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A Study of Molecular Genetic Changes and Common Environmental Risk Factors Associated with Pulmonary Thromboembolism

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Abstract

Aims: Gene variations in different genes that are associated with homeostasis and thrombosis have a significant effect on the incidence of idiopathic venous thromboembolism in the future. Thromboembolism is a complex disease in which genes are an important component of the risk. The present research investigates the gene variations in pulmonary thromboembolism with a healthy group.

Method: During a cross-sectional descriptive study, we evaluated a sample of 53 patients with a pulmonary thromboembolism and 32 healthy individuals. We performed DNA analysis for variations of different genes involved in pulmonary thromboembolism. Finally, we assessed environmental factors in patients through their history.

Results: We compared 53 patients with a mean age of 41.13±2.7 years including 29 men and 24 women with 32 healthy individuals as control group with a mean age of 39.6±8.4 years including 17 men and 15 women. There was no statistically significant difference between the two groups based on age and gender of patients. In genetic variations in prothrombin (G20210A), there was a statistically significant difference between the patient and healthy groups (p = 0.01). The prothrombin gene mutation (G20210A) had significant predictability with relative risk (CI 0.1-0.9). Younger patients with gene mutations had a sensitivity of 0.65 for pulmonary thromboembolism.

Conclusions: The results showed that mutation of prothrombin gene increases the risk of pulmonary thromboembolism. According to this research, the Factor V Leiden mutation has no effect on pulmonary thromboembolism, contrary to other reports.

Keywords: Pulmonary thromboembolism, mutation, risk factor

INTRODUCTION

Pulmonary embolism is a very common and very deadly condition that is the leading cause of death in all age groups. Pulmonary embolism is the third most common cause of death in the United States. In most age groups, this condition is the first or second cause of unexpected mortality.[1] Many genetic causes remain silent in combination with environmental causes[2, 3]. The most important key risk factors for thromboembolism based on previous studies are trauma, surgery, old age, prolonged immobility, and pregnancy.[4]

According to the articles, the most important genetic mutations involved in pulmonary thromboembolism are expressed as factor V Leiden, prothrombin factor II (G20210A), deficiency of protein C and protein S and antithrombin III.[3] Heterozygous carriers due to mutation of this gene are important genetic risk factors for pulmonary thromboembolism, which increases its prevalence in the population by 7 times. It is more severe in people with this mutated gene as homozygous so that its incidence is higher by 80 times.[5] Some studies have reported hyperhomocystinuria as an independent risk factor for pulmonary thromboembolism.[6,7] In the

study of Segal et al, the study of factor V Leiden and mutation of prothrombin G20210A gene in people with a previous history of thrombosis and embolism in themselves or in the family shows that heterozygosity of factor V Leiden of this gene has a relative risk of 1.5 and has been homozygous for 2.6.[8] In a study conducted by Maginicka et al. on 146 patients with pulmonary thromboembolism and 100 healthy controls, there was a significant association between thromboembolism and factor V Leiden.[9]

The research of Zee et al. showed that factor V Leiden had a relative risk of about 3.2 with significant predictability in the incidence of pulmonary thromboembolism and prothrombin mutation with a risk of 2.5 and significant predictability was in the next rank.[10] In a study conducted by Colazzio et al., the results showed that carriers of factor V Leiden and the mutation A20210 were more likely to develop lower extremity thromboembolism. Evaluation of JAK mutation in patients with myeloproliferative disorders is valuable.[11]

In a study carried out by Pardanani et al. on JAK2 V617F, they showed that mutations in the JAK gene are very rare and have the lowest prevalence of thromboembolism compared to other genetic changes and genetic variations.[12] A study on the events of reasonless pulmonary and venous thromboembolism by Ciuturuad et al. showed that the age under 45 years has a relative risk of developing thromboembolism, while the age over 71 years has genetically a very low prevalence of pulmonary thromboembolism.[13] A study of Aralingam Sunth et al. showed that there was a statistically significant difference in the mutation of fibrinogen genotypes of Thr312 allele and its alleles in patients with chronic pulmonary hypertension.[14] Considering the genetic differences in different populations and regions according to race and ethnicity of individuals, the present research investigates simultaneously the genetic changes and risk factors of patients with pulmonary thromboembolism in northwestern Iran in the educational center of Tabriz University of Medical Sciences.

METHODS

The present research was methodologically descriptive-analytical. We conducted it in Imam Reza (AS) Educational and Medical Center in Tabriz and in the Department of Internal Medicine. The sample includes patients who are examined in the pulmonary ward of the center with a definitive diagnosis of pulmonary thromboembolism within 6 months after the approval of the project. According to previous statistics, about 30 patients will be included in the study. 30 age- and sex-matched healthy individuals were also considered as a control group.

In this research, during 6 months, we included in the research about 53 patients who were admitted to the study site with a definitive diagnosis of pulmonary thromboembolism by CT angiography. We performed intravenous blood sampling in all control patients and sent samples for genetic testing. We evaluated twelve genes after DNA extraction from leukocytes by salting out method using molecular analysis techniques including RFLP, PCR and ARMS-PCR. We also examined the known environmental factors associated with VTE and PTE in both groups.

We used Spss.17 software and chi-square test to analyze the data. In this research, p value less than 0.50 was supposedly significant.

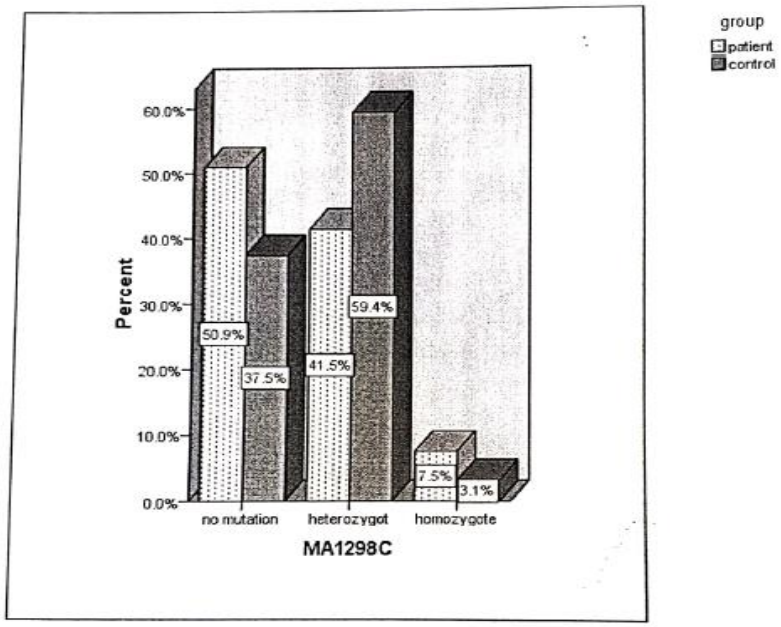
RESULTS

In this research, we compared 53 patients with pulmonary thromboembolism with a mean age of 41.2±13.7 years with 32 healthy individuals as a control group with a mean age of 39.6±8.4 years; in terms of age, there was no statistically significant difference between the two groups. Patients with pulmonary thromboembolism in 29 cases were male and in 24 cases female; in the control group, 17 cases were male and 18 cases were female. There was no statistically significant difference between the two groups in terms of gender.

Examination of Genetic Variations:

MA1298C gene in patients was in 28 cases without mutation and in 26 cases with mutation and in control group it was in 12 cases without mutation and in 20 cases with mutation. There was no statistically significant difference. Figure 1 shows the degree of heterozygosity in two groups. The distribution of genetic variations is the same between the two groups.

Figure 1. Frequency of MA1298C gene alleles between patients and healthy individuals.



The FVG1916A gene was non-mutant in 17 cases and mutant in 36 cases.it was non-mutated in 15 cases and mutated in 17 cases in the healthy group. There was no statistically significant difference between the two groups in terms of heterozygosity and homozygosity in Figure 2. G20210A gene (factor II) in patients was with mutation in 36 cases (64.1%) and in 17 cases (35.9%) without mutation. In healthy group. It was in 30 cases (93.8%) without mutation and in 2 cases (6.2%) had mutation. It was heterozygous in both groups and we did not observed the homozygosity of this gene and there was a statistically significant difference between the two groups (P = 0.01).

The PAI-146/56 gene was negative in 51 cases (96.2%) and positive in 2 cases (3.8%) in terms of mutation, but we observed no mutation in this group of healthy individuals. Two cases of mutation that were positive for thromboembolism patients were heterozygous. Due to the low frequency of variables, we performed statistical analysis on this gene in a descriptive manner.

The FVHR22 gene was negative in 40 cases (75.5%) of patients with mutations and in 13 cases (24.5%) was negative. In the opposite group, ie healthy individuals, it was positive in most cases, so that in 26 cases (81.3%), it had mutation and in 6 cases (18.8%) it had no mutations. Regarding the distribution of alleles in each group in Figure 3, we can see that there is not much difference in terms of different variations between the two groups.

The factor XIII gene was with mutation in 7 cases (13.2%) in patients and without mutation in 46 cases (86.8%). In the healthy group, it was positive in 13 cases (40.6%) and had a gene mutation and in 19 case (59.4%), it was without mutation. We observed a statistically significant difference with P <0.005 between the two groups. The frequency of mutation was higher in healthy individuals, but in the group of patients, the mutation was homozygous but in the group of healthy people, they were all heterozygous.

Lycoprotein gene had mutation in patients more than healthy individuals, so that in 13 cases (24.5%) it had mutation and in 40 cases (75.5%) it was negative. In the group of healthy individuals, in 7 cases (21.9) it had mutation and in 254 cases (78.1%) had no mutations. In all mutations in both groups, it was heterozygous and there was no statistically significant difference between the two groups.

FV2 gene was positive in 27 cases (50.9%). In 26 cases (49.1%), it was without mutation. In the opposite group, in 20 cases (62.5%), it had mutation and in 13 cases (37.5%), it was without mutation. We can see the distribution of heterozygosity and homozygosity in Figure 4.

There was no statistically significant difference in terms of gene distribution between the two groups.

The ACE gene was mutated in 23 cases (43.4%) of patients and it was without mutation in 30 cases (56.6%). In 17 cases (53.1%) of healthy individuals, it was with mutation and in 15 cases (46.9%), it was non-mutant. The distribution of alleles can be observable in Figure 5. There was no statistically significant difference in the distribution of alleles of this gene between the two groups.

As for TPA gene in patients, in 41 cases (77.4%) it was with mutation and in 12 cases (22.6%) without mutation; in healthy individuals, in 26 cases (81.3%) it was with mutation and in 6 cases (18.8%) without mutation. The distribution of alleles can be observable in Figure 6. There was no statistically significant difference between the two groups for this gene.

Beta Fib gene was with mutation in 33 cases (60.4%) of patients and without mutation in 21 cases (39.6%); in the opposite group, in 22 cases (68.9%) it had mutation and in 10 cases (31.3%) no mutations. The allele distribution of this gene in the patients under study was heterozygous in 18 cases (34.9%) and homozygous in 14 cases (26.4%). Heterozygous and homozygous individuals were 11 cases. There is no statistical difference between the two groups in terms of variations in this gene.

According to the regression model for the genes under study, only the case of G20210A gene (factor II) had significant predictability in terms of the occurrence of thromboembolism with a relative risk of 0.1 (CI 0.3-0.9) with a P of 0.40 and the other genes were not of significantly prediction.

Figure 2. Frequency of FVG1691A gene alleles between patients and healthy individuals.

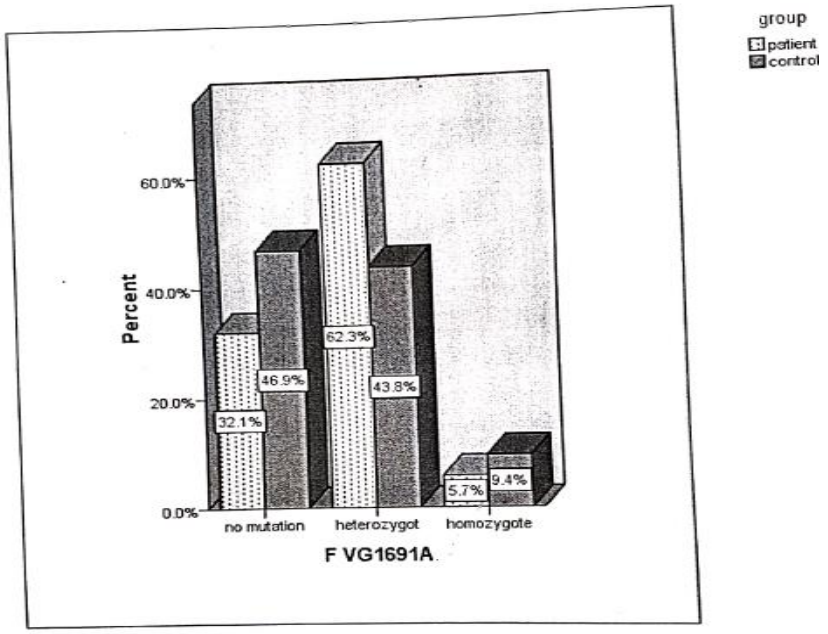


Figure 3. Frequency of FVHR22 gene alleles between patients and healthy individuals.

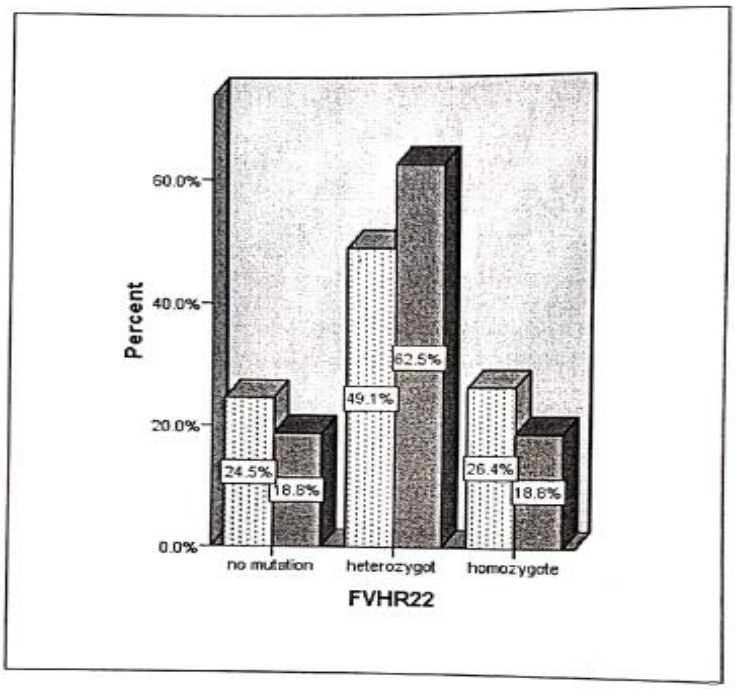


Figure 4. Frequency of FV2 gene alleles between patients and healthy individuals.

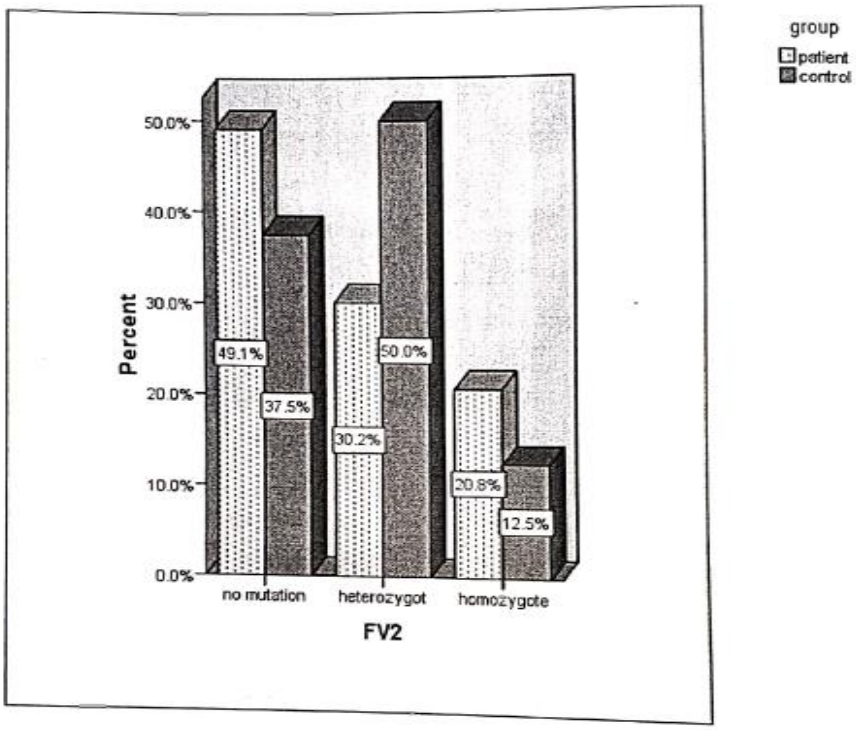


Figure 5. Frequency of ACE gene alleles between patients and healthy individuals.

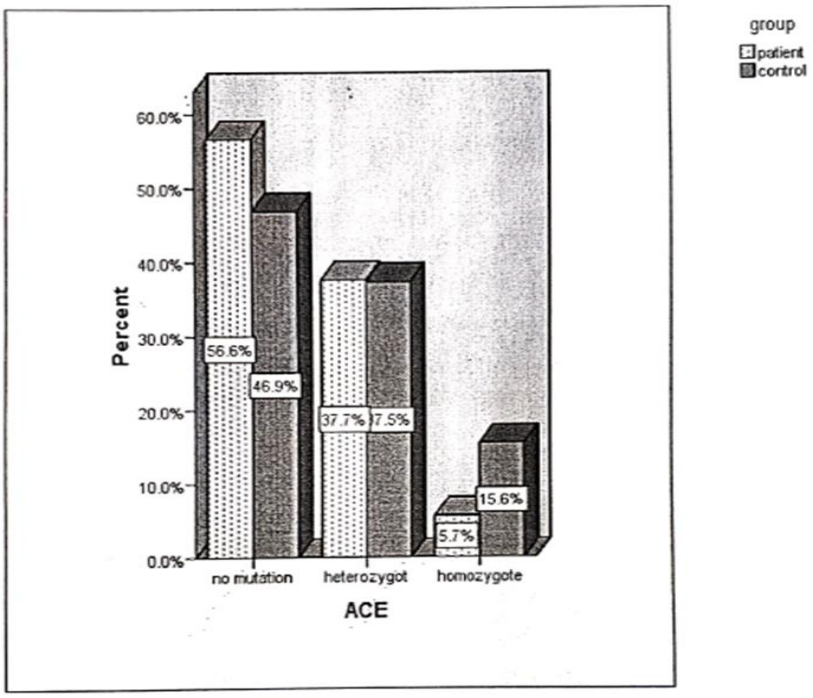
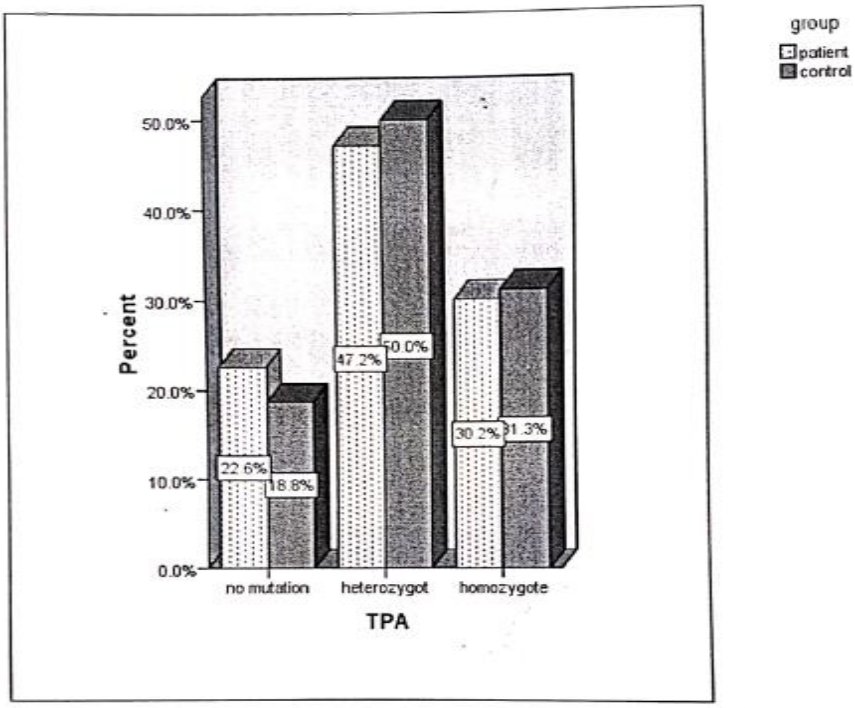


Figure 6. Frequency of TPA gene alleles between patients and healthy individuals.



Examination of Risk Factors in the Patients under Study:

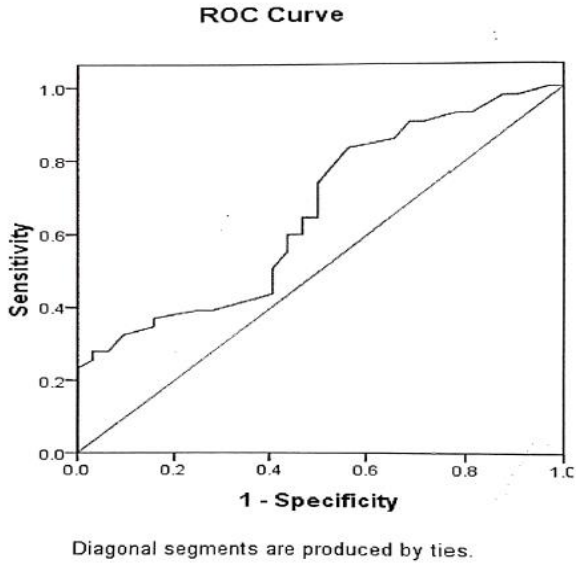
In the patients under study, in terms of types of risk factors generally, in 35 cases (66%), the risk factor of thromboembolism was positive and in 18 cases (34%), we did not observed any risk factor.

In patients with thromboembolism, the following was the case: surgery in 6 cases (11.3%), Absolute rest in 2 cases (3.7%), Positive family history in 9 cases (11.6%), Lower vascular thrombosis in 2 cases (3.7%), use of oral anti-pregnancy drugs (OCP) in 10 cases (18.9%), use of various drugs in 15 cases (28.3%), previous history of thrombosis in 20 cases (37.3%), smoking in 9 cases (17%), history of abortion in 4 cases (17.5%), recent fracture in 4 cases (7.5%), long-term travel in 4 cases (7.5%), malignancy in 2 cases (3.7%) and chemical veteran in 1 case. Environmental risk factors in cases where there were mutations in the genes under study were 29 (69.4%) for mutation in FVHR2 gene and TPA gene. The lowest mutated gene in cases with risk factor of FXIII gene was 4 (7.1%). In surgery, the most important mutated genes are FVG, Beta, TPA and FVHR2 each in 5 cases (12.5%). Family history of factor V Leiden gene, FVG and FVHR2, was mutated in 8 cases (22%).

Consumption of OCP in combination with mutation of factor V Leiden gene (FVHR2) and the gene TPA was positive for gene mutation in 8 cases (22%). In cases of smoking accompanied by mutation of factor V Leiden gene (FVHR2) and TPA gene were positive in 6 cases (15%) and 7 cases (18%), respectively. In cases of rest for more than 3 days, there were FVII and TPA gene mutations of 11 (40.7%) and 14 cases (34.1%), respectively. In the previous history of abortion, factor V Leiden gene mutation was positive in 4 cases (10.9%) and in previous cases of thrombosis, the gene mutation of factor V Leiden gene, namely FVHR2, was positive in 11 cases (3%). It was positive in fracture and pulmonary thromboembolism in 2 cases (5%). In long-term travel, the gene mutation in TPA was positive in 4 cases (9.8%) and in malignancy, the gene mutation in factor V Leiden and Lycoprotein gene was positive in 1 case (2.5%) and in 2 cases (5%), respectively. Other mutations had a low percentage associated with environmental risk factors. Due to the low frequency of data, it was not possible to perform statistical analysis. We expressed descriptively the data and the most important gene mutations in each of the environmental risk factors.

Regarding age and thromboembolism based on the level, Figure 7 shows a significant role in the development of thromboembolism. The cut-off point is 40 years old and has a relatively high sensitivity of about 65% (53-78% CI).

Figure 7. Age sensitivity to pulmonary thromboembolism associated with gene variations.



DISCUSSION

This research evaluated the risk genetic factors in the development of thrombosis and pulmonary thromboembolism; we tried to investigate the genetic causes involved in the development of this disease in the northwestern region of Iran. In the present study, performed on healthy patients with pulmonary thromboembolism, the data showed that the prevalence and frequency of mutations in this gene were similar to those in other populations, with a frequency of 6.3%; but the Heterozygosity of factor V Leiden was higher in the study population than in other studies.

In one study, homogeneity of factor 5 Leiden was highly correlated with heterozygosity of this gene in thrombosis, but in other studies, heterozygosity of this gene was also mentioned as a risk factor for thromboembolism. Based on the findings of the present study, there was no difference in heterozygosity and homozygosity of factor 5 Leiden between the patients and healthy group.[13]

In a study, Kearon et al. introduced factor 5 Leiden and prothrombin G20210A as the most important mutations in causing thromboembolism.[16] In the study of Moginicka et al, in addition to classical risk factors in idiopathic cases, gene mutations played an important role in causing thromboembolism. There was a statistically significant relationship between thromboembolism and factor V Leiden and mutations in the prothrombin G20210A gene[7], but there was no significant relationship between methylene tetrahydrofolate reductase and thromboembolism.

In a study conducted by Zee et al., factor V Leiden had a relatively high risk of idiopathic thromboembolism, followed by a mutation in the prothrombin gene with a relative risk twice higher than other genes. In the case of this gene, there was a significant predictability in thromboembolism and thrombosis, but in the case of other genes, this predictability was not true.[8]

In a study conducted by Colaizzo et al., Factor II, prothrombin, was more positive in patients with thromboembolism, and carriers of the factor V Leiden mutation and prothrombin mutation as heterozygotes were more likely to develop thromboembolism and lower extremity thrombosis.[9]

According to the findings of the present research, idiopathic cases without risk factor were present in 18 cases, ie 34% of patients with thromboembolism. It corresponds to the prevalence of idiopathic cases in other studies of other countries because in these studies it was about 30%. According to the findings of our study, the most important gene mutation in association with environmental risk factors was about the gene mutation for factor V Leiden; in other reports, the factor V Leiden was one of the most important mutated genes.

In terms of different gene mutations in our population under study, prothrombin G20210A gene mutation was significantly different between patients and healthy individuals and the frequency of the mutation of this gene was 6.3% higher in healthy individuals and 64.1% higher in healthy individuals. It is similar with the results of other studies, but in the case of factor V Leiden, as mentioned, it differs from other studies.

Moreover, mutation in the prothrombin gene according to the present study has a more important role in the development of thromboembolism with significant predictability than other genes. However, factor V Leiden, contrary to other studies, has not significant role in predicting thrombosis in terms of frequency of the mutation between patients and healthy individuals, so it could not predict the development of thromboembolism based on the regression model. In the case of other genes, as according other studies, based on which they did not play a significant role in the development of thromboembolism, in the present study also, other genes did not play a role in the frequency of mutations and the ability to predict the development of pulmonary thromboembolism.

In our study, the incidence of pulmonary thromboembolism for the age under 40 years of age has been higher, so that people under the age of 40 have an age sensitivity for pulmonary thromboembolism of about 65%.

CONCLUSION

Based on the evidence obtained from this research, the gene mutation in prothrombin is one of the most important gene variations involved in the development of pulmonary thromboembolism in the present population. In young people, gene variations are most commonly associated with pulmonary thromboembolism, and other gene variations are less likely to predict pulmonary thromboembolism.

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Conflict of interest: The authors declare that they have no conflict of interest.

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