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Factor V G1691A and prothrombin G20210A gene polymorphisms among Iranian patients with cerebral venous thrombosis

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Abstract

Objective: Cerebral venous thrombosis (CVT) is an important cause of stroke, especially in young adults, that has many predisposing factors. G20210A mutation in prothrombin gene (Factor II) and G1691A mutation in Factor V Leiden (FVL) are two common hereditary causes of CVT. This study aimed to study the rate of these mutations in patients with CVT from Fars Province in southern Iran. *Methods:* In a case-control study, 57 case patients with definite diagnosis of CVT, confirmed clinically and by MRI and MRV, and 50 sex and age matched healthy controls, with no family history of thrombosis, were enrolled from March 2008 to March 2010. G1691A mutation of FVL and G20210A mutation of factor II were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. *Results:* Mutation in G20210A of factor II was found in 3.6% of patients and 4% of the controls (P=1). For FVL mutation, 7% of the patients carried the mutant allele while this mutation was not found in the controls (P=0.12). Two and 4 patients were heterozygous for prothrombin G20210A and FVL G1691A mutations, respectively.
Conclusions: It seems that G20210A mutation in Factor II and G1691A mutation in FVL are not responsible for CVT in the southern Iran population with predominant Fars ethnicity.

INTRODUCTION

Cerebral venous thrombosis (CVT) is an uncommon cause of cerebral infarction especially in young adults that usually causes hemorrhagic lesion. The prevalence of CVT is 5 people per million and it accounts for 0.5% of all strokes.[1] The main causes of CVT are usually multifactorial and aquiared and genetic thrombophilia, pregnancy and puerperium, infections, hematologic disorder, and taking drugs such as oral contraceptive pills. However, it can occur with no definite cause in about 15% of cases.[1] The combination of non-invasive methods of magnetic resonance imaging (MRI) and magnetic resonance venography (MRV) are gold standard for diagnosis of CVT.[1,2] Increased awareness of the diagnosis and more effective treatment improve the prognosis in such a way that the outcome of CVT is favourable, more than 80% of the patients have a good neurologic outcome and mortality is below 10%.[1,3-5]

Among hereditary causes of venous thrombosis, G20210A polymorphism in prothrombin gene (Factor II) and Factor V Leiden (FVL) G1691A polymorphism are the two most common. Spontaneous or secondary venous thrombotic event occurs in younger and older carriers of these polymorphisms.[6–10] However, the frequency of these factors may vary in different populations.

We designed this study to investigate the rate of G20210A polymorphism of Factor II and G1691A polymorphism of FVL in patients with CVT in Fars province, southern Iran, and to compare it with normal population.

METHODS

Patients and controls

Fifty-seven patients were enrolled in this study from March 2008 to March 2010. All the cases were admitted to the Namazee Hospital, affiliated to Shiraz University of Medical Sciences. CVT was diagnosed based on the clinical presentations and the results of MRI and MRV that were evaluated by a Neurologist. Fifty age and sex matched healthy people from the same geographical area and ethnicity, who had no family history

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of thrombosis were selected as controls. The demographic, clinical and imaging data as well as possible risk factors of CVT of the cases and the demographic data of the controls were recorded by a questionnaire. All patients and controls gave their informed consent in writing and the medical research ethics committee of Shiraz University of Medical Sciences approved the study.

Sample collection

Blood sample (1 mL in EDTA) was collected from each patient. We used Qiagen kit from each blood sample to isolate DNA. G1691A mutation of factor V and G20210A mutation of factor II were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

FVL mutation detection

G to A substitution at nucleotide 1691 which is located in exon 10 of factor V was examined by PCR-RFLP method. A region with 241 bp of exon 10 was amplified by using following primers: forward primer 5'TCAGGCAGGAACAACACCAT3' and reverse primer 5'GGTTACTTCAAGGACAAAA TACCTGTAAAGCT3'.[11] PCRs were performed in a total volume of 25 µl consisting of 100 ng DNA, 0.5 µl of 10 pM/µl each primer, 1 µl of dNTP mix (10 mM), 1 unit of Taq DNA polymerase, 1 X PCR Buffer and 1.5 mM $MgCl_2$. Reactions were carried out in a thermal cycler (Eppendorf, Hamburg, Germany). Thermal profile consisted of 5 min at 94°C followed by 35 cycles including 94°C for 30 s, 57°C for 30 s, and 72° for 45 s. Samples were maintained at 72°C for further 10 min. PCR amplification was confirmed by gel electrophoresis (2% agarose gel). Then 10 µl of each PCR product was mixed with 10 units of Hind III (Fermantas International Inc.) for 18 hours at 37°C. Digested products were 12% polyacrylamide gel. There was no restriction site for Hind III in the 1691G allele and the fragment of 241 bp was remained undigested, whereas 1691A allele was digested into two fragments of 209 bp and 32 bp. Both patterns were detected when the subjects were heterozygous.

Prothrombin gene (FII G20210A) mutation detection

G to A substitution at nucleotide 20210 which is located in exon 14 of factor II was also examined by PCR-RFLP method. A region with 345 bp of exon 14 was amplified using following primers: forward primer 5'TCTAGAAACAGTTGCCTGGC3' and reverse primer 5'ATAGCACTGGGAGCATTGAAGC3'[12] PCRs were performed in the same condition for factor V. After digestion with Hind III, 20210A allele was digested into two fragments of 322 and 23 bp while 20210G remained undigested (345 bp).

Statistical analysis

The data were analyzed with SPSS software version 15 (SPSS Inc., Chicago, Illinois) and Fisher exact test was used to compare quantitative data.

RESULTS

Of 57 case patients, 38 (66.7%) were females with mean age of 34.0±8.3 SD and 19 (33.3%) were males with mean age of 32.9±9.6 SD. Mean age of total population was 33.7±8.7 SD.

General characteristics of the patients and common clinical features of CVT are shown in Tables 1 and 2, respectively. The main possible predisposing factors of CVT were taking oral contraceptives (OCPs) and peripartum condition in the female group and history of previous deep vein thrombosis (DVT), that was the first associative factor in the male group (Table 1). The most common clinical signs and symptoms were headache, seizure, and impaired consciousness (Table 2). Seven patients (12.28%) out of 57 died. Most of them had developed CVT because of peripartum conditions (Table 2). All of the patients with CVT were treated with intravenous heparin at first few days and then with oral anticoagulant, warfarin for at least 6 months.

In this study, 3.6% of patients had mutation in Factor II G20210A while 4% of the individuals in the control group showed this mutation (P=1). FVL allele was observed in 7% of the patients, but it was not found in the controls (P=0.12). The frequency of mutations is shown in Table 3.

DISCUSSION

The demographic characteristics, common clinical presentations and possible risk factors of the patients in this study was the same as other studies.[2,13–15] The mortality of CVT in this study was 12.3% that is more than what was reported by an international study.[2] However, it is relatively similar to the mortality rate of CVT in two previous studies in the same geographic area.[13,15]

Table 1: Characteristics of patients with CVT.

Parameters	Female (n=38)	Male (n=19)
Age (years)	34.0 ± 8.3	32.9 ± 9.6
OCPs	25 (65.8%)	-
Pregnancy, Post partum	4 (10.5%)	-
History of DVT	-	3 (15.6%)
Anabolic steroid	-	1 (5.3%)
Behcet's disease	-	1 (5.3%)
Spinal cord injury and prolonged bedridden	-	1 (5.3%)
Homocystinuria and Protein C deficiency	-	1 (5.3%)
Hypertension and hyperviscosity	-	1 (5.3%)
Unknown	9 (23.7)	11 (57.9)

Age is expressed as Mean ± SD. OCPs**=** oral contraceptives, DVT**=** deep vein thrombosis**.**

The prevalence of prothrombin G20210A gene mutation varies widely worldwide; it is common in Europe but uncommon in Africa, Asia, and natives of America.[16] It seems that although the prothrombin G20210A and factor V G1691A gene mutations are common thrombophilic polymorphisms in Caucasians, their frequencies are different in other populations. Some studies have shown that the prevalence of factor V leiden mutations in Middle Eastern populations is high.[17,18] Angchaisuksiri *et al*.[19] reported that the frequency of FVL and prothrombin gene mutation in Thai population was lower than other populations. In Iran, Zeinali *et al*.[20] reported allel frequencies of 2.7% and 1.5% for factor V leiden and prothrombin G20210A mutations, respectively, among 161 healthy individuals from Tehran. Rahimi *et al*.[21] studied individuals of Kurdish ethnic background from the Kermanshah Province, Iran, and reported that the prevalence of prothrombin (G20210A) and factor V (G1691A) mutations were 1.6% (95% CI; 0.5 -2.7) and 2.97% (95% CI; 1.3-4.6), respectively. They concluded that these mutations are not rare among populations of western Iran.[21]

Inherited thrombophilia has significant role in pathogenesis of CVT that FVL and prothrombin G20210 A mutations are the main ones. FV-Leiden mutation has shown to be a major inherited risk factor of venous thrombosis, particularly in young adults.[22] In addition, some studies conducted in patients with venous thrombotic event (either peripheral or central) showed that the researchers are interested in investigating prothrombin gene mutation as a potent risk of thrombosis with or without acquired risk factors.[7,23] A meta-analysis that was conducted in patients with CVT showed that the G20210A mutation of Factor II is significantly associated with the risk of CVT (OR 9.27:95% CI:5.85 to 4.67; P<0.0000).[24] The risk of developing CVT is also higher in heterozygous individuals with prothrormbin gene mutation than general population with different degrees of prevalence in the literature.[25]

In women who take oral contraceptives and have concomitant hereditary thrombophilic

Table 2: Frequency of neurological features and mortality in 57 patients with CVT.

	Headache	Papilledema	Seizure	Motor deficit	Impaired consciousness	Death
Female	37 (97.2%)	30 (79%)	10 (26.3%)	9 (23.6%)	11 (30.0%)	7 (12.3%)
Male	18 (94.7%)	10 (53%)	9 (47.0%)	5 (26.3%)	4 (21.0%)	_
Total	55 (96.0%)	40 (70%)	19 (33.0%)	14 (24.5%)	15 (26.3%)	7 (12.3%)

Table 3: Allele and genotype frequency of factor II and FVL genes among 57 CVT patients and 50 matched healthy controls.

	20210A Allele	FVL Polymorphism	1691A Allele	G20210A Polymorphism
Patients	2 (1.75%)	4 (3.5%)	2 (3.6%)	4 (7%)
Controls	2 (2%)	0 (0%)	2 (4%)	0 (0%)

condition, the risk of CVT increases greatly.[7,26] Martinelli *et al*.[7] studied 40 patients with CVT and 80 with DVT of lower extremities and showed higher prevalence of prothrombin G20210 and Factor V G1691 mutations in these patients (OR 10.2:95% CI: 2.3 to 31.0). They also reported that the odd ratio for CVT rose to 149.3 (95% confidence interval, 31 to 711) in women who had taken concomitant oral contraceptive.

There are many studies which have evaluated the relationship between venous thrombosis and common inherited FVL and Factor II mutations as a predisposing factor, but they have reported various result. A study that was performed in Turkish patients with thrombosis showed that the prevalence of factor II and FVL mutations was not significantly different to its prevalence in the general population.[27] Bauduer et al.[28] conducted a case-control study in 103 French Basque patients with thrombotic conditions and showed lower G20210A mutation compared with other reports.[28] Nagaraja et al.[16] studied a large series of patients with puerperal CVT and showed that the prothrombin G20210A variant was not found in south Indian women and was not associated with puerperal CVT.[16]

Rahimi *et al*.[29] found a significant correlation between factor V leiden mutation and CVT in 24 Kurdish CVT patients with odd ratio 9.8, but prothrombin G20210A and methylene tetrahydrofolate reductase (MTHFR C677T) had no significant correlation with CVT. Our findings showed the frequency of the prothrombin G20210A and factor V G1691A gene mutations were not significantly increased in CVT patients who lived in the southern of Iran with predominant Fars ethnicity. We supposed that the difference between our results and the findings reported by Rahimi et al.[29] might be because of different ethnicity and geographical area in the two groups. However, it needs future studies with more cases in various ethnical and geographical areas of Iran to confirm the role of inherited thrombophilia in CVT.

In conclusion, both racial and environmental factors can be considered as risk factors for thrombothic diseases. Our study revealed that G20210A mutation in Factor II and G1691A mutation in FVL are not responsible for CVT in our population. This study sheds new light on the subject and suggests that more detailed future studies may be warranted to further investigate other possible causes of hereditary thrombophilia and genetic defect as predisposing factors of CVT.

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DISCLOSURE

Conflicting interest: None

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