Investigation of Plasminogen Activator Inhibitor-1 4G/5G Gene Polymorphisim in Patients with Pre-Diagnosed Cerebrovascular Disease, Coagulation Disorder and Hypertension

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Eur J Basic Med Sci 2013;3(3): 56-60

Received: 02-07-2013

Accepted: 18-11-2013

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ABSTRACT

Plasminogen activator inhibitor-1 (PAI-1); gene polymorphism is the main inhibitor of fibrinolysis, and high levels may increase the risk of cardiovascular disease. The polymorphism of the 4G/5G gene is located in the PAI-1 gene promoter region. In this study, our objective is to investigate the genotype and allele distributions of PAI-1 gene polymorphisms in patients with cerebrovascular disease, coagulation disorder and those pre-diagnosed with hypertension. The results of the analysis PAI-1 gene polymorphism in 109 patients were evaluated retrospectively. These patients were divided into three groups as cerebrovascular disease, hypertension and coagulation disorder. In the analysis, CVD T (cardiovascular disease thrombosis) stripassay kit which is based on reverse hybridization technique was used. No statistically significant difference was found between three groups in terms of genotype and allele frequencies. PAI-1 4G/5G genotype in cerebrovascular disease and hypertension groups were found to be statistically significant compared with 4G/4G genotype, in terms of intra group comparison of genotype distrubutions of the group. In the group pre-diagnosed with coagulation disorder PAI-1 4G/5G genotype were found to be statistically significant compared with 4G/4G and 5G/5G. In conclusion; PAI-1 4G/5G genotype may be evaluated a risk factor in patients cerebrovascular disease, coagulation disorder and hypertension.

Key Words: *Plasminogen activator inhibitor-1, PCR, polymorphism, 4G/5G*

Serebrovasküler Hastalık, Koagülasyon Bozukluğu ve Hipertansiyon Ön Tanısı Alan Hastalarda Plazminojen Aktivator İnhibitör-1 4G/5G Gen Polimorfizminin Araştırılması

ÖZET

Plazminojen aktivator inhibitor-1 (PAI-1) gen polimorfizmi fibrinolizis oluşmasında başlıca inhibitördür ve artan PAI-1 seviyesi kardiyovasküler hastalıkların riskini arttırabilir. 4G/5G gen polimorfizmi PAI-1 genin başlangıç bölgesinde lokalize olmuştur. Bu çalışmada amacımız; serebrovasküler hastalık, koagülasyon bozukluğu ve hipertansiyon ön tanısı alan hastalarda PAI-1 gen po-

limorfizminin genotip ve allel dağılımlarını araştırmaktır. 109 hastanın PAI-1 gen polimorfizm analiz sonuçları retrospektif olarak değerlendirildi. Bu hastalar serebrovasküler hastalık, koagülasyon bozukluğu ve hipertansiyon olarak üç gruba ayrıldı. Analizde ters hib-ridizasyon yöntemini esas alan CVD T (cardiovascular disease thrombosis) stripassay kiti kullanıldı. Serebrovasküler hastalık, hipertansiyon, koagülasyon bozukluğu ön tanısı alan hastaların PAI-1 4G/5G polimorfizminin genotip frekansı açısından her üç grubun karşılaştırılmasında istatistiksel olarak bir fark olmadığı tespit edildi. Grupların genotip dağılımlarının grup içi karşılaştırmaları için serebrovasküler hastalık ve hipertansiyon gruplarında; PAI-1 4G/5G genotipi, 4G/4G genotipine göre istatistiksel olarak anlamlı olduğu tespit edildi. Koagülasyon bozukluğu ön tanısı alan grupta ise; PAI-1 4G/5G genotipi 4G/4G ve 5G/5G genotiplerine göre istatistiksel olarak anlamlı olduğu tespit edildi. Sonuç olarak; serebrovasküler hastalık, koagülasyon bozukluğu ve hipertansiyon ön tanısı alan hastalarda PAI-1 4G/5G genotipi bir risk faktörü olarak değerlendirilebilir.

Anahtar Kelimeler: *Plazminojen aktivator inhibitör-1, PCR, polimorfizm, 4G/5G*

INTRODUCTION

Functional capacity of fibrinolytic system of plasminogen activator inhibitor-1 is an important factor on developing thrombus. Reduced fibrinolytic activity is correlated with increased PAI-1 level (1). Plasminogen activator inhibitor-1 is a single bonded glycoprotein and has a molecular weight of 52 000 kD. It consists of 379 amino acid. Because of the fact that it doesn't have cysteine, it doesn't have disulphid bridge. It is the most important physiologic inhibitor of tissue plasminogen activator and urokinse-type plasminogen activator on human plasma. It belongs to the super family of serin proteaz inhibitor (serpin).

PAI-1 waves as an active molecule, however under physiologic circumstances it becomes functionless spontaneously (2). Plasminogen activator inhibitor-1 gene polymorphism is formed by 9 exon and 8 intron which are localized on 7th (7q21.3-22) chromosome. 4G/5G gene polymorphism localizes PAI-1 gene on promoter zone. It is a single based insertion (5G) deletion (4G) polymorphism generated from the starting zone transcription of PAI-1 gene. PAI-1 gene has three sub genotypes. These are; 4G/4G (homozygous mutant), 4G/5G (heterozygous) and 5G/5G (normal type). 4G/4G allele carriers always carry higher level of plasmic PAI-1 activity than 4G/5G and 5G/5G carriers (3). In the study which was carried on coroner arter patients in Indian population, Ashavaid et al. (4) found that PAI-1 4G/5G genotypes didn't effect the patients severely. In the study which was carried on patients who had myocardial infarction in Tunisian population by Abbout et al. (5) it was claimed that 4G allele carrier density and plasmic PAI-1 activity was in a high level.

In this work, our aim is to study genotype and allele frequency PAI-1 gene polymorphism on patients pre-diagnosed with cerebrovascular illness, coagulation disorder and hypertension in the city of Van and its around.

MATERIALS AND METHODS

In this work, peripherical blood samples of the patients who came from different clinics (internal disease clinic, neurology clinic etc.) to biochemistry laboratory of medical faculty of the University of Yüzüncü Yıl were collected. All of the patients' blood samples were obtained in terms of declaration of Helsinki. 109 patients were included in the study. In one year's time (October 2009-November 2010), the results of the cardiovascular disease thrombosis (CVD T) gene mutation analysis of the 109 patients (75 female, 34 male) were evaluated retrospectively. These patients were divided in three different groups.

Group I: The patients who were pre-diagnosed with cerebrovascular illness (n= 37),

Group II: The patients who were pre-diagnosed with hypertension (n= 31),

Group III: The patients who were pre-diagnosed with coagulation disorder (n= 41).

This study was carried out by using PCR method, using a ready commercial kits, (CVD T Stripassay, ViennaLab Labordiagnostika GmbH, Austria) based on reverse hybridization with strip test method. 3 ml blood obtained from cases were put in tubes with EDTA. The stripassay method is carried out in four levels. DNA was extracted from peripherical leucocyte using standard method. Genomic DNA was obtained. The exon mutations which we were scanned with multiplex PCR broadened by using primers labeled with biotin. After amplification with PCR, outcomes PCR were controlled by using 3 %

***Table 1**. Inter-group comparisons of genotype ranges of groups.*

Genotype frequency	Cerebrovascular illness	Hypertension	Coagulation disorder	p value		
	(n= 37)	(n= 31)	(n= 41)	Ci-H	Ci-Cd	H-Cd
4G/4G	7 (19%)	7 (22%)	9 (22%)	0,710	0,741	0,949
4G/5G	18 (49%)	16 (52%)	23 (56%)	0,808	0,705	0,547
5G/5G	12 (32%)	8 (26%)	9 (22%)	0,297	0,705	0,297

Ci: Cerebrovascular illness H: Hypertension Cd: Coagulation disorder

agarose gel. One wild type which is immobilized on the hybridization phase of outcomes of PCR and outcomes of PCR with oligonucleotide were hybridized in one mutant zone. The hybrits were become apparent by the reaction of streptavidin alkali phosphatase and colour substrate.

Statistical Analysis

Genotype and allele frequencies were stated as numbers and percentage. In the way of genotype and allele frequencies in the comparison of groups who were pre-diagnosed with cerebrovascular illness, hypertension and coagulation disorder; Independent ratio comparison test was used with Z test. Statistic meaningfullness degree was taken 5 % in calculations and MINITAB for windows statistics pocket program was used for calculations.

RESULTS

The study was included 109 (75 female, 34 male) patients. 23 of these 109 patients were homozygous (4G/4G, 21.10 %), 57 of these 109 patients were heterozygous (4G/5G, 52.29 %) and 29 of these 109 patients had normal (5G/5G, 26.61 %) genotype. The patients who were pre-diagnosed with cerebrovascular illness, hypertension and coagulation disorder were examined in the way of PAI-1 gene polymorphism genotype frequency and it was determined that these three groups had no difference statistically ($p>0.05$) (Table 1). The groups were observed in terms of allele frequence; 4G allele frequence was 43 % (cerebrovascular illness), 48 % (hypertension), 50 % (coagulation disorder), respectively. 5G allele frequence was 57 % (cerebrovascular illness), 52 % (hypertension), 50 % (coagulation disorder) respectively (Table 2).

When the groups' genotype ranges were compared with in-group comparisons, the groups who were pre-diagnosed with cerebrovascular illness and hypertension had normal heterozygous genotype of PAI-1 gene polymorphism and in comparison with homozygous mutant group, they were statistically more meaningfull. And for the group of patients who pre-diagnosed with coagulation disorder, PAI-1 gene polymorphism was statistically more meaningfull in comparison with both 4G/4G and 5G/5G genotypes ($p<0.05$) (Table 3).

DISCUSSION

Main function of plasminogen activator inhibitor-1 is to decrease fibrinolysis, start the accumulation of fibrin which is correleated with thrombotic illnesses (6). The studies on people and mice has shown that PAI-1 gene polymorphism is a candidate gene for arterial hypertension. Martinez-Calatrava et al. (7) researched whether PAI-1 gene polymorphism is risk indicator for arterial hypertension or not. When the results were evaluated it was found out that, without considering other factors of hypertension and plasmic PAI-1 level, the fact that high levels of incidence on PAI-1 4G/4G genotype's arterial hypertension is a risk. In the study of Jeng (8), it was stated that hypertension patients who have genotype of 4G/4G, there was a raise on PAI-1 enzyme activity, but they needed to be studied on a larger population of people.

***Table 2**. Allele frequency of PAI-1 gene polymorphism.*

Allele frequency	Cerebrovascular illness (n= 37)	Hypertension (n= 31)	Coagulation disorder (n= 41)
4G	32/74 (43 %)	30/62 (48 %)	41/82 (50 %)
5G	42/74 (57 %)	32/62 (52 %)	41/82 (50 %)

***Table 3**. Intra-group comparisons of genotype ranges of groups (p value).*

Genotype ranges	Cerebrovascular illness	Hypertension	Coagulation disorder
4G/5G/4G/4G	0,004	0,013	0,001
4G/5G/5G/5G	0,150	0,767	0,001

Song et al. (9) researched the effect of environmental and genetical component on PAI-1 level. They suggested that plasmic PAI-1 level wasn't only under control of genetic structure, but also some environmental factors. Thus there were an intristic interaction between environmental factors and genetical structure. In the study of Margaglione et al. (10) on coroner arterial patients in South Italy region, they stated that PAI-1 4G/5G polymorphism had an effect on the progression of illness with other risk factors. In the study which Catto et al. (11) worked on it was stated that PAI-1 enzyme activity on patients who had stroke was a lot higher than healthy people. In their study, after they had studied 2565 patients, Gardemann et al. (12) stated that PAI-1 gene polymorphism was an independent risk factor on coroner atherosclerosis. In the study of Ding et al. (13) which was carried out on both white and black stroke patients, they were stressed the fact that there was no connection between PAI-1 gene polymorphism and stroke but genotypes of this polymorphism was effective on plasmic PAI-1 enzyme activities. Onalan et al. (14) studied Turkish population and they asserted that PAI-1 4G/4G genotype was an independent indicator on proggession of myocardial infarction. In the study of Zhan et al. (15) carried out in China, the patients who were diagnosed miyocardial and cerebral infarction, PAI-1 4G/5G genotype had a high risk on these patients. In the study on swedish population by Wiklund et al. (16) between two control case, it was concluded that PAI-1 4G/4G and 4G/5G genotypes had enhanced risk factor on ischemic stroke. There are lots of researches in literature for PAI-1 gene polymorphism which was the case in this study. And these researches are the researches which seek for a connection between a present illness. In the literature review we did, we didn't find any comparison of PAI-1 gene polymorphism between three illnesses which had different etiopathogenesis. Therefore the research of three different illnesses in this study is fairly important and it has been the first research. In this study, we expressed the genotype and allele frequencies of patients groups as percentage. When we look at the genotype and allele frequencies of groups as paired comparison, we determined that there were no statistically difference between these three groups ($p>0.05$). In a study on patients, who had different types of thrombosis, Balta et al. (17) showed that PAI-1 4G/4G and 4G/5G genotypes might be correlated with thrombosis in the vessels of inner organs and especially portal vein thrombosis. In the study of Kim et al. (18) which was carried on female patients who were diagnosed with hypertension, they asserted that PAI-1 gene polymorphism was a polymorphism which could contribute to the hypertension, gene-environment interaction had no effect on patients who were diagnosed with hypertension and there needed to be a larger population to study. Akhter et al. (19) researched the relationship between deep vein thrombosis and PAI-1 gene polymorphism in Asian-Indian population. They stated that there were a higher prevelance of 4G allele between the deep vein thrombosis patients and control group and the data was compatible with Caucasian population.

In our study, it was determined that in-group comparisons of genotype range, the groups who were pre-diagnosed with cerebrovascular illness and hypertension had a normal heterozygous PAI-1 gene polymorphism and statistically much more meaningfull than homozygous mutant group, but the group who were pre-diagnosed with coagulation disorder had a statistically meaningfull PAI-1 gene polymorphism than both 4G/4G and 5G/5G genotypes. In all of the groups we studied the 4G/5G patients were more than others but among the patients with coagulation disorder, there were more 4G/5G patients than other groups. In this sense, the research which were carried out before support our research. The most important handicap of our research is that we do not have a control group. As a result; 4G/5G genotype of PAI-1 gene polymorphism can be evaluated as a risk factor on the patients pre-diagnosed with cerebrovascular illness, hypertension and coagulation disorder in the city of Van and its around.

REFERENCES

1. *Hamsten A, Wiman B, de Faire U, Blomback M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. N Engl J Med 1985; 313: 1557-63.*
2. *Dobrovolsky AB, Titaeva EV. The fibrinolysis system: regulation of activity and phsiologic functions of its main components. Biochem 2002; 67: 99-108.*
3. *Eriksson P, Kallin B, van't Hooft FM, Bavenholm P, Hamsten A. Allele specific increase in basal transcription of the plasminogen-activator inhibitor-1 gene is associated with myocardial infarction. Proc Natl Acad Sci USA 1995; 92: 1851-5.*
4. *Ashavaid TF, Todur SP, Kondkar AA, et al. Platelet poly-*

morphisms: frequency distribution and association with coronary artery disease in an Indian population. Platelets 2011; 22: 85-91.

5. *Abboud N, Ghazouani L, Saidi S, et al. Association of PAI-1 4G/5G and -844G/A gene polymorphisms and changes in PAI-1/tissue plasminogen activator levels in myocardial infarction: a case control study. Genet Test Mol Biomarkers 2010; 14: 23-7.*
6. *Pawse AR, Tarachand U. Clot lysis: role of plasminogen activator inhibitors in haemostasis and therapy. Indian J Exp Biol 1997; 35: 545-52.*
7. *Martínez-Calatrava MJ, Gonzalez-Sanchez JL, Zabena C, et al. Is the plasminogen activator inhibitor-1 gene a candidate gene predisposing to hypertension? Results from a population-based study in Spain. J Hypertens 2007; 25: 773-7.*
8. *Jeng JR. Association of PAI-1 gene promoter 4G/5G polymorphism with plasma PAI-1 activity in Chinese patients with and without hypertension. American Journal of Hypertension 2003; 16: 290-6.*
9. *Song Y, Xu W, Chen Y, et al. The influences of genetic and environmental factors on plasma plasminogen activator inhibitor-1 levels in patients with essential hypertension. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 1999; 16: 374-6.*
10. *Margaglione M, Cappucci G, Colaizzo D, et al. The PAI-1 gene locus 4G/5G polymorphism is associated with a family history of coronary disease. Arteriosclerosis Thrombosis Vascular Biology 1998; 18: 152-6.*
11. *Catto AJ, Carter AM, Stickland M, et al. Plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphism and levels in subjects with cerebrovascular disease. Thromb Haemost 1997; 77: 730-4.*
12. *Gardemann A, Lohre J, Katz N, et al. The 4G4G genotype of the plasminogen activator inhibitor 4G/5G gene polymorphism is associated with coronary atherosclerosis in patients at high risk for this disease. Thromb Haemost 1999; 82: 1121-6 (Abstract).*
13. *Ding J, Nicklas BJ, Fallin MD, et al. Plasminogen activator inhibitor type-1 gene polymorphisms and haplotypes are associated with plasma plasminogen activator inhibitor type 1 levels but nor with myocardial infarction or stroke. American Heart Journals, 2006; 152: 1109-15.*
14. *Onalan O, Balta G, Oto A, et al. Plasminogen activator inhibitor-1 4G4G genotype is associated with myocardial infarction but not with stable coronary artery disease. J Thromb Thrombolysis 2008; 26: 211-7.*
15. *Zhan M, Zhou Y, Han Z. Plasminogen activator inhibitor-1 4G/5G gene polymorphism in patients with myocardial or cerebrovascular infarction in Tianjin. China Chin Med J 2003; 116: 1707-10.*
16. *Wiklund P, Nilsson L, Ardnor SN, et al. Plasminogen activator inhibitor-1 4G/5G polymorphism and risk of stroke: Replicated findings in two nested case-control studies based on independent cohorts. Stroke 2005; 36: 1661-5.*
17. *Balta G, Altay Ç, Gurgey A. PAI-1 Gene 4G/5G Genotype: A Risk Factor For Thrombosis in Vessels of Internal Organs. Am J Hematol 2002; 71: 89-93.*
18. *Kim KN, Kim KM, Kim BT, et al. Relationship of plasminogen activator inhibitor 1 gene 4G/5G polymorphisms to hypertension in Korean women. Chin Med J 2012; 125: 1249-53.*
19. *Akhter MS, Biswas A, Ranjan R, et al. Plasminogen activator inhibitor-1 (PAI-1) gene 4G/5G promoter polymorphism is seen in higher frequency in the Indian patients with deep vein thrombosis. Clin Appl Thromb Hemost 2010; 16: 184-8.*