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Prothrombin, Factor-V Leiden, and Plasminogen Activator Inhibitor Type 1 Gene Polymorphisms in Hemodialysis Patients with/without Arteriovenous Fistula Thrombosis

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Abstract

Background and Aims: Factor V Leiden mutation (G1691A) has been recognized to be the most prevalent genetic risk factor for venous thrombosis. Other common risk factor for venous thrombosis is Prothrombin 20210 G-A alteration, which causes a gain of function in the coagulation system with an increase of prothrombin levels, associated with an increased potential to form thrombin. Plasminogen activator inhibitor type 1 (PAI-1) 4G/5G polymorphism is the most frequently studied in thrombotic events. Aim of this study was to investigate the relationship between these polymorphisms and arteriovenous fistula (AVF) thrombosis in hemodialysis (HD) patients.

Methods: The study included 31 HD patients with AVF thrombosis and 51 HD patients without AVF thrombosis. DNA was extracted from peripheral blood samples from the patient and control groups. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods were used to identify the polymorphisms.

Results: There were no significant differences between HD patients with and without AVF thrombosis in terms of Factor V Leiden and Prothrombin G20210A mutations. PAI-1 4G allele in heterozygous state have an effect on the thrombosis risk in HD patients (O.R= 4.271).

Conclusions: Only carrying PAI-1 4G/5G genotype had an additional risk for thrombosis in HD patients.

Keywords: Hemodialysis, Factor V Leiden mutation, Plasminogen Activator Inhibitor Type 1 4G/5G Polymorphism, Prothrombin G20210A Mutation

Introduction

Several inherited factors that predispose to thrombosis have been identified (1). Factor V Leiden mutation (G1691A) has been recognized to be the most prevalent genetic risk factor for venous thrombosis (2). Factor V plays a major role in homeostasis by converting prothrombin to thrombin. The mutation at the nucleotide 1691 of Factor-V gene results in an amino acid substitution of arginine to glutamine (3). Factor V Leiden is highly prevalent in eastern Mediterranean countries including Turkey (4).

The mutation of prothrombin gene, due to a G to A transition at nucleotide 20210 in the 3'-untranslated region, is associated with an increased risk for venous thrombosis (5). The mutation is the second most frequent factor for venous thrombosis

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(with prevalence varying between 5% and 19%) (6). It has been found that an association between the 20210A allele and elevated prothrombin levels, which can be a risk factor for venous thrombosis (7). Factor V Leiden, which changes Factor V activity, and Prothrombin G20210A polymorphisms, which enhance prothrombin accumulation, are independent risk factors for thrombosis. These mutations consequently promote blood coagulation and precipitate a thromboembolic state (4).

Plasminogen activator inhibitor type 1 (PAI-1) regulates the homeostasis of the fibrinolytic system (8). The human PAI-1 gene is located on the long arm of chromosome 7 and contains 9 exons and 8 introns (9). A few polymorphisms of the PAI-1 gene have been discovered. The 4G/5G polymorphism in the promoter region of the gene, 675 bp upstream of the transcriptional start site, is the most frequently studied in thrombotic events (10). Reduced fibrinolytic activity due to elevated plasma PAI-1 activity is common in patients with venous thromboembolism (11). It has been reported that plasma PAI-1 concentration in homozygous subjects for the 4G allele (4G/4G genotype) was approximately 25% higher than that of homozygous subjects for the 5G allele (5G/5G genotype) (9).

Aim of this study was to investigate whether the Prothrombin G20210A, Factor V G1691A, and PAI-1 4G/5G genotypes influence the risk of thrombosis in hemodialysis (HD) patients with/without arteriovenous fistula (AVF) thrombosis.

Materials and Methods

Thirty-one (20 male and 11 female) HD patients with AVF thrombosis, who had no apparent cause for thrombosis, were enrolled in this study. On the other hand, 51 (22 male and 29 female) HD patients without AVF thrombosis were selected as control group. The study protocol was approved by the local ethics committee. The study procedures were approved by all patients. All patients were enrolled from Nephrology Department in Erciyes University Medical School between 2007 and 2008 for this study which was performed in Medical Genetics Department in Erciyes University Medical School.

In both groups, genomic DNA was extracted from peripheral blood by using DNA isolation machine (Roche Magna Pure LC). We used polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods to determine all the polymorphisms.

We performed a PCR procedure in a total volume of 50 µl using 5 µl genomic DNA, 10xPCR Buffer, dNTP (2,5mM), MgCl2 (1,5mM), Taq DNA polymerase (1U/ml, Sigma), 5'- TCTAGAAACAGTTGCCTGGC-3' forward primer and 5'- ATAGCACTGGGAGCATTGAAGC-3' reverse primer (10 pmol primer concentration was used) to detect the Prothrombin G20210A mutation. PCR was subjected to 35 cycles at 94°C for 5 min in denaturation period, 94°C for 1 min, 56°C for 1 min and 72 °C for 1 min with 35 cycles, the final extension step at 72 °C was extended by 1 min. PCR product was 345 bp and detected by 2% agarose. Hind III restriction enzyme was used. The enzymatic digestion generates one fragment of 345 bp from the normal allele and two fragments of 322 bp and 23 bp from the mutant allele. We analyzed the fragments by using 3% agarose gel electrophoresis.

5'- TCTAGAAACAGTTGCCTGGC-3' forward primer and 5'-ATAGCACTGGGAGCATTGAAGC-3' reverse primer were used to detect Factor V Leiden (G1691A) mutation. A PCR mix has been prepared as previously described in Prothrombin mutation detection. PCR product was 223 bp and detected by 2% agarose. After amplification, the PCR product was digested overnight at 37 °C with Mnl I restriction enzyme and analyzed by 3% agarose gel electrophoresis.

PAI-1 4G/5G polymorphism was performed with forward primer 5'- CACAGAGAGAGTCTGGCCACGT-3' and

Table 1. Genotype and allele frequencies for the Factor V Leiden G1691A in patients with AVF thrombosis and those without AVF thrombosis.

Factor-V G1691A Genotype	Patients with AVF thrombosis (n: 31)	Patients without AVF thrombosis (n: 51)	OR (CI 95%)	P value
G1691G	22 (71%)	36 (70.6%)	1.019 (0.382-2.719)	0.588
G1691A	9 (29%)	15 (29.4%)	0.982 (0.368-2.621)	0.588
A1691A	-	-	-	-
G allele	53 (85.5%)	87 (85.3%)	1.015 (0.415-2.483)	0.582
A allele	9 (14.5%)	15 (14.7%)	0.985 (0.403-2.408)	0.582

AVF, arteriovenous fistula; **OR**, odds ratio; **CI**, confidence interval.

5'- CCAACAGAGGACTCTTGGTCT-3' reverse primer. Amplification was performed for 30 cycles with denaturation temperature of 94°C for 3 min, 94°C for 30 sec, 60°C for 30 sec and 72 °C for 30 sec, the final extension step at 72 °C was extended by 1 min. Amplified 98 bp product was digested overnight with Bsl I at 55°C and subjected to 4% agarose gel electrophoresis.

Statistics:

The Chi-square test and Fisher's exact test were used to compare the distributions of Prothrombin, Factor V Leiden and PAI-1 genotypes and allele frequencies between the patients and control groups. The results were expressed as Odds ratio (OR) and 95% confidence intervals (CI 95%) (11, 12). OR and 95% CI were calculated in terms of the relative risk of venous thromboembolism (VTE) in the presence of the mutations in homozygous, heterozygous and normal states, compared to that in their absence. A p value <0.05 was accepted as statistically significant.

Results

None of the subjects were found to have prothrombin G20210A mutation (heterozygous and homozygous form was not detected) in both groups.

The allele frequency of the Factor V Leiden G1691A and the distribution of the Factor V Leiden G1691A genotypes in 31 patients with AVF thrombosis were compared with 51 patients without AVF thrombosis (Table I). Factor V G1691A mutation with homozygous genotype was not detected in both groups. There were no significant differences between HD patients with and without AVF thrombosis in terms of the distribution of Factor V G1691A mutation and G and A allele frequencies (p>0.05).

The frequency of the PAI-1 4G allele and the distribution of PAI-1 4G/5G genotypes in 31 patients with AVF thrombosis were compared with 51 patients without AVF thrombosis (Table II). Presence of PAI-1 5G/5G genotype was significantly lower in patients with AVF thrombosis compared to those without AVF thrombosis (16.1% vs. 37.3%, p=0.035). On the other hand, presence of PAI-1 4G/5G genotype was significantly higher in patients with AVF thrombosis compared to those without AVF thrombosis (83.9% vs. 54.9%, p=0.006). There was no meaningfully difference between two groups in terms of 4G/4G genotype (p > 0.05). Genotype 4G/4G was not detected in the patients with AVF thrombosis. However, it was detected in 4 of the patients without AVF thrombosis. There was no meaningful difference between two groups in terms

Table 2. Genotype and allele frequencies for the PAI-1 4G/5G polymorphism in patients with AVF thrombosis and those without AVF thrombosis.

PAI-1 Genotype	Patients with AVF thrombosis (n: 31)	Patients without AVF thrombosis (n: 51)	OR (CI 95%)	P value
PAI-1 5G/5G	5 (16.1%)	19 (% 37.3)	0.324 (0.106-0.986)	0.035
PAI-1 4G/5G	26 (83.9%)	28 (% 54.9)	1.415-12.892))4.271	0.006
PAI-1 4G/4G	-	4 (% 7.8)	1.660 (1.386-1.987)	0.143
PAI-1 4G allele	26 (41.9%)	36 (% 35.3)	1.324 (0.693-2.530)	0.246

PAI-1, Plasminogen Activator Inhibitor Type 1; **AVF**, Arteriovenous Fistula; **OR**, Odds Ratio; **CI**, Confidence Interval.

of 4G allele, although its frequency was higher in patients with AVF thrombosis compared to those without AVF thrombosis ($p > 0.05$).

Discussion

Cardiovascular disease is the most cause of the mortality in dialysis patients (13-15). In the fibrinolytic system there are two important enzymes; tissue plasminogen activator (t-PA) and PAI (16). In HD patients, the thrombotic events are higher because of t-PA and PAI-1 balance is altered. Vascular thrombosis is one of the most important complications in HD patients (17). PAI-1 enzyme levels are higher in chronic renal failure patients than in healthy persons. Therefore thrombogenesis and thrombotic events are more frequent in these patients (18).

Segui et al detected that PAI-1 polymorphism affects PAI-1 levels. PAI-1 4G genotype increases the PAI-1 level and also increases the thrombosis risk in thrombophilic patients (19). PAI-1 4G allele carriers potentially decrease fibrinolytic activity (20). However, some studies revealed that PAI-1 4G allele in homozygous or heterozygous state does not affect thrombosis risk (21-23). In our study, presence of PAI-1 normal genotype is significantly lower in patients with AVF thrombosis compared to those without AVF thrombosis while PAI-1 heterozygous genotype was significantly prevalent in patients with AVF thrombosis than those without AVF thrombosis. In our study, we observed that carrying the 4G allele in heterozygous state have an effect on the thrombotic risk. Also PAI-1 in homozygous state has an effect on the thrombosis risk between groups. In addition, carrying 4G allele increases this risk. According to this data thrombosis risk seemed to be increased. Our results and those from the other some studies (19, 24) are in contrast with the study was performed by Irish et al They found that there was no significant difference in the patients with chronic renal disease not requiring dialysis and healthy controls (23).

Another study was performed by Visanji et al in 99 patients with Factor V Leiden carriers and 99 controls and it was found that PAI-1 4G allele is more prevalent in patients than in controls (11). Similarly, in our study 4G allele frequency was found more prevalent in patients with AVF thrombosis than in controls without AVF thrombosis.

Factor V G1691A mutation is one of the most common mutations reported in the Turkish population (25). It was revealed that Factor V G1691A has significant effect on the development of thrombosis (12). Gürgey et al studied with Factor V G1691A and Prothrombin G20210A mutations in thrombosis

patients. They found 45 patients with G1691A mutation and 10 patients with G20210A mutation. According to their results thrombosis was determined an important genetic risk factor for Turkish population (26). But in our study no Prothrombin G20210A mutation was detected among the patient groups who were similar for carrying Factor V G1691A mutation. No difference was found between the groups for normal genotype. Also homozygous genotype was not found. There was also no difference for Factor V G1691A mutation between heterozygous and normal genotypes in the groups. This result contrasts with Gürgey et al. Therefore we are not sure that there is an increased risk factor for thrombosis in chronic renal failure patients with/without Factor V Leiden and Prothrombin mutations. We conclude that large groups must be studied to provide more significant and conclusive data.

In this study we did not find any relationship between thrombosis and the mutations of Prothrombin or Factor V Leiden in hemodialysis patients with/without AVF thrombosis. Only carrying PAI-1 4G/5G genotype had an additional risk for thrombosis. We also conclude that in future researches PAI-1 4G/5G polymorphism must be studied as a risk factor for thrombosis.

Conflict of Interest

None declared.

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