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FREQUENCY OF VENOUS THROMBOSIS RELATED DNA POLYMORPHISMS IN A HEALTHY TURKISH POPULATION

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ABSTRACT

Environmental and genetic factors play important role for the development of venous thromboembolic diseases. This study investigated the prevalence coagulation factor V Leiden (FVL) and factor II G20210A (FII) in a sample (N=96) of the Turkish population depending on age and weight. Participants were stratified into two age groups: 20-39 years, 40-59 years and their body mass index: normal (<24.9), obese (>25). The heterozygous FII genotype was identified in 2 (8.33%) of normal donors in 20-30 years while it was found in 1 (4.16%) of obese donors in 40-59 years. The mutant homozygous FVL genotype was found in 1 (4.16%) of obese donors in 20-30 years. The distribution of allele and genotype frequencies of FVL and FII polymorphisms did not differ significantly between normal and obese groups. Also, the frequencies of carriage rates of FVL and FII polymorphisms in 20-39 and 40-59 years were similar. In our study, observed FII allelic frequencies are higher than other healthy populations whereas the prevalence of factor V Leiden gene polymorphism was found to be consistent. Knowledge of the prevalence of these coagulation factor variations in a given population may contribute to the design of effective preventive measures against venous thrombosis.

KEYWORDS:
Factor II, Factor V Leiden, Healthy Population, Polymorphism, Venous thrombosis

INTRODUCTION

Obesity is a serious health and social problem that leads to haemostatic system and thrombosis-related diseases. Unfortunately, the number of obese adults have increased significantly over the last 25 years across all racial/ethnic and age groups [1]. Aging is very complex and multifactorial process that progresses to the gradual deterioration in normal function and leads to degenerative diseases and death [2]. Previous studies have shown that aging is one of the strongest and most prevalent risk factor for venous thrombosis [3]. But, venous thromboembolism is a multicausal disease as consequence of environmental and genetic risk factors [4]. Factor V Leiden (FVL) G1691A and factor II G20210A are the most common inherited factors for thrombosis. Single nucleotide substitution G1691A in factor V gene leads to an amino acid substitution, therefore, active protein C can't neutralize activated factor Va which acts as a cofactor in the conversion prothrombin to thrombin [5]. Based on venous thrombosis patients' data, it has been suggested that heterozygous FVL carriers have 7 fold higher risk of deep venous thrombosis [6]. Factor II G2021A is the second most common mutation after Factor V Leiden mutation. This mutation is associated with the increase of plasma prothrombin quantity and resulting from the substitution of guanidine adenine at the 20210 locus in the untranslated 3' region of the FII gene [7]. Studies reported that heterozygous FII carriers have 3 fold higher risk of deep venous thrombosis.

Most of the genetic factors are associated with venous thrombosis in selected patient groups, but the relationship between genetic and environmental risk factors still is not fully understood in healthy population. Therefore, in this study, we investigated FII G20210A and Factor V Leiden genes related to venous thrombosis diseases in a healthy Turkish population for different age and weight and compared the frequencies of these coagulation factor variations with other populations.

MATERIALS AND METHODS

Experimental design. This prospective study was carried out in the Regional Blood Center of Cukurova University. The study's protocol was approved by the Human Subject Ethics Committee of the Cukurova University (2016, 24) and was conducted in accordance with Helsinki Declaration. Peripheral blood was collected in disodium EDTA

from 96 male Caucasian healthy blood donors living in Cukurova Region. Participants were stratified into two age groups: 20-39 years, 40-59 years and their body mass index: normal (<24.9), obese (>25).

Molecular Assays. Genomic DNA was isolated from buffy coat using Qiagen DNA extraction kit. The Factor II G20210A and Factor V Leiden genotypes were determined by Real Time PCR (Roche, Cobas Z 480) using commercial kits from TIB, MOLBIOL, LightMix (CN: 40-0593-64, 40-0594-64).

Data analysis. The differences in genotype were analysed statistically using Fischer's exact test. The associations between allele frequencies in all groups were evaluated by computing the odds ratios (OR) and their confidence intervals using Hardy-Weinberg equilibrium. P<0.05 was considered statistically as significant.

RESULTS AND DISCUSSION

All donors of obese in 20-39 age group and normal in 40-59 age group were found to have homozygous for FII G20210A while 2 (8.33%) of normal donors in 20-39 age group and 1 (4.16%) of obese donors in 40-59 age group were found to have heterozygous (TABLE 1-2). Cardiovascular diseases represented by 30% of global deaths in 2008 and still remain the most important cause of morbidity and mortality in worldwide. In the studies conducted, it was observed that more than 80% of cardiovascular disease-related deaths occurred in low- and middle-income countries, and the incidence was similar in males and females. The polymorphisms in coagulation factors are among the unchangeable risk factors of cardiovascular diseases [8]. Previous studies with healthy populations in different regions reported that, the prevalence of factor II G20210A was 0.6-2.4% in North Europe, 1.1-4.0% in South Europe, 0.7-2.5% in North America, 0.0-3.7% in Africa, 1.3-2.0% in Hispanic, 0.0-1.8% in West Asia, 6.8% in Chilean native, 0.0% in East Asia and 0.0% in Indians of Malesia [9-20]. In this study, results showed that the prevalence of heterozygous prothrombin factor II G20210A genotype was higher than other populations but it was not related to the age and body mass index (p=0.361 between normal and obese donors in 20-39 years, p=1 between normal and obese donors in 40-59 years). Higher prevalence of heterozygous may be due to differences in the genetic background due to heavy/fatty dietary habits and environmental factors as well as the constant migration status.

TABLE 1
Genotype frequencies of FII G20210A gene in normal and obese donors of two age groups

Genotype	Age Groups			
	20-39 (n/%)		40-59 (n/%)	
	Normal Donors	Obese Donors	Normal Donors	Obese Donors
GG	22 (91.66%)	24 (100%)	24 (100%)	23 (95.83%)
GA	2 (8.33%)	-	-	1 (4.16%)
AA	-	-	-	-

TABLE 2
Allele frequencies in peripheral blood FII G20210A gene in normal and obese donors of two age groups

Allele	Age Groups			
	20-39 (n/%)		40-59 (n/%)	
	Normal Donors	Obese Donors	Normal Donors	Obese Donors
G	46 (95.83%)	48 (100%)	46 (95.83%)	46 (95.83%)
A	2 (4.16%)	-	2 (4.16%)	2 (4.16%)
Odds Ratio	*0.191*		*0.416*	

TABLE 3
Genotype frequencies of FVL G1691A gene in normal and obese donors of two age groups

Genotype	Age Groups			
	20-39 (n/%)		40-59 (n/%)	
	Normal Donors	Obese Donors	Normal Donors	Obese Donors
GG	22 (91.66%)	23 (95.83%)	22 (91.66%)	22 (91.66%)
GA	2 (8.33%)	-	2 (8.33%)	2 (8.33%)
AA	-	1 (4.16%)	-	-

TABLE 4
Allele frequencies in peripheral blood FVL G1691A gene in normal and obese donors of two age groups

	Age Groups			
	20-39 (n/%)		40-59 (n/%)	
Allele	Normal Donors	Obese Donors	Normal Donors	Obese Donors
G	46 (95.83%)	46 (95.83%)	46 (95.83%)	46 (95.83%)
A	2 (4.16%)	2 (4.16%)	2 (4.16%)	2 (4.16%)
Odds Ratio	*1.0*		*1.0*	

The most common genetic defect of venous thrombosis is Factor V Leiden G1691A polymorphism. In this study, the heterozygous and mutant homozygous FVL genotypes were identified in 2 (8.33%) of normal and 1 (4.16%) of obese donors in the 20-39 age group. In 40-59 age group, the heterozygous FVL were found in 2 (8.33%) of normal and 2 (8.33%) of obese donors (TABLE 3-4). The prevalence of heterozygous FVL G1691 in the Aegean region of Turkey was declared as 7.3% in healthy population [21]. The prevalence of the FVL carriers in different regions were reported to be 1.1-7.3% in North Europe, 0.6-5.1% in Central Europe, 0.9-4.0% in South Europe, 2.7-3.0% in North America, 1.0-10.2% in Africa, 0.4-1.4% in Hispanic and 2.1- 3.8, 2.7% in Australia, 5.5% in Chilean residents, 1.9% in Northern India and 5.5% in South India [22-31]. In our study, the prevalence of heterozygous FVL was found to be consistent with the reported studies. However, it is understood that is not depending on age and body mass index (p=0.244 between normal and obese donors in 20-39 years, p=1 between normal and obese donors in 40-59 years). Factor V Leiden mutation is estimated to have occurred in the vicinity of Anatolia approximately 15.000 to 30.000 years ago [33]. This explains the geographic distribution of this polymorphism on the general population and the high prevalence rate in our region.

CONCLUSION

In this study, the prevalence of factor II G20210A was higher than other healthy populations, whereas the prevalence of FVL was found consistent. The genetic structure of the Turkish population is complicated by the demographic history and the reason for its location in Central Asia, Europe and the Middle East. There are differences between the genetic of Turkish and European populations, which contribute to the explanation of the health problems and the pharmacogenetic differences observed in Turkish populations living in Europe. Also, comprehensive knowledge about the lifestyle and eating habits of the Turkish population will strengthen understanding of the relationship between environmental and genetic risk factor of cardiovascular diseases. A comparison of genotype frequencies among healthy populations at different age groups is a useful strategy for evaluating polymorphisms associated with vascular diseases. In addition, having knowledge of the prevalence of these coagulation factor variations in a given population may contribute to the design of effective preventive measures against venous thrombosis.

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