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Commonness of Factor V Leiden, Prothrombin G20210A and Methylenetetrahydrofolate Reductase C677T Mutations among Patients with Thromboembolism in the Western Black Sea (Zonguldak) Region of Turkey

Muammer Bilici[1]*, Basak Delikanli Corakci[1], Yasin Ozturk[1] and Sehmus Ertop[2]

[1]*Department of Internal Medicine, Bülent Ecevit University, School of Medicine, Turkey*
[2]*Department of Hematology, Bulent Ecevit University, School of Medicine, 67600 Zonguldak, Turkey*

Abstract

Introduction: Venous thromboembolism (VTE) remains a life-threatening disease. Screening for inherited risk factors in selected patients may have an impact on treatment approaches to VTE. In this study, we aimed to assess the frequency of factor V Leiden (FVL), prothrombin G20210A and MTHFR C677T gene mutations among idiopathic VTE patients and healthy population in Zonguldak province and the Western Black Sea Region of Turkey.

Methods: The present study included 157 patients with VTE and 135 healthy individuals. FVL, prothrombin G20210A and MTHFR C677T gene mutations were investigated in patients hospitalized for VTE and healthy subjects.

Results: In agreement with the previously reported publications in Turkey and Europe, the prevalence of FVL, prothrombin G20210A and MTHFR C677T gene mutations were 6.7%, 2.9% and 43.7%, respectively, among healthy population. In VTE patients, it was observed that the rates of FVL, prothrombin G20210A increased significantly as well as the frequency of homozygosity for MTHFR in VTE patients was higher than controls.

Conclusıon: Screening for genetic disorders such as FVL, prothrombin G20210A and MTHFR C677T gene mutations should be evaluated in patients presented with unexplained VTE.

Keywords: Venous thromboembolism; Factor V Leiden; Prothrombin G20210A; MTHFR C677T

Introduction

Although the exact incidence of venous thromboembolism (VTE), consisting of deep vein thrombosis (DVT) and pulmonary embolism (PE), is unknown. In general population, it has been estimated to be 1-2 per 1000 annually and increases with age [1-4]. And also it remains a serious health problem with an unacceptable mortality rate that the 30-day mortality was reported to be 6.4% for all patients with VTE [5]. Overall, idiopathic (unprovoked) VTE comprises 25% to 50% of all cases with first-time VTE. A significant number of people who have a first episode of VTE will have a recurrent event, particularly in the days or weeks following the first episode, then the risk of recurrence declines in time [2,3,6]. The provoked VTE by acquired risk factors has lower risk of recurrence than unprovoked VTE whose recurrence risk for VTE is approximately 5-7% per year [7]. Cushman et al. reported that the first-year incidences of recurrent VTE among patients with VTE and idiopathic VTE were 7.7% and 7.8%, respectively [8]. Previous studies have revealed that the annual risk of recurrence due to thrombophilia is not found to be significantly higher than the others without any thrombophilic defects, among patients with an initial idiopathic VTE [9-11].

Thrombosis does not occur in most people with thrombophilia. The acquired risk factors such as advanced age (>75 years), immobilization, inflammation, trauma, surgery, pregnancy, oral contraception use, obesity (body mass index [BMI] >30 kg/m2), diabetes, hormonal replacement therapy, cancer, lupus anticoagulant, sickle cell anemia and other hemolytic anemias are the important provoking factors for VTE [12,13]. In cases of unprovoked VTE, VTE at young age (<45 years), recurrent VTE, VTE in unusual sites, VTE in the setting of a strong family history of VTE and recurrent pregnancy loss, laboratory testing for hypercoagulable states should be considered to uncover any inherited abnormalities [14]. Although, testing for inherited hypercoagulable states (including antithrombin deficiency, protein C and S deficiency, hyperhomocysteinemia, FVL, prothrombin G20210A and methylene tetrahydrofolate reductase (MTHFR) C677T gene mutations) can uncover an abnormality in more than 60% of patients presenting with a first VTE, it minimally influences VTE management in most of these patients. Thus, laboratory testing for hypercoagulable states is recommended if the results of individual tests will affect the choice of anticoagulant agent, duration and intensity of anticoagulation therapy, therapeutic monitoring, family screening, family planning and choice of concomitant medications [3,7,12,14]. It is obvious that thromboprophylaxis is perceived as being important to patients in at moderate or high risk for VTE [15].

In this study, we aimed to evaluate demographic and clinical features of our patients with idiopathic VTE and assess the frequency of factor V Leiden (FVL), prothrombin G20210A and MTHFR C677T gene mutations among idiopathic VTE patients and healthy population as a screening test.

***Corresponding author:** Bilici M, Department of Internal Medicine, Bülent Ecevit University, School of Medicine, Zonguldak, Turkey, Zip Code: 67600, Tel: +905387220925; E-mail: drmbilici@hotmail.com

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Materials and Methods

Study population

Screening for Factor V Leiden, prothrombin G20210A and MTHFR C677T gene mutations was performed in patients hospitalized for venous thromboembolism (DVT, PE, Cerebral and Abdominal vein thrombosis) and healthy subjects, from October 2010 to May 2017. The patients from neighboring provinces in this region are admitted to our hospital as well. The study population consisted of the 157 consecutive patients with idiopathic VTE and 135 healthy subjects. The patients who have acquired risk factors for VTE such as advanced age (>75 years), malignancy, trauma, immobilization and obesity (BMI>30 kg/m^2) were excluded from the study. The diagnosis of venous thromboembolism was confirmed by Doppler ultrasonography or computed tomography (CT) angiography [16]. The study protocol conforms to the ethical guidelines of the world medical association's declaration of Helsinki as reflected in a priori approval by the Local Ethics Commitee of Bulent Ecevit University, Zonguldak, Turkey. All participants were fully informed about the study protocol and informed consent was obtained from all individuals.

Laboratory tests

From each patient, peripheral venous blood samples were drawn from antecubital vein into tubes containing ethylenediaminetetraacetic acid (EDTA) using standard operational procedure. Hemoglobin (Hgb), Platelet Count (PLT) and Mean Platelet Volume (MPV) were obtained from the automated complete blood count using a Coulter LH 780 Hematology Analyzer (Beckman Coulter Inc. Brea., CA, USA). For detecting Factor V Leiden, prothrombin G20120A and MTHFR C677T gene mutations, following procedures were used: Genomic DNA was extracted from peripheral blood leucocytes (200 µl of total blood) by using a Macherey-Nagel (MN) Nucleospin blood® DNA extraction kit (cat. no. 740.951.250) according to the manufacturer's instructions. Factor V Leiden, prothrombin G20210A and MTHFR C677T gene mutations were detected by Dr. Zeydanli Type I PCR System (Ankara, Turkey) based TaqMan® 5′ nuclease assay method in ABI 7500 (Applied Biosystems, Foster City, CA, USA). PCR reaction was performed according to the manufacturer's instructions. The reactions were started at 95°C for 10 min, followed by 32 cycles of 95°C for 15 s and 60°C for 1 min.

Statistical analyses

Statistical analysis was performed with SPSS (Version 19.0 for Windows, Chicago, Illinois). Descriptive statistics of continuous variables are given as mean, standard deviation, median, minimum, and maximum values. Categorical variables are presented as frequency and percentage. The Shapiro-Wilk test was used as a test of normality. The Mann Whitney U test was used for non-parametric two-group comparisons. Pearson chi-square, Yates chi-squre and Fisher exact chi-square tests were used for group comparisons of categorical variables. For all statistical comparisons a p value of below 0.05 was assumed to be statistically significant.

Results

Demographic characteristics of patients and controls are shown in Table 1. In terms of age and gender, there were no significant differences between the patients with VTE and controls (p>0.05). The distributions of Factor V Leiden, prothrombin, G20210A and MTHFR C677T gene mutations among all participants were given in Figure 1. Factor V Leiden and prothrombin G20210A mutations were found to be significantly higher in patients than controls (p<0.05), whereas there was no significant difference between the groups regarding MTHFR C677T gene mutation (p=0.619). One hundred eighteen of the 157 patients with VTE (75.2%) and 66 of the 135 healthy subjects

	VTE patients n=157 mean ± sd	Controls n=135 mean ± sd	p
Age (year)	41.25±12.86	38.56 ± 7.30	0.215
Gender % (Male/Female)	84 (53.5)/73 (46.5)	62 (46)/73 (54)	0.197
Hgb (g/dL)	13.50 ± 1.52	13.30 ± 0.95	0.277
Plt (×1000/mm^3)	260.95 ± 81.61	251.58 ± 58.29	0.612
FVL (%)	69 (43.9)	9 (6.7)	<0.001
Prothrombin G20210A (%)	19 (12.1)	4 (2.9)	0.004
MTHFR C677T (%)	66 (42)	59 (43.7)	0.619

Abbreviations: VTE: Venous Thromboembolism; Plt: Platelet Count; MPV: Mean Platelet Volume; FVL: Factor V Leiden; MTHFR: Methylene Tetrahydrofolate Reductase; sd: Standard Deviation.

Table 1: Characteristics of 137 consecutive patients with VTE and 135 controls.

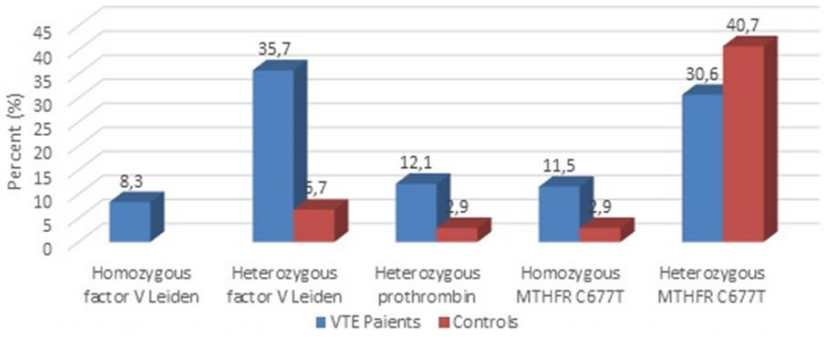

Figure 1: Frequency of factor V Leiden, Prothrombin G20221A and MTHFR C677T among VTE patients and controls.

(48.9%) had at least one genetic mutation assessed in the study. Among 135 controls, all of the FVL (6.7%) and PT (2.9%) mutations were heterozygous. Additionally, frequencies of heterozygous and homozygous mutations for MTHFR were 40.7 and 2.9% in the controls, respectively. Six subjects of controls had combined mutations. Four of them had both FVL and MTHFR mutations, and the next two had both PT and MTHFR mutations, whereas none of them had all of the three mutations. Among the 157 patients with VTE, 56 (35.7%) patients were heterozygous and 13 (8.3%) homozygous for FVL. Nineteen (12.1%) were heterozygous and none homozygous for the PT mutation. Forty-nine (30.6%) patients were heterozygous and 18 (11.5%) homozygous for MTHFR mutation. Thirty-four patients had combined mutations. Two patients had both FVL and PT mutations, 23 patients had both FVL and MTHFR mutations, 7 patients had both PT and MTHFR mutations, and a combination of FVL and PT G20210A and MTHFR C677T gene mutations was detected in 2 cases (Figure 2). No significant differences existed between genders.

Discussion

Factor V Leiden (FVL), prothrombin gene mutation and MTHFR mutation are the most common genetic disorders associated with VTE [17,18]. The prevalence of FVL varies according to geography and ethnicity as well as it is approximately 5% in general population. It can also account for 20-25% of patients with a first idiopathic VTE and 40-50% of those with recurrent VTE. Homozygotes (about 0.06 to 0.25% of the population) are much less common than heterozygotes but homozygous for factor V leiden is associated with much higher relative risk for VTE. Although, the prevalence varies from 3 to 8% in common US and European populations. FVL is relatively uncommon in African, Australian and Asian populations [19,20]. In Turkish population, the prevalence of FVL mutation varies in the range of 4.2% and 15.2%. The lowest frequency of FVL mutation was found to be 4.28% in the Thrace region and the results of surveys conducted in the regions of Southeastern, Aegean and the cities of Ankara and Tokat were 4.6%, 8.4%, 9.8%, and 15.2% respectively [21-25]. The location of Turkey between Europe and Asia as if a bridge allows the distribution of inherited thrombophilia to show some variation in different regions of Turkey. The prevalence of FVL in our control group (6.7%) was consistent with the previously reported publications in Turkey and Europe. Furthermore, in our study, FVL was found in 69 patients (43.9%) with VTE and 56 were heterozygous and 13 were homozygous with a rate of 35.7% and 8.3%, respectively. Roldan et al. reported that 597 (31.3%) of the 1907 patients with a first idiopathic VTE were tested positive for thrombophilia in their study, as well as 149 (7.8%) of patients had FVL mutation [26]. In a study of a Croatian population, Alfirević et al. found that the frequency of FVL mutation was 16% among VTE patients and all of these were heterozygous mutations [19]. In Turkish population, the results of the studies conducted on the frequency of FVL mutation among VTE patients vary. Akar et al. found that the prevalence of FVL mutation in thrombosis cases was 9.8% [27]. Furthermore, the prevalence of FVL mutation was reported as 37.5% by Gul et al. [28]. In another study conducted on patients with a history of DVT, it was found as 24.6% by Kalkanli et al. 22.9% and 1.6% of the patients bearing FVL mutation were heterozygous and homozygous, respectively [29]. On the other hand, there are some reports from Turkey that found the FVL mutation in 20% of patients with VTE [30]. In our study, the frequency of FVL in patients with idiopathic VTE is higher than the previous reports.

The second most common cause of inherited thrombophilia is PT gene mutation. It has been reported to increase the risk of VTE almost threefold [31]. In our study, the frequency of PT gene mutation in healthy group was 2.9%, while it was higher in the group of patients with thromboembolic disease (12.1%). All of them were heterozygous. These results were in accordance with the findings already reported in other Caucasian populations which is present in 2-4% [32]. PT gene mutation is more common in European countries that it was reported to be present in 3-17% of patients with VTE and 1-8% in healthy controls [33]. There are some reports from our geographic area (Bulgaria, Greece, Azerbaijan and Romania) that showed the prevalence of PT gene mutation in the range of 2.77% and 7.0% [22,34,35]. In Turkish population, Ayyildiz et al. reported that the prevalence of PT gene mutation was 6.5% in patients with VTE and 1.2% in the healthy group [36]. Akar et al. found the mutation as 2.7% among healthy group and 6.25% in patients with DVT [37]. In another study, the PT gene mutation was found to be 27.5% in patients with VTE, by Yilmaz et al. [38].

Besides the above mentioned genetic factors, MTHFR mutation is recognized to be one of the most common inherited risk factors for VTE. However, the role of MTHFR polymorphisms in VTE is controversial [37,40]. In our study, the frequency of heterozygous and homozygous MTHFR mutations appeared to be 40.7% and 2.9%, respectively, in healthy subjects. Sazci et al. reported that the frequencies of heterozygosity and homozygosity for MTHFR (C677T) in Turkish population were 47.4% and 9.6%, respectively [41]. Our ratios were lower than this study, this difference may be related to

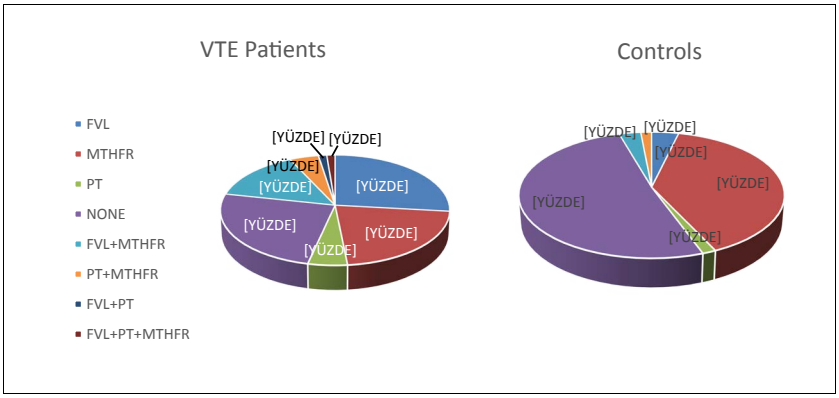

Figure 2: Frequencies of genetic mutations associated with thromboembolism including FVL (Factor V Leiden), MTHFR (Methylene tetrahydrofolate reductase C677T), PT (Prothrombin G20210A) among patients with VTE and controls.

the study group size. In our study, we observed that the frequencies of heterozygous and homozygous MTHFR mutations were 30.6% and 11.5%, respectively, in patients with idiopathic VTE. There was no significant difference between the patients and controls in terms of MTHFR mutation whereas, homozygous MTHFR mutation rate was higher in patients with VTE than healthy controls. In patients with VTE, Sahin et al. showed that the frequency of MTHFR mutation was 60% (47.3% heterozygous and 12.7% homozygous) [25]. In another study including 1507 patients with recurrent pregnancy loss conducted by Incebiyik et al. reported that the frequency of heterozygous and homozygous was found to be 40.61% and 8.29%, respectively [42].

One or more inherited risk factor can be detected in almost half of patients who have suffered venous thrombosis [1]. In addition, the coexistence of several inherited risk factors accounting for VTE, increases the risk of recurrent VTE [43]. In our study, FVL, prothrombin and MTHFR mutations were found in 43.9%, 12.1% and 42%, respectively, of the patients, whereas 20.8% of patients had no mutations. Furthermore, two or more mutations were detected in 21.7% of patients with idiopathic VTE. Our findings were consistent with the previous studies in which the high rate of the presence of any of inherited risk factors among patients with venous thrombosis was stated [19,25,42,44].

The results of our study give us some new information on commonness of thrombosis related genetic mutations in Turkish population. Nevertheless, it is not sufficient to generalize our findings to the whole population, due to the small sample size and limited power of the study.

Conclusion

Our findings showed that the prevalence of FVL, MTHFR and PT gene mutations among healthy subjects who are living in the Western Black Sea region of Turkey, were consistent with the previously reported publications in Turkey and Europe. We observed that the patients with idiopathic VTE had significantly more FVL and PT gene mutations than healthy individuals. Thus, these genetic disorders should be evaluated in patients presented with unexplained VTE. We believe that the results of our study will contribute in part to indicate the distribution and prevalence of FVL, MTHFR and PT gene mutations in Turkish population.

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