The Role of Plasminogen Activator Inhibitor-1 Polymorphism, Factor-V-Leiden, and Prothrombin-20210 Mutations in Pulmonary Thromboembolism

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Polymorphism in plasminogen activator inhibitor-1 gene is suggested to be associated with an increased risk of venous thromboembolism. The aim of this study was to investigate the association of plasminogen activator inhibitor-1 gene polymorphism and its coexistence with factor-V-Leiden and prothrombin-20210 mutations in pulmonary thromboembolism. The authors investigated plasminogen activator inhibitor-1 4G/5G polymorphism, factor-V-Leiden, and prothrombin-20210 mutations in 143 pulmonary thromboembolism patients and 181 controls. Plasminogen activator inhibitor-1 4G/4G, 4G/5G, and 5G/5G gene polymorphisms and prothrombin-20210 mutations were not different between cases and controls. Factor-V-Leiden mutation was present in 21.0% and 7.7% of the cases and controls, respectively, \(P = .001\). Neither different plasminogen activator inhibitor-1 genotypes and 4G allele nor coexistence of the allele with factor-V-Leiden or prothrombin-20210 was associated with the risk of recurrence. As a result, plasminogen activator inhibitor-1 gene polymorphism or its concomitant presence with mentioned mutations was not found to be associated with increased risk for pulmonary thromboembolism or recurrent disease in this study.

Keywords: pulmonary thromboembolism; genetics; plasminogen activator inhibitor-1; factor-V-Leiden; prothrombin-20210

Plasminogen activator inhibitor-1 (PAI-1) is the primary inhibitor of plasminogen activators in plasma, inactivating both tissue plasminogen activator and urokinase plasminogen activator.\(^1_2\) Its high levels could lead to a hypofibrinolytic state, which causes a thrombotic tendency. Levels of PAI-1 depend on genetic factors. 4G/5G insertion/deletion polymorphism in the promoter region of the PAI-1 gene located on chromosome 7 is one of the most frequently studied polymorphisms of the gene. In some studies, it is suggested that the 4G allele was associated with high PAI-1 levels, and its presence could be a predictor of increased thrombotic risk.\(^3_5\) In contrast, there are conflicting results with larger cohorts.\(^6_9\) In this study, we investigated the association of PAI-1 gene polymorphism together with factor-V-Leiden (FVL) and prothrombin-20210 (PT\(^{20210}\)) mutations in pulmonary thromboembolism (PTE).
Methods

Subjects
One hundred fifty patients with documented PTE and 200 control subjects were initially enrolled in the study. Because of missing data in 7 cases, we studied a total of 143, consecutive documented PTE patients (61 men, 82 women) with a mean age of 55.1 ± 14.5 (20-88 years). Pulmonary thromboembolism was confirmed by high-probability, ventilation-perfusion lung scanning or spiral computed thorax tomography. Current or previous deep-venous thrombosis (DVT) was confirmed by Doppler ultrasound. Because of the missing data, out of 200 subjects, 181 age-matched and sex-matched subjects without a history of venous thromboembolism (VTE) were enrolled in the control group. This control group was selected from people attending the outpatient laboratory for other reasons (accompanying healthy people or patients with other pulmonary disorders). All patients and controls were people of Turkish descent who were born in Turkey. Written consent was obtained from each individual.

Laboratory Studies
Blood samples were obtained from the antecubital vein from all patients at the time of diagnosis before the start of anticoagulant therapy. Deoxyribonucleic acid was extracted by conventional techniques. Allele-specific polymerase chain reaction was performed to amplify 4G and 5G alleles according to the previously described method. The PAI-1 4G/5G polymorphism was determined for each subject using primers 5′-CACAGAGAGTCTGGCCACGT-3′ and 5′-CCAACAGAGGACTCTTGGTCT-3′. The mixture was subjected to 35-step cycles, with annealing temperature of 60° C (Ericomp, San Diego, California). Amplified 98/99-bp product was digested with BseLI (Fermentas, Vilnius, Lithuania) at 55°C and subjected to 6% polyacrylamide gel electrophoresis. The G-to-A transition at nucleotide position 1691 within the factor V gene and the G-to-A transition at nucleotide position 20210 within the prothrombin gene locus were analyzed according to previously reported techniques.

Statistical Methods
Data were analyzed by using SPSS for Windows release 10.0.1 (SPSS, Chicago, Illinois). Values were expressed as mean ± SD. The odds ratio (OR) was used to estimate the relative risk of PTE for persons with a specific genetic profile compared with the risk of these diseases for a person having a referent genetic profile. The chi-square test was used to compare categorical variables. All P values were 2-tailed, and a P value of <.05 was considered statistically significant.

Results
The cases and the controls did not differ significantly by age and sex. The frequency of polymorphisms in PAI-1 gene, PAI-1 4G allele, and PT20210 mutation did not differ significantly between cases and controls. Factor-V-Leiden mutation was significantly higher in the PTE group than in controls. Frequencies of PAI-1 gene polymorphism and FVL and PT20210 mutations in cases and controls are shown in Table 1.

Coexistence of PAI-1 4G allele and PT20210 mutation was present in 4.9% and 3.3% of cases and controls, respectively (OR = 1.5, 95% confidence interval [CI] 0.4-4.5, P > .05), whereas coexistence of PAI-1 4G allele and FVL was present in 13.3% and 6.1% of cases and controls, respectively (OR = 2.3, 95% CI 1.0-5.1, P = .033).

Results for Subgroup Analysis
Results of patients with or without a risk factor for PTE. Thirty-three (23.1%) patients had no documented risk factors for PTE and no FVL and PT20210 mutations. This group was called the idiopathic group. The remaining patients had 1 or more predisposing factors for PTE such as DVT (n = 51, 35.7%), immobilization (n = 52, 36.4%), recent travel history of more than 6 hours (n = 9, 6.3%), malignancy (n = 8, 5.6%), pregnancy (n = 6, 4.2%), stroke (n = 4, 2.8%), estrogen therapy (n = 10, 7%), operation (n = 33, 23.1%), trauma (n = 9, 6.3%), FVL mutation, and PT20210 mutation. When we compared these patients with documented risk factors with the patients in the idiopathic group and both groups with the control group, there was no significant difference in PAI-1 genotypes and allele frequencies (Table 2).

Results of patients having concurrent DVT or isolated PTE. A total of 92 patients had isolated PTE, of whom 51 had current DVT complicated with PTE. When we compared these 2 groups with each other and with the controls, no significant difference was found in different PAI-1 genotypes, PAI-1 allele frequencies, and PT20210 mutation rate. However, FVL
Results in recurrent disease. Out of 143 patients, 20 had recurrent documented thromboembolic disease (16 with DVT and 4 with PTE). No difference was found in different PAI-1 genotypes and alleles in relation to the rate of recurrence. Having FVL and PT20210 mutations appears to increase the risk of recurrence (OR = 2.3, 95% CI 0.8-6.6 and OR = 1.4, 95% CI 0.2-7.0, respectively) but not at a statistical
level. Distribution of gene polymorphisms and the risk assessments of double-gene alterations on recurrence are given in Table 3.

Discussion

In this study, we showed that PAI-1 4G/5G polymorphism is not a risk factor for PTE. It is clear from the previous investigations from the West that higher plasma PAI-1 levels associated with PAI-1 4G allele are responsible for a hypofibrinolytic state, which could serve as a risk factor for arterial and venous thromboses. However, few reports found the presence of PAI-1 4G allele as a solitary risk factor for VTE. The largest cohort investigating the association of PAI-1 polymorphism and VTE was from the United States. Ridker et al examined nearly 15,000 men and found no association with PAI-1 genotype among patients with venous thrombosis and controls. Later, Slovenian and other US studies supported this result.

After comparing the negative results in our study group with controls, we performed subgroup analysis (idiopathic patients and patients with documented risk factors) to investigate if PAI-1 4G/5G polymorphism could serve as a risk factor in idiopathic PTE cases; however, the results failed to show any significance. Again, the results were negative in distinguishing the risk of isolated PTE patients from DVT patients complicated with PTE.

In recent years, increasing numbers of reports emerged investigating the concurrence of PAI-1 gene polymorphism and other thrombophilic factors. According to these data, more than being a solitary risk factor for VTE, PAI-1 gene polymorphism, especially the 4G allele, was found to be acting as an "enhancer" to many other genetic risk factors such as FVL mutation reported by Visanji et al and Junker et al, PT20210 mutation reported by Barcellona et al, protein S deficiency reported by Zöller et al, and antiphospholipid syndrome reported by Tassies et al, where different forms of VTE were studied.

Plasminogen activator inhibitor-1 gene polymorphism was previously studied in another Turkish cohort in our laboratory, where only the concomitance of PAI-1 4G allele and FVL mutation was found to be associated with increased DVT risk. Our current study on two gene alterations showed an increased risk for PTE in the concurrent presence of FVL mutation and PAI-1 4G polymorphism though this was not an "enhancer" effect for FVL mutation.

The results again failed to show an association for the concurrent presence of PAI-1 4G allele and PT20210 mutation as a risk factor for PTE or recurrent disease. The difference between the study population of our study and those of other studies is that we enrolled mainly isolated PTE patients and only some had DVT, whereas the other studies mostly included DVT patients with or without PTE. This difference is important in interpreting the results because the frequency of some thrombotic factors can differ between PTE and DVT patients.

Increased levels of PAI-1 were shown to be correlated with recurrent VTE in the DURAC study. We did not measure PAI-1 levels in this study, but we found no correlation between PAI-1 gene polymorphism and recurrent disease.

One of the major clinical implications of having a genetic thrombophilic factor is its impact on the decision of anticoagulation duration. Those patients with high incidence of recurrence warrant consideration of long-term durations of anticoagulant therapy. We would like to emphasize that the results of the studies with limited number of subjects are not enough to arrive at a conclusion on this topic. As a result, we suggest that before deciding on routine investigation of PAI-1 4G allele in patients affected by both PTE and FVL or PT20210 mutations, further investigations in larger cohorts are needed to better define their role in recurrent disease.

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References


