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Association of +505A>G Polymorphism at TAFI Gene with Recurrent Miscarriage in Iranian Women

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ABSTRACT

Background: Recurrent miscarriage (RM) is one of the major problems of public health globally. The thrombin-activatable fibrinolysis inhibitor (TAFI) gene is a plasma zymogen that regulates both fibrinolysis and inflammation. Genetic variants within TAFI gene are presumed to be associated with development of RM. This case-control study aimed to investigate the association of TAFI +505A>G polymorphism with RM in Iranian women referred to Meybod Genetic Center.

Methods: Fifty women with RM (at least 2 miscarriages) and 50 healthy women with no history of miscarriage or other fertility complications were participated in this study. The TAFI +505A>G polymorphism was genotyped by allele specific PCR (AS-PCR) assay.

Results: The mean age of cases with RM and controls was 27.25 ± 4.31 and 28.42 ± 3.22 years, respectively. The frequency of GG genotype and G allele was 0.00% in patients and controls. There was no significant difference between RM cases and controls in terms of +505A>G genotypes and alleles.

Conclusion: This study results indicated that there was no significant relationship between the TAFI +505A>G polymorphism and RM risk in Iranian women. However, further rigorous, studies with a larger sample size and different ethnicity are necessary to confirm our findings.

Introduction

Pregnancy loss is characterized as a medically identified pregnancy unintentional ending before 20 weeks or when the fetus weighs lower than 500g and increasing with maternal age.[1,2] Pregnancy loss is the most current problem of pregnancy, affecting about 10%-15% of clinically recognized pregnancies.[3] Moreover, miscarriage can be arranged as embryonic loss (or early miscarriage) when it takes place before 10 gestational weeks and fetal loss (or fetal miscarriage) when it takes place after 10 gestational weeks, because factors related to each may differ.[1,4] Recurrent pregnancy loss (RPL) is traditionally described as the event of two or more continuous pregnancy losses. RPL is one of the most common fertility complications and the exact prevalence of RPL is difficult to estimate, most studies report that RPL affects 1-5% of women during reproductive ages.[5,6] It is a multifactorial condition involving the interaction of genetic factors and environmental factors. Today several factors of RPL such as genetic thrombophilia, endocrinological factors, abnormalities in chromosomes, uterine abnormalities, thrombotic tendency, hormone or metabolic disorders have been identified. Moreover, environmental and psychological factors include infection, malefactors, autoimmunity, age, and lifestyle problems.[7,8] Also RPL can be caused by endocrine, immunological, vascular, and metabolic imbalances.[9] Although the causes have been studied deeply, more than 50% of cases remain unexplained.[3,10,11] Several articles provide evidence that genetic factors display a significant element of human fertility.[12]

Thrombophilia is one of the most reasons for RPL. Thrombophilia could be either acquired or inherited. Approximately 40% of cases displaying thrombosis are inherited. variation in the amount or the function of the proteins which are in the coagulation system pathway leads to hereditary thrombophilias.[13] Hereditary thrombophilia has been displayed to be a risk factor for reproductive diseases including infertility, RPL, and obstetrical complications.[11] The balance between coagulation and fibrinolysis is an essential part of early pregnancy.[14] At pregnancy, alterations in the mother's body due to changes in the homeostasis lead to the hyper-coagulable state of pregnancy. A hyper-coagulable state is described by exceeded levels of prothrombotic factors and reduced antithrombotic factors. Such thrombophilias enhance the prothrombotic condition of pregnancy, resulting in insufficient fetomaternal circulation, and affect the function of placentation in the developing embryo.[3] The risk of thrombophilia becomes larger which can reflect many genetic factors like polymorphisms which then affect the coagulation system. Of late, the relation of RPL with maternal thrombophilic or hypofibrinolytic gene variants has collected growing evidence. Many previous studies evaluated polymorphism of several thrombotic genes that had a role in RPL such as prothrombin 20210G/A, FVL 1691G/A, MTHFR 1298A/C, MTHFR 677C/T, and PAI-1 4G/5G polymorphisms and RPL risk in the Iranian population.[15]

Thrombin activatable fibrinolysis inhibitor (TAFI) factor, is a basic carboxypeptidase with strong antifibrinolytic and anti-inflammatory activity.[16] Practically TAFI is an unstable carboxypeptidase of plasma zymogen which forms a molecular link between coagulation and fibrinolysis.[17] The physiological function of TAFI is to dilute fibrinolysis secondary to activation of plasminogen by tPA into plasmin on the surface of a fibrin clot.[18] The proposal of most studies about TAFI is that increase in TAFI levels takes part in arterial thrombus and venous thrombus formation.[18] Also TAFI has a role in the regulation of early human trophoblastic invasion. Reportedly TAFI levels in the maternal circulation slowly increased during gestation with a peak in the last trimester, especially in complicated

pregnancy, and later come back to natural levels after the first day postpartum.[19]

In the two decades, the effects of several polymorphisms influencing TAFI level on these thrombotic events have been evaluated. Several applications such as +505A/G SNP and 1040C/T SNP in the programming region and a -438G/A SNP in promoters are associated with plasma antigen levels.[18] Also, multiple kinds of research have shown an association between thrombophilia abnormalities and their correlations to pregnancy loss. Therefore, in our study we investigated the association of TAFI +505A/G polymorphism with RPL in Iranian women.

Materials and Methods

Subjects: All procedures in the study were carried out under the ethical standards of the institutional or national research committee of Ashkzar Branch, Azad University, Ashkzar, Yazd and with the 1964 Declaration of Helsinki and its later modifications or similar ethical standards. The aims of the study were fully explained to all participants and written consent was obtained. A total of 50 women with unexplained RPL before 20 weeks' gestation were included in the study. The control group included 50 healthy women without a history of miscarriage or other fertility complications. All participants were examined by an expert gynecologist and they were checked for chromosomal abnormalities and thrombophilic factors. Both cases and healthy subjects were originally Iranian and were recruited from Meybod Genetics Center, Yazd.

DNA Isolation and Genotyping*:* Five cc of peripheral blood from peripheral blood of all subjects was collected in EDTA-containing tubes and genomic DNA was isolated using an extraction kit (Rojeh Company's Co., LTD). The quality of DNA samples was evaluated by 0.8% agarose gel electrophoresis and NanoDrop and then stored at -20C (Figure 1 and 2).

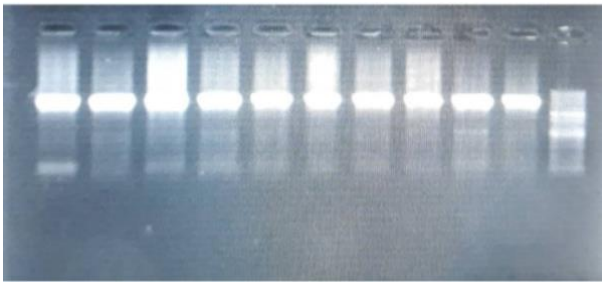

Figure 1. The quality of DNA samples was assessed by performing the 0.8% agarose gel electrophoresis

The +505A/G polymorphism at the TAFI gene was genotyped using tetra-primer amplification refractory mutation system-polymerase chain (ARMS-PCR) assay. The primers were designed using Oligo software and NCBI BLAST search engine and synthesized by Fazapjooh Tehran Company (Tehran, Iran). The size of PCR products and primer sequences for each single nucleotide polymorphism are displayed in Table 1.

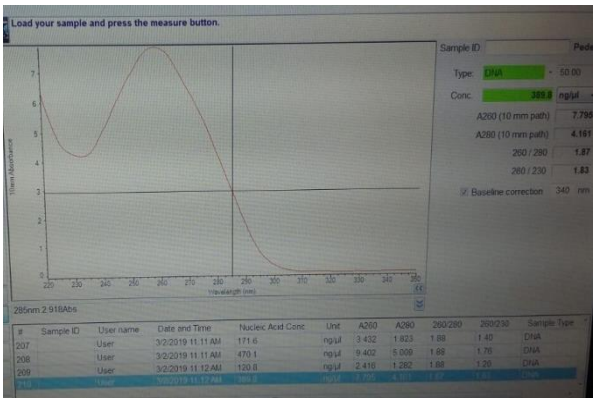

Figure 2. Assessing the quality of DNA extracted with NanoDrop

The PCR amplification was performed in a total volume of 27 µL reaction mixture, contain 5 µL genomic DNA, 10 µL Master mix 2x, 1 µL of each primer and 10 µL sterilized water. The reaction mixtures were denatured at 96°C for 5 min, followed by 36 cycles of 94°C for 50 s, 55°C for 50 s, and 72°C for 1 min, with a final elongation at 72°C for 7 min. The PCR products were separated at 37°C overnight by 1% agarose gel electrophoresis and visualized under UV light (Figure 3).

Table 1. Primer sequences for detection of TAFI +505A/G polymorphism

SNP-ID	Sequence	PCR product size (bp)
rs3742264	F-5′-CTTCCACATGCAGCTCTGAC-3′ R-5′-ATAGCCCAGTTGAGTCTGACAC-3′	180
	F-5′-GGTTTCTGGAAAAGAACTAG 3′ R-5′-CATATGGCATTTTTGGCCGT-3′	520

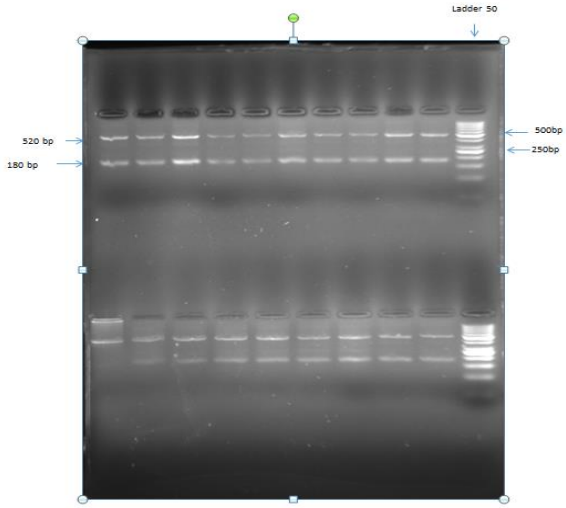

Figure 3. Scheme of electrophoresis of the PCR-RSM assay for genotyping of TAFI +505A/Gpolymorphism

Statistical analysis: All Statistical analyses were performed using SPSS version 19.0 (SPSS Co., Chicago, IL, USA) for Windows. A $P < 0.05$ was considered to demonstrate statistical significance. The chi-square test was applied to examine the differences between RPL cases and healthy controls in terms of mean age. The distribution of the genotype and allele frequency for the polymorphism between cases and controls was examined by the chi-square test. Hardy-Weinberg equilibrium (HWE) for the distributions of TAFI +505A/G genotypes was performed in healthy subjects by the chi-square (χ^2) test.

Results

Characteristics of participants: The demographic and clinical features of the participants are given in Table 2. The mean age of patients and control group was 27.25 ± 4.31 and 28.42 ± 3.22 years, respectively. Patients were divided into two groups based on the number of abortions, of which 27 patients (54%) had two abortions, 23(46%) patients had three and more than three recurrent abortions (Table 2). In the control group, they had an average of 2.34 children. In addition, in the group of patients with recurrent miscarriage, 23(46%) cases had familial marriages and 27(54%) cases had non- consanguineous marriages. Patients were also divided based on type of miscarriage. Miscarriage in 24 patients took place before 10 gestational weeks (early miscarriage) and in 26 patients took place after 10 gestational weeks (late miscarriage).

Table 2. Characteristics of cases with RPL and healthy controls

Variables	URPL Cases (n = 50)	Healthy Control (n = 50)	*P*
Age (year)			
Mean (±SD)	27.25 ± 4.31	28.42 ± 3.22	0.721
Contagious Marriage			
Familial	23	-	
Non familial	27	-	
Number of miscarriage			
2	27	-	
≥ 3	23	-	
Type of miscarriage			
Early(< 10 weeks)	24	-	
Late(> 10 weeks)	26	-	

Table 3. The frequencies of genotypes/alleles of the TAFI +505A/G polymorphism in subjects

SNPs	RPL Cases (n = 50)		Healthy Control (n = 50)		P
	Frequency	Percent	Frequency	Percent	
Genotypes					
AA	31	62%	26	52%	
AG	19	38%	24	48%	NA
GG	0	0	0	0	
Alleles					
A	81	81%	76	76%	NA
G	19	19%	24	24%	

Association between TAFI polymorphism and RPL susceptibility: Genotype and allele frequencies of TAFI +505A/G polymorphism were examined in case and control groups. Genotypes frequencies of CC for +505A/G polymorphism in women with an unexplained recurrent miscarriage were not seen. These findings showed that there was no significant difference between case and control groups. Moreover, no significant association was observed in allele frequencies and genotype distributions for +505A/G (Table 3).

Discussion

The connection of RPL with maternal thrombophilic or hypofibrinolytic gene variants has collected developing documents.[20] Such thrombophilias enhance the prothrombotic state of pregnancy, lead to insufficient fetomaternal circulation, and influence the action of placentation in the developing embryo.[20] It is recommended to investigate polymorphisms of the genes playing roles in thrombophilia in different communities so that approaches for early determination and treatment are suggested. In the two decades, several emerging candidate genes have been reported in association with RPL. One of them is the TAFI gene, which is involved in thrombosis.

A previous study evaluated polymorphism of several thrombotic genes that had a role in RPL. In 2018, Kamali et al., in a meta-analysis demonstrated that there is a significant relation between thrombotic genes including FVL 1691G/A,MTHFR 677C/T, MTHFR 1298A/C, Prothromb in 20210G/A, and PAI-1 4G/5G polymorphisms and risk of RPL in the Iranian population.[15] Chatzidimitriou et al, in a study, evaluated genetic variants at 12 thrombophilicin the Greek population. Their results indicated that ten genetic loci are mainly correlated with an augmented risk of RPL. Remarkablely, FV Leiden, FV HR2, Factor II prothrombin20210G/A, Factor XIII V34L, b-fibrinogen -455G/A, PAI-1 4G/5G, GPIIIa L33P (HPA-1a/b L33P), MTHFR 1298A/C, MTHFR 677C/T, Apo B R3500Q, ACE I/D, and Apo E2/E3/E4 have been incriminated to state mild or more severe thrombotic risks.[3]

TAFI, as markable anti-fibrinolytic factor, moderate partly the coagulation and fibrinolysis system, which is associated with an expansion incidence of venous thrombosis disorders. TAFI controls both fibrinolysis and inflammation, which both can contribute to RPL occurrence.[21]

There is no report about the association between TAFI polymorphism and miscarriage possible in Iranian women. In this study, we investigated the relationship of +505A/G polymorphism within TAFI gene with susceptibility to RPL. Our findings demonstrated that the TAFI +505A/G polymorphism was not correlated with an increased risk of RPL in Iranian women. Our findings are consistent with previous case-control studies among Spanish women. Martı´nez-Zamora et al., evaluated the decreased plasma fibrinolytic potential in cases with recurrent implantation failure after IVF and embryo transfer. Their results did not show a significant difference in the distribution of TAFI polymorphism between IVF and fertile women groups.[22] ElDanasori

et al., assessed the TAFI gene polymorphism (TAFI1040C/T) in women with RPL among Egyptian patients. They reported a higher frequency of C allele in the control group and a higher frequency of T allele in the case group with no statistical significance. Their study showed that TAFI 1040C/T could not be counted as a molecular predictive factor for RPL in Egyptians.[23] However, previous studies among European and Egyptian women showed that the polymorphism was associated with RPL risk. Masini et al., assessed the association between TAFI polymorphism and RPL among Italian women. They showed that the +505 and +1583 polymorphisms at TAFI gene were linked with RPL risk. Their results also demonstrated that SNPs directed toward increased circulating TAFI antigen levels are related to a decreased risk of RPL.[24] Nelly et al., evaluated the prevalence of VEGF, eNOS and TAFI polymorphisms among Egyptian women with RPL. They showed eNOS genetic variant associated with TAFI 1040C/T confirmed an almost one and half fold increase risk of RPL.[19] Moreover, Pruner et al., assessed R1040 C/T polymorphism in the coding region of TAFI gene and the risk of idiopathic RPL in Serbia. They recognized an enhanced frequency of R1040T/T of TAFI genotype in a patient group, recommending that this genotype could be a potential risk factor for idiopathic RPL.[21] In our study the prevalence of TAFI polymorphism was not significant and demonstrated that this polymorphism did not happen in any of the patients and control group. Therefore, well-designed epidemiological studies with a greater sample size and various subgroups are needed.

Conclusion

This study results showed that there was no obvious evidence of association between +505G/A polymorphism at TAFI gene and the risk of RPL in the Iranian women. Further rigorous designed studies with adequate sample size and in different ethnicity are needed to confirm our findings.

Conflict of Interests

Authors have no conflict of interests.

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