The Association of Plasma PAI-1 and tPA Antigen Concentrations with Related Cardiac Risk Factors in Patients with Acute Coronary Syndrome and Their First Degree Relatives

AKUT KORONER SENDROMLU HASTALARDA VE BİRİNCİ DERECEDEN AKRABALARINDA PLAZMA PAI-1 VE tPA ANTİJEN DÜZEYLERİNİN KARDİYAK RİSK FAKTÖRLERİ İLE İLİŞKİSİ

Dilek SOYSAL, MD,[a] Tuğba EMÜROĞLU, MD,[b] Serdar BİÇEROĞLU, MD,[c] Çetin ÇEVİK, MD,[a] Sadık ULUSOY, MD,[d] Nihal K. CENGİZ, MD,[a] Gülbin S. ÇETİNKAYA, MD[a]

Departments of [a]Internal Medicine, [c]Cardiology, [d]Biochemistry, Atatürk Research Hospital, İZMİR
[b]Clinics of Internal Medicine, Merzifon State Hospital, AMASYA

Abstract

Objective: To assess the association of plasma plasminogen activator inhibitor 1 (PAI-1) and tissue plasminogen activator (tPA) antigen concentrations and related cardiac risk factors in patients with acute coronary syndrome and their first degree relatives.

Material and Methods: Thirty-eight patients with acute coronary syndrome (33 men and 5 women, mean age 58 ± 9 years) and their 20 first-degree relatives (16 men and 4 women, mean age 52±7 years) were included in the study. The mean period of time that elapsed between the last episode of chest pain and blood sampling from the patients was 3.5 ± 1 hours. Blood samples were collected from patients and their relatives simultaneously. All patients underwent coronary angiography. Thirty patients had multivessel disease.

Results: The two groups did not differ in gender (p= 0.218). Patients were older (p< 0.05) and smoked more (p< 0.001) than their relatives. There were no significant differences in plasma tPA and PAI-1 antigen concentrations between patients and their relatives (10.4 ± 5.6 vs 11.4 ± 4.6 ng/ml, p= 0.452 and 68.9 ± 23.5 vs 68.8 ± 17.3 ng/ml, p= 0.583), and, between patients with multivessel disease and their relatives (11 ± 6 vs 11.4 ± 4.6 ng/ml, p= 0.168 and 70.6 ± 24.1 vs 68.8 ± 17.3 ng/ml, p= 0.996, respectively). In the first degree relatives group there was a moderate, inverse correlation between PAI-1 antigen and HDL cholesterol (rho= -0.47; p< 0.05). In the patient group, LDL cholesterol and triglycerides significantly correlated with plasma PAI-1 antigen concentrations (t= 2.084, p= 0.045 and t= 2.365, p= 0.024) with strong partial correlation coefficients (β= 0.983 and β= 1.13 respectively).

Conclusion: The insignificance of the difference between patients with acute coronary syndrome and their healthy first-degree relatives in plasma levels of tPA and PAI-1 antigen and the poor relation of these markers with cardiac risk factors may be attributed to the multifactorial mediation of the release and functioning of tPA and PAI-1.

Key Words: Myocardial infarction, tPA antigen, PAI-1

Özet

Amaç: Akut koroner sendromlu hastalar ve birinci dereceden akrabalarında plazma PAI-1 ve tPA antijen konsantrasyonlarını değerlendirmek, kardiyak risk faktörleri ile ilişkisini araştırmaktır.

Gereç ve Yöntemler: Çalışmaya yaş ortalaması 58 ± 9 yıl olan, 33 erkek 5 kadın toplam 38 akut koroner sendromlu hasta ve hastalara refakat eden yalnızca birinci dereceden akrabalarından, yaş ortalaması 52 ± 7 yıl, 16 erkek 4 kadın toplam 20 kişi alındı. Venöz kan örnekleri tüm hastalardan son iskemik kökenli göğüs ağrısını takip eden ortalama 3.5 ± 1 saat içerisinde ve akrabalarından da eş zamanlı olarak usulüne uygun biçimde alındı. Hastaların tamamına anjiyografi yapıldı ve 30'unun çok damar hastası olduğu saptandı.

Bulgular: Her iki grupta da cinsiyet açısından fark yoktu (p= 0.218). Hastaların yaşı ve kullandıkları sigara paket yılı akrabalarına göre anlamlı olarak yüksekti, sırasıyla (p< 0.05 ve p< 0.001). Plazma tPA antijen konsantrasyonu tüm hastalarda 10.4 ± 5.6 ng/ml, akrabalarında 11.4 ± 4.6 ng/ml (p= 0.452) ve çok damar hastalarında 11±6 ng/ml, akrabalarında 11.4 ± 4.6 ng/ml (p= 0.168) bulundu.Plazma PAI-1 antijen konsantrasyonu tüm hastalarda 68.9 ± 23.5 ng/ml, akrabalarında 68.8 ± 17.3 ng/ml (p= 0.583) ve çok damar hastalarında 70.6 ± 24.1 ng/ml, akrabalarında 68.8 ± 17.3 ng/ml (p= 0.996) bulundu. Akrabaların oluşturduğu grupta plazma PAI-1 antijen ile HDL kolesterol arasında orta derecede güçlü, ters korelasyon saptandı (rho= -0.47, p< 0.05). Hastaların oluşturduğu grupta, LDL kolesterol ve trigliseridler ile plazma PAI-1 antijen arasında güçlü bölümsel korelasyon katsayısı (sırasıyla, β= 0.983 ve β= 1.13) ve pozitif korelasyon (sırasıyla, t= 2.084, p= 0.045 ve t= 2.365, p= 0.024) saptandı.

Sonuç: Plazma tPA ve PAI-1'in yapımında ve fonksiyonunda birçok faktörün rol oynaması, tPA ve PAI-1 antijenin plazma düzeyleri arasındaki farkın akut koroner sendrom hastalarında ve onların sağlıklı birinci dereceden akrabalarında anlamsız olmasını ve kardiyak risk faktörleri ile arasındaki zayıf ilişkiyi açıklayabilir.

Anahtar Kelimeler: Akut koroner sendrom, tPA antijeni, PAI-1

Turkiye Klinikleri J Cardiovasc Sci 2006, 18:4-12

Geliş Tarihi/*Received*: 06.01.2005 **Kabul Tarihi/*Accepted*:** 07.04.2005

Yazışma Adresi/*Correspondence*: Dilek SOYSAL, MD
Atatürk Research Hospital,
Department of Internal Medicine, İZMİR
dileksoysal@hotmail.com

The development of coronary artery disease, and specifically myocardial infarction extends from hyperplasia of arterial smooth muscle to thrombus formation and vessel occlusion.[1] These changes are in part genetically

determined, as demonstrated by the fact that the risk of myocardial infarction in persons who have a first degree relative with myocardial infarction is seven-fold higher than the risk in persons who do not, but the extent of the contribution of a shared environment to the risk must also be considered. [2]

Incidence of acute myocardial infarction follows a circadian rhythm with a peak in the morning and a number of different factors may play a role in the circadian variation of arterial thrombosis. Heart rate, arterial blood pressure, catecholamine levels, cortisol levels and platelet aggregability were reported to peak in the morning hours, thus increasing the risk of thrombus formation.[3] The fibrinolytic system is among the major determinants of thrombus turnover, and the key components of the fibrinolytic system are tissue plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1).[4] PAI-1 determines the amount of free tPA that is available for actual plasminogen activation and fibrinolysis, and thus the concentration of PAI-1 is important for the fibrinolytic potential.[4] Considering possible cardiac risk factors, plasma PAI-1 and tPA levels are known to be influenced by age, sex, serum triglyceride levels, insulin levels, diabetes mellitus, hypertension, smoking and obesity.[4]

The purpose of this study was to determine the plasma levels of tPA and PAI-1 antigens in patients with acute coronary syndrome as well as in their first degree relatives and their association with other cardiac risk factors.

Material and Methods

Patients

The study population consisted of 38 consecutive patients, 29 with acute myocardial infarction (AMI) and 9 with unstable angina pectoris (UAP), admitted to our emergency unit between 4:00 and 8:00 AM within two weeks. The clinical diagnosis of AMI was based on the concurrence of chest pain, elevated myocardial enzymes (cardiac troponin T above normal, total and MB creatine kinase more than twice the upper normal limit) and electrocardiographic changes (ST segment elevation or depression) in 2 or more contiguous leads. Diagnosis of UAP included the presence of typical angina at rest associated with acute and transient ST segment or T wave changes, with or without progressive exercise-induced angina without enzyme elevation.[5,6] The AMI group consisted of 24 males and 5 females (mean age 58±9 years; range 45-75 years), UAP group consisted of 9 males (mean age 57±8 years; range 45-69 years). In the UAP group, 4 patients were Braunwald's Class IIIB1 and 5 patients were class IIIB2.[7] In the AMI group 5 patients were non ST elevated MI (NSTEMI) and 24 patients were ST elevated MI (STEMI).[5,6] Twenty nine patients (76.3%) were current smokers, 12 patients (31.6%) had high blood pressure and 5 patients (13.2%) were diabetic.

Exclusion criteria

The exclusion criteria were age>75 years, presence of Killip class III or IV, left bundle branch block, cardiomyopathy, patients with valvular heart disease, previous myocardial infarction, coronary artery bypass grafting or coronary angioplasty and patients on corticosteroid or anticoagulant therapy.

Relatives

Among the first degree relatives of the patients, 20 subjects (4 females, 16 males, mean age 52±7 years; range 45-67 years) were included in the study. All subjects were screened for signs of coronary artery disease (CAD) or peripheral vascular disease by a physician and their resting 12-lead ECGs were obtained. They had no clinical evidence of coronary heart disease. Lifestyles and health status were assessed using a detailed questionnaire on former and current diseases, use of medication, smoking habits and alcohol consumption. Three (15%) cases were current smokers, two (10%) had diabetes mellitus and three (15%) had high systemic blood pressure. One of the relatives with hypertension had diabetes also. The current smokers were neither diabetic nor hypertensive. The clinical characteristics of the study population are described in Table 1.

Table 1. Demographic and basic characteristics of the study groups.

		Patients (n= 38)		1st degree relatives (n= 20)	p Value
Age (years)		58 ± 8.9*		52 ± 6.7	0.012
Sex (male %)		87		80	0.218
Hypertension, n (%)		12 (31.6 %)		3 (15 %)	0.318
SBP (mmHg)		122.18 ± 20.26 (90-180)		129.75 ±10.6 (120-150)	0.063
DBP (mmHg)		77.42 ± 13.70 (50-120)		81.00 ± 9.40 (70-100)	0.248
DM, n (%)		5 (13.2 %)		2 (10 %)	1.00
Cigarettes (package/year)		48.79 ±29.71** (5-120)		10.00 ± 6.61 (2.5-15)	0.003
ECG, n (%)	STEMI	NSTEMI	UAP		
	24 (63%)	5 (13.2%)	9 (23.7%)		
Coronary angiography, n (%)	One vessel	Multıvessel	None		
	5 (13.2%)	30 (78.9%)	3 (7.9 %)		

Values are mean ± SD and n (%). *p < 0.05 and **p < 0.01 vs control subjects (unpaired t test or χ^2 test)
Hypertens; hypertension, SBP; systolic blood pressure, DBP; diastolic blood pressure, DM; diabetes mellitus, ECG; electrocardiography

Blood sampling

In addition to the initial blood sampling of cardiac enzymes for diagnosis and of serum lipids, blood samples for tPA and PAI-1 antigens were also obtained between 4:00 and 8:00 AM to minimize the effect of diurnal variation [3,8] after 15 minutes of supine rest and without applying a venous compression with a tourniquet.[9] Patients who received thrombolytic therapy had their blood samples drawn before administration. The mean period of time that elapsed between the last episode of chest pain and blood sampling was 3.5 ± 1 hours (range 1.5-4 hours). Nine ml of venous blood was drawn into a standardized Vacutainer tubes containing 1 ml sodium citrate and they were immediately centrifuged at 3000 g for 15 minutes at room temperature. The plasma samples were stored at -20ºC until analyzed. Both plasma tPA and PAI-1 antigen were measured using a commercially available enzyme-linked immunosorbant assay kit (Asserachrom tPA and PAI-1 reagent kit, Diagnostica Stago, France). Results were expressed in nanograms per milliliter. Intra-assay normal range was 1-12 ng/ml for tPA antigen and 4-43 ng/ml for PAI-1 antigen.

Blood samples from patients and their relatives were collected simultaneously. Fasting blood samples were obtained after an overnight fast from patients and relatives for analysis of routine clinical chemical parameters. Plasma fibrinogen, blood glucose, serum cholesterol and triglyceride concentrations, as well as HDL and LDL cholesterol and cardiac enzymes were determined by conventional techniques in routine use at the hospital.

Study protocol

According to the main outcome measures, high blood pressure was defined according to JNC VI as systolic blood pressure (BP)>139 mmHg and diastolic BP>89 mmHg or to be on an antihypertensive medication.[10] Diabetes mellitus was defined according to ADA as fasting blood glucose > or =126 mg/dl in 2 separate measurements or to be on an antidiabetic medication.[11] Twelve patients (31.6%) and 3 relatives (15%) had high BPs, 5 patients (13.2%) and 2 relatives (10%) had high fasting blood glucose levels. Patients and relatives with high BPs were using either an angiotensin converting enzyme inhibitor (ACEI) or a long acting dihydropiridin calcium channel blocker and cases with high fasting blood glucose were using oral antidiabetic agents. None of the subjects were using antilipidemic agents. Twenty one patients in the AMI group underwent thrombolytic therapy with streptokinase and all patients were treated with a 5000 U bolus of heparin and heparin infusion at 600-800 U/hour for 48 hours as indicated. All patients received treatment that included, if appropriate, coronary dilating agents such as nitrates, β-blockers, aspirin (100 mg/day), statins and ACEIs.

Coronary angiography was performed within 10 days of hospitalization in UAP patients and within 4 to 6 weeks after recovery in AMI patients.

A >70% stenosis of a major coronary vessel was considered significant.[3,12] Five (13.2%) patients had one-vessel disease (two were UAP, one was NSTEMI and two were STEMI), and 30 (78.9%) patients had at least two-vessel disease or multi-vessel disease. Three (7.9%) patients who did not fulfill the angiographic criteria were UAP patients. The study was approved by the Ethics Committee of Atatürk Research Hospital and all subjects gave consent for the study.

Statistical analysis

The statistical evaluation was performed by SPSS Windows 9.1.3 (SPSS, Chicago, IL). Between-group differences in mean values for unpaired data were compared by Student's t test and the categoric variables were compared by the chi square test, where applicable. Data were expressed as the mean ± SD for numerical variables and as numbers (%) for categoric variables. Significance was defined as a p value < 0.05. Correlation coefficients between different variables were calculated by Spearman rank correlation analysis in the relatives group and by linear regression analysis in the patient group, after adjusting for age.

Results

Demographic and basic characteristics of the study groups

Table 1 and Table 2 summarize the relevant information. The relatives were younger than the patients (p< 0.05). The two groups did not differ in gender (p= 0.218), in the incidence of diabetes mellitus (p= 1.00), hypertension (p= 0.318) and in the serum levels of fasting blood glucose (p= 0.087), HDL cholesterol (p= 0.589), LDL cholesterol (p= 0.77), lipoprotein (a) (p= 0.347) and fibrinogen (p= 0.690). High levels of serum triglycerides and cigarettes smoked (package/year) were more common among patients than in their relatives, (p= 0.032 and p= 0.003). The levels of systolic and diastolic pressures did not differ between the two groups (p=

Table 2. Basic characteristics and plasma concentrations of tPA and PAI-1 antigen in the study groups.

	Patients (n=38)	1st degree relatives (n=20)	p Value
BMI (kg/m^2)	25.89 ± 4.04* (18.5-35)	29.55 ± 4.90 (22-40)	0.004
FBG (mg/dl)	121.42 ± 54.9 (59-292)	100.6 ± 35.47 (44-202)	0.087
HDL-C (mg/dl)	34.36 ± 10.04 (17-59)	35.80 ± 9.23 (25-63)	0.589
LDL-C (mg/dl)	127.36 ± 41.12 (23-194)	130.70 ± 41.80 (28-225)	0.773
Triglycerides (mg/dl)	160.38 ± 38.42* (90-215)	119.23 ± 21.52 (82-168)	*0.032*
Lp (a) (mg/dl)	16.54 ± 18.42 (1.90-85.80)	15.63 ± 11.17 (1.90-46.20)	0.347
CK (IU/ml)	529.55 ± 625.93** (46-2767)	88.30 ± 43.96 (20-182)	0.003
CK-MB (IU/ml)	71.13 ± 113.09** (5-575)	13.55 ± 3.56 (7-20)	0.003
TnT (ng/ml)	1.50 ± 0.67 (0.19-2.10)	0.01 ± 0.005 (0.004-0.025)	0.000
Fibrinogen (mg/dl)	290.4 ± 70.10 (180-400)	278,95 ± 66,80 (198-344)	*0.690*
TPA (ng/ml)	10.40 ± 5.63 (4-30)	11.43 ± 4.65 (4.5-23)	0.452
PAI-1 (ng/ml)	68.92 ± 23.56 (17-101)	68.85 ± 17.3 (30-98)	0.583

Values are mean ± SD. *p< 0.01 and **p< 0.001 vs control subjects (unpaired t test)
BMI; body mass index, FBG; fasting blood glucose, HDL C; high density lipoprotein cholesterol, LDL C; low density lipoprotein cholesterol, CK; creatine kinase, CK-MB; creatine kinase myocardial band isoenzyme, TnT; cardiac troponin T, TPA; tissue plasminogen activator, PAI-1; plasminogen activator inhibitor 1

0.063 and p= 0.248). Although unexpected, body mass index was higher in the first-degree relatives than in the patients (p= 0.004).

Except for one of the relatives with a high CK concentration of 170 U/ml, who had had an intramuscular injection recently, cardiac enzymes, such as serum creatinine kinase, -MB fraction and cardiac troponin T concentrations were significantly high in patients as expected.

Plasma levels of tPA and PAI-1 antigen

In this study, plasma mean tPA and PAI-1 antigen concentrations among the two groups did not differ significantly (p= 0.452 and p= 0.583). The mean plasma concentration of tPA antigen was 10.4 ± 5.6 ng/ml in the patient group and 11.4 ± 4.6 ng/ml in the relatives group, and the mean plasma concentration of PAI-1 antigen was 68.9 ± 23.5 ng/ml in the patients group and 68.8 ± 17.3 ng/ml in the relatives group (Figure 1).

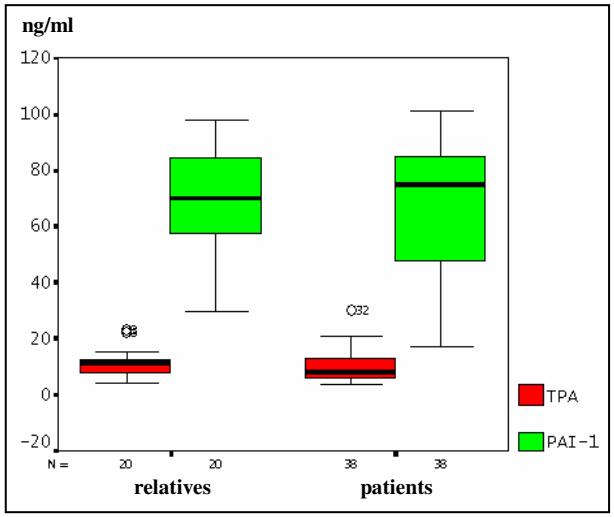

Figure 1. Case processing summary for plasma tPA and PAI-1 antigen concentrations in patients and their first degree relatives. The black thick lines express the median values.

Plasma levels of tPA and PAI-1 antigen according to the angiographic findings

The mean plasma concentrations of tPA and PAI-1 antigens in patients with multivessel disease and their first degree relatives did not differ (11.0 ± 6.05 ng/ml vs 11.43 ± 4.65 ng/ml; p= 0.168 and 70.6 ± 24.13 ng/ml vs 68.85 ± 17.3 ng/ml; p= 0.996), respectively (Table 3). Five patients with one vessel disease had a mean plasma tPA antigen concentration of 9.14±3.04 ng/ml and a PAI-1 antigen concentration of 63.4 ± 21.85 ng/ml. They were not included in the statistical analysis because of the low number of subjects.

Correlations between the plasma concentrations of tPA and PAI-1 antigens and other risk factors

Correlations between plasma concentrations of tPA and PAI-1 antigens and other cardiac risk factors were evaluated by Spearman rank correlation analysis in the relatives group, and by linear regression analysis in the patient group, after adjusting for age. There was a moderate, inverse correlation between PAI-1 antigen and HDL cholesterol (rho= -0.47, p= 0.033) in the relatives group. Smoking, serum lipids and multivessel disease were found to be effective on plasma concentrations of tPA by 13.2% and PAI-1 antigen by 19.4% (r^2= 0.132 and r^2= 0.194) in the patient group. However, in the evaluation of each data separately, none of the variables had a significant correlation with plasma tPA antigen concentration except the serum LDL cholesterol and triglyceride levels, which were significantly correlated with plasma PAI-1 antigen concentration (t= 2,084, p= 0.045 and t= 2.365, p= 0.024) with strong partial correlation coefficients (for LDL cholesterol; β= 0.983 and for triglycerides; β= 1.13).

Table 3. Comparing plasma tPA and PAI-1 antigen concentrations in patients with regard to their angiographic findings and in their first degree relatives.

	Multivessel disease (n=30)	One vessel disease (n=5)	Without significant narrowing (n=3)	1st degree relatives (n=20)	*p Value
TPA (ng/ml)	11.00 ±6.05	9.14 ±3.04	6.46 ±2.33	11.43 ±4.65	0.168
PAI-1(ng/ml)	70.6 ±24.13	63.4 ±21.85	61.33 ±25.71	68.85 ±17.3	0.996

Values are mean ±SD. * p value is for defining significance between multivessel disease and 1st degree relatives

Discussion

Coronary thrombosis was demonstrated in many patients with unstable angina and in those with acute myocardial infarction.[1-4] The increased tendency to thrombosis can be explained by several mechanisms including a defective fibrinolytic system. The reduction of fibrinolytic activity may be due to two major mechanisms -a reduced release of tPA or an increased release of PAI-1.[4]

Since previous studies showed that overall fibrinolytic activity followed a diurnal variation with a peak in PAI-1 activity and tPA antigen in the morning,[3,4,9] blood samples were drawn between 4:00 and 8:00 AM following admission to the hospital, to minimize the effect of diurnal variation [3,8]

In the present study, while plasma PAI-1 antigen concentrations were high in acute coronary syndrome patients (both in UAP and AMI patients) as well as in their first-degree relatives, plasma tPA antigen concentrations were within normal limits.

Although most studies report that tPA and PAI-1 antigens appear to increase with age,[3,13] we did not find a significant difference in plasma tPA and PAI-1 antigen concentrations between patients and their relatives; in fact, patients were older than their first degree relatives. Most of the patients were current smokers in this study; however, no association between smoking and related variables was present, which was not consistent with the literature suggesting that most of the hemostatic variables increase with age and smoking habit.[13,14] This confusing result may depend on our sample size which was too small. In a recent study, an impaired coronary release of active tPA was associated with cigarette smoking. Current and ex-smokers released less active tPA than nonsmokers, but current smokers had higher basal plasma tPA antigen concentrations than ex-smokers despite similar plasma PAI-1 concentrations and coronary arterial plaque burden.[15] We know that visceral obesity, assessed by the body mass index, is one of the risk factors like smoking and other confounding variables in coronary artery disease patients.[13,16-1] Studies show that enhanced expression of the PAI-1 gene in visceral fat may increase the plasma PAI-1 concentration and have a role in the development of vascular disease in visceral obesity.[9] However, we could not find an association between BMI and plasma tPA and PAI-1 antigen concentrations in our study, consistent with the findings of Soeki [9] and Akanji et al.[19] In different studies, strong relationships were found between plasma concentrations of tPA, PAI-1, nephelometric fibrinogen with serum cholesterol and triglycerides.[13,16,18] In the same studies, no correlation was present with serum Lp(a) concentrations, which compete with plasminogen for binding to fibrin, thus resulting in decreased lysis of intravascular fibrin in acute coronary syndrome patients, as in our study. However, Fujino et al.[20] noted a trend toward a positive but nonsignificant correlation between Lp(a) and PAI-1 and a negative but nonsignificant correlation between Lp(a) and tPA in acute myocardial infarction patients admitted to the coronary care unit between 6:00 and 12:00 AM. In the Stockholm Heart Epidemiology Program,[17] tPA antigen, PAI-1 activity and tPA/PAI-1 complex were higher in men and women with recurrent MI than those without. The tPA/PAI-1 complex was correlated with serum triglycerides and BMI in all of groups except women with reinfarction. Another study reported that plasma PAI-1 activity was influenced by factors such as body mass index, triglycerides, insulin sensitivity and glycemic status.[19] In our study, smoking, serum lipids and angiographic extent of disease were found to have a slight and positive cumulative effect on plasma concentrations of tPA and PAI-1 antigens in the patient group. Geppert et al.[21] found that, during the progression of coronary artery disease, angiographic extent of disease, hypercholesterolemia, hypertriglyceridemia and PAI-1 activity were significantly and positively related to plasma tPA antigen concentrations and that all were indicators of a prothrombotic state. Hamsten et al.[22] evaluated tPA antigen and its inhibitor PAI-1 in patients who had survived a myocardial infarction before the age of 45, three years after the infarction. The data in this study suggested that reduced fibrinolytic capacity due to increased plasma levels of PAI-1 might have pathogenetic importance in myocardial infarction, particularly in patients with hyper-

triglyceridemia due to the positive and significant correlation found between the levels of plasma PAI-1 and serum triglycerides. Concerning our study, none of the variables had a significant correlation with plasma tPA antigen concentration except that only serum LDL cholesterol and triglycerides were found to be significantly correlated with plasma PAI-1 antigen concentration in the patients and there was a strong inverse correlation between plasma PAI-1 antigen and serum HDL cholesterol concentration in the relatives.

Negri et al.[23] evaluated PAI-1 levels in patients with coronary artery disease and in normal subjects and looked for a correlation, if any, with severity and diffusion of coronary lesions documented by angiography. He found that patients with multivessel disease had significantly higher levels of plasma PAI-1-not consistent with our results-and serum triglycerides than patients with 1-vessel disease had. There was also a positive correlation between plasma PAI-1 levels and serum triglycerides and between plasma PAI-1 levels and coronary artery disease severity and diffusion scores of angiographic lesions. However, according to Soeki et al.[9] there was no association between plasma levels of PAI-1 and angiographically documented coronary artery disease. Also, Carter et al.[24] stated that in subjects with coronary artery disease, tPA was associated with the number of coronary arteries with > or = 50 % stenosis, but this association was lost after adjustment for PAI-1, which was found to be the largest determinant of tPA levels in linear regression models and accounted for as much as 38% of the variation in levels. In our study, the number of vessels with a luminal diameter reduction > 70 % did not have any correlation with the other risk factors as well as with tPA and PAI-1 antigens.

Ridker,[25] found that the relative risk of myocardial infarction in the highest quintile of tPA antigen was reduced if all available atherosclerotic risk factors, especially serum total cholesterol and HDL cholesterol was controlled for. In the prospective Atherosclerosis Risk in Communities (ARIC) study of middle-aged adults, the association of incident coronary heart disease (CHD) with several fibrinolytic factors, including tPA antigen and PAI-1 antigen were examined. Both plasma tPA and PAI-1 antigen levels were associated positively with CHD incidence after adjustments were made for age, race and sex but not for other risk factors.[26]

In summary, this study, in agreement with previously published data showed that, the incidence of acute coronary syndrome in the early morning was associated with higher levels of plasma PAI-1 antigen levels. As multiple factors mediate the release of plasma tPA and PAI-1 it may explain the poor relationship between the plasma levels of tPA and PAI-1 antigen and the extent of coronary artery disease. Studies suggest a genetic contribution to circulating levels of hemostatic factors in healthy families with effects of known genetic polymorphisms on heritability. The influence of known polymorphisms contributing to the variance of PAI-1 was 2% and heritability was 26%, after adjustment for age and sex.[27] Age, sex, body mass index, lifestyle and metabolic covariates explained 44% of phenotypic variance for PAI-1.[27] In our study, plasma PAI-1 antigen concentrations of both patients and their first-degree relatives showed similar high results that may be due to familial influence.

Furthermore, we measured PAI-1 antigen while numerous earlier studies assayed PAI-1 activity, which is more susceptible to variations in reaction characteristics and this may account for the differences found in our study and some other studies.[28] It is thus necessary to reanalyze results after correction for specific assay methodology to ensure meaningful inter-study comparability.

Study Limitations

The small sample size in the present study precludes making any firm conclusions from the data and lacks sufficient power to address the influence of all the individual variables associated with each other and with acute coronary syndrome. Especially, the number of current smokers in control subjects and the number of patients and control subjects with diabetes mellitus and hypertension were so small that this study might have failed to detect associations between these factors and the others.

In conclusion, the high levels of plasma PAI-1 antigen in the first-degree relatives of acute coronary syndrome patients without any signs of coronary artery disease were of major clinical importance pointing at the prothrombotic state.

Acknowledgements

We are grateful to Dr. Hatice Üstün, Ege University, Department of Statistics, for her support to the statistical analysis.

REFERENCES

1. Fuster V, Badimon L, Badimon JJ, Chesebro JH.The pathogenesis of coronary artery disease and the acute coronary syndromes. N Eng J Med 1992;326:242-50, 310-8.
2. Kohler HP, Grant PJ. Plasminogen activator inhibitor type I and coronary artery disease. N Eng J Med 2000;342: 1792-801.
3. Angleton P, Chandler WL, Schmer G. Diurnal variation of tissue-type plasminogen activator and its rapid inhibitor (PAI-1). Circulation 1989;79:101-6.
4. Masuda T, Yasue H, Ogawa H, et al. Plasma plasminogen activator inhibitor activity and tissue plasminogen activator levels in patients with unstable angina and those with coronary spastic angina. Am Heart J 1992;124:314-9.
5. The Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial infarction redefined. A consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. Eur Heart J 2000;21:1502-13.
6. Braunwald E, Antman EM, Beasley JW, et al. ACC/AHA Guidelines for the management of patients with unstable angina and non-ST segment elevation myocardial infarction: Executive summary and recommendations. Circulation 2000; 102:1193-209.
7. Braunwald E. Unstable angina: A classification. Circulation 1989;80:410-4.
8. Hoffmeister HM, Jur M, Wendel HP, Heller W, Seipel L. Alterations of coagulation and fibrinolitic and kallikrein-kinin systems in the acute and postacute phases in patients with unstable angina pectoris. Circulation 1995;91:2520-7.
9. Soeki T, Tamura Y, Fukuda N, Ito S. Plasma and platelet plasminogen activator inhibitor-1 in patients with acute myocardial infarction. Jpn Circ J 2000;64: 547-53.
10. From Joint National Committee: The sixth report of the Joint National Committee on Prevention, Detection and Treatment of High Blood Pressure (JNCVI). Arch Inten Med 1997;157:2413-46.
11. American Diabetes Association: Standarts of medical care for patients with diabetes mellitus (Position Statement). Diabetes Care 1998;21:23-31.
12. Fiqueros J, Monasteria Y, Lidon RM, Nieto E, Soler J. Thrombin formation and fibrinolytic activity in patients with acute myocardial infarction or unstable angina: In-hospital course and relationship with recurrent angina at rest. J Am Coll Cardiol 2000;36:2036-43.
13. Yarnell John WG, Sweetnam PM, Rumley A, Lowe Gordon DO. Lifestyle and hemostatic risk factors for ischemic heart disease. The Caerphilly study. Arterioscler Thromb Vasc Biol 2000;20:271-80.
14. Eliasson M, Asplund K, Evrin P-E, Lundblad D. Relationship of cigarette smoking and snuff dipping to plasma fibrinogen, fibrinolitic variables and serum insulin: the Northern Sweden MONICA Study. Atherosclerosis 1995;113:41-53.
15. Newby DE, Mc Leod AL, Uren NG, et al. Impaired coronary tissue plasminogen activator release is associated with coronary atherosclerosis and cigarette smoking. Circulation 2001; 103:1936-41.
16. Eliasson M, Evrin PE, Lundblad D. Fibrinogen and fibrinolytic variables in relation to antropometry, lipids and blood pressure: the Northern Sweeden MONICA Study. J Clin Epidemiol 1994;46:513-25.
17. Wiman B, Andersson T, Hallquist J, Reuterwall C, Ahlbom A, de Faire U. Plasma levels of tissue plasminogen activator/plasminogen activator inhibitor-1 complex and von Willebrand factor are significant risk markers for recurrent myocardial infarction in the Stockholm Heart Epidemiology Program (SHEEP) study. Arterioscler Thromb Vasc Biol 2000;20:2019-23.
18. Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature. Ann Intern Med 1993;118:963-5.
19. Akanji AO, Al-Shayji I. The relationships between insulin and plazminogen activator inhibitor 1 levels: assesment in groups of subjects with dyslipidemia and hypertension. Clinica Chimica Acta 1998;274:41-52.
20. Fujino T, Katou J, Fujita M, Ohta T, Hasebe N, Kikichi K. Relationship between serum lipoprotein (a) level and thrombin generation to the circadian variation in onset of acute myocardial infarction . Atherosclerosis 2001;155: 171-8.
21. Geppert A, Graf S, Beckmann R, et al. Concentration of endogenous tPA antigen in coronary artery disease: relation to thrombotic events, aspirin treatment, hyperlipidemia and multivessel disease. Arterioscler Thromb Vasc Biol 1998;18:1634-42.
22. Hamsten A, Wiman B, de Faire U, Blombäck M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. N Eng J Med 1985;313:1557-63.
23. Negri M, Sheiban I, Arigliano PL, et al. Interrelation between angiographic severity of coronary artery disease and plasma levels of insulin, C-peptide and plasminogen activator inhibitor-1. Am J Cardiol 1993;72:397-401.
24. Carter AM, Catto AJ, Grant PJ. Determinants of tPA antigen and associations with coronary artery disease and acute cerebrovascular disease. Thromb Haemost 1998; 80:632-6.

25. Ridker PM, Vaughan DE, Stampfer MJ, Manson JE, Hennekens CH. Endogenous tissue type plasminogen activator and risk of myocardial infarction. Lancet 1993; 341:1165-8.
26. Folsom AR, Aleksic N, Park E, Salomaa V, Juneja H, Wu KK. Prospective study of fibrinolytic factors and incident coronary heart disease:the Atherosclerosis Risk in Communities (ARIC) study. Arterioscler Thromb Vasc Biol 2001;21:611-7.
27. Freeman MS, Mansfield MW, Barret JH, Grand PJ. Genetic contribution to circulating levels of hemostatic factors in healthy families with effects of known, genetic polymorphisms on heriability. Arterioscler Thromb Vasc Biol 2002;22:506-10.
28. Gram J, Declerk PJ, Sidelman J. Multicenter evaluation of commercial kit methods:Plasminogen activator inhibitor activity. Thromb Haemost 1993;70: 852-7.