

## Plasma t-PA and PAI-1 Antigen Concentrations in Non-Insulin Dependent Diabetic Patients: Effects of Treatment Modality on Fibrinolysis

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Basal plasma tissue type plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1) antigen levels were studied in 49 non-insulin dependent diabetic patients (23 men, 26 women: ages  $51.3 \pm 14.9$  years) and 16 age matched non-diabetic subjects (9 men, 7 women: ages  $49.8 \pm 12.2$  years) as a control group. Compared to a control group, the diabetic patients had a significantly higher mean t-PA antigen ( $5.15 \pm 3.02$  vs  $3.20 \pm 2.30$  ng/ml) and PAI-1 antigen ( $35.89 \pm 18.59$  vs  $17.60 \pm 15.36$  ng/ml) levels ( $p < 0.05$ ). Plasma t-PA antigen level was not influenced by each treatment modality. There was a significant decrease of plasma PAI-1 antigen level after Metformin administration compared to that of before Metformin administration ( $39.74 \pm 19.39$  vs  $25.14 \pm 16.18$  ng/ml) ( $p < 0.05$ ), and the insulin-treated group showed a tendency for a decrease of plasma PAI-1 antigen levels after insulin administration but this did not reach statistical significance ( $29.93 \pm 15.37$  vs  $17.32 \pm 10.60$  ng/ml). Sulfonylurea did not change both plasma t-PA and PAI-1 antigen levels.

In conclusion, diabetic patients have high t-PA and PAI-1 antigen levels. Biguanide reduced plasma PAI-1 antigen levels, which might play some helpful role in the improvement of chronic complications in NIDDM.

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**Key Words:** t-PA, PAI-1, NIDDM, Treatment Modality

### INTRODUCTION

Diabetic patients are predisposed to micro and macro-angiopathy which occurs earlier and more frequently than in comparable non-diabetic patients<sup>1)</sup>. Thromboembolic occlusion of the microvasculature might be caused by a disturbance of the delicate equilibrium between coagulation and fibrinolysis. The endothelial cells produce the t-PA and also an inhibitor (PAI) of this activator<sup>2)</sup>. After vascular occlusion, t-PA is immediately released

and activates plasminogen to plasmin, which starts to digest the fibrin mesh work of the thrombus<sup>3)</sup>. Recently Auwerx et al. reported that the low fibrinolytic activity presented by NIDDM patients was related to elevated concentration of t-PA and PAI activity<sup>4)</sup>. Defective fibrinolysis in diabetic patients might constitute a factor of pathogenetic significance for the development of the vascular manifestations of diabetes<sup>5-8)</sup>. Implicit in such a concept of the cause and effect of an impaired fibrinolysis is the possibility that stimulation of fibrinolysis by the administration of drugs might delay the development of angiopathy in diabetes. Sulfonylurea has been reported to enhance fibrinolysis and to improve the microcirculation in diabetes<sup>9-12)</sup>. Insulin stimulates the synthesis of PAI-1 by the human hepatocellular cell line<sup>13)</sup> and metformin decreases the PAI<sup>14)</sup>. The aim of this

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study is, in addition to evaluating the fibrinolytic system compared with non-diabetic controls, to know what effects conventional treatment modalities (insulin, sulfonylurea and biguanide) have on plasma t-PA and PAI antigen levels in diabetic patients.

**MATERIALS AND METHODS**

49 NIDDM patients (23 men, 26 women), mean age (SD) 51.3 years (14.9), were studied. The mean duration of diabetes was 7.8±6.1 years. They were treated with insulin (Humulin-N, Eli Lilly, mean 28.2±9.4 U/day) in 17 cases, sulfonylurea (Gliclazide, 80-240 mg/day) in 10 cases, and biguanide (Metformin, 500-1500 mg/day) in 22 cases. Their mean follow up period was 36.1 days (range 16-62 days). During the trial of each treatment, subjects were kept on weight maintaining diet, and there were no significant blood sugar and body weight changes. Clinico-biochemical profiles of diabetic patients were listed on Table 1. 16 age-matched subjects (9 men, 7 women), mean age (SD) 49.8 years (12.2), served as controls.

Blood samples for blood sugar, HbA1c, serum total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride were obtained at a fasting state. Glycosylated hemoglobin (HbA1c) was measured by cation-exchange chromatography. Serum C-

peptide was assayed by radioimmunoassay and serum samples were extracted with polyethylene glycol. Proteinuria was assessed by estimating the 24 hour urinary protein with the trichloroacetic acid method. For t-PA and PAI-1 antigen determination, blood from the antecubital vein was collected from resting state at 8:00 a.m., after at least 8 hours fasting, and was collected into trisodium citrate (final concentration 0.011 M). Platelet poor plasma (PPP) was obtained by centrifuging at 4000 g (for 15 min) within 60 min after blood collection. The PPP was aliquoted and stored at -70°C. t-PA and PAI-1 antigen was measured by an enzyme linked immunosorbent assay (ELISA) using a commercially available kit (Asserachrom t-PA, Imubind PAI-1, respectively), and each assay was run in one session in order to eliminate interassay variation.

Statistical analysis was achieved by the student's t-test on paired or unpaired value results, expressed as the mean±SD, were considered as significantly different when p value was lower than 0.05. Simple linear regression analysis was used to evaluate the univariate relationships between fibrinolytic indices (t-PA and PAI-1) and various parameters of diabetes.

**RESULTS**

Table 1 shows the means and standard deviation

**Table 1. Clinico-biochemical Profiles of Diabetic Patients Studied (mean±SD)**

Profiles	Total diabetics (n=49)	Insulin-treated group (n=17)	Sulfonylurea-treated group (n=10)	Biguanide-treated group (n=22)
Men: Women	23:26	10:7	7:3	6:16
Age (year)	51.2±14.9	50.6±30.7	49.9±16.6	52.2±13.2
Duration (year)	7.8±6.1	9.2±6.2	8.4±7.6	7.5±5.5
BMI (kg/m <sup>2</sup> )	23.2±3.4	20.8±1.7	21.3±3.6	26.0±2.3*
HbA1c (%)	8.2±1.5	8.4±1.9	7.4±1.2	8.4±1.1
Blood sugar (mg/dl)				
Fasting 1*	160.9±69.5	175.0±90.8	156.1±59.2	152.2±57.8
Fasting 2*	156.0±53.6	153.8±69.3	150.0±83.4	151.5±27.9
C-peptide (ng/ml)				
Basal	2.01±1.62	1.77±1.87	1.67±1.04	2.56±1.46
Serum lipid (mg/dl)				
Total cholesterol	187.3±65.3	171.5±72.7	173.6±52.1	204.7±62.7
HDL cholesterol	45.2±10.9	43.1±9.9	45.3±17.1	46.6±9.2
LDL cholesterol	114.6±49.5	100.4±65.4	103.1±35.7	128.5±37.7
Triglyceride	168.8±78.2	142.9±73.9	172.7±117.4	186.5±62.1
Proteinuria (mg/24 hr)	239.5±555.4	417.4±803.21	169.2±164.1	134.1±228.7
Follow-up perio (day)	36.1±16.7	33.8±18.5	35.7±16.6	38.0±15.4

\*Fasting blood sugar (FBS) before treatment, \*FBS after treatment

\*p<0.05 compared with insulin and sulfonylurea-treated groups

tions of various parameters in diabetic patients. No significant differences between men and women were observed. There was no difference between each group of diabetic patients, except body mass index which is higher in biguanide-treated group than other groups.

In diabetic patients, the basal plasma concentration of t-PA ( $5.15 \pm 3.02$  ng/ml) and PAI-1

antigen ( $35.89 \pm 18.59$  ng/ml) were significantly higher than in the controls ( $3.20 \pm 2.30$ ,  $17.60 \pm 15.36$  ng/ml), respectively ( $p < 0.05$ ) (Table 2, Fig. 1).

There was no difference of plasma t-PA antigen levels between before and after each treatment modality and plasma t-PA showed an increase in the insulin-treated group, but this did not reach statistical significance ( $p = 0.19$ ) (Table 3). Metfor-

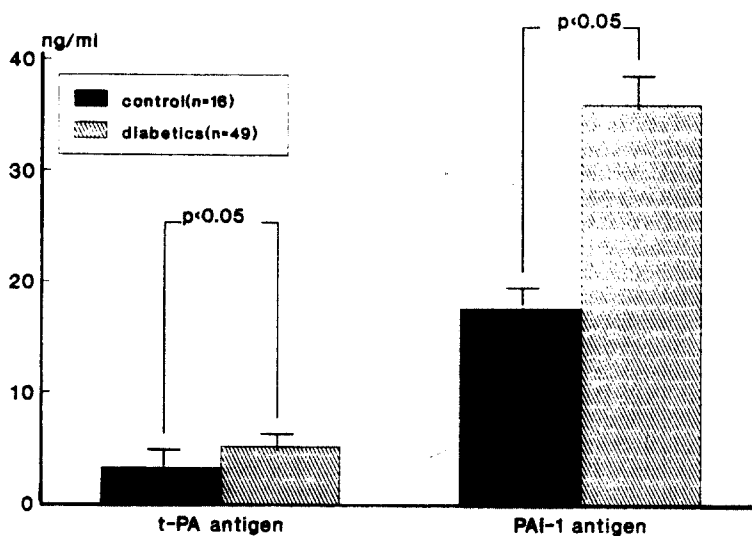


Fig. 1. Comparison of t-PA and PAI-1 antigen levels diabetic and non-diabetic subjects.

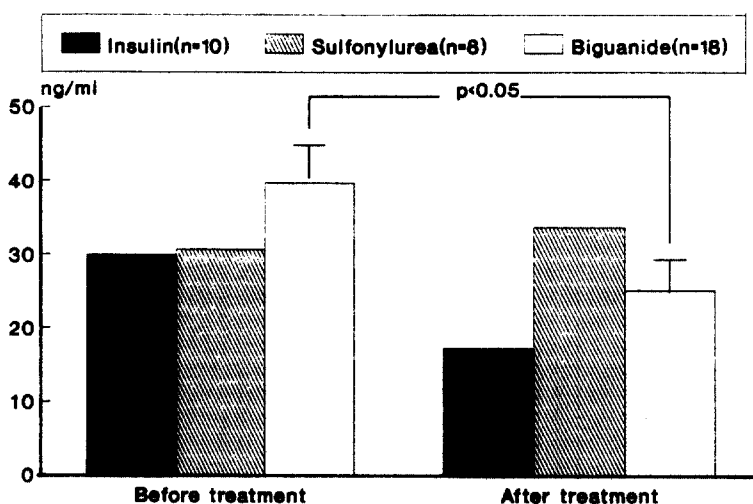


Fig. 2. Changes of plasma PAI-1 antigen level according to treatment modality.

**Table 2. Comparison of Plasma t-PA and PAI-1 Antigen Levels between Diabetic and Non-diabetic Subjects**

Subject	t-PA (ng/ml)	PAI-1 (ng/ml)
Diabetics (n=49)	5.15±3.02*	35.89±18.59*
Non-diabetics (n=16)	3.20±2.30	17.60±15.36

\*p<0.05 compared with non-diabetic subjects

**Table 3. Changes of Plasma t-PA Antigen Levels According to Treatment**

Treatment	t-PA (ng/ml)	
	Before	After
Insulin (n=10)	4.07±3.28	6.13±3.00
Sulfonylurea(n=8)	4.07±1.90	3.93±2.91
Biguanide (n=18)	6.47±2.80	7.10±6.97

**Table 4. Changes of Plasma PAI-1 Antigen Levels According to Treatment**

Treatment	PAI-1 (ng/ml)	
	Before	After
Insulin (n=10)	29.93±15.37	17.32±10.60
Sulfonylurea(n=8)	30.70±23.17	33.73±20.55
Biguanide (n=18)	39.74±19.39	25.14±16.18*

\*p<0.05 compared with before treatment

min treated group exhibited a significant decrease in PAI-1 antigen level (p<0.05) while other treatment groups showed no alteration in PAI-1 antigen level (Table 4, Fig. 2).

## DISCUSSION

The pathogenesis of the micro-vascular complications in diabetes is still obscure. Among the possible etiologic factors in the development of diabetic angiopathy are the disturbances of the fibrinolytic system.

Almer et al reported that a poor defense mechanism against deposits in the vessel walls in diabetes might contribute to the development of diabetic microangiopathy<sup>3</sup>. Final outcome of fibrinolytic activity is determined by balance in the blood between t-PA and PAI, and it is difficult to differentiate free t-PA and/or their complexes in current methods. And there are no indices that

reflect the exact fibrinolytic activities of the blood. Cash et al reported that the mean basal levels of t-PA in the diabetic population appeared to be higher than those of the controls and high level of t-PA is a physiological response to this relative hypercoagulativity<sup>15</sup>. Deficient fibrinolysis is mostly due to the increase in plasma PAI-1 level<sup>16</sup> and several reports have pointed that the reduced plasma fibrinolytic activity showed a negative correlation with PAI activity<sup>4,6,17,18</sup>. The elevated level of PAI activity causes inhibition of t-PA<sup>18</sup>. Garcia Frade et al reported that elevated PAI activity and decreased fibrinolytic response to stimulus may contribute to vascular disease in diabetic patients<sup>9</sup>. In this study, diabetic patients showed an elevation of t-PA and PAI antigen levels. These findings are similar to the results reported by others<sup>4,6,18</sup>. Increased t-PA antigen levels in the presence of decreased fibrinolytic activity can be explained on the basis of the occurrence of inactive t-PA-PAI complexes in blood, resulting in reduced free t-PA levels<sup>4,20</sup>. But it is impossible to decide if the elevation of PAI in diabetics is secondary to angiopathy, or if it is primarily a metabolic defect of the endothelial synthesis and release of the inhibitor<sup>9</sup>.

Several studies reported that sulfonylurea induced a significant increase in the t-PA activity<sup>9-12</sup>, while another study reported that treatment of newly diagnosed maturity-onset diabetic patients with gliclazide did not raise the fibrinolytic activity in spite of a significant improvement of the glycemic control<sup>21</sup>. In this study, sulfonylurea did not alter both t-PA and PAI-1 antigen levels. Discrepancy between other and our result is not clear and we speculated that insufficient subjects, and difference in patient selection and/or treatment protocol, may contribute to this discrepancy. Long-term prospective and longitudinal studies should be required.

A reduction of the plasma insulin levels was associated with a decrease in PAI and high insulin levels might stimulate the synthesis of PAI<sup>13,22</sup>. The existence of a significant correlation between plasma PAI and insulin concentration and the simultaneous variation of these two parameters in intervention studies aimed at resulting in the insulin level suggest that insulin may act directly and indirectly on the cells which synthesize PAI-1<sup>23,24</sup>. Another mechanism by which insulin influences the release of PAI-1 may be either direct or indirect through modification in plasma lipoproteins. Very

low density lipoprotein stimulates PAI-1 secretion by endothelial cells<sup>25</sup>). Recently, Modrescek et al reported that PAI-1 was significantly decreased after introduction of insulin<sup>26</sup>), while another study reported acute hyperinsulinism does not affect the fibrinolytic activity in control and obese subjects<sup>27</sup>). In our results, the insulin-treated group showed an increase of plasma t-PA and PAI-1, but this did not reach statistical significance.

In agreement with our results, there are some previous reports that biguanide stimulate fibrinolysis<sup>28-30</sup>). Chakrabarti et al reported Metformin-ameliorated fibrinolysis in some patients with occlusive vascular disorders<sup>28</sup>) and Hocking et al reported that biguanide had a beneficial effect on euglobulin fibrinolytic activity<sup>30</sup>).

The mechanism by which Metformin decreases PAI is not clear. Primarily inhibitory effect of Metformin on PAI production at the liver and endothelial cell should be considered. The stimulating effect of insulin on PAI-1 synthesis by HepG2 cell line was abolished when Metformin was added to the cell cultures at the same time as insulin, and this inhibitory effect of Metformin was dose dependent<sup>31</sup>).

In conclusion, diabetic patients are hypofibrinolytic compared with non-diabetic patients. Administration of Metformin could lead to an enhancement of fibrinolysis in NIDDM patients by reduction of a decrease in plasma PAI levels, which might contribute as a useful role to reduce the progression of diabetic microangiopathy, but long-term prospective studies should be required in order to determine if the change of the fibrinolytic system induced by Metformin is favourably associated with a delayed development of the vascular complications of diabetes.

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