RESEARCH ARTICLE

Incidence of the MTHFR polymorphisms in patients with varicose veins

Ekim M[1], Ekim H[2]

[1]Department of Emergency Aid and Disaster Management School of Health
[2]Department of Cardiovascular Surgery, School of Medicine
Bozok University, Yozgat, Turkey

Abstract

Background: Varicose vein disease is one of the most common inherited disorders worldwide that causes mental, cosmetic, medical, and socio-economic problems. Varicose vein formation is thought to be multifactorial and often develops through the interaction of environmental and genetic risk factors. Its incidence displays a trend parallel to the distribution of methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism worldwide. The objective of this study was to determine the incidence of MTHFR C677T and A1298C mutations in Turkish patients with varicose veins.

Material and Methods: Our study included 98 patients with varicose veins; twenty-nine (29.6 %) males and 69 (70.4 %) females, with a mean age of 52.9 ± 14.7 (age range: 12-83) years. Polymorphisms were investigated by polymerase chain reaction (PCR) which is an enzymatic reaction of DNA amplification, and allele-specific hybridization.

Results: The homozygous MTHFR C677T polymorphism was detected in 13 (13.3 %), and heterozygous MTHFR C677T polymorphism in 37 (37.7 %) patients. The homozygous and heterozygous MTHFR A1298C polymorphisms were found in nine (9.2 %), and 47 (47.9 %) patients, respectively. The combined double heterozygous genotype MTHFR C677T/MTHFR A1298C was identified in 19 (19.4 %) patients. The rates of factor V Leiden (FVL) and prothrombin gene (PT G20210A) polymorphisms were found as 11.2 %, and 4.1 %, respectively. Additionally, the majority of patients with thrombosed varicose veins were accompanied by FLV polymorphism.

Conclusion: Our findings display that the rates of MTHFR C677T and A1298C genotypes are similar between patients with varicose veins and healthy subjects in Turkish society. However, should FLV or PG G20210A polymorphisms accompany these polymorphisms, then there might be a tendency to development of superficial venous thrombosis. Further studies are required to support these findings. HIPPOKRATIA 2017, 21(4): 175-179.

Keywords: Varicose vein, factor V Leiden, FVL, prothrombin gene, PT G20210A, methylenetetrahydrofolate reductase, MTHFR

Corresponding author: Prof. Dr. Hasan Ekim, Bozok Universitesi Tıp Fakültesi, Department of Cardiovascular Surgery, Yozgat, Turkey, tel: +905354176539, e-mail: drhasanekim@yahoo.com

Introduction

Varicose vein disease is one of the most common inherited disorders worldwide that causes mental, cosmetic, medical and socio-economic problems[1]. Varicose veins are abnormally twisted, elongated and dilated veins, and often are associated with insufficient venous valves[2]. They are characterized by intimal hypertrophy and increased luminal diameter[3]. The main etiological factors in the development of varicose veins are venous dilatation and valvular insufficiency which are initiated by factors that are still not fully understood[4]. Venous incompetence leads to increased venous pressure and subsequently skin changes in lower extremities[5]. Biochemical and structural alterations in the vessel wall play a role in the pathogenesis of varicose veins[3]. The quality of life has been shown to decrease due to symptoms of varicose venous disease. Therefore, varicose veins can contribute to significant health care problems[3].

Although environmental risk factors are important, a number of epidemiological studies have shown that genetic factors may play a role in determining susceptibility to vascular disease[4]. Thrombophilic risk factors have been shown to be more prevalent amongst patients with varicose vein[6]. For this reason, the detection of thrombophilic mutations is valuable for the prognosis and care of varicose veins. The methylenetetrahydrofolate reductase (MTHFR) enzyme has two significant polymorphisms (C677T and A1298C). It has been previously reported that these polymorphisms may be related to varicose venous disease[7]. The objective of the current study was to determine the incidence of these polymorphisms in Turkish patients with varicose veins.

Material and Methods

This prospective study was conducted in the Bozok University Hospital, in Yozgat, Turkey between January

2015 and December 2016. The study was approved by the Ethical Committee of Bozok University (13.10.2015/112) and was conducted in accordance with the principles of the Declaration of Helsinki. All patients signed informed consent. We included in the study 98 consecutive patients eligible for surgical intervention. The presence of varicose veins was assessed by clinical examination and venous duplex ultrasound scanning which carefully examined for patency, compressibility, and reflux both the deep and the superficial venous systems. Reflux was accepted as a reverse flow of more than 0.5 seconds. All patients had both truncal and perforator venous insufficiency. According to the Clinical, Etiological, Anatomical, and Pathophysiological (CEAP) classification, 86 patients were in the C2 class and the remaining patients were in the C3 class. Patients with secondary varicose veins, ulceration or lipodermatosclerosis were excluded from the study.

Laboratory Study

Ten milliliters of peripheral venous blood was drawn from each patient into an ethylenediaminetetraacetic acid (EDTA) tube. The DNA was extracted from the peripheral blood samples according to the manufacturer's protocol (Qiagen Inc., Germany). The MTHFR (C677T, A1298C), factor V Leiden (FVL), and prothrombin gene (PT G20210A) mutations were investigated by polymerase chain reaction (PCR) which is an enzymatic reaction of DNA amplification, and allele-specific hybridization in all patients. Polymorphism screening was carried out with a SNaPshot® multiplex system (Applied Biosystems Inc., Switzerland). Wild, heterozygous, and homozygous genotypic distributions of these polymorphisms were defined as numbers and percent frequencies.

Statistics

Results were reported as mean ± standard deviation (SD). The prevalence of polymorphisms was shown as percent (%). For statistical analysis, the paired sample t-test was used. A p-value of less than 0.05 was accepted as significant.

Results

Twenty-nine (29.6 %) male and 69 (70.4 %) female patients with a mean age of 52.9 ± 14.7 (age range: 12-83) were included in the study. Seventy (71.5 %) patients had a family history of varicose vein disease.

The homozygous and heterozygous MTHFR C677T polymorphisms were found in 13.3 % (n =13; four males and nine females) and 37.7 % (n =37; seven males and 30 females) of patients, respectively, and gave an overall prevalence of 51 %. A similar prevalence (49.1 %) was also found in patients without superficial venous thrombosis (SVT). The homozygous and heterozygous MTHFR A1298C polymorphisms were found in 9.2 % (n =nine; five males and four females) and 47.9 % (n =47; 15 males and 32 females) of the patients, respectively, and revealed an overall prevalence of 57.1 % (Table 1). A similar prevalence (57.6%) was also found in patients without SVT. None of the patients carried the double homozygous genotype (MTHFR 677TT/MTHFR 1298CC). The combined double heterozygous genotype MTHFR C677T/MTHFR A1298C was identified in 19 (19.4 %) patients. There were no significant differences in the distribution of polymorphisms according to gender ($p < 0.001$).

There was no detected homozygote PT G20210A mutation. The heterozygote PT G20210A mutation was identified in four (4.1 %) female patients. The homozygous FVL genotype was found in two (2 %) patients (one male, one female), while the heterozygous FVL genotype was found in 9.2 % (n =9; one male and eight females) of the patients. There were two patients with a double heterozygous genotype for the FVL and the PT G20210A mutations. On the other hand, 10.2 % (n =10) of patients carried none of these polymorphisms.

Of the patients, 13 had thrombosed varicose veins; eight of them had MTHFR C677T polymorphism (three homozygous, five heterozygous) and seven had heterozygous MTHFR A1298C polymorphism. Additionally, the majority of the patients with thrombosed varicose veins were accompanied by FLV polymorphism (two homozygous, four heterozygous) and PT G20210A polymorphism (three heterozygous) (Table 2). There was no difference in the MTHFR polymorphisms between our patients with or without SVT.

Discussion

Most epidemiological studies have reported that varicose veins are observed among females at a rate of 25-35 % and 10-20 % among males, while the incidence increases with age[8]. The development of the varicose vein is thought to be multi-factorial and caused by an interaction between inherited and acquired risk factors. Although its etiopathogenesis is still not entirely understood[9], some mechanisms leading to varicose vein formation have been proposed. Valvular insufficiency causing reflux has been hypothesized as the primary cause of venous wall weakness and dilation. However, this hypothesis has been challenged by evidence proposing that primary venous wall changes may precede valvular insufficiency[10]. It has been suggested that the etiology of this disease is largely due to luminal pathology such as venous hypertension and thrombosis rather than a defect on the vascular wall[8]. As a matter of fact, the level of D-dimer (fibrin degradation product) was found to be elevated in varicose veins, indicating luminal thrombosis and thrombus turnover[11]. However, the debate is continuing.

Alterations in the function of enzyme MTHFR may lead to endothelial dysfunction and oxidative stress[12]. Endothelial dysfunction plays a role in the progression of varicose vein formation and its thrombotic complications[13]. The single nucleotide C677T lies in the catalytic domain of the MTHFR enzyme and has been associated with a reduction of enzymatic activity leading to mild hyperhomocysteinemia[14].

Table 1: Distribution of polymorphisms in the 98 patients (29 males and 69 females) with varicose veins that were included in the current study.

Polymorphism	Male	Female	Total (%)
HOM MTHFR 677	4 (13.8 %)	9 (13 %)	13 (13.3 %)
HET MTHFR 677	7 (24.8 %)	30 (43.47 %)	37 (37.7 %)
HOM MTHFR 1298	5 (17.2 %)	4 (5.79 %)	9 (9.2 %)
HET MTHFR 1298	15 (51.7 %)	32 (46.37 %)	47 (47.9 %)
HET PT G20210A	0 (0 %)	4 (5.79 %)	4 (4.1 %)
HOM FVL	1 (3.4 %)	1 (1.45 %)	2 (2 %)
HET FVL	1 (3.4 %)	8 (11.59 %)	9 (9.2 %)
No Polymorphism	3 (10.3 %)	7 (10.14 %)	10 (10.2%)

HOM: mutant homozygous, HET: heterozygous, MTHFR: Methylenetetrahydrofolate reductase, PT 20210A: Prothrombin gene mutation, FVL: factor V Leiden.

Table 2: Distribution of the polymorphisms detected in the 13 patients with superficial venous thrombosis.

Age / gender	FVL	PT G20210A	MTHFR 677	MTHFR1298
44 F	HOM	WT	HET	WT
56 M	HOM	WT	WT	HET
70 M	WT	WT	HOM	WT
51 F	WT	HET	HET	WT
59 F	HET	HET	HET	HET
50 F	WT	WT	HOM	WT
50 F	WT	WT	WT	HET
67 F	WT	WT	HOM	WT
54 F	HET	WT	WT	WT
64 M	WT	WT	HET	HET
48 F	HET	WT	WT	HET
62 F	HET	HET	WT	HET
49 M	WT	WT	HET	HET

F: female, M: male, FVL: Factor V Leiden, PT 20210A: Prothrombin gene mutation, MTHFR: Methylenetetrahydrofolate reductase, HOM: mutant homozygous, HET: heterozygous, WT: wild type.

Although MTHFR (C677T, A1298C) polymorphisms may cause mild or moderate hyperhomocysteinemia, not all subjects will develop this complication[15]. Furthermore, adequate folate intake prevents the unfavorable effects of these polymorphisms on homocysteinemia levels[4].

The incidence of varicose vein disease displays a trend parallel to the distribution of MTHFR C677T polymorphism worldwide. This suggests that MTHFR C677T polymorphism has a significant role in the development of varicose veins[13]. A few studies investigating the role of this polymorphism in the varicose vein formation have been conducted. Sverdlova et al[7] detected a significantly higher prevalence of MTHFR C677T polymorphism in patients with varicose veins compared with healthy controls. Fernández-Peralta and González-Aguilera[16] suggested that there may be an association between the pathogenesis of varicose disease and MTHFR polymorphism. Similarly, Rafetto et al[12] suggested that there may be a link between the MTHFR C677T polymorphism and the pathogenesis of the varicose vein formation. In contrast, Kapısız et al[4] and Shadrina et al[9] reported that MTHFR C677T polymorphism has no contribution to the varicose vein formation. The different findings obtained from the various studies may be related to ethnic and geographic differences.

In our previous study, rates of homozygous and heterozygous MTHFR C677T polymorphism carriers were detected to be 8.8 % and 33.3 %, respectively, in the healthy Turkish population[17]. Angelopoulou et al[18] reported that the prevalence of homozygous and heterozygous MTHFR C677T polymorphisms were 17.8 % and 44.4 % in the healthy Greek Cypriot population, respectively. In the current study, homozygous and heterozygous MTHFR C677T carriers were found to be 13.3 % and 37.7 %, respectively, suggesting patients with varicose veins and healthy subjects have a similar frequency of MTHFR C677T polymorphism.

The second important mutation of the MTHFR enzyme which causes the A1298C polymorphism is located in the C-terminal end of the enzyme and associated with a lesser activity of the enzyme[16]. Unlike homozygous MTHFR C677T carriers, homozygous MTHFR A1298C carriers are not associated with increased homocysteinemia levels[19]. In our previous study, the rates of homozygous and heterozygous MTHFR A1298C polymorphism carriers were detected to be 13.3 % and 33.3 % in the healthy Turkish population, respectively[17]. Sazci et al[20] reported that the prevalence of homozygous and heterozygous MTHFR A1298C polymorphisms were 10 % and 46.3 % in the healthy population, respectively. Similarly, in the current study, homozygous and heterozygous MTHFR A1298C carriers were found to be 9.2 % and 47.9 %, respectively.

Sazci et al[20] found that the prevalence of compound MTHFR C677T/A1298C genotypes was 21.6 % in the healthy Turkish population. Similarly, the rate of C677T/

A1298C compound heterozygosity was detected as 19.4 % in the current study.

Although damaged venous wall and stasis are important risk factors predisposing to venous thrombosis in patients with varicose veins, the presence of thrombophilic risk factors is also significant among these patients[21]. FVL mutation is the most common genetic thrombophilic defect of the coagulation system and characterized by a missense mutation in the factor V gene causing an arginine to glutamine substitution at position 506[22]. Although the FVL polymorphism has been found as a thrombophilic risk factor for venous thromboembolism, it also seems a risk factor for SVT[23]. Similarly, the frequency of FVL polymorphism was found to be higher in our patients with SVT (Table 2). Furthermore, it has been identified that varicose venous disease and SVT are independent risk factors for venous thromboembolism[24]. Therefore, patients with varicose veins and thrombophilic mutations should be closely monitored as long as they live. Fansa et al[25] suggested that thromboprophylaxis might be advantageous in pregnant women with FVL polymorphism. Prophylactic precautions should be taken in risky situations such as surgical intervention, trauma, pregnancy, and long-term bed rest in patients with thrombosed varicose veins.

Although there has been no consensus on the optimal treatment of SVT, some treatment approaches have been proposed, such as surgical intervention, nonsteroidal anti-inflammatory drugs (NSAIDs), elastic stockings, and a prophylactic dose of low-molecular-weight heparin (LMWH)[26]. In patients with SVT, 45-day anticoagulant therapy resulted in a significant reduction in venous thromboembolism and recurrent SVT attacks[27]. Also, no complications were observed in any of our treated patients with LMWH.

Although mutations in the methylenetetrahydrofolate reductase (MTHFR) gene have been shown to be associated with hypertension and increased risk of cardiovascular disease[28], the MTHFR C677T mutation has not been shown to play a role in the development of venous thrombosis alone[29]. However, it has been reported that there is a risk for venous thrombosis of MTHFR C677T mutation is accompanying with FVL mutation[29].

Thrombophilic defects were found significantly associated with SVT in patients with varicose veins[30]. Moreover, genetic polymorphisms play an essential role in the extension of the thrombotic process from the superficial to the deep veins[31]. Although SVT arises on the varicose vein probably because of venous wall damage independent from genetic defects, these defects significantly operate in the possible extension into the deep venous system and in the eventual pulmonary embolism[31].

The second most common inherited thrombophilic risk factor is PT G20210A polymorphism (located 3'-untranslated region of the prothrombin gene) which is associated with elevated prothrombin levels[32]. The worldwide frequency of PT G20210A polymorphism was determined as 1.7 to 4 %[33]. Darvall et al[6] found that there were no significant differences between patients with varicose veins and control subjects for FVL and PT G20210A mutations. The rates of FVL and PT G20210 polymorphisms were determined to be 19 % and 5.5 % in healthy Turkish people, respectively[17]. In this study the rates of FVL and PT G20210A polymorphisms were found as 11.2 % and 4.1 %, respectively, suggesting no association between these polymorphisms and varicose venous disease.

Conclusion

Our findings display that the rates of MTHFR C677T and MTHFR A1298C genotypes are similar between patients with varicose veins and healthy subjects in Turkish society. However, FVL and PT G20210A polymorphisms accompanying to MTHFR polymorphisms were detected more often in patients with SVT. Therefore, if FLV and/or PG G20210A polymorphisms accompany these polymorphisms, then there may be a tendency for the development of SVT in patients with varicose veins. Further studies are required to support our findings.

Conflict of interest

The authors declared no conflicts of interest with respect to this article.

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