Reports of Biochemistry & Molecular Biology
Vol. 2, No. 2, Apr 2014

Original article

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Inherited Genetic Markers for Thrombophilia in Northeastern Iran (a Clinical-Based Report)

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Abstract

Background: Thrombophilia is a main predisposition to thrombosis due to a procoagulant state. Several point mutations play key roles in blood-clotting disorders, which are grouped under the term thrombophilia. These thrombophilic mutations are methylenetetrahydrofolate reductase (MTHFR, C677T, and A1298C), factor V Leiden (G1691A), prothrombin gene mutation (factor II, G20210A), and plasminogen activator inhibitor (PAI). In the present study, we assessed the prevalence of the above thrombophilia markers in patients with recurrent pregnancy loss or first and second trimester abortions, infertility, and failed in vitro fertilization (IVF).

Methods: This study was conducted among 457 cases those were referred to detect the inherited genetic markers for thrombophilia. Markers for MTHFR, Factor II, and Factor V were assessed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), and PAI was assessed by Amplification Refractory Mutation System (ARMS-PCR).

Results: Two hundred sixty cases (56.89%) were diagnosed as having at least one thrombophilia marker, whereas 197 cases (43.11%) had no thrombophilia markers and were normal.

Conclusion: According to the current study, the pattern of abnormal genetic markers for thrombophilia in northeastern Iran demonstrates the importance of genetic evaluations in patients who show clinical abnormalities with recurrent spontaneous abortion (RSA) or other serious obstetric complications.

Keywords: Factor II, Factor V, Thrombophilia, MTHFR, PAI, Thrombophilic markers

Introduction

Thrombophilia (hypercoagulability or prothrombotic state) is an abnormality in blood coagulation that increases the risk of thrombosis (1). Such abnormalities can be recognized in 50% of people who have thrombotic episodes (2). Thrombophilia is the tendency to develop thromboses due to inherited defects in the coagulation system. A significant proportion of the population has detectable abnormalities, but most people develop thromboses in the presence of additional risk factors.

Women with thrombophilia are at risk for pregnancy loss and other serious obstetric complications. Recently, studies have focused on thrombophilic factors associated with pregnancy complications. The four most common genetic markers for thrombophilia are; factor V Leiden (FVL, G1691A), methylene tetrahydro folate reductase mutations (MTHFR, C677T and A1298C), prothrombin gene mutation (FII, G20210), and plasminogen activator inhibitor 1 (PAI-1) (3).

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Received: Nov 03, 2013; Accepted: Dec 11, 2013

In FVL, at amino acid residue 506, arginine is substituted by glutamine. Due to this substitution factor V becomes resistant to degradation by activated protein C, increasing the risk of venous thromboembolis. In factor II, at position 20210 of the 3´ untranslated region (UTR) of the factor II gene, a G to A transition has been found to be associated with increased prothrombin levels and increased risk for venous thrombosis. The homozygous state for the C to T transition at position 677 and A to C transition at position 1298 of MTHFR gene is associated with hyperhomocysteinaemia, which predisposes the carrier to thrombosis (4).

Plasminogen activator inhibitor-1 (PAI-1) is a glycoprotein with a molecular weight of approximately 50 kDa and a member of the serine protease inhibitor super-family. PAI-1 is a rapid and specific inhibitor of both tissue-type plasminogen activator and urokinase, and is considered as the primary regulator of plasminogen activation in vivo. Abnormally high concentrations of PAI-1 in blood observed in atherothrombotic and other diseases seem to result from changes in the rate of PAI-1 gene expression in tissues rather than from release of stored PAI-1 in the cells. The 4G/5G polymorphism in the PAI-1 gene promoter seems to be one of DNA sequence variation, which has functional importance in regulating expression of the PAI-1 gene. This polymorphism, located 675 base pairs upstream of the PAI-1 gene transcription start site, is characterized by a single guanosine deletion/insertion, resulting in two alleles containing either 4 or 5 consecutive guanosines.

Our ability to detect genetic abnormalities responsible for thrombotic propensity has improved in recent years. However the evidence for the role of inherited thrombophilias such as factor V Leiden, prothrombin G20210A mutation, the methylenetetrahydrofolate reductase (C677T, A1298T) mutation, and the plasminogen activator inhibitor is less clear.

As it is conceivable that thrombophilias were diagnosed in these series due to thromboembolic phenomena, we considered it necessary to assess the prevalence of thrombophilias in patients with recurrent miscarriages and no previous thrombophilic phenomena. In the present study, we assessed the prevalence of the above thrombophilia markers in patients with recurrent pregnancy losses or first and second trimester abortions, infertility, and failed in vitro fertilization (IVF).

Materials and Methods

In this study, over a three years period (2011-2014), 468 cases were attended by specialists in the Genetic Counseling Services in the Pardis Clinical and Genetics Laboratory (PCGL) or other facilities.

Genomic DNA was extracted from peripheral blood samples by the salting out method (5). Samples with optical density (OD) 260nm/280 ratios of 1.5 – 1.8 and final concentrations of 5 ng/µl were used for amplifications.

The G1691A polymorphism in factor V Leiden was detected by PCR amplification of a 206 bp fragment and MnlI digestion, as previously described (6). The C677T and A1298T substitutions in the MTHFR gene were identified using HinfI and MboII cleavage of 494 and 237bp PCR products, respectively (7). For identification of the G20210A substitution in the factor II gene, a 345bp fragment from the 3´ UTR was amplified by PCR using the same primers as described (8) and digested with HindIII. For PAI, Amplification Refractory Mutation System (ARMS-PCR) was used to detect the 4G/5G polymorphism.

Statistical analysis was performed using SPSS version 16. The genotype distributions of each mutation and their frequencies were compared between patients.

Results

Two hundred sixty nine patients (57.48%) were diagnosed with at least one thrombophilia marker, whereas 199 patients (42.52%) had no thrombophilia markers and were normal. The prevalence of the thrombophilias assessed is shown in Table 1.

Table 1. Frequency of thrombophilia markers in subjects.

Thrombophilia Marker	*Frequency*	
	Normal	**Abnormal**
MTHFR	29	244
Factor II	157	4
Factor V	12	4
PAI	1	17
Total	199 (42.52%)	269 (57.48%)

Figure 1 shows some of amplified and digested products for MTHFR, Factor II, Factor V, and PAI following agarose gel electrophoresis.

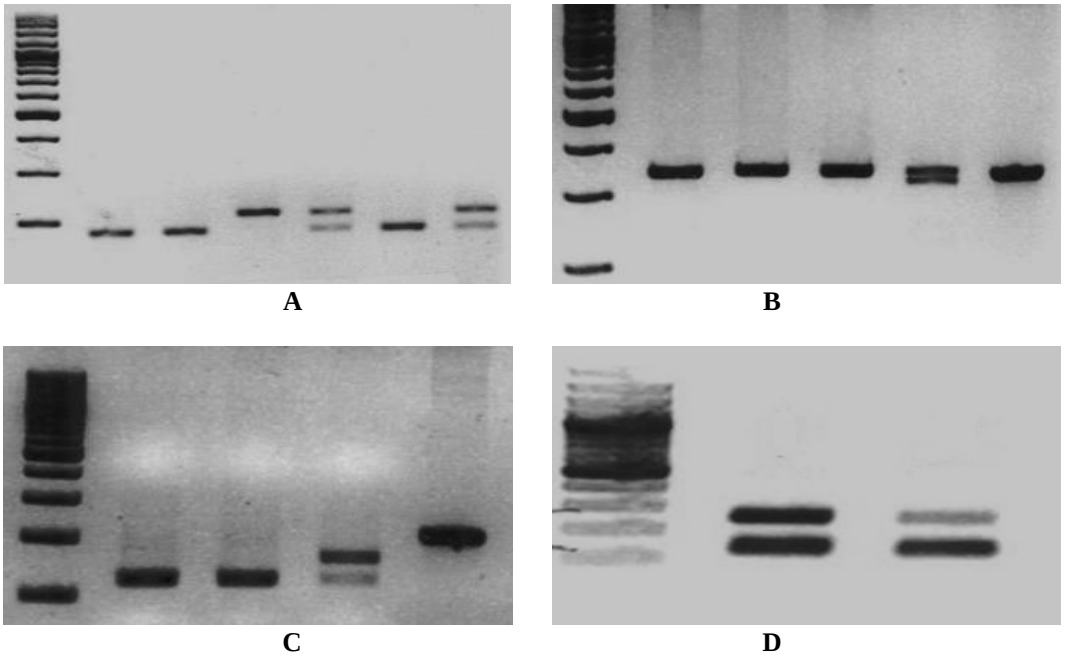

Fig. 1. Agarose gel electrophoresis of samples from thrombophilia patients; (A) Analysis of the A1298C polymorphism in the MTHFR gene. The samples were PCR-amplified and digested with MboII. Samples in lanes 1, 2, and 5 came were isolated from normal subjects. Samples in lanes 4 and 6 were from subjects who were heterozygous, and the sample in lane 3 came from a subject who was homozygous for the A1298C polymorphism. (B) Analysis of the G20210A polymorphism in Factor II. The samples were PCR-amplified and digested with HindIII.: Lanes 4 is heterozygous; 1, 2 and 3 are normal; and 5 is uncut. Lanes 1, 2, and 3 were isolated from normal subjects. Lane 4 was isolated from a subject who was heterozygous and lane 5 is undigested. (C) Analysis of the G1691A polymorphism in Factor V Leiden. The samples were PCR-amplified and digested with Mnl1: The samples in lanes 1 and 2 were islated from normal subjects. The sample in lane 3 was from a heterozygous subject, and lane 4 is undigested. (D) Analysis of PAI polymorphisms using ARMS-PCR: The samples in lanes 1 and 2were isolated from subjects with the 4G and 5G polymorphisms, respectively.

MTHFR

The genotype distribution of the MTHFR mutations in subjects and their case presentations are shown in Table 2. Of 273 subjects analyzed, 29 (10.62%) were normal for MTHFR.

The heterozygous and homozygous C677T polymorphisms were found in 18.32% (50/273) and 9.52% (26/273) of subjects, respectively. The heterozygous and homozygous A1298C polymorphisms were found in 20.88% (57/273) and 12.45 % (34/273), respectively. The heterozygous C677T and A1298C mutations were found in 28.21% of subjects (77/273).

Factor II

The genotype distribution of the G20210A polymorphism in subjects and the genotype distribution based on RSA, infertility, and failed IVF are shown in Table 3. The frequency of the heterozygous G20210A mutation was 1.91% (4/209) and 98.09% (205/209) had normal genotypes.

Factor V

The genotype distribution of the G1691A mutation in subjects and the genotype distribution based on RSA and infertility shown in Table 4. The heterozygous and homozygous G1691A mutation was found in 5.0 % (2/40) and normal individuals were 90.0% (36/40).

Table 2. Distribution and case presentations for subjects with MTHFR mutations

Mutation	*Presenting*	*Frequency*	*Percentage %*
Heterozygous C677T	RSA	47	18.32
	Infertility	2	
	Failed IVF	1	
	Total	50	
Homozygous C677T	RSA	25	9.52
	Infertility	1	
	Failed IVF	0	
	Total	26	
Heterozygous A1298C	RSA	51	20.88
	Infertility	5	
	Failed IVF	1	
	Total	57	
Homozygous A1298C	RSA	30	12.45
	Infertility	3	
	Failed IVF	1	
	Total	34	
Heterozygous C677T and A1298C	RSA	72	28.21
	Infertility	4	
	Failed IVF	1	
	Total	77	
Normal	RSA	23	10.62
	Infertility	4	
	Failed IVF	2	
	Total	29	
Total		**273**	**100**

Table 3. Distribution and case presentations for subjects with factor II (G20210A) mutations.

Mutation	*Presenting*		*Frequency*	*Percentage %*
Heterozygous G20210A	RSA		4	1.91
	Total		4	
Normal	RSA		134	75.60
	Infertility		19	
	Failed IVF		5	
	Total		158	
Normal for factor II, but with MTHFR polymorphisms	Heterozygous C677T		8	22.49
	Homozygous C677T	RSA	5	
	Heterozygous A1298C		17	
	Homozygous A1298C	RSA	4	
		Infertility	2	
	Heterozygous C677T and A1298C	RSA	11	
	Total		47	
Total			**209**	**100**

PAI

The genotype distribution of the PAI polymorphism in subjects and the genotype distribution based on RSA and infertility shown in Table5. The frequency of the heterozygous 4G/5G mutation was 96.77% (30/31) and the frequency of homozygous 5G/5G mutation was 3.23% (1/31).

Table 4. Distribution and case presentations for subjects with Factor V (G1691A) mutations

Mutation	*Presenting*		*Frequency*	*Percentage %*
Homozygous	RSA		1	5.0
	Infertility		1	
	Total		2	
Heterozygous	RSA		1	5.0
	Infertility		1	
	Total		2	
Normal	RSA		12	30.0
	Total		12	
Normal for Factor II and Factor V	RSA		6	42.5
	Infertility		1	
	Total		7	
Normal for Factor V, but with MTHFR polymorphisms	Heterozygous C677T	RSA	3	12.9
	Homozygous C677T		3	
	Heterozygous A1298C		5	
	Homozygous A1298C		1	
	Heterozygous C677T and A1298C		5	
	Total		17	
Total			**40**	**100**

Table 5. Distribution and case presentations for subjects with PAI mutations.

Mutation	*Presenting*	*Frequency*	*ercentage %*
Heterozygous 4G/5G	RSA	15	51.62
	Infertility	1	
	Total	16	
Homozygous 5G/5G	RSA	1	3.23
Homozygous 4G/4G Normal	-	0	0
	-	0	
Hetero 4G/5G with Factor II	RSA	4	19.35
	Mutation analysis	1	
	Embolism	1	
	Total	6	
Hetero 4G/5G with MTHFR	Heterozygous C677T and A1298C	1	12.9
	Heterozygous A1298C	1	
	Normal	2	
	Total	4	
Hetero 4G/5G with Factor V	RSA	4	12.9
	Total	4	
Total		**31**	**100**

Discussion

Several studies have reported that factor V Leiden, which is responsible for more than 75% of inherited activated protein C resistance, is a common thrombotic risk factor affiliated with RPL (10-12).Other studies have reported an association between factor V and late pregnancy loss (13). Goodman CS et al. reportedan association between 4G/5G genotype of PAI-1 and recurrent pregnancy loss (14). The relationship of methylenetetrahydrofolate reductase (MTHFR) gene C677T, factor V (FV) gene G1691A and prothrombin (PT) gene G20210A polymorphisms to unexplained recurrent early spontaneous abortion were examined by Xu et al. They showed that the genetic polymorphisms of MTHFR C677T are associated with spontaneous abortion. These results supported those of previous studies. (15-18). It was reported that there is no increased prevalence of FVL, FII, and MTHFR in recurrent early pregnancy loss (19). The presumed relationship between thrombophilia and recurrent pregnancy loss has become sufficient to allow the presence of thrombophilias to be an indication for treatment with anticoagulant drugs (20).

Acknowledgements

This project was financially supported by Pardis clinical and genetic laboratory. We would like to thank referral cases of PCGL for contributing in this project.

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