PAI-1 and Factor VII Activity Are Higher in IDDM Patients With Microalbuminuria

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Microalbuminuria is associated with an increased risk of cardiovascular disease (CVD) in insulin-dependent diabetes mellitus (IDDM) patients, but the pathophysiological basis of this association is not clear. To see whether or not hemostatic dysfunctions might contribute to explain this association, we measured tissue plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), factor VII activity, plasma fibrinogen, and plasma endothelin-1 (ET-1) in 13 microalbuminuric (albumin excretion rate [AER], 20–200 μg/min) and in 13 comparable normoalbuminuric (<20 μg/min) IDDM patients. t-PA and ET-1 were similar in the two groups, whereas PAI-1 activity (5.65 ± 1.92 vs. 0.85 ± 0.58 IU/ml, $P < 0.05$), factor VII (87.85 ± 4.94 vs. 76.54 ± 2.31%, $P < 0.05$), and plasma fibrinogen (3.38 ± 0.21 vs. 2.65 ± 0.13 g/l, $P < 0.05$) were significantly higher in microalbuminuric than in normoalbuminuric patients. Plasma fibrinogen was related to AER ($r^2 = 0.23$, $P < 0.05$), whereas triglycerides and factor VII were related to PAI-1 ($r^2 = 0.39$, $P < 0.001$ and $r^2 = 0.10$, $P < 0.05$). These results suggest that microalbuminuria is associated with a hypercoagulative and hypofibrinolytic state. Hemostatic dysfunctions might be a pathogenetic link between microalbuminuria and CVD. *Diabetes* 43:426–29, 1994

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IDDM, insulin-dependent diabetes mellitus; CVD, cardiovascular disease; PAI-1, plasminogen activator inhibitor-1; t-PA, tissue plasminogen activator; ET-1, endothelin-1; BMI, body mass index; AER, albumin excretion rate; BP, blood pressure; sBP, systolic blood pressure; dBP, diastolic blood pressure; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RIA, radioimmunoassay.

Microalbuminuria is not only a predictor of diabetic nephropathy but also a potent marker of risk of cardiovascular disorder (1). The conventional cardiovascular risk factors such as hypertension, elevated plasma cholesterol, and fibrinogen are seen more frequently in microalbuminuric, diabetic, and nondiabetic patients than in normoalbuminuric patients (2). However, the presence of these risk factors cannot fully explain the high cardiovascular mortality of microalbuminuric subjects (1).

Studies have shown that hemostatic factors can be related to cardiovascular disease (CVD) (3–5). In insulin-dependent diabetes melitus (IDDM) patients, many hemostatic dysfunctions have been reported (6–8), but little is known about hemostasis and fibrinolysis in microalbuminuric patients.

Plasminogen activator inhibitor-1 (PAI-1), the fast inhibitor of tissue plasminogen activator (t-PA), is synthesized by vascular endothelial cells, hepatocytes, and megakaryocytes. PAI-1 is released into the bloodstream by endothelial cells, but is mainly stored in platelets. High PAI-1 plasma levels have been found in several disorders characterized by thrombotic events, such as acute myocardial infarction, deep venous thrombosis, and essential thrombocythemia (9–11). Furthermore, significantly higher plasma levels of factor VII, another marker of thrombotic risk (12), and PAI-1 have been found in hypertriglyceridemic patients than in normotriglyceridemic patients (13,14).

Interactions of prothrombotic factors with endothelium and hyperlipidemia are of particular interest in microalbuminuric patients, because microalbuminuria is considered to be an indicator of vascular endothelial damage (2) associated with lipid abnormalities (1). This study aimed to measure PAI-1, factor VII, and fibrinogen in IDDM patients with microalbuminuria and to compare these results with those for a comparable group of IDDM

patients with normoalbuminuria. t-PA and endothelin-1 (ET-1) also were measured as indirect indexes of endothelial damage.

RESEARCH DESIGN AND METHODS

From 157 IDDM outpatients, we selected 13 microalbuminuric and 13 normoalbuminuric subjects who had an absence of acute or chronic infections, negative familial and personal histories of nondiabetic renal disease, no echographic evidence of renal abnormalities, normal urine sediments, and negative bacterial urine cultures. In addition, these subjects were taking no drugs other than insulin and had normal values for creatinine clearance, prothrombin time, and partial thromboplastin time. Normoalbuminuric patients were selected to obtain a group comparable for age, sex, body mass index (BMI), smoking habit, HbA_{1c}, daily insulin dose, and duration of diabetes to the microalbuminuric group (Table 1).

Patients were considered normoalbuminuric if albumin excretion rate (AER) was <20 μg/min and microalbuminuric if AER was 20–200 μg/min in 2 of 3 overnight urine collections. The level of AER was defined as the median value in these three collections.

Blood pressure (BP) was measured three times on the right arm with a random zero sphygmomanometer (Baumanometer, W.A. Baum, Copiagne, NY) after 20 min of rest, and the mean value was used for comparison (Table 1). Retinopathy was assessed by fundus examination after pupil dilation.

Blood samples for tPA and PAI-1 activity and ET-1 assay were collected without venous stasis with the first 5 ml of blood discarded. Venous occlusion was conducted for 10 min using sphygmomanometer pressure midway between systolic blood pressure (sBP) and diastolic blood pressure (dBP), and an additional blood sample was then taken. Blood samples for t-PA and PAI-1 were drawn into precooled Biopool Stabilyte tubes (Umea, Sweden) containing a low pH citrate anticoagulant, put immediately into melting ice, and centrifuged within 20 min at 4,000 rpm at 2°C (14). Blood samples for ET-1 were collected into a chilled syringe, transferred into a polypropylene tube containing EDTA and aprotinin at 0°C, and then centrifuged at 3,000 *g* for 15 min.

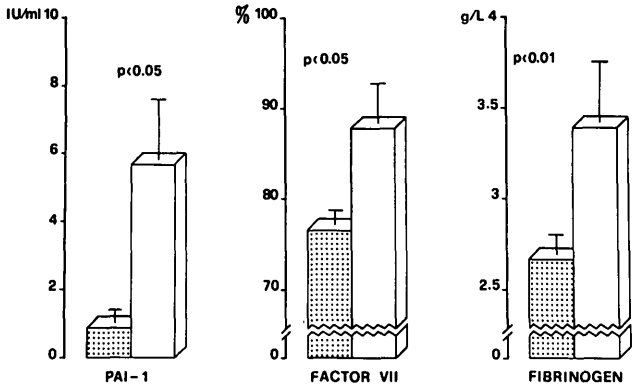

FIG. 1. PAI-1 and factor VII activities and plasma fibrinogen in 13 normoalbuminuric (▥) and 13 microalbuminuric (□) IDDM patients. Data are means ± SE. The Mann-Whitney test was used.

HbA_{1c} was determined by high-performance liquid chromatography (DIAMAT, Bio-Rad, Richmond, CA) and fasting plasma glucose by a Beckman (Fullerton, CA) Glucose Analyzer II. Plasma triglycerides (TG) and total cholesterol were measured enzymatically (Boehringer Mannheim, Mannheim, Germany), and high-density lipoprotein (HDL)-cholesterol was measured by precipitation with heparin and $MgCl_2$ on whole plasma. Low-density lipoprotein (LDL)-cholesterol was calculated by Friedenwald's formula. Fibrinogen was mea-

TABLE 1
Clinical and metabolic characteristics of the patients

	Normoalbuminuric patients	Microalbuminuric patients
n	13	13
Age (years)	29 ± 2	32 ± 4
Sex (M/F)	9/4	8/5
Smokers (yes/no)	3/10	3/10
BMI (kg/m^2)	23 ± 1	23 ± 1
Duration of diabetes (years)	14 ± 1	16 ± 1
Insulin dose ($IU \cdot kg^{-1} \cdot day^{-1}$)	0.7 ± 0.1	0.7 ± 0.1
Retinopathy (*n*) (none/simple/proliferative)	9/4/0	6/7/0
HbA_{1c} (%)	9.0 ± 0.4	9.2 ± 0.5
Fasting glucose (mM)	8.1 ± 0.7	7.7 ± 0.8
BP (mmHg)		
sBP	118 ± 3	132 ± 7
dBP	75 ± 2	83 ± 3*
Creatinine clearance (ml/s)	1.71 ± 0.04	1.78 ± 0.06
TG (mM)	0.69 ± 0.07	1.44 ± 0.27*
Cholesterol (mM)		
Total	4.16 ± 0.18	5.07 ± 0.36*
HDL	1.35 ± 0.27	1.41 ± 0.29
LDL	2.48 ± 0.51	2.99 ± 0.90
AER (μg/min)	3.3 ± 0.6	46.0 ± 6.8†

Data are means ± SE. Student's *t* test or Mann-Whitney test were used.
*$P < 0.05$ vs. normoalbuminuric.
†$P < 0.001$ vs. normoalbuminuric.

TABLE 2
Stepwise multiple regression analysis

	Log AER			Log PAI-1	
	Partial (r^2)	Model (r^2)		Partial (r^2)	Model (r^2)
Log fibrinogen	0.24	0.24*	Log TG	0.39	0.38†
Log TG	0.08	0.32	Log factor VII	0.10	0.49*
Total cholesterol	0.04	0.36	Log fibrinogen	0.6	0.55
Log PAI-1	0.02	0.38			
dBP	0.02	0.40			

Data are significant independent correlations.
*$P < 0.05$.
†$P < 0.001$.

sured by the Clauss method (16), and t-PA and PAI-1 activity were measured with a commercial chromogenic kit (Spectrolyse [fibrin] and Spectrolyse [poly-Lysine] PAI, Biopool, Umea, Sweden). Factor VII activity was determined by a clotting time assay using factor VII-deficient plasma. ET-1 was determined by a competitive radioimmunoassay (RIA) after extraction from plasma (ET-1, Peninsula, Belmont, CA). Urinary albumin concentrations were measured by RIA (Pharmacia, Uppsala, Sweden), and the AER (μg/min) was calculated as the timed urine volume multiplied by the albumin concentration. All the procedures followed were in accord with the Helsinki Declaration of 1975. Informed consent was obtained from all subjects.

Statistical analysis. Data are given as means ± SE. The Student's *t* test for unpaired data was used for comparisons of groups when values were normally distributed; otherwise the Mann-Whitney test was used. AER and PAI-1 activity were taken as dependent variables and the other parameters as independent variables in stepwise multiple regression analysis. For regression analysis, factor VII, t-PA, PAI-1 AER, fibrinogen, and TG were converted to log values because of their skewed distribution. Analyses were performed with SAS statistical software (17). The threshold of statistical significance was taken as $P < 0.05$.

RESULTS

AER (46.04 ± 6.84 vs. 3.31 ± 0.55 μg/min, $P < 0.001$), dBP (83.4 ± 3.33 vs. 75 ± 2.04 mmHg, $P < 0.05$), plasma total cholesterol (5.07 ± 0.36 vs. 4.16 ± 0.18 mM, $P < 0.05$), and plasma TG (1.44 ± 0.27 vs. 0.69 ± 0.07 mM, $P < 0.05$) were significantly higher in microalbuminuric than in normoalbuminuric patients (Table 1). Plasma fibrinogen (3.38 ± 0.21 vs. 2.65 ± 0.13 g/l, $P < 0.01$), factor VII (87.85 ± 4.94 vs. 76.54 ± 2.31%, $P < 0.05$), and PAI-1 activity (5.65 ± 1.92 vs. 0.85 ± 0.58 IU/ml, $P < 0.05$) were significantly higher in microalbuminuric than in normoalbuminuric patients (Fig. 1). Basal (0.12 ± 0.03 vs. 0.17 ± 0.04 IU/ml) and after stasis (0.16 ± 0.03 vs. 0.19 ± 0.14 IU/ml) t-PA activity and ET-1 values (5.37 ± 0.3 vs. 4.71 ± 0.42 pg/ml) were similar in the two groups. Stepwise multiple regression analysis shows significant positive correlations between the dependent variable AER and plasma fibrinogen (partial $r^2 = 0.23$, $P < 0.05$) and between the dependent variable PAI-1 activity and plasma TG (partial $r^2 = 0.39$, $P < 0.001$) and the factor VII activity (partial $r^2 = 0.10$, $P < 0.05$; Table 2).

DISCUSSION

The main finding of our study was that plasma factor VII and PAI-1 activity are significantly higher in microalbuminuric than in normoalbuminuric IDDM patients (Fig. 1). These data suggest that microalbuminuria is associated with an hypofibrinolytic and hypercoagulative state. It would be interesting to clarify whether hemostatic dysfunctions are peculiar of microalbuminuria independently from diabetes. However, this point cannot be evaluated in this study.

Increased factor VII and PAI-1 activities have been generally reported in diabetic patients (6–8,18,19), but their AER were not measured. In only one study was PAI-1 activity normal in microalbuminuric IDDM patients (20). The different anticoagulant used in collecting blood samples or possible heterogeneity of patient characteristics or both might explain the apparent discrepancy between this study and our results. In fact, the use of a low pH citrate has been recommended instead of the current citrate anticoagulant to avoid the underestimation of PAI-1 caused by the rapid accumulation of inactive tPA/PAI-1 complexes, especially for patients with high PAI-1 activity (15). In our microalbuminuric group, PAI-1 activity may be considered high, because in our laboratory the mean value for healthy subjects is 1.90 IU/ml.

Increased morbidity and mortality for CVDs has been noted in microalbuminuric patients (1) but the mechanism of this association is still not known. Increased prevalence of conventional atherosclerotic risk factors such as hypertension and lipid abnormalities in microalbuminuric patients has been suggested as a possible cause (1,21). In our study, dBP, plasma total cholesterol, and TG were significantly higher in the microalbuminuric than in the normoalbuminuric group. However, although these atherosclerotic risk factors contribute to cardiovascular risk, they cannot by themselves explain why microalbuminuria is a potent cardiovascular risk marker, and additional abnormalities probably exist that favor the rapid development of atherosclerosis (1). Our results confirm the hyperfibrinogenemia reported previously (21) and indicate that two other known cardiovascular risk

factors, increased PAI-1 and factor VII activity (12,13,22), are two additional abnormalities in microalbuminuric patients.

In stepwise multiple regression analysis, we found a significant positive correlation between AER and plasma fibrinogen. Plasma TG, total cholesterol, PAI-1, and dBP also enter into the best model for explaining the variance of AER, but these variables individually were not significant. On the other hand, when PAI-1 was taken as the dependent variable, we found a significant positive correlation with plasma TG and factor VII (Table 2). These results suggest that fibrinolysis and coagulation disorders could be other links between microalbuminuria and the cardiovascular risk, as proposed previously for lipid abnormalities (1,21). Furthermore, the relationship between hemostatic and lipid disorders suggests that they may be involved in a mechanism leading to increased atherothrombotic risk (23–26).

The cause of hemostatic alterations in microalbuminuric patients is unclear. Microalbuminuria has been considered to be an expression of widespread endothelial damage (2). Our t-PA and ET-1 results are not in line with this hypothesis, even though an impairment of the endothelial antithrombotic function cannot be excluded merely on this basis. On the other hand, lipid abnormalities associated with microalbuminuria have been implicated in hemostatic alterations (27,28). The correlation between plasma TG and PAI-1 we found in our patients supports this hypothesis.

In conclusion, PAI-1, factor VII activity, and fibrinogen are increased in microalbuminuric patients, which suggests that microalbuminuria might be the expression of vascular endothelial damage associated with a detectable hypofibrinolytic and hypercoagulative condition. The links between endothelial dysfunction or lipoprotein abnormalities or both and these hemostatic alterations are being investigated further.

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