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ORIGINAL ARTICLE

Factor V Leiden, MTHFR C677T and Prothrombin Gene Mutation G20210A in Iranian Patients with Venous Thrombosis

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ABSTRACT

Background: Factor V Leiden, Prothrombin gene (G20210A) and MTHFR (C677T) polymorphism are the main biomarkers for evaluation of tendency for venous thromboembolism. We aimed to investigate the frequency of mutations in factor V Leiden, Prothrombin G20210A and MTHFR C677T and identify the genetic status for these mutations in patients with venous thrombosis.
Methods: This study was carried out in 312 patients with venous thrombosis who were referred to "Thrombosis Clinical center", Imam Khomeini Hospital, Tehran, Iran and "Sarvar Clinic", Mashhad, Iran. Identification of gene mutations was performed using PCR-restriction fragment length polymorphism (RFLP)-based method.
Results: The prevalence of Factor V Leiden mutation was 35.8%, while 8.9% of them were homozygous for AA allele and 26.9% had the GA allele in heterozygous state. The prevalence of MTHFR (C677T) mutation was 17.9% of which 7.1% had the TT mutant allele in homozygous and 10.8% had CT allele in heterozygous state. The prevalence of mutation in prothrombin gene G20210A was 8.9% with all cases heterozygous for GA mutant allele.
Conclusion: In our study from two referral centers for thrombotic disorders, the prevalence of mutations in gene encoding factor V Leiden was higher than Prothrombin 20210A and MTHFR C677T polymorphisms. Therefore, assay for factor V Leiden mutation has the first priority in the evaluation of patients with hereditary thrombophilia in these geographical regions.

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Introduction

Venous thromboembolism (VTE), manifesting as deep vein thrombosis (DVT) and pulmonary embolism (PE) is associated with considerable morbidity and mortality.[1-4] According to the literature, positive family history for VTE increases the likelihood of having an inherited thrombophilic defect.[5-7] Risk factors for venous thrombosis are classified into two main categories as genetic and acquired.[8,9] The former includes quantitative or qualitative abnormalities of protein C, protein S and anti-thrombin III.[10,11] There are also other hereditary thrombophilic conditions including mutations in Factor V (Factor V Leiden), prothrombin G20210A and MTHFR enzyme (C677T).[12,13] Among genetic risk factors, Factor V Leiden is the most common with a prevalence of 20%-25% in patients with VTE and 50% in those with "hereditary thrombophilia".[11] Prothrombin gene mutation G20210A is the second most prevalent prothrombotic

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polymorphism. As a result of this mutation, Prothrombin level rises by 150%, which significantly increases thrombin production.[14] Methylene tetrahydrofolate reductase (MTHFR) mutation decreases enzyme activity by 50% and increases blood homocysteine level, predisposing to thromboembolic events.[15-17]

We aimed to assess the frequency of Factor V Leiden, Prothrombin gene mutation (G20210A) and MTHFR (C677T) in patients with VTE.

Materials and Methods

In this cross-sectional study, patients with VTE referring to "Thrombosis Clinical center", Imam Khomeini Hospital, Tehran, Iran or "Sarvar Clinic", Mashhad, Iran during 2014-2017 were studied. The vascular thrombotic events were confirmed by Doppler Ultrasound examination. Patients with Systemic lupus erythematous or those with a history of warfarin or heparin consumption were excluded from the study.

The protocol of the study was approved by the Ethics Committee of the "Thrombosis Research Center" and informed consent was obtained from each participant.

Anticoagulated blood was collected from all the participants. Genomic DNA was isolated using SinaClone DNA extraction kit. Primers with the sequences mentioned in Table 1 were purchased from Invitrogen (USA). RFLP technique was used to determine the presence of the mutations and also to characterize whether the mutation was heterozygous or homozygous. Hinfl, Mnl1 and HindIII enzymes (Invitrogen, USA) were used for assessment of mutations in MTHFR (C677T), Factor V Leiden and Prothrombin G20210A, respectively. The PCR products were run on agarose gel electrophoresis (2%). Table 1 shows the primers and digestive enzymes for each individual factor.

Results

312 participants including 146 men and 166 women were analyzed. The age range was 5-50 years old (28.9±10.76 years). RFLP was carried out not only to detect the mutation, but also to determine hetero- or homozygosity state of the individuals for the three target genes. Prevalence of Factor V Leiden mutation was 35.8% (112 patients), where 8.9% (28 patients) and 26.9% (84 patients) had the AA (homozygote individuals) and GA mutant allele (heterozygote individuals), respectively. The prevalence of MTHFR (C677T) mutation was 17.9% (56 patients) of which 7.1% (22 patients) had the TT mutant allele (homozygote individuals) and 10.8 % (34 patients) had CT allele (heterozygote individuals). Meanwhile, the prevalence of prothrombin G20210A mutation was 8.9% (28 patients), where all had GA mutant allele and were heterozygotes. The results are listed in Table 2. The PCR- RFLP results are shown in Figures 1-3.

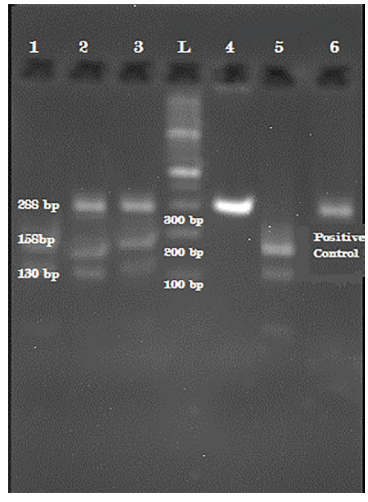

Figure 1: RFLP for factor V leiden by MnlI enzyme
Agarose gel electrophoresis of PCR products for identification of Factor V Leiden:
Wells number 1 and 5: patients without the mutation of Factor V Leiden. (158-130 bp)
Wells number 2 and 3: heterozygous patients for Factor V Leiden. (288-158-130 bp)
Well number 4: homozygous patient for the Factor V Leiden. (288bp)
Well number 6: Positive Control for Factor V Lieden mutation (Homozygous) (288bp)
L: DNA lader (100 bp)

Table 1: Genes, Primer sequences and amplified fragment length for mutations of MTHFR (C677T), Factor V Leiden and Prothrombin G20210A

Gene name	Primer sequence		Amplified Fragment Length
MTHFR(C677T)	For	5′TGAAGGAGAAGGTGTCTGCGGGA3′	198 bp
	Rev	5′AGGACGGTGCGGTGAGAGAGTG3′	
F V Leiden	For	5′GGAACAACACCATGATCAGACCA3′	288 bp
	Rev	5′TAGCCAGGAGACCTAACATGTTC3′	
Prothrombin G20210A	For	5′TCTAGAAACAGTTGCCTGGC3′	345 bp
	Rev	5′ATAGCACTGGGAGCATTGAAGC3′	

Table 2: Prevalence of factor Five Leiden, MTHFR (C677T) and Prothrombin G20210A mutations

Mutation	The Overall Prevalence	Heterozygote & Homozygote status	
Factor Five Leiden	112(35.8%)	Heterozygote	84(26.9%)
		Homozygote	28(8.9%)
MTHFR (C677T)	56(17.9%)	Heterozygote	34(10.8%)
		Homozygote	22(7.1%)
Prothrombin G20210A	28(8.9%)	Heterozygote	28(8.9%)
		Homozygote	0

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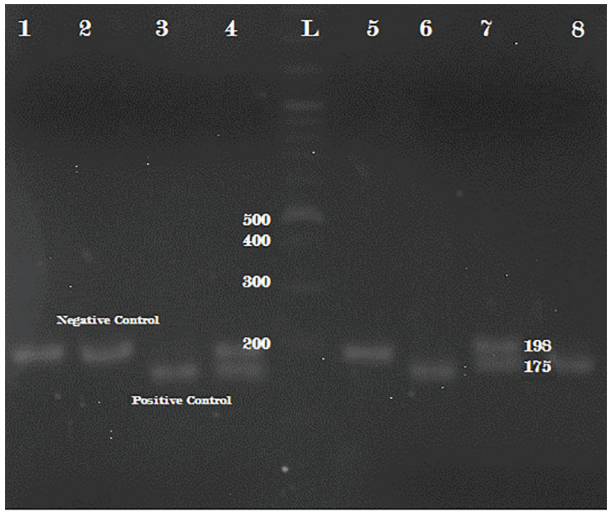

Figure 2: RFLP for MTHFR (C677T) with Hinf1 enzyme
Agarose gel electrophoresis of PCR products for identification of mutation of MTHFR (C677T):
Wells number 1 and 5: Patients without the mutation of MTHFR (C677T) (198 bp)
Well number 2: Negative Control (198 bp)
Well number 3: Positive Control for MTHFR (C677T) (175bp)
Wells number 4 and 7: Heterozygous patient for MTHFR (C677T) (198bp - 175bp)
Wells number 6 and 8: Homozygous patient for MTHFR (C677T) (175 bp)
L: DNA lader (100 bp)

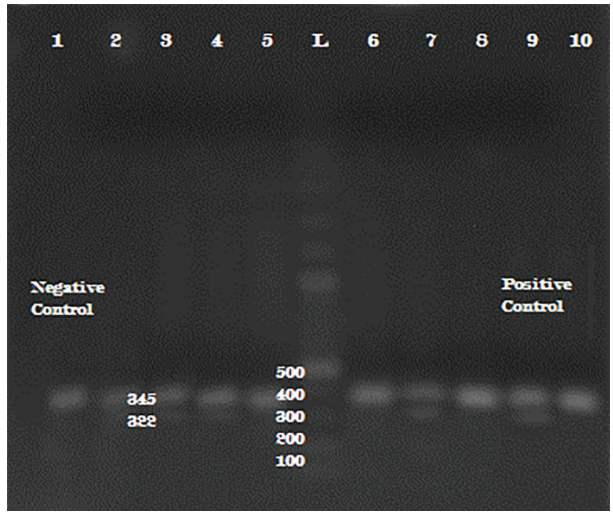

Figure 3: RFLP for Prothrombin (G20210A) with HindIII enzyme
Agarose gel electrophoresis of PCR products for identification of mutation of Prothrombin (G20210A):
Well number 1: Negative Control (345 bp)
Wells number 2 and 3 and 4 and 7: Heterozygous patients for Prothrombin (G20210A) (345bp-322bp)
Wells number 5 and 6 and 8 and 10: Patients without the mutation of Prothrombin (G20210A) (345bp)
Well number 9: Positive Control for Prothrombin (G20210A) (345bp-322bp)
L: DNA lader (100 bp)

Discussion

Factor V Leiden mutation is the most frequent hereditary risk factor associated with venous thrombosis. This mutation has the highest prevalence (15%) in european population with thrombosis, while it occurs in almost 5 percent of white Americans and Canadians.[18,19] In Asia, the prevalence of this mutation was reported to be 1.9% and 2.5% in healthy population of northern India and Saudi Arabia, respectively.[20]

Our study showed that the prevalence of Factor V Leiden was 35.8% among 312 patients with VTEs. Our study was compatible with the existing statistics in terms of the frequency of Factor V Leiden particularly for thrombotic events in early ages (mean: 28.9±10.76 years). In the study conducted on Lebanese patients with thrombosis, the prevalence of Factor V Leiden was 56.9% and 43.1% in patients younger and older than 50 years old, respectively.[21] It means that the prevalence of Factor V Leiden is higher in younger patients with thrombotic events. In a case-control study, the prevalence of mutations in 198 patients with DVT was 52% for Factor V Leiden, 19.2% for prothrombin G20210A and 20.7% for MTHFR C677T mutations.[22] The prevalence of Factor V Leiden was remarkably higher in a group of patients with arterial thrombosis in comparison with control; however, the two other mutations (MTHFR C677T and prothrombin G20210A) were not significantly more common.[23] The prevalence of factor V Leiden mutation in the normal population in western Iran was reported to be around 2.3%.[22] We realized that the severity of symptoms such as pain and redness was associated with homozygosity for mutation of Factor V Leiden. Factor V Leiden mutation causes the deletion of cleavage site of "activated protein C" (APC). As a result, Factor V Leiden would not be inactivated, remains activated in the prothrombinase complex and consequently, more thrombin will be produced. Therefore, it increases the risk of thrombosis. Factor V Leiden also loses its property as a cofactor to inactivate factor VIII. Therefore, it increases the activity of factor VIII as a cofactor in intrinsic tenase complex leading to more thrombin production.[14] Zappacosta et al. assessed the prevalence of MTHFR C677T mutation in Italian newborns. They reported the prevalence of homozygous and heterozygous mutation for MTHFR (TT genotype) to be 25% and 50.5%, respectively.[24] Moreover, Marco Cattaneo et al. reported the prevalence of homozygous MTHFR mutation in DVT patients to be 20.8% while its frequency in a group of 134 individuals representing healthy population was 22.7%.[25] Our results showed that the prevalence of MTHFR (C677T) mutation among our subjects was 17.9% (56 patients) of which 7.1% (22 patients) had the TT mutant allele in homozygous state and 10.8 % (34 patients) had CT allele in heterozygous state. According to our study, the prevalence of MTHFR C677T homozygous mutation was higher in women. However, it does not apply to the heterozygous state of the mutation. Our study also showed that women with MTHFR mutation have a higher risk of thrombotic events during pregnancy such as preeclampsia and consecutive abortions.[25,26]

The prevalence of prothrombin mutation is geographically widespread. Prevalence of its heterozygous mutation is reported to be 0.7-6.5% in Caucasian populations and the highest prevalence has been reported in Spain.[14]

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In our study, the prevalence of prothrombin G20210A mutation was 8.9% (28 patients) and none of them were homozygotes.

In addition to prothrombin G20210A, VTE risk will be increased 2 or 3 folds when it is accompanied by additional inherited or acquired risk factors.[27] It is important to mention that assessing these mutations is valuable in terms of prevention, particularly when they are accompanied by other acquired risk factors such as consumption of OCP pills, pregnancy, immobilization or surgery which are associated with increased risk of thrombosis.[28]

Conclusion

In our study from two referral centers for thrombotic disorders, the frequency of mutations in gene encoding factor V Leiden was higher than Prothrombin 20210A and MTHFR C677T polymorphisms. Therefore, assay for factor V Leiden mutation has the first priority in the evaluation of patients with hereditary thrombophilia in these geographical regions.

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Conflict of Interest: None declared.

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