İzmir Göğüs Hastanesi Dergisi 2020;34(1):43-9
doi:10.5222/IGH.2020.1746

Association Between Plasminogen Activator Inhibitor type-1 Gene (PAI-1) Polymorphism and Pulmonary Embolism

Plazminojen Aktivatör İnhibitörü tip-1 Gen (PAİ-1) Polimorfizmi ve Pulmoner Emboli Arasındaki İlişki

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Cite as: Çulfacı Karasu F, Er Mükremin, Hasanoğlu CH, Ceylan G. Association between plasminogen activator inhibitor type-1 gene (PAI-1) polymorphism and pulmonary embolism. İzmir Göğüs Hastanesi Dergisi. 2020;34(1):43-9.

ABSTRACT

Objective: Pulmonary thromboembolism (TPE) may develop due to hereditary and acquired risk factors but idiopathic cases may face us. Plasminogen activator inhibitor-1 (PAI-1) is the primary inhibitor of the plasminogen activator in plasma. It inhibits tissue plasminogen activator which involves in conversion of plasminıgen to plasmin, and urokinase. The objective of this study is to investigate the effectiveness of PAI-1 gene polymorphism in the development of PTE alone and in combination with other genetic mutations.
Method: Sixty-four patients with PTE and a control group of 60 individuals were enrolled in our study. Among hereditary risk factors, PAI-1 gene polymorphisms, Factor V Leiden mutation, Factor V 1299, methylene tetrahydrofolate reductase C677 and A1298, and Factor II G20210A were studied. Besides acquired risk factors of the patients were identified. Levels of HDL, homocysteine, and D-dimer were analyzed.
Results: The mean ages of the patient, and the control groups were 60.8±15.4, and 56.6±16.9 years, respectively. In the patient group, PAI-1 gene 5G/5G polymorphism (normal) (n=18), 4G/5G polymorphism (n=27) and 4G/4G polymorphism (n=19) were detected in indicated number of participants. In the control group, PAI-1 5G/5G 11 polymorphism (normal) (n=11), 4G/5G polymorphism (n=34) and 4G/4G polymorphism (n=15) in respective number of patients. There was no statistically significant difference between patients and the control group when comparisons were made in double and triple combinations in terms of PAI-1 gene polymorphisms, and other risk factors (p>0.05). Also, we found a weak association between PAI-1 gene polimorphism and pulmonary embolism.
Conclusion: In our study we couldn't arrive at data suggesting increase in the risk of pulmonary trhromboembolism in the presence of PAI-1 gene polymorphism alone or in combination with other mutations.

Keywords: pulmonary embolism, plasminogen activator inhibitör-1 gene polimorphism, Factor V mutation, methylene tetrahydrofolate reductase mutation, prothrombin gene mutation

ÖZ

Amaç: Pulmoner tromboemboli (PTE) kalıtsal ve kazanılmış risk faktörlerine bağlı gelişebileceği gibi idiopatik olarak ta karşımıza çıkabilir. Plazminojen aktivatör inhibitör-1 (PAİ-1) plazmada plasminojen aktivatörünün esas inhibitörüdür. Plasminojenin plazmine dönüşümünde aktivatör görev yapan doku plasminojen aktivatörü ve ürikinaz' ı inhibe eder. Bu çalışmanın amacı PTE gelişiminde PAİ-1 gen polimorfizminin tek başına ve diğer genetik mutasyonlarla birlikte etkinliğini araştırmaktır.
Yöntem: Çalışmaya 64 PTE hastası ve 60 sağlıklı kontrol grubu dahil edildi. Kalıtsal risk faktörlerinden PAİ-1 gen polimorfizmi, Faktör V leiden mutasyonu, Faktör V 1299, Metilen tetrahidrofolat redüktaz C677 ve A1298, Faktör II G20210A (protrombin gen mutasyonu) çalışıldı. Ayrıca hastaların kazanılmış risk faktörleri belirlendi. Hasta grubunda HDL, homosistein, D-dimer düzeyleri çalışıldı.
Bulgular: Hasta grubunda yaş ortalaması 60,8±15,4 ve kontrol grubunda yaş ortalaması 56,6±16,9 idi. Hasta grubunda PAİ-1 gen polimorfizmi 5G/5G (normal) olan 18 kişi, 4G/5G olan 27 kişi, 4G/4G olan 19 kişi vardı. Kontrol grubunda PAİ-1 gen polimorfizmi 5G/5G (normal) olan 11 kişi, 4G/5G olan 34 kişi, 4G/4G olan 15 kişi vardı. Hasta ve kontrol grubu karşılaştırıldığında fark istatistiksel olarak anlamlı değildi (p>0.05). PAİ-1 gen polimorfizmleri ile diğer genetik risk faktörlerinin ikişerli ve üçerli birlikteliği hasta ve kontrol grubunda karşılaştırıldığında fark istatistiksel olarak anlamlı değildi. Sonuç olarak çalışmamızda; PAİ-1 gen polimorfizminin diğer mutasyonlarla birlikte veya yalnız olarak pulmoner tromboemboli riskini artırdığına dair veriye ulaşılamamıştır.
Sonuç: Çalışmamızda, PAİ-1 gen polimorfizminin diğer mutasyonlarla birlikte veya yalnız olarak pulmoner tromboemboli riskini artırdığına dair veriye ulaşılamamıştır.

Anahtar kelimeler: pulmoner tromboemboli, plazminojen aktivatör inhibitör-1 gen polimorfizmi, Faktör V mutasyonu, metilen tetrahidrofolat redüktaz mutasyonu, protrombin gen mutasyonu

Alındığı tarih: 18 Nisan 2019
Kabul tarihi: 27 Ağustos 2019
Yayınlandığı tarih: 30 Nisan 2020

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INTRODUCTION

Acquired and/or inherited risk factors are identified in 75% of venous thromboembolism (VTE) cases [1]. Endothelial damage, hypercoagulability and venous stasis are the underlying pathophysiological mechanisms of thrombosis. There are also concomitant acquired risk factors in half of hereditary thrombophilia cases. Acquired risk factors including advanced age, long-term travel, trauma, major surgery, immobilization, cancer, oral contraceptive use, hormone replacement therapy can be seen in patients with VTE. Protein C and S deficiencies, active protein C resistance (Factor V Leiden [FVL]), mutation of prothrombin (PTM) G20210A, antithrombin (AT) III deficiency, hyperhomocysteinemia / mutation of methylene tetrahydrofolate reductase (MTHFR) enzyme, increment of Factor VIII, congenital dysfibrinogenemia, anticardiolipin antibodies, increment of plasminogen activator inhibitor-1 (PAI-1) activity, plasminogen deficiency, Factor VII deficiency and increased Factor IX are the hereditary risk factors of patients with PE. Although available data about the prevalence of inherited thrombophilia in patients with VTE are controversial, thrombophilia is shown in at least 30% to 40% of these patients [2-4].

Two enzyme systems play an important role in the regulation of hemostasis in the fibrinolytic system. Plasminogen activators which include the tissue plasminogen activator (t-PA), have primary roles in the enzyme system. PAI which include PAI-1 plays a role in the second enzyme system and inhibits t-PA and urokinase (uPA), which are activators involving in the conversion of plasminogen to plasmin [5].

The most common genetic variant of PE is the 4G/5G polymorphism formed by PAI-1, which is a single guanosine insertion/deletion in 675 base pairs in the promoter region of PAI-1 gene transcription and it is considered that 5G/5G homozygous is normal and 4G/4G homozygous is mutant. The levels of PAI in the plasma are about 25% higher in 4G/4G genotype individuals than in 5G/5G genotype individuals [5,6].

Different results have been observed in previous studies. The levels of PAI-1 and 4G/5G polymorphism were found to be significantly increased in patients with deep vein thrombosis (DVT) in some studies [6,7], although other studies reported lack of any difference between the healthy group and the patients with DVT [8,9].

The aim of the study is to investigate the role of PAI-1 gene polymorphism alone and together with FVL, MTHFR and PTM gene mutations in the development of pulmonary thromboembolism.

MATERIALS and METHODS

We conducted this retrospective study in patients who were diagnosed with PE in a Training and Research Hospital, Pulmonary Diseases Department during six months. A control group was created by randomly selecting healthy individuals in nearly equal number of participants in the patient group. The study was initiated after the approval of local ethics committee. Approval for this study was received from the ethics committee of Ankara Atatürk Research and Training Hospital (05/11/2012-74). PE was diagnosed by pulmonary angiography with computed tomography. Information about age, gender and family history of the patients were recorded. Deep and superficial vein thrombosis was investigated via Doppler ultrasonography of the lower and upper extremities. The acquired and hereditary risk factors were determined.

Laboratory Studies

Blood samples were obtained from the antecubital veins of all patients at the time of diagnosis before initiation of anticoagulant therapy. Samples for genetic studies were placed in standard tubes containing 0.072 ml of 7.5% K3-ethylenediaminetetraacetic acid (EDTA) and

were sent to the hospital's medical genetic laboratory. Samples were kept at 4°C. These samples were kept at -20°C after isolation of deoxyribonucleic acid (DNA) by the Qiacube brand isolating device. PAI polymorphism and the other parameters of the thrombosis panel (MTHFR C677T, MTHFR A1298, factor V G1691A, factor V 1299, FactorII G20210A) were studied using the real-time polymerase chain reaction (PCR) method using the Euroclore kit. D-dimer was studied by using the enzyme-linked fluorescent assay (ELFA) technique on a VIDAS device using quantitative methods.

Statistical Analysis

Statistical analyses were performed with SPSS for Windows version 20.0 (SPSS Inc., Chicago, IL, USA). A P-value <0.05 was regarded as statistically significant in all analyses. Characteristics of patients were evaluated with basic statistics. Means±standard deviations for metric variables and frequencies as percentages for categorical variables were used.The Student's t test was used for comparison between the groups in terms of age. The distribution of qualitative variables in the groups was compared using Pearson's chi-square test and Fisher's exact test.

RESULTS

Sixty- four patients with PE and 60 healthy subjects consisted the study group. The mean age of the patient group was 60.8±15.4, and the mean age of the control group was 56.6±16.9 years (p>0.05). There were 32 females and 32 males in the study group, while there were 33 females and 27 males in the control group. No significant difference was found between groupsin terms of age and gender.

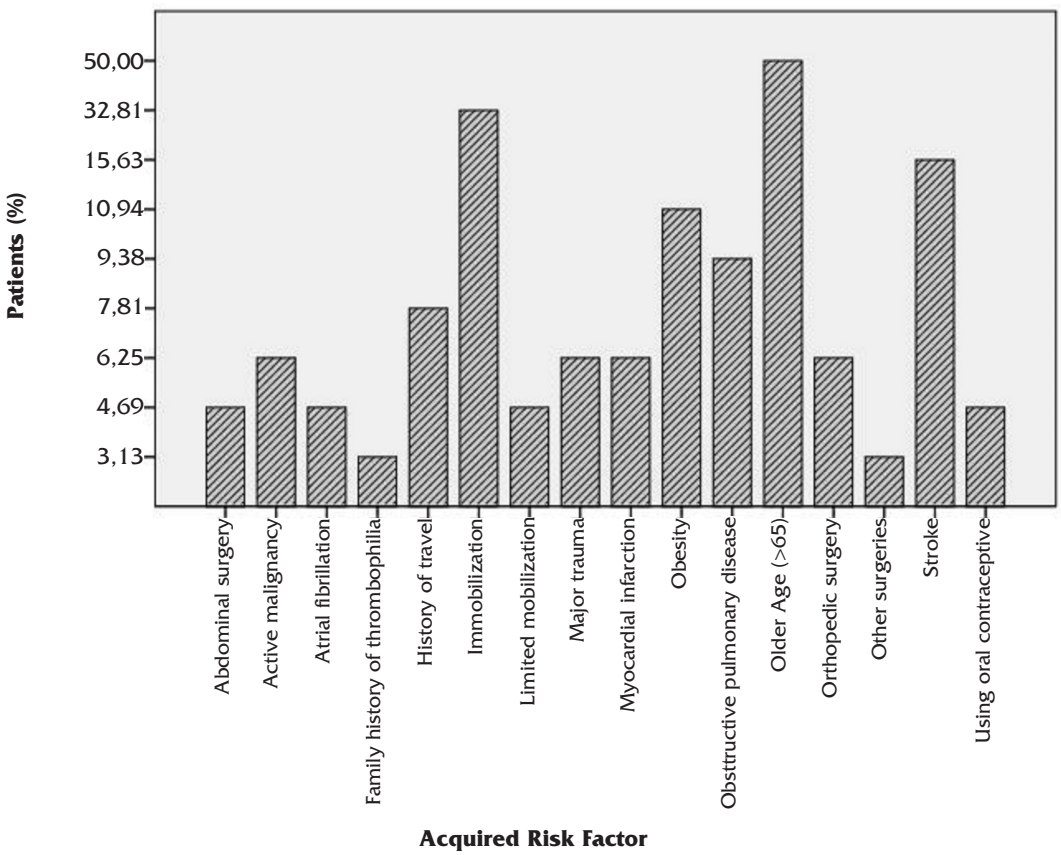

Figure 1. The distribution of acquired risk factors in the study group.

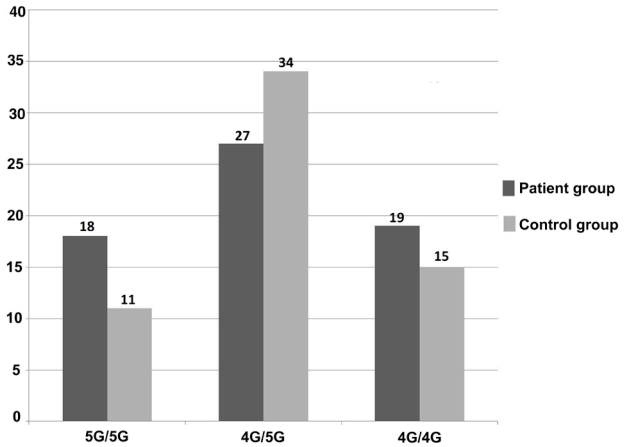

Figure 2. Rate of PAI-1 gene polymorphisms between the study and control groups.

Venous Doppler ultrasound imaging of the lower extremities was performed on 61 patients (95.3%) in the study group and thrombi were detected in 33 of them.

The main risk factor was determined as older age in the study group. Fifty percent (50%) of the participants in study group were 65 years of age or older. In addition, the most common risk factor was immobilization in patients over the age of 65 (17 patients). Any risk factors were not found in 16 patients. The acquired risk factors in the study group are shown in Figure 1.

In the patient group, PAI-1 gene 5G/5G polymorphism (normal) (n=18), 4G/5G polymorphism (n=27)and 4G/4G polymorphism (n=19) were detected in indicated number of participants. In the control group, PAI-1 5G/5G 11 polymorphism (normal) (n=11) , 4G/5G polymorphism (n=34) and 4G/4G polymorphism (n=15) in respective number of patients. There was no significant difference in types of PAI-1 gene polymorphism between the study and control groups (p>0.05) Table 1.

Sixteen of 64 patients in the study group had not any acquired risk factors. Five patients had 5G/5G, seven had 4G/5G and four had 4G/4G

Table 1. The distribution of the other genetic factors in patients with 4G/4G and 4G/5G PAI-1 gene polymorphisms in the study and control groups.

		PAI-1 4G/4G			PAI-1 4G/5G		
		Study group (19) n (%)	**Control group (15) n (%)**	**p**	**Study group (27) n (%)**	**Control group (34) n (%)**	**p**
FV G1691A (Factor V Leiden)	No mutation	15 (78,90)	13 (86,70)	0,286	18 (66,70)	26 (76,50)	0,433
	Heterozygous mutation	4 (21,10)	1 (6,70)		8 (29,60)	8 (23,50)	
	Homozygous mutation	0	1 (6,70)		1 (3,70)	0	
FV 1299	No mutation	18 (94,70)	12 (80,00)	0,299	26 (96,30)	30 (88,20)	0,371
	Heterozygous mutation	1 (5,30)	3 (20,00)		1 (3,70)	4 (11,80)	
	Homozygous mutation	0	0		0	0	
MTHFR C677	No mutation	8 (42,10)	4 (26,70)	0,406	15 (55,60)	17 (50,00)	0,881
	Heterozygous mutation	9 (47,40)	7 (46,70)		9 (33,30)	12 (35,30)	
	Homozygous mutation	2 (10,50)	4 (26,60)		3 (11,10)	5 (14,70)	
MTHFR A1298	No mutation	7 (36,80)	5 (33,30)	0,646	15 (55,60)	16 (47,10)	0,781
	Heterozygous mutation	9 (47,40)	9 (60,00)		11 (40,70)	16 (47,10)	
	Homozygous mutation	3 (15,80)	1 (6,70)		1 (3,70)	2 (5,90)	
FII G20210A	No mutation	19 (100,00)	15 (100,00)		24 (88,90)	31 (91,20)	
	Heterozygous mutation	0	0		3 (11,10)	3 (8,80)	
	Homozygous mutation	0	0		0	0	

Table 2. The distribution of association of the genetic mutation 4G/4G and 4G/5G PAI-1 gene polymorphism in the study and control groups.

Gene mutations and polymorphism	Study (46) n (%)	Control (49) n (%)	P value
4G/4G,4G/5G+ Factor V G1691A+ any of MTHFR mutation	10 (21.7%)	8 (16.3%)	0,65
4G/4G,4G/5G+ MTHFR C677+MTHFR A1298	13 (28.3%)	14 (28.6%)	0,42
4G/4G,4G/5G+ Factor V G1691A (Factor V Leiden)	13 (28.3%)	10 (20.4%)	0,42
4G/4G,4G/5G+ ANY Factor V mutation	15 (32.6%)	14 (28,6%)	0,08

PAI-1 gene polymorphisms amongst these patients. Graphical demonstration of the results is shown in Figure 2.

There was no significant difference regarding the mutations of FV G1691A, FV 1299, MTHFR C677, MTHFR A1298 and FII G20210A in patients with 4G/4G PAI-1 gene polymorphism (p>0.05). There was no significant difference between the mutations of FV G1691A, FV 1299, MTHFR C677, MTHFR A1298 or FII G20210A in patients with 4G/5G PAI-1 gene polymorphism (p>0.05) Table 1.

The association of MTHFR C677 and MTHFR A1298 mutations were compared in patients with 4G/4G and 4G/5G PAI-1 gene polymorphism in the study and control groups , and any statistically significant intergroup difference was not found (p>0.05). The mutation factor V G1691A was compared in patients with 4G/4G and 4G/5G PAI-1 gene polymorphism in the study and control groups, and any statistically significant intergroup difference was not found (p>0.05). The mutation of Factor V G1691A and any coexistence of the MTHFR mutation was compared in patients with 4G/4G and 4G/5G PAI-1 gene polymorphism in the study and c ontrol groups, and no significant difference was observed between them (p>0.05). When comparing the association of any coexistent factor V mutation in patients with 4G/4G and 4G/5G PAI-1 gene polymorphism in the study and control groups, no statistically significant difference was found between them (p>0.05) Table 2.

DISCUSSION

In this study, FVL mutation was more frequently found in the patient than the control group, when compared as for PAI-1 4G/4G and 4G/5G polymorphisms and FVL mutations without any statistically significant intergroup difefrence (p>0.05). In addition, there was no significant difference in terms of the presence of FV 1299 mutation (p>0.05).

Also we found an insignificant and weak difference between the patients and control subjects in terms of the types of PAI-1 gene polymorphism (p>0.05). So it can be suggested that 4G/5G and 4G/4G polymorphisms of PAI-1, do not have an important, but weakly positive role in the development of VTE in our country. It should be noted that PAI-1 gene polymorphism indicates ethnic and geographical differences. Further studies involving more people may provide more accurate results.

Some studies have presented the association of DVT with the levels and 4G/5G polymorphism of PAI-1 in different populations. Segui et al. studied PAI-1 4G/5G polymorphism in 190 patients with DVT and 152 healthy controls, and a significant difference was not observed in the distributions of 4G and 5G alleles between the patient and control groups. However, the risk of thrombosis significantly increased in the presence of the 4G allele in patients with other thromboembophilic defects [10].

Studies are available in our country concerning

PAI-1 gene polymorphism in patients with VTE. Kupeli et al. did not find any intergroup difference in terms of PAI-1 gene polymorphism in their study, which included 80 patients with VTE (deep vein thrombosis and pulmonary thromboembolism) and 104 healthy controls [9].

Oguzulgen et al. did not observe any correlation between the PAI-1 gene polymorphism and pulmonary embolism in their study including 143 patients with PE and 181 healthy controls [8]. Moreover, Kaya et al. observed that frequency of the 4G allele in 80 patients with VTE was greater than in 79 controls, but this difference was not statistically significant [11].

Oguzulgen et al. compared the 4G/4G and 4G/5G polymorphisms of the PAI-1 gene and PTM gene mutations in 143 patients with PE and 181 healthy controls, but there was no statistically significant difference [8]. Barcellona et al. compared the 4G/4G allele of the PAI-1 gene and the prothrombin mutation gene in patients and controls, and a significant difference between groups was observed. They found that in patients carrying the prothrombin mutation with allele 4G (odds ratio for VTE of 6.1 (C.I. 95% 3.2-11.4; $p<0.001$), the risk increased up to 13.0 (C.I. 95% 3.0-60.4; $p<0.001$) [12].

Methylene tetrahydrofolate reductase is a key enzyme in the metabolism of folate [13]. A mutation in the MTHFR gene (C677T polymorphism is the most common) reduces the activity of enzyme. Clinical features such as stroke and thrombosis occur in the presence of MTHFR deficiency. However, a mild deficiency of MTHFR is also quite common in the general population [12].

As far as our knowledge, no study has investigated the association of PAI-1 polymorphism with MTHFR gene mutation in the literature. The association of 4G/4G and 4G/5G polymorphisms of PAI-1 with MTHFR gene mutation was compared in patients and controls and the difference was not significant ($p>0.05$). The association of 4G/4G and 4G/5G polymorphisms of the PAI-1 gene (4G alleles) with the mutations of FVL (FV G1691A) and MTHFR was compared binary and tertiary in the patient and control groups; still there was no significant difference between groups.

Limitations of our study were that the levels of PAI-1 were not analyzed and the subgroups contained smaller number of cases.

CONCLUSION

Results of our study showed that, PAI-1 gene polymorphism was not a significant hereditary risk factor for PE in our country. Multicenter studies with higher number of cases and controls may be planned in order to obtain more accurate data.

Conflict of Interest: There is no conflict of interest.

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