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Frequency of factor V Leiden (G1691A) and prothrombin (G20210A) polymorphisms in Population of Kerman Province, Iran

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Abstract

Background: Thromboembolism is an acute cardiovascular disease that ranges from clinically unimportant to massive embolism. Both acquired and hereditary risk factors contribute to the disease. We aimed to determine the prevalence of two hereditary predisposing factor of the disease, prothrombin G20210A and factor V Leiden (G1691A) polymorphisms, in Kerman population.

Methods: factor V and factor II genes of 112 healthy individuals were examined to detect factor V Leiden (G1691A) and prothrombin G20210A variants. Genomic DNA of subjects was isolated from leukocytes of the whole blood using salt-saturation method. We used amplification refractory mutation system technique to find G1691A and G20210A variations.

Results: We found two subjects with prothrombin G20210A mutation and three individuals with factor V Leiden variant, both in heterozygote state. The frequency of the polymorphisms were 1.79 and 2.68, respectively. No homozygote or compound heterozygote individual was detected for these two variants in this study.

Conclusion: our findings about the polymorphisms frequency were different from what was detected in other provinces of Iran or in some region of neighboring countries. This discrepancy of the variant frequency can be explained by gene flow phenomenon.

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Introduction

Venous thromboembolism (VTE) is one of the most common cardiovascular diseases that includes deep vein thrombosis and pulmonary embolism. The disease, also called thrombophilia, results from hereditary and acquired risk factors. Factor V Leiden (FVL) and prothrombin G20210A variants, as hereditary risk factors, may result in VTE events (1, 2). The polymorphisms are used as molecular biomarkers in the evaluation of hereditary venous thromboembolism (3).

Factor V Leiden, a well-known genetic variation, is a single point mutation at position 1691 that cause substitution of glutamine to arginine (G1691A or R506Q) (4). The variant is inherited in an autosomal dominant pattern with incomplete penetrance and Leiden is a city in the Netherlands where FVL was first identified. Normally, factor V gene encodes a protein

called coagulation factor V that functions as a cofactor to enhance the activation of prothrombin to thrombin. The glutamine to arginine change causes resistance of factor V to the inhibitory action of activated protein C (APC). This yield subsequent overproduction of thrombin that leads to generation of excess fibrin and formation of a clot (5, 6).

The second hereditary risk factor is a point mutation (G20210A) at the 3'UTR region of prothrombin gene result in a moderate liability for thrombosis. This gain of function mutation cause overexpression of the gene that consequently increases plasma level of prothrombin (7). The prothrombin acts as a clotting factor that is enzymatically cleaved to thrombin by activated factor X (FXa). Thrombin converts soluble fibrinogen into insoluble strands of fibrin.

Different ethnic groups have various prevalence of prothrombin G20210A and FVL mutations. The majority of Iranian population is ethnically Persian, but sizeable minorities are from various ethnic, religious, and linguistic backgrounds. Determining the patterns of the two polymorphism prevalence in different ethnic groups provides valuable information about population genetic background, gene pool, and individual specific biomarkers (8).

The aim of this study was to detect frequency of the two coagulation factor variants in healthy individuals for the first time in Kerman province of Islamic Republic of Iran. Our results will be compared with such other studies to find the possible frequency differences among populations with various ethnicity background.

Materials and methods

In the present work, one hundred and twelve healthy unrelated individuals including 59 men and 53 women from Kerman province of the Islamic Republic of Iran were studied to find the frequency of prothrombin G20210A and FVL polymorphisms. The blood donor subjects were medical students, hospital employees, and healthy volunteers who come to hospital for other reasons than disease.

A genetic counsellor explained the objectives and aims of the study to participants. A written consent for molecular study was obtained from all subjects and 5 ml whole blood from of their brachial vein was collected in tubes containing 200 µl EDTA (Ethylene Diamine Tetra-acetic Acid).

Genomic DNA was isolated from leukocytes of the whole blood using salt-saturation method (9). The quality and quantity of extracted DNA checked on agarose gel for all the samples. Briefly in this procedure, the whole blood sample is washed three times with cold water in order to lyses and discards the red blood cells. Then, the remaining white blood cells are incubated at 37^{o}C at the presence of proteinase K that denatures proteins. Then, saturated NaCl is added to separate unwanted parts. Finally, the upper phase is centrifuged at the presence of isopropanol and the remaining sediment DNA is dissolved in elusion buffer or deionized water. The quality and quantity of extracted DNA checked on agarose gel for all the samples.

Genome of the subjects were screened for the two identified variants by amplification refractory mutations system-polymerase chain reaction (ARMS-PCR) method (10). Under the ARMS-PCR condition, DNA amplification reaction was carried out using sequences of normal and mutant primers as described by Ranguelov et al (11). A β-globin gene fragment was amplified as control band in each test tube using a forward primer (5`- CAA TGT ATC ATG CCT CTT TGC ACC -3`) and a reverse primer (5`- GAG

TCA AGG CTG AGA AGA TGC AGG A -3`). In order to confirm the accuracy of tests, DNA sample of subjects with diagnosed FVL and G20210A were sequenced.

Results

In this study, the genotype and allele frequencies of prothrombin G20210A and FVL variants were studied in Kerman province using DNA sample of 112 healthy individuals. The average age of the studied subjects was 28.03 ± 5.78 years with a median age of 26 years and individuals with a history of known systemic diseases were excluded from the study.

We obtained a 340bp and a 152bp PCR products in each wild type and/or mutant test tubes during amplification of factor II and factor V genes, respectively. An 861bp control band was found in each test tube from PCR product of β-globin gene (figure 1).

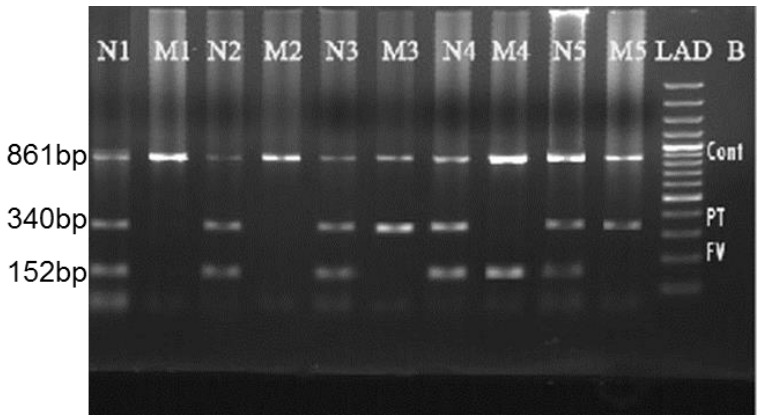

Figure 1. Gel electrophorese analysis of ARMS-PCR products. N; wild type bands, M; mutant bands. Cont; 861bp control band, Lad; ladder, B; control test tube without DNA, PT; 340bp Prothrombin band, FV; 152bp Factor V band. N1M1 and N2M2 columns show normal sample for the variants. N3M3 and N5M5 columns show heterozygous for prothrombin G20210A. N4M4 show heterozygous for FVL.

In the overall investigated samples, we found two subjects with G20210A prothrombin mutation in heterozygote state (Table 1). The genotype and allele frequencies of this mutation were 1.79 and 0.009, respectively. Our results showed three individuals who carry the FVL variant, giving the genotype frequency of 2.68 and allele frequency of 0.13 (Table 2).

Based on the obtained allele frequencies, we expect the frequency of 0.000081 to have G20210A prothrombin mutation in homozygote state (AA). This homozygote genotype frequency (AA) is believed to be 0.0169 for FVL, while the frequency of compound heterozygote inheritance of the two variants will be 0.00117.

Table 1. Genotype prevalence of prothrombin G20210A and FVL

Gene	Prothrombin	Factor V
Genotype	Number (%)	Number (%)
GG	110 (98.21)	109 (97.32)
AG	2 (1.79)	3 (2.68)
AA	0	0
Total	112 (100)	112 (100)

Table 2. Allele frequency of prothrombin G20210A and FVL

Gene	Prothrombin	Factor V
Allele	Number (frequency)	Number (frequency)
G	222 (0.991)	221 (0.987)
A	2 (0.009)	3 (0.13)
Total	224 (1)	224 (1)

Discussion

Heterozygosity of FVL increases fourfold to eightfold the chance of hyper-coagulation, but homozygous people may have up to 80 times the usual risk of developing a clot (12). We found according to Hardy-Weinberg law, the chance of homozygote genotype (AA) for FVL is 0.0169 in Kerman province. It was reported that most heterozygotes of prothrombin G20210A mutation never develop VTE in their lifetimes (13). Because of the more frequency of the factor V Leiden than prothrombin G20210A carriers in Kerman province, heterozygotes and homozygotes have a slightly increased risk for venous thrombosis in this region.

The most common hereditary hypercoagulability disorder amongst ethnic Europeans is FVL (14-16). About 5 percent of Caucasians in North America have FVL. This variant is less common in Latin Americans and African-Americans, while it is extremely rare in people of Asian descent.

The frequency of FVL and prothrombin G20210A mutations have previously been studied in Kermanshah, Fars, and Western Azerbaijan (Urmia) (17-19) provinces of Iran.

Our results showed Kerman has the lowest frequency of prothrombin G20210A among these provinces, while Western Azerbaijan has the highest (Table 3). Different frequency of G20210A mutation in this area can be explained by dissimilar ethnic background of their populations.

Fars province has reported the highest carriage rate of FVL among the provinces, while Kermanshah announced the lowest in their study. Decreasing of the mutation frequency from Fars to Kermanshah may reflect the gene flow phenomenon.

Also, carriage rate for each variant of factor V Leiden (FVL) and FII genes constituted 2.96% in Saudi Arabia (٢٠), while these values were at 6% and 2% among Iraqi people, respectively (٢١). High prevalence of FVL (6.42%) was found in Kuwait (٢٢), the neighbor of Saudi Arabia and Iraq, but it was at a lower rate (1-3%) in Pakistan (2٣). It was not surprising to find a similar prevalence of the FVL in Pakistan and Kerman, since this country is closer to Kerman than Iraq or other Arabic countries. But it seems unexpected that turkey had the highest prevalence of FVL (19%) and FII (5.5%) among neighbors of the Islamic Republic of Iran (24).

We conclude the frequencies of the polymorphisms reported here are different from those found in other part of the world and even other Iranian provinces. Reporting a number of these mutations in the neighboring countries and provinces can be explained by gene flow phenomenon.

Study the frequency of prothrombin G20210A and FVL variants in Kerman Province was done on a limited samples. Extension of the research with more subjects in future studies must be considered.

Table 3. Genotype prevalence of prothrombin G20210A and factor V Leiden in different provinces of Islamic Republic of Iran

Province	Kerman		Fars(19)		Kermanshah (18)		Western Azerbaijan (17)	
Genotype	FII%	F V%	FII %	FV%	FII %	FV%	FII %	FV%
GG	98.21	97.32	96.93	95.9	97	98.4	95.8	-
AG	1.79	2.68	3.07	4.1	2.7	1.6	4.2	-
AA	0	0	0	0	0.3	0	0	-
Total	100%	100%	100%	100%	100%	100%	100%	100%

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Ethical Consideration

The study was approved by Kerman University of Medical Sciences board of ethics. The ethical code is: IR.KMU.REC.1391.346

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