Fibrinolytic activity in type II diabetes mellitus and the effect of glycemic control on fibrinolytic parameters

Alî KESKİN[1], Yurdaer SERMEZ[2], Gökhan ÖZGEN[2], Filiz BÜYÜKKEÇECİ[3], Candeğer YILMAZ[2]

[1]Dept. of Internal Medicine Medical School of Pamukkale University, Denizli,
[2]Depts. of Endocrinology and hematology, Medical School of Ege University, Izmir, TURKEY

Fibrinolytic parameters including tissue-type plasminogen activator (t-PA) antigen and plasminogen activator inhibitor-1 (PAI-1) antigen were studied in 34 poorly controlled type 2 diabetic patients. Age and sex matched 23 healthy subjects with normal glucose concentration comprised the control group. The diabetic patients had a significantly higher mean PAI-1 and t-PA antigen levels compared to control group ($p<0.05$), but elevation of PAI-1 was higher than t-PA antigen resulting decrement In fibrinolytic capacity. t-PA and PAI-1 was significantly correlated with body mass index ($p<0.05$), but not with age, triglyceride, cholesterol, HDL, LDL, and HbA1C. To determine the effect of improved glycemic control on fibrinolytic activity, we measured changes in plasma t-PA and PAI after 3 months treatment with insulin (14 patients) or gliclazide (14 patients). Although there was a trend to falling in PAI-1 and increasing in t-PA resulting in a slight improvement of the fibrinolytic impairment, these changes were not statistically significant.
[Turk J Med Res 1995; 13(2): 74-77]

Key Words: Diabetes mellitus, Fibrinolytic activity, Plasminogen activator inhibitor

Atherosclerosis and atherosclerosis-related complications represent the primary cause of morbidity and premature mortality in patients with diabetes mellitus (1-3). Changes in coagulation, fibrinolysis, platelet and vessel wall function are accepted to contribute to micro and macroangiopathy (4,5). Fibrin deposit could play a role in the development of atherosclerotic lesions possibly an initiating factor of endothelial cell injury. Since it leads to the decreased removal of fibrin deposit, hypofibrinolysis would be a prime candidate for a role in the initiation and development of atherothrombosis (6,7).

Studies of the fibrinolytic system in patients with diabetes have yielded contradictory results. Fibrinolytic activity in basal conditions was reported to be decreased (8-11), normal (12), or increased (13,14). In view of these discrepancies in data reported by various laboratories, we decided to examine fibrinolytic parameters in patients with type 2 diabetes mellitus and to prospectively determine the effects of improved glycemic control on fibrinolytic parameters.

Received: Dec. 4,1994 **Accepted:** Jan. 18,1995

Correspondence: Ali KESKİN
174.Sok. 36/11
Hatay, Izmir, TURKEY

MATERIALS AND METHODS

Thirtyfour type 2 diabetic patients, consisting of 17 males and 17 females were included in the study. The known duration of the disease ranged from 1 to 25 years. Before this study, 13 patients were being treated with insulin and 21 patients with diet and oral hypoglycemic drugs. But they were in poor glycemic control because of many reasons. During this study, patients separated into two treatment groups. 14 patients were treated with insulin, and 20 patients with gliclazide. They were controlled at weekly intervals and adjustments in insulin and gliclazide dosages were made as needed. 6 patients who were in the gliclazide group were excluded from the study because of inadequate controls.

A group of 23 healthy volunteers, matched for age and sex, with normal glucose concentration and no family history of diabetes and no clinical or electrocardiographic evidence of ischemic heart disease or hypertension comprised the control group.

Blood from an antecubital vein for estimation of fibrinolytic parameters was collected from resting patients or controls between 8.00 and 9.00 a.m. after a 12 hours fast into plastic tubes containing sodium citrate (nine parts of blood were mixed with one part of 3.8% sodium citrate) and centrifuged for 15 minutes at 3000 g and the plasma was then stored at -70°C

until analyzed. All samples were assayed in one session in order to reduce inter assay variations.

Fibrinolytic parameters including plasminogen activator inhibitor antigen (PAI-1) and tissue plasminogen activator (t-PA) were measured by enzyme linked immunoserbert assay (ELISA) using commercial test kits (Asserachrom t-PA and asserachrom PAI-1, Diagnostica Stago, France).

Serum parameters such as albumin, creatinine, glucose, cholesterol, triglyceride, LDL and HDL cholesterol and HbAiC were measured using standard procedures.

All of the parameters were repeated after three months of therapy in diabetic patients. Pre treatment and post treatment values were compared.

Statistical analysis: Correlations were determined by linear regression analysis. Comparison were performed using the Student's t test (between patients and control groups) Mann-Witney test (between subgroups of patients) and Wilcoxon matched-pairs signed rank test (between pre-treatment and post-treatment vlaues).

RESULTS

Table 1 shows the means and standard errors of various parameters in diabetic and control subjects. There were no differences in age, sex, total cholesterol, blood pressure, and LDL cholesterol between the two groups. As expected, diabetic subjects had significantly higher body mass index (BMI), higher fasting plasma glucose and triglycerdie levels and lower HDL cholesterol levels than control group.

The mean concentration of PAI-1 antigen was significantly higher in the diabetic subjects compared with the control subjects (26 ng/ml vs 9.4 ng/ml). t-PA antigen levels were also higher in diabetic compared with the control group but elevation of t-PA was lower than PAI-1 antigen. As a result, PAI/t-PA was higher in diabetic patients than controls. t-PA and PAI were further analyzed with respect to presence of retinopathy. Fourteen patients had retinopathy. There were no significant differences between the two groups.

Table 1. Parameters in diabetic subjects and normal control groups (mean standardterror)

	Diabetic patients n:34	Control group n:23	P value
Age (years)	52±2.0	46±1.9	NS
Sex (M/F)	17/17	12/11	NS
BMI (kg/m2)	27.4±0.9	23.9±0.7	<0.05
SBP (mmHg)	140±6.1	135±4.2	NS
DBP (mmHg)	83±2.4	80±2.1	NS
HbA1C	8.8±0.3	—	
Cholesterol (mg/dl)	222±7.3	210±6.1	NS
Triglyceride (mg/dl)	178±18	130±9.8	<0.05
Fasting plasma glucose (mg/dl)	140+8.1	90+6.4	O.001
LDL Chol, (mg/dl)	131 ±8.7	120±3.7	NS
HDL chol. (mg/dl)	43±5.4	54±3.3	<0.05
t-PA (ng/ml)	11.7±0.9	7.8±0.6	<0.05
PAI-1 (ng/ml)	26.1 ±4.0	9.4±1.5	<0.001

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, NS: Not significant

Table 2. Correlation coefficients between parameters of diabetes and fibrinolysis

	Tissue plasminogen activator		Plasminogen activator inhibitor	
	r-.	P	r	P
Age	0.16	NS	0.001	NS
BMI	0.49	<0.05	0.36	<0.05
HbAiC	0.18	NS	0.29	NS
Cholesterol	0.20	NS	0.23	NS
Triglyceride	0.19	NS	0.01	NS
HDL	0.09	NS	0.20	NS
LDL	0.16	NS	0.10	NS

NS: Not significant

Table 3. Comparison of pre and post-treatment values in all patients with diabetes mellitus

	Pretreatment values	Posttreatment values	P
t-PA	11.7±0.9	12.8±1.5	NS
PAI-1	26.1 ±4	22.5±2.8	NS
HbA1C	8.8±0.3	6.9±0.2	<0.05
Cholesterol	222±7.3	215±7.9	NS
Triglyceride	178±18	150±15	NS
LDL	138±8.7	136±10	NS
HDL	43±5.4	45.6±2.3	NS

NS: Not significant

Table 2 shows correlation coefficients between some characteristics of patients and fibrinolytic parameters. It can be seen that PAI-1 and t-PA was significantly correlated with BMI ($p<0.05$), but not with age, triglyceride, cholesterol, HDL, LDL and HbAiC.

Table 3 shows pre and post treatment values. At the end of treatment, HbA1C decreased in both treatment groups and changes were statistically significant. Changes in other assays were not statistically significant. There were trends to increasing t-PA antigen and decreasing PAI-1 antigen resulting minor improvements in fibrinolytic activity. Changes in t-PA and PAI-1 were not different between insulin and gliclazide treatment groups.

DISCUSSION

In this study, fibrinolytic parameters were assessed in 34 type 2 diabetic patients with varying degrees of

metabolic control. We found that diabetic patients had significantly higher PAI-1 and t-PA antigen levels compared with nondiabetic control subjects. These results demonstrated that a reduced fibrinolytic activity is present in basal condition that agrees with some previous reports (8-11).

Endogenous fibrinolysis is modulated physiologically by two activators of plasminogen, t-PA and u-PA. PAI is considered to be a major regulating factor of fibrinolysis by inhibiton of the plasminogen activators (15,16). A dynamic equilibrium exits between t-PA and PAI-1 on the luminal surface of vessels that determines net local fibrinolytic activity. An increase in expression of PAI-1, a decrease in the expression of t-PA, or both lead to decreased net fibrinolytic activity. In this study, diabetic subjects had threefold elevations of plasma concentrations of PAI-1 compared with values In control subjects. Whereas elevations of t-PA compared to normal subjects were lower than PAI. The elevated level of PAI-I causes inhibition of t-PA, resulting in a decreased fibrinolytic capacity.

Fibrin deposited on injured endothelial cells is resolved by an activation of the fibrinolytic system. Therefore, a defective fibrinolysis will delay the resolution of fibrin and cause an increased formation of thrombus and scar tissue. Patients with diabetes mellitus is known to have high incidence of atherosclerosis.

Plasma fibrinolytic activity has been found to be diminished in many studies, including those of patients with hypertension (17), obesity and hypertriglyceridemia (18,19) and angina pectoris (20). This defect has been attributed to the increased plasma concentration of PAI-1 accompanying these disorders. We found a positive correlation of BMI with t-PA and PAI-1. This observation further supports documented decreased fibrinolytic activity in obesity. Elevated BMI predisposes to insulin resistance and hyperinsulinism rather than to insulin deficiency (10). More recently, Juhan-Vauge and coworkers have reported a positive correlation between plasma levels of insulin and PAI-1 in both normal and obese individuals and in patients with type 2 diabetes, and these authors consider insulin to be an Important determinator for the regulation of endothelial cell related fibrinolysis (9).

The concentrations of t-PA and PAI-1 antigen seem to be independent of diabetes control. Since none of these measured parameters were correlated with the HbAiC level. To determine the effect of improved glycemic control on fibrinolytic activity we also measured changes in plasma t-PA and PAI after 3 months treatment with insulin or gliclazide. Although there was a trend to falling in PAI and increasing in t-PA resulting in slight improvement of fibrinolytic system, results in our patients indicate that glycemic control alone in type 2 diabetes mellitus does not remarkably improve fibrinolytic activity.

Tip II diabetes mellitusta fibrinolitik aktivite ve glisemik kontrolün fibrinolitik parametrelere etkisi

Glisemik kontrolü iyi olmayan 34 tip 2 diabetes mellituslu hastada doku plazminojen aktivatörü (t-PA) ve plazminojen aktivatör inhibitörü (PAI-1) bakılarak fibrinolitik aktivite araştırıldı. Kontrol grubu olarak kan glukoz düzeyi normal olan 23 sağlıklı kişi alındı. Diabetli hastalarda PAI-1 ve t-PA kontrol grubuna göre anlamlı derecede yüksek bulundu (p<0.05). Fakat PAI-1'nin artışı t-PA'dan daha fazla olduğu için hastalarda net fibrinolitik kapasite azalmış olarak değerlendirildi. t-PA ve PAI-1 ile vücut kütle oranı (BMI) arasında anlamlı düzeyde korelasyon tespit edilirken; yaş, trigliserid, kolesterol, HDL, LDL ve HbA 1C ile korelasyon tespit edilmedi. İyi glisemik kontrolün fibrinolitik aktivite üzerine etkilerini araştırmak için hastalara uygulanan 3 aylık insulin (14 hasta) ve gliclazide (14 hasta) tedavisinden sonra plazma t-PA ve PAI-1 düzeyindeki değişiklikler araştırıldı. Her ne kadar t-PA'da hafif yükselme, PA-I'de hafif düşme ve buna bağlı olarak fibrinolitik bozuklukta kısmi bir düzelme görülmüşse de, oluşan değişiklikler istatistiki olarak anlamlı bulunmadı.
[TurkJMedRes 1995; 13(2): 74-77]

REFERENCES

1. Kannel WG, D'Agostino RB, Wilson PW et al. Diabetes, fibrinogen and risk of cardiovascular disease: The Framingham experience. Am Heart J 1990; 120:672-976.
2. Bierman EL. Atherogenesis in diabetes. Arterioscler Thromb 1992; 12:647-56.
3. Uusitupa MI, Niskanen LK, Siitonen O et al. 5-year incidence of atherosclerotic vascular disease in relation to general risk factors, insulin level and abnormalities in lipoprotein composition in non-insulin dependent diabetic and non diabetic subjects. Circulation 1990; 82:27-36.
4. Garcia Frade U, Calle H, Alava I et al. Diabetes mellitus as a hyper coagulable state: its relationship with fibrin fragments and vascular damage. Thromb Res 1987; 47:533-40.
5. Christe M, Fristchi J, Lammle B et al. Fifteen coagulation and fibrinolysis parameters in diabetes mellitus and in patients with vasculopathy. Thromb Haemostas 1984; 52:138-43.
6. Wiman B, Hamsten A. The fibrinolytic enzyme system and its role in the etiology of thrombo-embolic disease. Semin Thromb Haemost 1990; 26:207-16.
7. Gram J, Jespersen J. Induction and possible role of fibrinolysis in diabetes mellitus. Semin Thromb Haemost 1991; 17:412-6.
8. Rydzewski A, Kawamura H, Watanabe I et al. Plasminogen activators and plasminogen activator inhibitor (PAI-1) in type 2 diabetes mellitus. Fibrinolysis 1990; 4:183-8.

9. Vague I J, Alessi MC, Vague P. Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothiombosis. Diabetologia 1992; 34:457-62.

10. Auwerx J, Bouillon R, Collen D et al.. Tissue type plasminogen activator inhibitor in diabetes mellitus. Arteriosclerosis 1988; 8:68-72.

11. McGill JB, Schneider DJ, Arfken CL et al. Factors responsible for impaired fibrinolysis in obese subjects and NIDDM patients. Diabetes 1994; 43:104-9.

12. VD Knaap JH, De Boer AJ, Pannenbakker MAG et al. The effects of diabetes regulation on platelet release, fibrinolysis and coagulation tests before and after stimulation with DDVAP. Thromb Haemost 1985; 3:118-21.

13. Sharma S. Platelet adhesiveness, plasma fibirnogen and fibrinolytic activity in juvenile onset and maturity onset diabetes mellitus. J Clin Pathol 1981; 34:501-3.

14. Ostermann H, Tschope D, Greper H et al. Impaired fibrinolytic system in diabetic microangiopathy. Fibrinolysis 2 (suppM) 1988:174.

15. SchleifferT, Hellstern P, Freitag M et al. Plasminogen activator inhibitor 1 activity and lipoprotein (a) in nephropathic patients with non-insulin dependent diabetes mellitus versus patients with nondiabetic nephropathy. Haemostasis 1994; 24:49-54.

16. Schneider DJ, Nordt TK, Sobel BE. Attenuated fibrinolysis and accelerated atherogenesis in type 2 diabetic patients. Diabetes 1993; 42:1-7.

17. Keskin A, Tombuloglu M, Büyükkececi F. Fibrinolytic activity and platelet release reaction in essential hypertension. Japan Heart J 1994; 35:757-63.

18. Landin K, Stigendal L, Erikson E et al. Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. Metabolism 1990; 39:1044-8.

19. Vague P, Juhan-Vague I, Aillaud MF et al. Correlation between blood fibrinolytic activity, PAI level plasma insulin level and relative body weight in normal and obese subjects. Metabolism 1986;

20. Juhan-Vague I, Alessi MC, Joly P et al. Plasma plasminogen activator 1 in angina pectoris. Arteriosclerosis 1989; 9:362-7.