Journal of Natural Sciences and Engineering, Vol. 2, (2020)
DOI number: 10.14706/JONSAE2020216

Distribution of inherited thrombophilia markers in Bosnian-Herzegovinian population: a review of previous studies

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***Abstract* – Thrombophilia is a condition that is associated with an individual's risk for venous or arterial thrombosis, as well as a risk of adverse pregnancy outcomes. Gene variants that are the most commonly associated with inherited thrombophilia are *F5* mutation 1691G>A (Factor V Leiden), *F2* 20210G>A (prothrombin mutation), *MTHFR* 677C>T, and PAI-1 variant 4G/5G. This paper aims to review currently available literature on the prevalence of heritable thrombophilia genetic markers and their association with thromboembolic events in Bosnia and Herzegovina. PubMed and PubMed Central databases of the National Center for Biotechnology Information (NCBI) and ResearchGate were searched to identify the most relevant studies. The results of the previously published studies show discrepancies when it comes to reported findings, thus implying that further research on this topic is necessary. It is suggested that new studies include greater sample size in order to confirm the correlation between the studied variants and conditions associated with heritable thrombophilia in the Bosnian-Herzegovinian population and to advance the understanding of these variants.**

Keywords - Bosnia and Herzegovina, Factor V Leiden, Inherited thrombophilia, MTHFR, PAI-1, Prothrombin mutation

1. Introduction

Thrombophilia is a condition putting an individual at risk of venous or arterial thrombosis and is mainly divided into hereditary and acquired. Hereditary thrombophilia is associated with genetic mutations influencing the level or activity of proteins involved in coagulation cascade and includes both loss-of-function and gain-of-function mutations [1,2]. Three genetic markers are the most commonly studied as indicators of inherited thrombophilia. *F5* gene variant 1691G>A, usually termed Factor V Leiden, gives rise to a peptide that is uncleavable by the activated protein C (APC) [3]. According to Inbal and Carp (2007), this mutation is responsible for 3-42% of pregnancy losses [4]. If present in homozygous state, this mutation increases a risk for venous thrombosis up to 50-100-fold. The second most studied variant is *F2* 20210G>A, also known as prothrombin mutation (PTM), that causes elevated levels of prothrombin and 2-5-fold increased risk of venous thrombosis as well as pregnancy loss [5]. *MTHFR* gene variant 677C>T codes for thermolabile variant of protein methylene tetrahydrofolate reductase which is a loss-of-function mutation and causes elevated levels of homocysteine in blood, the condition known as hyperhomocysteinemia, which is in turn considered a risk factor for venous thromboembolism [6-8]. The fourth variant indicative of inherited thrombophilia is 4G/5G in type 1 plasminogen activator inhibitor

(PAI-1) gene, which is a 4328G>T missense variant located 675 bp from the promoter that results in four or five guanine nucleotides in a row [9,10]. The protein product of this gene has a role in fibrinolysis and is associated with adverse pregnancy outcomes, such as intrauterine fetal death, intrauterine growth restriction, preeclampsia, recurrent miscarriage and placental abruption [11].

The aim of this paper is to provide a comprehensive review of the current knowledge regarding the prevalence of heritable thrombophilia markers and their correlation with thromboembolic events in Bosnia and Herzegovina (B&H) based on previously published population studies.

2. Methods

In order to investigate associations between the abovementioned genetic variants and inherited thrombophilia that may lead to adverse primary outcomes, National Center for Biotechnology Information (NCBI) databases PubMed and PubMed Central (PMC), and ResearchGate were searched to discover relevant studies published previously. Included were original research papers published in peer-reviewed journals that matched the search of the following keywords: "Factor V Leiden", "*F5* 1691G>A", "prothrombin", "*F2* 20210G>A", "*MTHFR* 677C>T", "*PAI-1* 4G/5G", "polymorphism", "thrombophilia", "Bosnia and Herzegovina". In order to enhance the search, Boolean "AND" and "OR" operators were used to investigate the association between two searched terms.

3. Prevalence of genetic markers of inherited thrombophilia in Bosnian-Herzegovinian population

The search results offered a total of seven studies related to the topic of the prevalence of genetic markers of inherited thrombophilia in B&H, all of them being conducted as of 2013.

The study of Karić and colleagues in 2013 was the first study which deals with prevalence of *MTHFR* 677C>T polymorphism in Bosnian-Herzegovinian population. They studied 102 men and 105 women, who were unrelated and healthy and originating from south-east B&H. At the time of blood sampling, the study participants ranged between 18 and 84 years with a mean age of 45.62 years. The results have shown that 44.44% were heterozygous and 11.11% were homozygous for the study allele. No significant difference was found in allele and genotype frequencies between male and female participants [12].

The first study aiming at analyzing Factor V Leiden prevalence in B&H was performed in 2013 by Valjevac and colleagues. A group of 67 women with mean age of 58.6 years (range 41 to 75 years) with no previous history of cardiovascular diseases and pregnancy loss was recruited and tested. The study failed to find any mutant allele, thus suggesting that, considering functional importance of this allele, there is a need to conduct more research on that topic, as the results of this study were heavily influenced with the small sample size [13].

The prevalence of polymorphisms Factor V Leiden, prothrombin mutation and *MTHFR* 677C>T was studied by Adler and colleagues (2014). The study involved a cohort of 100 healthy unrelated individuals from B&H (82 females and 18 males) with the year range of 24-82 years and the mean of 58.8 years. The analyzed loci were in Hardy-Weinberg equilibrium with the following minor allele frequencies (MAF): 6% for Factor V Leiden, 6% for *F2* 20210A allele, and 37.5% for *MTHFR* 677T allele. The authors noted the drawback of a small sample size and imbalanced sex ratio. The study, however, demonstrated the co-inheritance of thrombophilia markers, since nine participants had Factor V Leiden and *MTHFR* 677C>T mutant. Compared to 17 European countries, the prevalence of Factor V Leiden and *F2* 20210G>A variants was significantly higher in B&H [14].

Another study examined the prevalence of the same three variants and their association with deep venous thrombosis (DVT) in B&H [15]. The study group included 111 thromboembolic patients (59 females and 52 males ranging from 21 to 84 years) and 207 healthy controls (105 females and 102 males ranging from 18 to 84 years) with no history of venous thromboembolism (VTE). When it comes to Factor V Leiden prevalence, 18% of the study group patients were heterozygous and 2.7% were homozygous, while 3.86% of the control group participants were heterozygous for this variant, which a statistically significant difference between groups. Statistically significant difference was also found between men with DVT and the control group, as well as between women with DVT and the control women group. *F2* 20210G>A variant was detected in 2.7% of the study group patients and was absent in control group, which was not statistically significant. Frequencies of *MTHFR* C677T alleles and genotypes did not differ significantly between the two groups. Allele frequency and functional significance of Factor V Leiden variant detected in this study was in agreement with earlier studies in Caucasians [16-20]. Also, the results of this study were found consistent with the data from other neighboring countries [21-23]. The absence of functional significance of the studied *MTHFR* polymorphism was also in line with previously published literature [4,16,24,25]. Finally, the authors of this study found that 14.9% of the patients from the DVT group were compound heterozygotes for Factor V Leiden and *MTHFR* 677C>T variants, therefore proposing further studies that would aim to analyze whether such genotype combination might be a risk factor for DVT development [15].

A study of Mahmutbegović and colleagues (2017) enrolled 308 women, including 154 women who experienced pregnancy loss as the study group (mean age 33 ± 5.4 years) and 154 women with at least one live-born child and without pregnancy loss as the control group (mean age 31.4 ± 6.7 years) to investigate the correlation between three genetic markers and pregnancy loss. Detected allele frequencies were 3.9% in both study and control groups for Factor V Leiden, 1.9% and 1.6% in the study and control groups, respectively, for prothrombin mutation, and 35.7% and 29.9% in the study and control groups, respectively, for *MTHFR* 677C>T. Although allele frequencies obtained in this study were in accordance with allele frequencies obtained for other European countries, the authors, however, were not able to find the significant correlation between these three variants and pregnancy loss in Bosnian-Herzegovinian women, which may be due to small sample size of women with three or four pregnancy losses recorded [26].

The prevalence of Factor V Leiden, prothrombin mutation, *MTHFR* 677C>T and PAI-1 4G/5G in Bosnian-Herzegovinian women and their correlation with recurrent pregnancy loss was studied by Jusić and colleagues (2018). The study group included 60 women with two or more consecutive pregnancy losses that occurred before 20th gestation week with the same partner and without history of known causes of pregnancy losses due to chromosomal abnormalities, chronic diseases, or infections. The control group was consisting of 80 women with one or more successful pregnancy outcomes and without any pregnancy complication which could lead to the pregnancy loss. The results have shown that Factor V Leiden and *MTHFR* 677C>T were proved to correlate with recurrent pregnancy loss, while prothrombin mutation and PAI-1 4G/5G were not found to be significantly associated with the pregnancy loss. Reported allele frequencies were as follows: 7.5% for study and 1.88% for control group for Factor V Leiden, 2.5% for study and 0.63% for control group for prothrombin mutation, 39.17% for cases and 25% for control group for *MTHFR* 677C>T, and 30% for study and 20% control group for PAI-1 4G/5G. The authors are reporting allele frequencies that were in agreement with previous findings for all study polymorphisms. However, the role of genetic factors for inherited thrombophilia in recurrent pregnancy loss is still a matter of debate, especially when it comes to *MTHFR* and PAI-1 variants studied. Therefore, the authors are suggesting further studies with larger study and control groups, as well as the need to prevent recurrent pregnancy loss by assessing the status of these variants and calculating individual risk and optimum therapy for each patient [27].

In the most recent study by Ašić and colleagues in 2019, the prevalence of common thrombophilia markers was studied in a population of 130 unrelated healthy Bosnian- Herzegovinians of both sexes, from different age groups, with no recorded history of thrombotic events and originating from different parts of the country. Seven markers most commonly associated with the risk of heritable thrombophilia were investigated, namely Factor V Leiden, *F2* 20210G>A, *MTHFR* 677C>T, *MTHFR* 1286A>C, PAI-1 4G/5G, PAI-1 -844G>A and *F13* V35L. Whereas some of these markers were examined in previous studies as described above, this is the first study to include *MTHFR* 1286A>C, PAI-1 -844G>A and *F13* V35L polymorphisms in the population of B&H. The results have shown that the two main thrombophilia markers Factor V Leiden and prothrombin mutation appeared with MAF values of 0.023 and 0.008 respectively. For the remaining four loci, reported MAF values were 0.331 for *MTHFR* 677C>T, 0.323 for *MTHFR* 1286A>C, 0.446 for PAI-1 4G/5G, 0.588 for PAI-1 -844G>A, and 0.315 for *F13* V35L. This study provides the most extensive population data on the prevalence of main heritable thrombophilia risk factors in B&H and reported allele frequencies were consistent with those reported for other European populations [28].

4. Conclusion

Previously conducted studies in B&H represent a small and rather heterogenous group of studies, including either population studies or case-control studies with the aims to report heritable thrombophilia marker prevalence in the population or their potential association with DVT, pregnancy loss or recurrent pregnancy loss. While initial preliminary studies offered surprisingly low or high MAF values for the most well-known genetic variants Factor V Leiden and *F2* 20210G>A, later studies reported allele and genotype prevalence

that is in line with reported data for most European populations. While DVT was found to be positively associated with Factor V Leiden variants [15], the data for obstetric complications are more controversial since two studies reported conflicting results. The first study reported no statistically significant association between Factor V Leiden, *F2* 20210G>A and *MTHFR* 677C>T and pregnancy loss [26], while the second one reported a significant increase in mutant allele frequency for Factor V Leiden and *MTHFR* 677C>T in women with recurrent pregnancy loss [27]. Therefore, it is strongly suggested that further studies assess the functional importance of the most important markers for inherited thrombophilias in B&H by clearly defining study and control groups in order to assess the potential association of these variants with conditions such as (recurrent) pregnancy loss and venous thromboembolism with the goal of assessing the importance of genetic testing in Bosnian-Herzegovinian population and establishing the groundwork for the personalized treatment of inherited thrombophilia and conditions connected to it. In that effort, larger study cohorts including individuals from all parts of the country will be necessary.

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