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Distribution of prothrombin G20210A, factor V Leiden, and MTHFR C677T mutations in the middle Black Sea area (Tokat) of Turkey

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Aim: To determine the distribution frequencies of prothrombin (factor II) G20210A, factor V Leiden, and methylenetetrahydrofolate reductase (MTHFR) C677T mutations in Tokat Province and the middle Black Sea area of Turkey.

Materials and methods: The study group consisted of 243 patients who presented to our center with thrombosis and other complaints. Written permission from patients was obtained. DNA was isolated from the blood samples of these patients. Mutation analyses were conducted using the real-time polymerase chain reaction (RT-PCR) method. The data were subjected to statistical analyses.

Results: According to the results of the study, factor V Leiden was detected in 37 patients, prothrombin G20210A was detected in 5 patients, and MTHFR C677T was detected in 146 patients. On the other hand, 79 patients carried none of these mutations.

Conclusion: Factor V Leiden mutation was detected at a rate of 15.2%, which is much higher than the average in Turkey. The G20210A mutation rate in the prothrombin gene was determined as 2.1%. The MTHFR C677T mutation incidence was quite high (60%). The homozygous mutation rate was higher in diagnoses involving thrombosis, while the heterozygous mutation rate was higher in other diagnoses.

Key words: Thrombosis, factor V Leiden, prothrombin, G20210A, MTHFR, C677T

Introduction

Genetic and environmental factors affect the timing, severity, and recurrence of thrombosis formation. Thrombosis is an anomalous mass made of blood elements within the veins. It forms as a result of disruption of delicate balances among procoagulant, anticoagulant, and fibrinolytic factors. Endothelium damage and thrombocyte functions are important in arterial thrombosis, while stasis and clotting fibrinolytic system disorders become important in venous thrombosis (1-4). Factor V Leiden G1691A, prothrombin (factor II) G20210A, and methylenetetrahydrofolate reductase (MTHFR) C677T mutations are 3 common molecular biomarkers used in the evaluation of venous thrombosis predisposition (5-8).

Hereditary activated protein C resistance forms as a result of single point mutations at nucleotide position 1691 in the factor V gene. A change from A to G in this mutation causes a change from arginine to glutamine at amino acid residue 506 (9). Mutant factor V is known as factor V Leiden and is referred to as FVR506Q. The inactivation rate of factor V Leiden is slower than that of wild-type factor V. When incubated with active protein C, wild-type factor V becomes completely inactive in 10 min while factor

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V Leiden stays at 45% of its initial activity, resulting in hypercoagulability (10). Factor V Leiden could represent a sex-related survival advantage mutation by conferring on female carriers a lower risk of blood loss and profuse hemorrhage in association with delivery, resulting in a reduction of the maternal fatality rate (11,12).

Prothrombin or factor II is a vitamin K-dependent zymogen that is produced by the liver and is crucial in the conversion of fibrinogen into fibrin (13). Prothrombin G20210A mutation was discovered in 1996 during the search for candidate genes in families with thrombotic episodes. Poort et al. (14) found a single nucleotide change (G→A) at position 20210 in the 3'-untranslated region in 18% of the cases. This rate was 1 in 100 in a healthy control group. A recent study reported that the risk of a large artery disease event is increased in females, hypertensive subjects, cigarette smokers, or alcohol drinkers who are carriers of the prothrombin G20210A mutation (15).

C677T mutation occurs due to a thymidine-cytosine change at nucleotide position 677 of the MTHFR gene. Frequency of this mutation varies in different societies. As a result of C677T mutation in the MTHFR gene, alanine amino acid in the catalytic domain region of the MTHFR enzyme is converted to valine. Such a conversion makes the enzyme thermolabile, reducing in vitro MTHFR activities by 70% and 35% in homozygous and heterozygous conditions, respectively. Plasma homocysteine levels increase mildly in individuals who are homozygous for C677T mutation, which becomes remarkable in folate deficiency periods. C677T mutation in the MTHFR gene may lead to premature vascular disease or thrombosis (16). C677T mutation has been linked to increased risk for ischemic stroke in a recent population study (17).

Materials and methods

In the present study, 243 patients who presented to the Gaziosmanpaşa University Research and Training Hospital with thrombosis and other complaints were studied. Factor V Leiden, prothrombin G20210A, and MTHFR C677T mutations were studied in these patients. The patients were informed of the purpose of the practice applied. An approval form was signed by the patients, providing their written consent. From each patient, 4 mL of venous blood sample was collected into tubes containing ethylenediaminetetraacetic acid (EDTA). DNA was isolated from whole blood samples using the High Pure PCR Template Preparation Kit (Roche Applied Science, Penzberg, Germany). Mutation screening processes were performed using commercial kits: a factor V Leiden kit (Roche Molecular Systems, Branchburg, New Jersey, USA), a factor II (prothrombin) G20210A kit (Roche Molecular Systems), and the LightMix® MTHFR C677T kit (Tib Molbiol GmbH, Berlin, Germany). Mutation screening was performed based on the real-time polymerase chain reaction (RT-PCR) method using a LightCycler 480 RT-PCR thermal cycler (Roche Applied Science). The increase in the fluorescence of the product obtained through DNA amplification using RT-PCR was monitored in real time. Based on a detailed melting curve analysis of PCR products, gene polymorphism was detected. The factor V Leiden homozygous wild-type genotype melting temperature was 65 °C and the homozygous mutant genotype melting temperature was 57 °C. The prothrombin G20210A homozygous wild-type genotype melting temperature was 59 °C and the homozygous mutant genotype melting temperature was 49 °C. The MTHFR C677T homozygous wild-type genotype melting temperature was 63 °C and the homozygous mutant genotype melting temperature was 54.5 °C.

Results

Factor V Leiden, prothrombin G20210A, and MTHFR C677T mutations were studied in 243 patients. Based on the results, 37 patients had factor V Leiden (3 homozygous), 5 patients had prothrombin G20210A (0 homozygous), and 146 patients had MTHFR C677T (31 homozygous) mutations. On the other hand, 79 patients carried none of these 3 mutations. The results are given in the Table.

Discussion

Thrombosis occurs as a result of disruptions in the blood clotting system. Therefore, it is crucial to evaluate hereditary and acquired thrombotic risk factors, which may lead to prothrombotic pathological changes in normal coagulation systems (18).

Table. Distributions of factor V Leiden, prothrombin (Factor II) G20210A, and MTHFR C677T mutations.

		General rates of females and males (243)		Rates of thrombosis-related diagnoses (53)		Rates of other diagnoses (190)		General rates (243)
		Female	Male	Female	Male	Female	Male	
Factor V Leiden	Wild-type	116 (86.5%)	90 (82.5%)	28 (52.8%)	17 (32.1%)	88 (46.3%)	73 (38.4%)	37 (15.2%)
	Heterozygous	18 (13.4%)	16 (14.6%)	3 (5.6%)	3 (5.6%)	15 (7.8%)	13 (6.8%)	
	Homozygous	0	3 (2.7%)	0	2 (3.7%)	0	1 (0.5%)	
Prothrombin (factor II) G20210A	Wild-type	132 (98.5%)	106 (97.25%)	30 (56.6%)	22 (41.5%)	102 (53.6%)	84 (44.2%)	5 (2.1%)
	Heterozygous	2 (1.5%)	3 (2.75%)	1 (1.8%)	0	1 (0.5%)	3 (1.57%)	
	Homozygous	0	0	0	0	0	0	
MTHFR C677T	Wild-type	50 (37.3%)	47 (43.1%)	13 (24.5%)	10 (18.8%)	37 (19.5%)	37 (19.5%)	146 (60.08%)
	Heterozygous	64 (47.7%)	51 (46.7%)	12 (22.6%)	8 (15.1%)	52 (27.3%)	43 (22.6%)	
	Homozygous	20 (15%)	11 (10%)	6 (11.3%)	4 (7.5%)	14 (7.36%)	7 (3.68%)	

Factor V Leiden mutation is the most frequent inherited prothrombotic risk factor (12). When factor V Leiden mutation coexisted with protein C-S deficiency or antithrombin III deficiency, the thrombosis risk increased by several-fold (19,20). Its prevalence is higher in northern European countries, with a peak of 8% to 15% in Sweden, and lower in southern Europe, where it ranges between 2% and 4%. In the United States, prevalence between 5% and 8% was reported (21). Its prevalence in Turkey is as high as 10% (22). In the present study conducted in Tokat Province and the middle Black Sea area, even higher frequencies than the general average in Turkey were found for the factor V Leiden mutation. The mutation rate was 13.4% (all heterozygous) in females, 17.4% (14.6% heterozygous and 2.7% homozygous) in males, and 15.2% in general. Based on the diagnoses, the homozygous mutation rate in males was higher than in females.

Heterozygous forms of factor V Leiden and prothrombin G20210A are responsible only for a moderate increase of the risk of thrombosis (23). They-They et al. (15) showed that there is a significant relationship between the prothrombin G20210A genotype and the large artery disease subtype event. Rosendaal et al. (24) carried out a cohort study of 5527 cases (11 centers from 9 countries) and investigated the geographical distribution of the G20210A mutation. The investigators found 111 heterozygous carriers but no homozygous individuals. Prevalence varied from 0.7% to 4.0% (24). Similar to factor V Leiden, prevalence of the G20210A mutation outside of Caucasian people was found to be very low. In general, factor V Leiden is more prevalent in northern Europe, while prothrombin mutation is more common in southern Europe, especially in the Mediterranean region (13,24). In the present study, which included 243 patients, the G20210A mutation rate was 2.1% in the general population and was similar to that found by Nguyen (13), which was 1.5% and 2.75% in males and females, respectively. Based on the diagnoses made, the rates seem to be similar.

MTHFR mutations were investigated for their possible connection with many disease etiologies and some strong relationships were reported (25). For these diseases, which generally seem to have multifactorial etiology, the weight of the genetic etiology could vary considerably among different populations (26). This fact is apparent even within small geographic areas. Cases with slight MTHFR deficiencies are quite common in the general population. This is a risk factor for the occurrence of arterial diseases (27). In individuals carrying this allele in the heterozygous form, plasma homocysteine

levels are moderate. The frequency of heterozygosity and homozygosity in Turkish populations is 47.4% and 9.6%, respectively (28). A high frequency of MTHFR C677T mutations (60%) was observed in the present study. The frequency was 62.6% (47.7% heterozygous and 15% homozygous) in females and 56.8% (46.7% heterozygous and 10% homozygous) in males. The homozygous MTHFR C677T mutation rate in females was higher. Based on the diagnoses made, heterozygous and homozygous mutation rates were higher in females.

In conclusion, factor V Leiden and MTHFR C677T homozygous mutation rates were higher in patients with diagnoses involving thrombosis, while the heterozygous mutation rates were higher in patients with other diagnoses. The factor V Leiden mutation rate was higher in other studies. The general rate of MTHFR C677T and prothrombin G20210A mutations was in agreement with those of other studies. The present study is important because this is the first study of its kind in this region.

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