studied genes mutation.

Mutation	Primer Sequence	T_m	Products Size
FII G20210A	F: AGGCAGGAACAACACCAT	56˚C	506 bp
	R: AGGAATACAGGTATTTTGTCCTTGAAAGTA	60˚C	
FVL G1691A	F: GCACAGACGGCTGTTCTCTT	60˚C	241 bp
	R: ATAGCACTGGGAGCATTGAAGC	61˚C	

FII: Coagulation Prothrombin Factor II, FVL: Coagulation Factor V Leiden, T_m: Melting Temperature.

Results

Mean age of studied women was 31.12 ± 1.24 years (range, 20-40 years). All studied women were experienced 3.11 ± 0.41 (range, 2-5) successive abortions. The other demographic characteristics and clinical features of studied patients are presented in Table 2.

The allele frequencies and genotype distribution are presented in Table 3. We did not observe mutant homozygous in FII (G20210A) or FVL (G1691A) genes in the studied women with RPL. The heterozygous mutations of the FII and FVL genes were equally distributed among studied women (heterozygous for FVL: 1.0% and heterozygous for FII: 1.0%; normal homozygous for FVL: 99.0% and normal homozygous for FII: 99.0%). The allele frequencies of the FVL (G1691A) and FII (G20210A) mutations were 199 (99.5%) for G allele and 1 (0.5%) for A allele (Table 3).

Discussion

The role of thromogenic gene mutations in RPL pathogenesis remains unknown. So far, various environmental and genetic factors such as infection, immunological factors, coagulation factors, anatomical problems, and chromosomal abnormalities have been evaluated to identify cause of this event (6, 15, 16). This study focused on the thromogenic genes mutations to investigate the prevalence of FII (G20210A) and FVL (G1691A) mutations in Iranian Azeri women with RPL.

Thrombophilia is a coagulation disorder with predisposition to thrombotic, which associated with thrombophilia. The thrombosis cause to inhibition of trophoblast differentiation and placental insufficiency. The various mutations on FVL and FII genes are the most common causes of inherited thrombophilia (17).

RPL, venous thrombosis, and arterial disease (18). The heterozygous mutation (G20210A) on prothrombin gene was reported 1.5-2% in Iranian population (4). Also, in this study, frequency of G20210A heterozygous mutation on prothrombin gene was 1.0%, which was less than national prevalence.

The substitution of G to A at 20210 position in the 3’ untranslated region in the prothrombin gene cause to increase the prothrombin levels in serum. The increase of prothrombin leads to

The replacement of A to G at 1691 position in the FVL gene cause to resistant to activation of protein C (19).

Table 2. Demographic variables and characteristics of women with RPL.

Variable		Patients (n)	Percent (%)
Age			
	20-25	31	28%
	26-30	19	16%
	31-35	23	23%
	36-40	27	27%
Blood groups			
	AB	4	4%
	A	64	64%
	B	8	8%
	O	24	24%
Consanguinity degree			
	Degree 3	12	12%
	Degree 5	8	8%
Pregnancy loss			
	2 case	56	56%
	3 case	36	36%
	4 case	6	6%
	5 case	2	2%
Smoking (%)		12	12%
Number of pregnancies		4.71 ± 8.17	-
Menarche (years)		12.89 ± 9.28	-
Smoking (%)		12 (12%)	-
Mean BMI (Kg/m^2)		26.98 ± 2.38	-

BMI: Body Mass Index

Table 3. Genotype and allele frequencies of FVL (G1691A) and FII (G20210A) mutations in the studied women with RPL.

Mutations	Genotype and Allele	Frequencies
FII G20210A		
	Normal homozygous GG	99 (99.0%)
	Heterozygous GA	1 (1.0%)
	Mutant homozygous AA	0 (0.0%)
	G Normal	199 (99.5%)
	A Mutant	1 (0.5%)
FVL G1691A		
	Normal homozygous GG	99 (99.0%)
	Heterozygous GA	1 (1.0%)
	Mutant homozygous AA	0 (0.0%)
	G Normal	199 (99.5%)
	A Mutant	1 (0.5%)

Thus, increase of hyper-coagulable state during pregnancy cause to complications such as RPL (17). Prevalence of FVL gene mutation (G1691A) were reported in a wide range in Iran (20). Also, in this study, frequency of FVL gene mutation (G1691A) was 1.0%, which was less than national prevalence.

Differences in reported results by different studies might be due to other involved genes, and differences in geographic area, sample size and selection bias, ethnicity and race heterogeneity, and environmental factors (21, 22).

Conclusion

Generally, our study indicated a low prevalence of FVL (G1691A) and FII (G20210A) mutations in the Iranian Azeri women with RPL. However, these mutations involved in thrombophilia and can be a main cause of RPL in women. Furthermore, identification of gene mutations would change the treatment

strategy of the patients. Therefore, for better understanding prevalence and role of FVL (G1691A) and FII (G20210A) mutations with RPL, further studies are recommended on other populations and races with larger sample sizes.

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