FV LEIDEN, FII G20210A AND MTHFR C677T MUTATIONS IN PATIENTS WITH LOWER OR UPPER LIMB DEEP VEIN THROMBOSIS

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Deep vein thrombosis (DVT) is a multifactorial disease that occurs with frequency of 1/1000 per year. The FV Leiden, FII G20210A and MTHFR C677T mutations represent genetic factors for the occurrence of vein thrombosis. The goal of this study was to determine the frequency of these mutations in patients with DVT of upper and lower limbs. The study encompassed 119 patients divided in two groups. The group of patients with
the lower limbs thrombosis included 77 patients, while the upper limbs thrombosis group included 42 patients. The presence of FV Leiden, FII G20210A and MTHFR C677T mutations was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

In patients with DVT of lower limbs, the frequency of FV Leiden mutation was 26.0% in heterozygous form and 1.3% in homozygous form. In the group of patients with DVT of upper limbs, the frequency of heterozygous carriers was 7.2%. In patients with DVT of lower limbs, FII G20210A mutation occurred in heterozygous form in 15.6% subjects, and in the group with DVT of upper limbs the frequency was 7.2% in heterozygous and 2.3% in homozygous form. The frequency of MTHFR C677T mutation in patients with lower limbs DVT was 42.8% in heterozygous form and 13% in homozygous form, while in the group of patients with upper limbs DVT, the frequency was 52.4% in heterozygous form and 9.5% in homozygous form.

The FV Leiden and FII G20210A mutations represent significant risk factors for the occurrence of DVT of lower limbs. These mutations are less frequent in DVT of upper limbs and more extensive further studies are needed to determine their potential role. The MTHFR C677T mutation represents less significant risk factor for lower limb DVT and should be taken into account only in cases when it occurs in combination with other risk factors.

Key words: FV Leiden, FII G20210A, MTHFR C677T, risk factors, vein thrombosis, upper and lower limb

INTRODUCTION

Deep venous thrombosis (DVT) is a common disease with an annual incidence of 1 in 1000 individuals and represents leading cause of death in pregnancy, postpartum period and in surgical patients. The mechanisms of DVT formation are not completely elucidated, considering the fact that they are caused by joint effect of environmental and genetic risk factors (Bick, 2006).

Deep vein thrombosis (DVT) is a multifactorial disease which is predisposed by large number of acquired and genetic risk factors. Most common acquired risk factors are: pregnancy, surgery, trauma, long-term immobilization, immunization and malignancy (Bick, 2006). Since 1965, when the existence of familiar thrombosis has been shown, genetic risk factors have been extensively studied (Egeberg 1965).

A single point mutation (G→A) at nucleotide position 1691 in the factor V gene results in a mutated form of factor V is known as factor V Leiden (Chunilal and Bates, 2009). This mutation results in a replacement of arginine residue 506 with a glutamine at one of the factor V cleavage sites for activated protein C (APC). Mutated factor V is resistant to inactivation by APC (Bertina et al. 1994), resulting in increased thrombin generation and a hypercoagulable state. The prevalence of FV Leiden mutation in general population varies by 0% to 15% according to ethnicity
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The risk of venous thrombosis is increased by 3 to 8 fold if the heterozygous form of factor V Leiden mutation is present and 80 fold if factor V Leiden mutation is present in homozygous form (Rees et al. 1995). A common genetic variation (a guanine to adenine transition at position 20210) in the 3'-untranslated region of the prothrombin gene has been associated with elevated prothrombin blood levels and increased incidence of venous thrombosis (Zivelin et al. 1997). It is present in approximately 18% of patients with personal and familiar history of DVT and ~3% of healthy Caucasian population (Gadelha et al. 2010, Rosendaal et al. 1998). The presence of FII G20210A mutation is associated with 3 fold increased risk of deep venous thrombosis (Poort et al. 1996)

The mutation in the gene coding for 5, 10-methylenetetrahydrofolate reductase (MTHFR) is a cause of moderate hyperhomocysteinemia which has high prevalence in individuals with venous thrombosis. The genetic defect is a cytosine to thymine missense mutation at nucleotide 677, which substitutes a valine for an alanine residue (Chunilal and Bates 2009). The mutation in the heterozygous or homozygous state correlates with reduced enzyme activity and increased thermolability (Frosst et al. 1995). It is highly prevalent in general population, with 30-50% in heterozygous form and 5-20% in homozygous form (Frosst et al. 1995).

The aim of this study was to determine the prevalence of FV Leiden, FII G20210A and MTHFR C677T mutations in patients with upper and lower limb deep venous thrombosis. As this is the first study of this kind in Serbian population, the additional goal was to evaluate the importance of these mutations as risk factors for development of upper or lower limb deep venous thrombosis.

Materials and Methods

Our study included 119 patients (15-82 years, median 39 years). Patients were divided in two groups, depending on the localization of the thrombotic disorder. The group of patients with lower limb DVT consisted of 77 individuals (41 males and 36 females, 15-82 years, median 40 years), while the group of patient with upper limb DVT included 42 individuals (15 males and 27 females, 19-73 years, median 37 years). The study was approved by the hospital ethics committee.

Peripheral blood was taken on 3.8% Na-citrate as anticoagulant. Mutation detection was performed from the whole blood sample or purified genomic DNA. Genomic DNA was purified from 200µL of whole blood using QIAamp DNA blood mini kit (QIAGEN, Germany) according to manufacturer’s protocol.

The FV Leiden and FII G20210A mutations were detected by multiplex polymerase chain reaction (PCR) on whole blood or isolated DNA, followed by simultaneous digestion with specific restriction enzymes MnlI and HindIII (New England BioLabs) (Dordević et al. 2001). The fragment of the MTHFR gene that included nucleotide 677 was amplified by PCR using previously described primers (Frosst et al. 1995), followed by digestion with HinfI (New England BioLabs) endonuclease. Normal and mutated alleles were distinguished by the size of the
restriction fragments, using electrophoresis on 10% polyacrylamide gels and visualised by silver staining (RADOJKOVIC and KUSIC 2000).

Statistical analyses were carried out by using Statistical Package for Social Sciences 13.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The prevalence of each mutation was compared between patients and controls with the use of Fisher’s exact. Data for control group from Serbian population, which consisted of 120 healthy blood donors (84 males and 36 females), was previously published (DJORDJEVIC et al. 2004). The odds ratio (OR) and 95% CI (Confidence Interval) were estimated separately for each mutation. Value $p \leq 0.05$ was considered statistically significant.

RESULTS

The results of our study are shown in Table 1. Among the 77 patients with lower limb DVT, 20 patients (26%) were detected with FV Leiden mutation in heterozygous form and one patient (1.3%) was homozygous for this mutation. There were 12 carriers (15.6%) of FII G20210A mutation in heterozygous state. No homozygous carriers were detected. Thirty-three patients (42.8%) were heterozygous and 10 patients (13.3%) were homozygous for MTHFR C677T mutation. Seventeen patients were carriers of two mutations either in homozygous or heterozygous form, while one patient had all three analyzed mutation in heterozygous form. There is a statistically significant frequency increase of heterozygous carriers of FV Leiden and FII G20210A mutations in patients with lower limb ($p=0.0007$ and $p=0.02$, respectively). Carriers of FV Leiden mutation have 5.6-fold increased risk for development on DVT (95%CI 2.26-14.18) compared to healthy population, while carriers of FII G20210A have 4.25-fold risk increase (95%CI 1.43-12.59). The MTHFR C677T mutation showed increased frequency, but this increase was no statistical significance compared with controls. Patients heterozygous and homozygous for MTHFR C677T mutation have slightly increased risk for DVT development (by 1.16 and 1.13 fold, respectively).

In the 42 patients with upper limb DVT, 3 heterozygous carriers (7.2%) of FV Leiden were detected. Three patients (7.2%) carried FII G20210A mutation in heterozygous and one (2.3%) in homozygous form. MTHFR C677T mutation was detected in 22 patients (52.4%) in heterozygous form and 4 patients (9.5%) in homozygous form. Five patients carried two mutations in heterozygous and/or homozygous form. There is no statistically significant increase of analyzed mutations in the group of patients with upper limb DVT compared to controls (DJORDJEVIC et al. 2004). Carriers of FV Leiden exhibit 1.24-fold greater risk for DVT occurrence (95%CI 0.31-5.04), while carriers of FII G20210A and MTHFR C677T exhibit 1.77 and 1.71 fold increased risk, respectively (Table 1).

Comparative statistical analysis of mutation frequencies in patients with DVT of lower limb versus patients with DVT of upper limb determined that FV Leiden mutation represents 4.56 fold greater risk (95%CI 1.27-16.41) for development of lower limb DVT in homozygous form and 1.67 fold (95%CI 0.067-41.82) in heterozygous form. Carriers of FII G20210A mutation are 2.4 fold likely
to develop lower limb DVT rather than upper limb DVT (95% CI 0.64-9.04). Also, homozygous carriers of MTHFR C677T mutations would rather develop lower limb DVT than upper limb DVT (1.42-fold, 95% CI 0.42-4.83).

Table 1. Genotype frequencies of FV Leiden, FII G20210A and MTHFR C677T mutations in patients with upper and lower limb deep vein thrombosis

<table>
<thead>
<tr>
<th></th>
<th>FV Leiden</th>
<th>FII G20210A</th>
<th>MTHFR C677T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>heterozygous</td>
<td>homozygous</td>
<td>homozygous</td>
</tr>
<tr>
<td>LL DVT (n=77)</td>
<td>20 (26.0%)</td>
<td>1 (1.3%)</td>
<td>12 (15.6%)</td>
</tr>
<tr>
<td>UL DVT (n=42)</td>
<td>3 (7.2%)</td>
<td>/</td>
<td>3 (7.2%)</td>
</tr>
<tr>
<td>Control group (n=120)</td>
<td>7 (5.8%)</td>
<td>/</td>
<td>5 (4.2%)</td>
</tr>
</tbody>
</table>

P+: LL DVT compared to controls
OR+: OR compared to controls
95% CI+: 95% Confidence Interval compared to controls

DISCUSSION

In this study we examined the frequency of FV Leiden, FII G20210A and MTHFR C677T mutations in patients with DVT of upper and lower limb in Serbian population. We noted the increased frequency of FV Leiden, FII G20210A and MTHFR C677T mutations in study groups, which implies that these mutations could be potential risk factors for the occurrence of DVT.

All three examined mutations are more frequent in the group of patients with DVT of lower limb in relation to our healthy population. Statistically significant increase is present only in the case of FV Leiden and FII G20210A mutations, which is in accordance with published studies. The Almawi et al. study (ALMAWI et al. 2005) that included 198 patients has shown that the presence of FV Leiden and FII G20210A mutations increases the risk of DVT 4 to 7 fold, whereas the MTHFR C677T mutation does not represent a significant risk factor per se, except when it occurs combined with FV Leiden or FII G20210A mutations (ALMAWI 2005 et al.,
KOVAC et al. 2008, KOVAC et al. 2010). On the other hand, the Kreidy and Irani-Hakime study, performed on the group of 162 patients with diagnosed DVT of lower limb (KREIDY and IRANI-HAKIME, 2009) shows that the acquired risk factors like operations, age, increased body weight, hormone therapies and pregnancy are much more significant for the occurrence of disease. Only 25 patients in this study had the hereditary thrombophilia, of which 16 carried FV Leiden mutation, 8 carried the MTHFR C677T mutation while one was the FII G20210A mutation carrier. Although it was shown that the increased level of homocysteine in blood poses a serious threat for the occurrence of DVT (ELDIBANY and CAPRINI 2007), such correlation was not observed in the case of MTHFR C677T mutation. In the MEGA study (BEZEMER et al. 2007) that encompassed nearly 5000 patients, it has been shown that there is no link between MTHFR C677T mutation and the occurrence of DVT, unless there is also a lowered level of vitamin B.

DVT of upper limb accounts for about 14% of thrombotic disorders (SPENCER et al. 2007). It was described in 19th century by Paget and von Schroetter, who presumed that traumatic fissures in the intima, which develop as the consequence of physical strain, lead to the consecutive activation of coagulation cascade (ZELL et al. 2001). For the last several decades, due to the use of central venous catheter, bone marrow transplantation, dialysis and parenteral feeding which represent greatest risk factors, the frequency of DVT of upper limb has increased (JOFFE et al. 2002). Other additional risk factors include: malignity, the use of oral contraceptives and thrombophilia (BLOM et al. 2005). Unlike the DVT of lower limb, literature data about DVT of upper limb are scarce, whilst the link between the thrombotic disorder and the DVT of upper limb has not been completely elucidated.

Our study has shown that increased frequency of FV Leiden, FII G20210A and MTHFR C677T mutations in the patients with the DVT of upper limb, but with no statistically significant differences in relation to control group. According to literary data, the frequency of coagulational abnormalities in the upper limb DVT varies from 8-61% (HENDLER et al. 2004). In most of the studies, FV Leiden is emanated as a significant risk factor, while the data concerning FII G20210A and MTHFR C677T mutations is incomplete or contradictory. MARTINELLI et al. as well as FLINTERMAN et al. observed that FV Leiden and FII G20210A mutations increase the risk of DVT of upper limb 3-6 fold, while the hyperhomocysteinemia and the use of oral contraceptives are not related to the upper limb DVT(MARTINELLI et al. 2004, FLINTERMAN et al. 2008). On the other hand, VAYA et al. (VAYA et al. 2003) found FII G2010A mutation to be a significant risk factor for DVT of upper limb in women who use oral contraceptives. These contradictory results are most probably due to the small sample size, non-equal distribution of patients’ age, ethnic affiliation and different geographical prevalence of these mutations, as well as the presence of other acquired causes of DVT.

The comparative analysis of FV Leiden, FII G20210A and MTHFR C677T mutations frequency in the group of patients with DVT in upper and lower limb have shown that these mutations are a significant risk factors for the occurence of DVT of lower limb. One of the studies (LECHNER et al. 2008) has shown that the proportion
of FV Leiden carriers is significantly lower among those affected by DVT of upper limb compared to those affected by DVT of lower limb, while there was no significant difference in relation to the mutation in the prothrombin and MTHFR gene, which is in accordance with our results. Different frequency of these mutations in patients with DVT of both lower and upper limb implies the possibility of different pathophysiological mechanisms causing these two conditions. It has been reported that the patients with DVT of upper limb are younger and leaner compared to patients with DVT of legs. This observation is accordant with the findings that stress, age and body weigh are significant risk factors of DVT of lower limb (VAYA et al. 2003).

CONCLUSION

The FV Leiden and FII G20210A mutations represent significant risk factors for the occurrence of DVT of lower limb, while MTHFR C667T mutation is not a significant risk factor, but should be considered as an additional factor in case of occurrence along with other mutations and other risk factors for DVT. In cases of familiar thrombosis it is advised to perform routine testing for these mutations.

Based on our results, none of the examined mutations represent a statistically significant risk factor for the occurrence of upper limb DVT, but due to the increased frequency of these mutations in the group of patients and the contradictory literature data, it is needed to conduct the study that will include larger cohort of patients.

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MUTACIJE FV LEIDEN, FII G20210A I MTHFR C677T KAO FAKTORI RIZIKA ZA NASTANAK TROMBOZE DUBOKIH VENA GORNJIH I DONJIH EKSTREMITETA

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I z v o d

Tromboze dubokih vena (TDV) su multifaktorijalno oboljenje koje se javlja sa učestalošću 1/1000 stanovnika godišnje. Mutacije FV Leiden, FII G20210A i MTHFR C677T predstavljaju genetske faktoare za nastanak venskih tromboza. Cilj ove studije je da se utvrdi učestalost FV Leiden, FII G20210A i MTHFR C677T mutacija kod bolesnika sa TDV gornjih ili donjih ekstremiteta. Studija je obuhvatila 119 bolesnika podeljenih u dve grupe. Grupa bolesnika sa TDV donjih ekstremiteta brojila je 77, a grupa sa TDV gornjih ekstremiteta 42 bolesnika. Prisustvo FV Leiden, FII G20210A i MTHFR C677T mutacija dokazano je umnožavanjem odgovarajućeg fragmenta gena pomoću lančane reakcije umnožavanja polimerazom i digestijom dobijenih fragmenata restrikcionim enzimima (PCR-RFLP). Učestalost FV Leiden mutacije, u grupi bolesnika sa TDV donjih ekstremiteta, iznosila je 26,0% u heterozigotnom obliku i 1,3% u homozigotnom obliku. U grupi bolesnika sa TDV gornjih ekstremiteta, učestalost heterozigotnih nosilaca iznosila je 7,2%. Mutacija FII G20210A bila je prisutna kod 15,6% ispitnika u grupi bolesnika sa TDV donjih ekstremiteta u heterozigotnom obliku, dok je u grupi sa TDV gornjih ekstremiteta ova mutacija bila zastupljena sa 7,2% u heterozigotnom i 2,3% u homozigotnom obliku. Učestalost MTHFR C677T mutacije kod bolesnika sa TDV donjih ekstremiteta iznosila je 42,8% u heterozigotnom obliku i 13% u homozigotnom obliku, a kod TDV gornjih ekstremiteta 52,4% u heterozigotnom obliku i 9,5% u homozigotnom obliku. Mutacije FV Leiden i FII G20210A predstavljaju značajne faktoare rizika za nastanak TDV donjih ekstremiteta. Kod TDV gornjih ekstremiteta ove mutacije su manje zastupljene i potrebno je sprovedi dalja istraživanja koja obuhvataju veći broj bolesnika. MTHFR C677T mutacija je manje značajan faktor rizika i treba ga razmatrati samo u slučajevima kada se pojavljuje u kombinaciji sa drugim faktorima rizika.

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