The incidence of FVL and PT G20210A mutations in patients with idiopathic deep venous thrombosis

Meral Ekim¹, Hasan Ekim²

Ekim M, Ekim H. The incidence of FVL and PT G20210A mutations in patients with idiopathic deep venous thrombosis. J Phlebol Lymphol. 2018;11(1):21-24.

Background: Factor V Leiden (FVL), prothrombin gene (PT G20210A), and methylenetetrahydrofolate reductase (MTHFR) C677T mutations are known as molecular biomarkers to evaluate the predisposition of deep venous thrombosis (DVT). These hereditary risk factors affect the natural anticoagulant mechanisms and activate the coagulation mechanisms because of an imbalance between procoagulant and anticoagulant factors. Our study aimed to determine the prevalence of these mutations in Turkish patients presenting with idiopathic DVT.

Methods: A total of 135 patients with idiopathic DVT admitted to our clinic were investigated for FVL, PT G20210A, and MTHFR (C677T, A1298C) gene mutations. Screening of polymorphisms was carried out by using SNaPshot® multiplex system (Applied Biosystems Inc.). Wild, heterozygous and homozygous genotypic distributions of these mutations were defined as number and percentage frequency.

Results: There were 74 male and 61 female patients ranging in age from 18 to 85 years. FVL mutation was found in 66 (41.47%) patients (12 homozygotes 8.88%, 44 heterozygotes 32.59%), PT G20210A was found in 14 (10.36%) patients (1 homozygous 0.74%, 13 heterozygotes 9.62%). MTHFR C677T was found in 53 (39.25%) patients (10 homozygotes 7.40%, 43 heterozygotes 31.85%), and MTHFR A1298C was found in 84 (62.22%) patients (16 homozygotes 11.85%, 68 heterozygotes 50.37%). Key words: Factor V Leiden, Prothrombin Gene Mutation, MTHFR, Deep Venous Thrombosis.

Our study aimed to determine the prevalence of these mutations in Turkish patients presenting with idiopathic DVT.

INTRODUCTION

Venous thromboembolism (VTE) is now considered a serious and life-threatening disease in which interactions between hereditary and acquired or environmental factors contribute to clinical phenotype [1,2]. There are significant differences between the arterial and venous systems in terms of coagulation and plug formation. Arterial thrombosis is usually accompanied by underlying vascular abnormalities, typically atherosclerotic vascular disease and less frequently encountered in individuals with vasculitis. On the other hand, venous thrombosis usually occurs incidentally in individuals with genetic abnormalities coexistence with hypercoagulability [3].

In 1993, Dahlback et al. [4] identified a previously unrecognized mechanism for thromboembolism that is characterized by poor anticoagulant response to activated protein C (APC) due to a polymorphism of factor V (FV), in which glutamine replaces arginine, so cleavage of factor V by APC is inhibited [2]. This mutant FV molecule, called factor V Leiden (FVL), also known as factor V Q506 or Arg506Gln, is named after the city in the Holland where it was first identified [5]. FVL is the most common inherited risk factor for deep venous thrombosis (DVT).

The risk of venous thrombosis is also increased with prothrombin gene mutation (PT G20210A) via elevation of prothrombin levels [6]. This mutation within the 3' untranslated region of the prothrombin gene with a G to A transition at position 20210 was first identified by Poorts et al. [7]. Carriers of PT 20210A have higher plasma prothrombin concentrations than control subjects with the normal 20210GG polymorphism and have increased risk of venous thrombosis [7].

FVL, PT G20210A, and methylenetetrahydrofolate reductase (MTHFR) C677T are commonly used as molecular biomarkers to evaluate the predisposition of DVT [8]. These hereditary thrombophilic risk factors affect the natural anticoagulant mechanisms and activate the coagulation mechanisms because of an imbalance between procoagulant and anticoagulant factors [3].

¹School of Health, Bozok University, Yozgat, Turkey
²Department of Cardiovascular Surgery, School of Medicine, Bozok University, Yozgat, Turkey

Correspondence: Meral Ekim, MD, Department of Nutrition and Dietetics, Bozok Universitesi Saglik Bilimleri Fakultesi, Yozgat, Turkey, Tel: +90535 4176539; E-mail: meralekim@yahoo.com ; Doi: 10.11131/1983-8905.1000053

Received: March 27, 2018, Accepted: April 9, 2018, Published: April 15, 2018
out using the paired sample t-test and values of p were accepted to be significant when less than 0.05.

RESULTS

There were 74 male and 61 female patients ranging in age from 18 to 85 years with a mean age of 53.6 ± 15.9 years. The mean ages of PT G20210A and FVL carriers were 48.6 ± 16.0 and 52.4 ± 16.3 years, respectively. The mean age of patients without PT G20210A or FVL polymorphism was 55.9 ± 15.4 years (P<0.05).

The PT G20210A and FVL mutations were found in 14 (10.4%) and 66 (41.5%) patients, respectively (Table 1). The homozygous PT G20210A mutation was detected in only one female patient. The heterozygote PT G20210A mutation was detected in 13 (9.6%) patients (5 males, 8 females) (Table 2).

Table 1: Distribution of polymorphisms in patients with deep venous thrombosis.

| Type of gene mutation | Homozygote FVL | Heterozygote FVL | Heterozygote PT G20210A | Homozygous PT G20210 | Homozygote MTHFR 677TT | Heterozygote MTHFR C677T | Homozygote MTHFR 1298CC | Heterozygote MTHFR A1298C | No mutation | Total |
|-----------------------|----------------|------------------|------------------------|----------------------|------------------------|------------------------|------------------------|------------------------|-------------|-------|
|                       | 12 (8.9 %)     | 44 (32.6 %)      | 13 (9.6 %)             | 1 (0.7 %)            | 10 (7.4 %)             | 43 (31.8 %)            | 16 (11.8 %)            | 68 (50.4 %)             | 11 (8.1 %) | 147   |
|                       | M 9 (12.2 %)   | 27 (38.5 %)      | 5 (6.7 %)              | 0 (0 %)              | 5 (6.7 %)              | 24 (32.4 %)            | 10 (13.5 %)            | 28 (37.8 %)            | 4 (5.4 %)   |       |
|                       | F 3 (4.9 %)    | 17 (27.9 %)      | 8 (13.1 %)             | 1 (1.6 %)            | 8 (5.2 %)              | 19 (31.1 %)            | 6 (9.83 %)             | 40 (56.6 %)            | 7 (11.5 %)  |       |

FVL: Factor V Leiden; PT 20210A: Prothrombin gene mutation; MTHFR: Methylenetetrahydrofolate Reductase.

The homozygous FVL polymorphism was detected in 12 (8.9%) patients (9 males, 3 females). The heterozygous FVL mutation was found in 44 (32.6%) patients (27 males, 17 females). FVL was more frequently encountered in male patients (48.6% vs. 32.4%) (p<0.05). There were no combined homozygous FVL and PT G20210A carriers (Table 3). Double heterozygosity for the FVL and the PT G20210A genotypes was detected in 4 (2.9%) patients. Furthermore, a combination of the homozygous FVL and the heterozygous PT G20210A genotypes were detected in 1 (0.7%) patient.

Table 2: Distribution of the PT G20210A carriers according to gender and accompanying additional genetic mutations.

| Gender | PT G20210 | FVL | MTHFR C677T | MTHFR A1298C |
|--------|-----------|-----|-------------|--------------|
| M      | HET       | HET | WT          | WT           |
| F      | HET       | WT  | HOM         | WT           |
| F      | HET       | WT  | HOM         | WT           |
| M      | HET       | WT  | HET         |              |
| F      | HET       | WT  | WT          |              |
| F      | HOM       | WT  | WT          |              |
| M      | HET       | WT  | WT          |              |

The homozygote and heterozygote MTHFR 677 genotypes were identified in 10 (7.4%) and 43 (31.8%) patients, respectively, giving an overall incidence of 39.2%. Of the 53 patients with MTHFR 677 polymorphism, 27 (50.9%) had additional defects as follows: 21 (39.6%) had FVL, 4 (7.5%) had PT G20210A, and 2 (3.8%) had both FVL and PT G20210A. The homozygote and heterozygote MTHFR 1298 genotypes were detected in 16 (11.8%) and 68 (50.4%) patients, respectively, revealing an overall incidence of 62.2%. Of the 84 patients with MTHFR A1298C polymorphism, 40 (47.6%) had additional defects as follow: 35(41.7%) had FVL, 4 (4.8%) had PT G20210A, and 1 (1.2%) had both FVL and PT G20210A.

Table 3: Distribution of the homozygous FVL carriers according to gender and accompanying additional genetic mutations.

| Gender | FVL | PT G20210 | MTHFR C677T | MTHFR A1298C |
|--------|-----|-----------|-------------|--------------|
| M      | HOM | WT        | WT          | WT           |
| F      | HOM | WT        | WT          | WT           |
| M      | HOM | WT        | HOM         | WT           |
| F      | HOM | WT        | HET         | HET          |
| M      | HOM | WT        | HOM         | HET          |
| M      | HOM | WT        | WT          | WT           |
| M      | HOM | WT        | WT          | HET          |

The homozygote and heterozygote MTHFR 677 genotypes were identified in 10 (7.4%) and 43 (31.8%) patients, respectively, giving an overall incidence of 39.2%. Of the 53 patients with MTHFR 677 polymorphism, 27 (50.9%) had additional defects as follows: 21 (39.6%) had FVL, 4 (7.5%) had PT G20210A, and 2 (3.8%) had both FVL and PT G20210A. The homozygote and heterozygote MTHFR 1298 genotypes were detected in 16 (11.8%) and 68 (50.4%) patients, respectively, revealing an overall incidence of 62.2%. Of the 84 patients with MTHFR A1298C polymorphism, 40 (47.6%) had additional defects as follow: 35(41.7%) had FVL, 4 (4.8%) had PT G20210A, and 1 (1.2%) had both FVL and PT G20210A.

Although none of our patients had the double homozygous genotype of (MTHFR C677TT/ MTHFR A1298CC), there were 22 (16.3%) patients (15 males and 7 females) with double heterozygous for the MTHFR C677T/MTHFR A1298C genotypes. Twelve (54.5%) of these 22 patients had additional defects as follow: (10 (45.4%) were heterozygous for FVL and 2 (9.1%) were homozygous for FVL). On the other hand, (8.1% (n=11) of 135 patients carried none of these polymorphisms.
DISCUSSION

FVL polymorphism is the most common hereditary thrombophilic risk factor related to venous thrombosis [9]. The knowledge of FVL has represented a great advance in the understanding of the molecular mechanisms leading to venous thrombosis [10]. FVL is inactivated 10-20 times more slowly than the native form of factor V, leading to excessive thrombin generation and a presumed lifelong prothrombotic tendency [11].

Epidemiological and biochemical investigations support evidences that FVL should have emerged as a single event in the past. The Mediterranean basin has the higher prevalence of FVL than the remaining of the earth [12]. It has been estimated for 15000-30000 years since the Neolithic period in Middle East. Thus, the prevalence of FVL mutation is likely to be high among the Turkish population [13], as seen in the current study.

The prevalence of FVL mutation in population is variable according to the region and ethnicity. Some studies on the prevalence of the FVL showed that there was a relation between the mutations and ethnic and geographical distribution [14]. The high prevalence of FVL polymorphism was detected in Southeast Europe (9-15%) and the Middle East (13%) [15]. FVL prevalence in the healthy Turkish population has been published in different regions of Turkey and was reported to range between 3.5 to 15 % in different investigations. This wide range could be caused by the differences of geographical situations [15]. The FVL mutation was significantly higher in male patients compared with female patients in the current study. Additionally, we found that patients with FVL or PT G20210A mutation were younger than those without these polymorphisms.

Combination of acquired risk factors with inherited genetic factors significantly increases the risk of thrombosis [16]. Although FVL leads to an increased risk of VTE, many carriers of FVL may not show clinical findings of thrombosis unless they have combined risk factors, such as deficiency of protein C or protein S [17]. In certain subgroups that are already at increased risk for thrombosis (patients taking birth control pill, patients with hereditary protein C deficiency), the added presence of FVL may have a multiplicative effect on thrombophilic risk [18]. Women taking birth control pill who had FVL were found to have a 30-fold increase in risk for venous thrombosis compared with women who had no risk factor [18].

Carrier status of both FVL and PT G20210A may be advantageous to fertile females, reducing gynecologic hemorrhage, and protecting against anemia [19,20]. Also these polymorphisms may have been protective in the past, particularly in times when hunting-related injury was more common and medical management rather primitive [19]. Although these conditions seemed to be useful during Neolithic period, we think that these polymorphisms may be harmful at the present time owing to severe thromboembolic complications.

PT G20210A polymorphism has been found to be associated with increased level of prothrombin known as a risk factor for thrombosis [7]. The relative risk for DVT increases 2-3-fold for PT G20210A mutation alone and 26-30-fold for coinheritance of PT G20210A and FVL [16]. Although PT G20210A mutation is the second most prevalent thrombophilic risk factor, it is uncommon and reported in about 1%-2% in the general population [16]. Similar to FVL mutation, its prevalence has also been detected high in white population of European origin [21]. The FVL and PT G20210A mutations appear to be specific to Caucasians and virtually absent in those from Asia and Africa [22]. These mutations are associated with high risk of DVT [23].

The prevalence of PT G20210A mutation was detected as 2.6% in healthy Turkish population [24]. PT G20210A mutation was found as 8.1% in Turkish Cypriots patients presenting with DVT [24]. Similar prevalence (6.8%) for PT G20210A mutation was also detected by Gurgey et al. [9] in Turkish DVT patients. In our previous study, FVL and PT G20210A mutations were detected in 19% and 5.5%, respectively in healthy Turkish subjects [25]. In the current study, genotype frequencies of FVL and PT G20210A mutations were 41.47% and 10.36%, respectively, pointing to a possible association among these mutations and DVT.

Patients with both the FVL and the PT G20210A had increased risk of recurrent DVT. Thus, these patients should be candidates for lifelong anticoagulant treatment [26]. Carriers of heterozygous or homozygous FVL polymorphism have a thrombophilic tendency that may be enhanced during an inflammatory condition [27].

Although venous thrombosis is thought major clinical manifestation of the FVL, there is also evidence that FVL may play a role leading to unrecognized miscarriage due to thrombosis of placental vessels [5]. Sehirali et al. [28] suggest that PT G20210A mutation may also be the unexplained cause of recurrent miscarriage. Therefore, screening of both polymorphisms might be recommended for women with a history of recurrent miscarriage. Miscarriage history was present in two of our patients, of whom one had had a combination of heterozygous MTHFR C1298T and homozygous FVL mutations.

Especially, the homozygous MTHFR C677T polymorphism may lead to decreased enzymatic activity and increased homocysteinemia levels, whereas the MTHFR A1298C polymorphism presents a less well-defined effect, with a lesser decrease of the enzymatic activity [29]. The resultant mild hyperhomocysteinemia observed in homoygotes for MTHFR 677TT genotype is accentuated when such patients have reduced plasma folinic acid levels [30]. The relation between the risk of venous thrombosis and hyperhomocysteinemia is not clear. Homocysteine is likely to produce a thrombogenic effect by damaging the vascular endothelial cells [17]. During the past decade, epidemiologic studies have showed that mild to moderate homocysteinemia as an independent risk factor for VTE [21].

In a comprehensive study, homozygous MTHFR C677T genotype was found in 10% of patients with DVT and in 11% of healthy subjects, and the heterozygous MTHFR C677T genotype was found to be similar in both patients with DVT and control group as 43% [31]. In healthy Bosnian population, the prevalence of homozygous and heterozygous MTHFR 677 polymorphisms has been reported to vary between 9%-12%, and 30%-50%, respectively [32]. The prevalence of homoygote for MTHFR C677T genotype and double heterozygote for MTHFR C677T/ MTHFR A1298C genotypes was found 8.8% and 14.4%, respectively in our previous study in healthy Turkish subjects [25]; similar to the rates of this study 7.4% and 16.29%, respectively. Based on our finding and previous studies, we speculate that MTHFR polymorphisms are not playing a role in the development of DVT.

In this study, FVL and/or PT G20210A polymorphism were detected in 50.0% of patients with homozygous genotype for MTHFR C677T and in 54.5% of patients with combined double heterozygous genotype for MTHFR C677T/ MTHFR A1298C. Therefore, we support the notion that homozygous genotype of MTHFR C677T and combined double heterozygous genotype for MTHFR C677T/ MTHFR A1298C might be as synergistic risk factors for DVT in carriers of FVL or PT G20210A mutation.

CONCLUSION

The MTHFR C677T and the MTHFR A1298C genotypes could not be found associated with the predisposition of the development of DVT. In contrast, the FVL and the PT G20210A mutations were found to be associated with the development of DVT. For this reason investigation of these mutations will be particularly helpful in adjusting the duration of anticoagulant therapy in patients with DVT. However, our study should be continued by increasing the number of subjects and supported by further studies.

CONFLICT OF INTERESTS

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.
Ekim et al.

FUNDING
This study was supported by a grant from Bozok University, The Directorate of Scientific Research Projects (TF/A68, 6602c-TF/16-23).

REFERENCES
1. Aryurachai K, Archararit N, Sricote T, et al. Prevalence of factor V Leiden (G1691A) and prothrombin gene mutation (G20210A) among different ethnic groups in Thai Hospitals. J Hematol Transfus Med 2012;22:115-20.
2. Küpeli E, Verdi H, Simşek A, Ataç FB, et al. Genetic mutations in Turkish population with pulmonary embolism and deep venous thrombosis. Clin Appl Thromb Hemost 2011;17:E87-94.
3. Tug E, Aydin H, Kaplan E, et al. Frequency of genetic mutations associated with thromboembolism in the western black sea region. Intern Med 2011;50:17-21.
4. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to an previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Natl Acad Sci USA 1993;90:1004-8.
5. Khan S, Dickerman JD. Hereditary thrombophilia. Thromb J 2006;4:15.
6. Özmen F, Özmén M, Özálp N, et al. The prevalence of factor V Leiden (G1691A), MTHFR (C677T) and PT (G20210A) gene mutations in arterial thrombosis. Ulus Travma Acil Cerrahi Derg 2009;15:113-9.
7. Poort SR, Rosendaal FR, Reitsma PH, et al. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88:3698-703.
8. Şahin Ş, Benli I, Aydoğan L. Distribution of prothrombin G20210A, factor V Leiden and MTHFR C677T mutations in the middle black sea area (Tokat) of Turkey. Turk J Med Sci 2012;42:1093-7.
9. Gurgey A, Haznedaroğlu IC, Egesel T, et al. The common genetic thrombotic risk factors: Factor V Leiden and Prothrombin G20210A in adult Turkish patients with thrombosis. Am J Hematol 2001;67:107-11.
10. Franchini M. Utility of testing for factor V Leiden. Blood Transfus 2012;10:257-9.
11. Orban T, Carlsson M, Svensson PJ. Completed three-dimensional simulation and structural analysis. Biochemistry 2005;44:13082-90.
12. Jadaon MM. Epidemiology of activated protein C resistance and thrombophilia. Turk J Med Sci 2012;42:1625-9.
13. Eroğlu A, Sertkaya D, Akar J. The role of factor V Leiden in adult patients with venous thromboembolism: a meta-analysis of published studies from Turkey. Clin Appl Thromb Hemost 2012;18:40-4.
14. Ajem A, Slama A, Slama FBH et al. Prevalence of factor V Leiden mutation in patients with thrombosis in Tunisia. Eastern Med health J 2009;15:1483-8.
15. Akar N. Factor V 1691 G-A mutation distribution in a healthy Turkish population. Turk J Hematol 2009;26:9-11.
16. Kreidy R, Irani Hakime N. Is thrombophilia a major risk factor for deep vein thrombosis of the lower extremities among Lebanese patients? Vas Health Risk manag 2009;5:627-33.
17. Yurdakul AS, Atıçan Ş, Eren A. Coexistence of factor V Leiden and hyperhomocysteinemia. Turk Res J 2004;5:46-8.
18. Lee DH, Henderson PA, Blajchman MA. Prevalence of factor V leiden in a Canadian blood donor population. Can Med Assoc J 1996;155:285-8.
19. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet 1995;346:1133-4.
20. Mandala E, Lafaras C, Tsioni C, et al. Prevalence of thrombophilic mutations in patients with unprovoked thromboembolic disease. A comparative analysis regarding arterial and venous disease. Hippokratia 2012;16:250-5.
21. Anderson FA, Spencer FA. Risk factors for venous thromboembolism. Circ 2003;107:1-14.
22. Bourouba R, Houcger B, Djabi F, et al. The prevalence of methylenetetrahydrofolate reductase 677 C-T, factor V 1691 G-A, and Prothrombin 20210 G-A mutations in healthy populations in Setif Algeria. Clin Appl Thromb Hemost 2009;15:529-34.
23. Rahimi Z, Mozafari H, Shahriari-Ahmani A, et al. Deep venous thrombosis and thrombophilic mutations in Western Iran: association with factor V Leiden. Blood Coagul Fibrinolysis 2010;21:385-8.
24. Akar N, Misiriloğlu, Akar E, et al. Prothrombin gene 20210 G-A mutation in the Turkish population. Am J Hematol 1998;58:249.
25. Ekim M, Ekim H, Yılmaz YK. The prevalence of Factor V Leiden, prothrombin G20210A, MTHFR C667T and MTHFR A1298C mutations in healthy Turkish population. Hippokratia 2015;19:309-14.
26. De Stefano V, Martinelli I, Mannucci PM, et al. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med 1999;341:801-6.
27. Perez-Pujol, Aras O, Escolar G. Factor V Leiden and inflammation. Thromb 2012;59496:10.
28. Sehirali S, Inal MM, Yıldırım Y, et al. Prothrombin G20210A mutation in cases with recurrent miscarriage: a study of the Mediterranean population. Arch Gynecol Obstet 2005;273:170-3.
29. Hotoleanu C, Trifa A, Popp R, et al. The importance of homozygous 677C→T polymorphism and venous thrombosis. Arch Intern Med 2007;167:497-501.
30. Salomon O, Steinberg DM, Zivelin A, et al. Single and combined prothrombotic factors in patients with idiopathic venous thromboembolism prevalence and risk assessment. Arterioscler Thromb Vasc Biol 1999;19:511-8.
31. Bezemer ID, Doggen CJ, Yos HL, et al. No association between the common MTHFR 677 C→T polymorphism and venous thrombosis. Arch Intern Med 2007;167:497-501.
32. Jusic-Karic A, Terzic R, Jerkic Z, Avdíc A, Podanin M. Frequency and association of 1691 (G>A) FVL, 20210 (G>A) PT and 677 (C>T) MTHFR with deep vein thrombosis in the population of Bosnia and Herzegovina. BMJ 2016;19:43-50.