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Brief Report

Frequency of Factor V Leiden and Prothrombin Polymorphism in South of Iran

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Abstract

Normal hemostasis requires balanced regulation of prothrombotic and antithrombotic factors. Inherited alteration of factor V and prothrombin gene, the G20210A mutation, increases the resistance of factor V to degradation and booster production of prothrombin respectively. These alterations can increase hypercoagulability leading to thrombotic consequences. We aimed to assess the frequencies of these mutations in a group of the population of southern Iran. In total, 198 healthy volunteers with the age range of 1-64 years were selected and screened for factor V Leiden and prothrombin mutations using polymerase chain reaction and restriction fragment length polymorphism techniques. The carrier frequencies for factor V Leiden and prothrombin mutation in the studied cohort were 4.1% and 3.07%, respectively.

In the studied area, the allele frequency of factor V is higher than the prothrombin G20210A mutation (0.0204 *v* 0.0153). According to the data and Hardy-Weinberger equation, the total risk of thrombosis caused by homozygosity and heterozygosity of factor V Leiden, prothrombin G20210A mutation and compound heterozygosity of these mutations are about 1 in 500 individuals.

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Keywords ● Factor V Leiden ● thrombosis ● mutation ● prothrombin

Introduction

Balanced regulation between prothrombotic and antithrombotic factors is essential for the normal hemostasis.

Several inherited and acquired factors can alter these systems and lead to thrombosis.

Thrombosis can proceed with complications such as myocardial infarction, stroke, venous thromboembolism, preeclampsia, and abortion. It contributes significantly to morbidity and mortality. Susceptibility to thrombosis is conferred by both genetic and environmental factors.

Several genetic factors have been identified to be associated with an increased risk of venous thrombosis. The most frequent inherited risk factor is the G1691A mutation in the factor V gene (factor V Leiden).

Mutation in factor V Leiden is inherited as an autosomal dominant trait and results in substitution of aminoacid glutamine for arginine (Arg506Gln). The mutation makes factor V more resistant to proteolytic degradation by activated protein C (APC-R).[1,2] Factor V Leiden prolongs the activity of factor V_a in the prothrombinase complex (factors II, Va, Xa, phospholipid,

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and calcium) leading to increased thrombin formation (IIa). Functional resistance to activated protein C is found in 20% to 60% of patients with thrombophilia, of whom more than 90% are caused by the mutation of factor V Leiden.[3,4]

The second most common cause of familial thrombophilia is a mutation in the 3' untranslated region of the prothrombin gene (G20210A). This mutation leads to an elevated level of prothrombin because of increased synthesis but does not lead to an altered aminoacid sequence or altered function.[5]

The prevalence of the heterozygous G20210A mutation is 1% to 2% in general population, whereas this mutation in patients with a history of venous thromboembolism has been reported as 5% to 18%.[6] The frequency of each mutation in different ethnic groups with variable consanguinity and origin of mutation can be varying.

We aimed to assess the frequencies of mutations in factor V Leiden and prothrombin G20210A genes and evaluate the distribution of these mutations in a group of population in southern Iran as risk factors for thrombosis.

Patients and Methods

Having considered the prevalence of 5.5% for factor V Leiden in a study from central Iran,[7] and *confidence interval* = 95%, the sample size was estimated as 200.

Totally 198 volunteers from the healthy individuals referred to Hematology Research Center (affiliated to Shiraz University of Medical Sciences) for routine check-up were selected. Written informed consents were obtained from all the participants and the parents of children below 15.

The mean age of the cohort was 29.6 years (ranged from 1 year to 64 years and male to female ratio was 100/98 (table 1).

Table1: Frequency of age and sex distribution of 198 healthy volunteers

Age (year)	Number	Male/Female
1-10	45	23/22
11-20	40	21/19
21-30	42	20/22
31-40	31	17/14
>40	40	19/21
Total	198	100/98

The study population was mainly referred from Fars province (85%) and the remaining 15% were from the closed provinces including Khozestan, Hormozgan, Kohkeloye-Boyer Ahmad, Boshehr, and Lorestan. All the provinces are located in south of Iran.

Included individuals had no history of any systematic diseases such as myocardial infarction or cerebrovascular accident.

Exclusion criterion was positive history of any thromboembolic event.

Genomic DNA extraction was performed following the protocol described by Miller and co-workers.[8]

Polymerase chain reaction (PCR) amplification was done in 25 μl reaction volume containing 0.5 units Taq polymerase, 200 μM dNTP, 500 μM of each of previously described primers. PCR product digestion was carried out by 10 μl of product with MNII or Mbo restriction digestion enzymes [restriction fragment length polymorphism (RFLP) technique] and the results were analyzed by Agarose gel electrophoresis containing Ethidium Bromide.

Results

Genomic DNA analysis confirmed the mutation of factor V Leiden in eight (4.1%) individuals. The estimated gene frequency for factor V Leiden was 0.020. The 95% confidential interval (95%CI) for carrier frequency at the studied area is 4.00–4.07%. The calculated allele frequency for factor V Leiden varies from 0.0204 to 0.0207.

The G20210A mutation at the prothrombin gene was seen in six individuals (3.07%) as heterozygous form. The estimated allele frequency for the G20210A mutation was 0.015. The 95%CI for carrier frequency of this polymorphism at the region is 3.00 – 3.05%. The calculated allele frequency for G20210A is 0.0153 to 0.0155.

No homozygosis was seen for both mutations. Only one individual presented a double heterozygosis for factor V and prothrombin in this study.

According to the data and Hardy-Weinberger equation, about 1 in 500 individuals are at the risk of thrombosis as a result of homozygote state of factor V Leiden and prothrombin G20210A, and compound heterozygosity of these two mutations.

Discussion

The available reports about the role of factor V Leiden and PG20210A polymorphism in the pathogenesis of ischemic diseases provide conflict results. Individuals carrying the factor V Leiden allele have a 3- to 5-fold higher risk of developing thromboembolic diseases, and

homozygous individuals have 50-to 80-fold increased thrombosis risk.[9,10]

According to the present study, in the population of southern Iran, the carrier frequencies of factor V Leiden and G20210 were about 4.1% and 3.07% respectively. Based on Hardy-Weinberger equation, the expected homozygosis for factor V Leiden, prothrombin G20210A and compound heterozygosis of factor V Leiden and G20210A mutation were 0.00041, 0.00023, and 0.0003 respectively. The compound heterozygosis for factor V Leiden and G20210A mutation in each group was about 1 in 1000. Compared with the European reports, the frequency of prothrombin G20210A was more prevalent than factor V Leiden in our region.

Recently, some reports suggested that factor V gene might show different clinical presentations because of different polymorphism on exons of 13 and 16. The His199Arg polymorphism (HR2 haplotype) contributes to mild activated protein C resistance, particularly in the homozygous condition.[11]

Several studies of factor V Leiden allele in the European, Hispanic, and African and Asian Americans showed a frequency of 5% to 15%, 2%, and 1% respectively.[12]

Literature reviews of this mutation from Turkey (4.6% -12%),[13] the center and north parts of Iran (5.5%),[7] the present study (4.1%), Saudi Arabia (2.5%), India, and China,[12] which are all from Asian countries represented a declining pattern from the north through the south countries. This epidemiologic frequency of factor V Leiden may suggest a single origin of this mutation. However, there is no adequate genetic conformation to proof this hypothesis.

In case of prothrombin mutation of G20210A, high prevalent carriers have been reported from Cyprus and Greece,[14] with 8.1% and 4.4% frequencies respectively. Previous report of this mutation from central Iran,[7] and the present study (3.07%) did not show any significant difference between the north and south population of Iran.

According to the data and Hardy-Weinberger equation, about 1 in 500 individuals are at the risk of thrombosis as a result of homozygote state of factor V Leiden and PT G20210A, and compound heterozygosity of these two mutations. It should be taken into account that early diagnosis and prophylactic anticoagulant therapy will reduce recurrence risk, morbidity, and mortality in these individuals.

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Conflict of Interest: None declared

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