NOTE

Can We Use Plasma Fibronectin Levels as a Marker for Early Diabetic Nephropathy

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Abstract. Although increased plasma fibronectin (PF) levels have been found in diabetic patients with microalbuminuria, there is still controversy about its clinical implication for detecting early diabetic nephropathy. To evaluate the PF concentration as a possible marker for early diabetic nephropathy, three groups of sex- and age-matched patients were studied I) 22 insulin dependent diabetic (IDDM) patients with microalbuminuria (mean age $\pm$ SEM: 23.3 ± 3.6 years, mean urinary albumin excretion rate (AER) $\pm$ SEM: 47.1 ± 39.5 $\mu\text{g}/\text{min}$); II) 17 IDDM patients with normoalbuminuria (mean age: 23.4 ± 4.4 years, mean AER: 7.8 ± 2.1 $\mu\text{g}/\text{min}$) and III) 20 healthy control subjects (mean age: 22.6 ± 4.1 years, mean AER: 6.7 ± 2.1 $\mu\text{g}/\text{min}$). PF and urinary excretion of albumin were measured by an immunoturbidimetric method using commercially available kits (Boehringer Mannheim GMBH FRG, and Miles Lab., UK). The mean PF was significantly higher in the group with microalbuminuria (406.5 ± 122.9 $\mu\text{g}/\text{ml}$) than in the group with normoalbuminuria (295.6 ± 96.9 $\mu\text{g}/\text{ml}$, $P < 0.01$) or in the control group (299.54 ± 105.5 $\mu\text{g}/\text{ml}$, $P < 0.01$). A weak positive correlation was found between PF and urinary albumin values ($r = 0.35$, $P < 0.05$). There were no significant correlations between PF and the other variables such as age, duration of diabetes, body mass index, arterial blood pressure, fasting blood glucose, fructosamine and HbA1c in the diabetic patients or in the control group. Our results suggest that the PF concentration could be a weak marker for early diabetic nephropathy. We cannot therefore use PF instead of microalbuminuria because there is only a weak correlation between PF and microalbuminuria.

Key words: Diabetes mellitus, Fibronectin, Early diabetic nephropathy.

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FIBRONECTIN is a high-molecular-weight plasma glycoprotein that is mainly produced by fibroblasts and endothelial cells [1, 2]. Plasma fibronectin (PF) plays a role in erythrocyte or platelet adhesion to vascular endothelium or subendothelial collagen [3, 4].

Increased biosynthesis of fibronectin in endothelial cells and fibroblasts was reported in diabetes [5, 6], and it is also found in an increased amount in the capillary wall and mesangium of the glomeruli of diabetic subjects [6–8]. Glycated fibronectin

is characterized by altered properties, especially by decreased binding to collagen and endothelial cells [7–11]. This may contribute to disturbed vessel wall integrity [9, 11].

Conflicting data exist about the PF concentration in diabetic patients with microalbuminuria. In some previous studies, an increased PF concentration was found in diabetic patients with microalbuminuria [12, 13], but this was not confirmed by other investigators [3, 4].

The aim of this study was to clarify this controversial issue, and to evaluate the PF concentration as a possible marker for early diabetic nephropathy.

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Materials and Methods

Thirty-seven type 1 (IDDM) diabetic patients were selected for this study. Twenty insulin-dependent diabetic patients with microalbuminuria (3 female, 17 male, mean age: 23.3 ± 3.6 years) were compared with 17 sex- and age-matched insulin dependent diabetic patients (3 female, 14 male, mean age: 23.4 ± 4.4 years) and 20 control subjects (3 female, 17 male, mean age: 22.6 ± 4.1 years). Informed consent was obtained from all patients.

Diagnosis of IDDM was done according to World Health Organization (WHO) criteria [15, 16].

Exclusion criteria included albugin positive proteinuria; blood pressure $>160/90$ mmHg; serum creatinine >1.4 mg/dl, presence of macroangiopathy and laboratory and clinical signs of liver and renal diseases of neoplasm.

Blood samples were obtained without venostasis from patients and control subjects at 0800 h, after a 12-h fast to determine fasting plasma glucose, glycosylated hemoglobin (HbA1), plasma fibronectin and fructosamine.

PF was measured in EDTA-plasma by an immunoturbidimetric assay with a commercially available kit (Boehringer Mannheim, FRG). Plasma samples were stored at -70 °C until the fibronectin assays were performed.

The HbA1 concentration was measured by means of microcolumn chromatography at 25.5 °C (Sigma Diagnostics, St. Louis, USA) [18].

The fructosamine concentration was measured by a modified nitroblue-tetrazolium (NBT) reduction method on a Technicon RA-1000 autoanalyzer [19].

Urinary albumin excretion, expressed as the albumin excretion rate (AER), was measured by an immunoturbidimetric method with a "Urin-pak Micro Alb immunokit" (Miles Laboratory, UK). Timed 12-h nightly urine samples were collected from the patients and control groups. At least two overnight urine samples were obtained from each patient, except for the normal subjects who delivered only one sample. The specimens were collected between 1900 h and 0700 h with written instructions to avoid exercise, but slow walking indoors was allowed. The urine volumes was measured and the albumin concentration was analyzed after centrifugation from albugin-negative samples.

AER was calculated and expressed as micrograms per minute. Normoalbuminuria was defined as urinary albumin excretion of <15 $\mu\text{g}/\text{min}$, microalbuminuria as $15\text{--}200$ $\mu\text{g}/\text{min}$ [20, 31, 32].

All the patients underwent an ophthalmic examination of the fundi and fluorescein angiography of the retinal vessels, performed by an ophthalmologist unaware of the clinical details of the patients.

Blood pressure was calculated as the median value of three determinations carried out after 10 min of rest in the supine position.

The statistical method included one-way analysis of variance (ANOVA) with Duncan's studentized range test as the multiple comparison method and calculation of Pearson correlation coefficients. *P* values <0.05 were considered significant. Results are expressed as the means $\pm$ SD.

Results

Clinical details of the subjects and parameters under study are shown in Table 1.

There was no differences between microalbuminuric and normoalbuminuric groups in the mean duration of diabetes.

Patients with microalbuminuria were characterized by significantly higher systolic blood pressure than normoalbuminuric diabetic patients or control subjects.

The mean PF concentration was much higher in the group with microalbuminuria than in the control subjects (Table 1). Moreover PF was significantly increased in the group with microalbuminuria independent of the presence of retinopathy. The PF concentration in patients with only microalbuminuria ($n=18$; 377.5 ± 106.2 $\mu\text{g}/\text{ml}$) was significantly higher than in the normoalbuminuric group. The mean plasma fibronectin concentration in the whole group of diabetic patients did not differ greatly from the controls (377.5 ± 106.2 $\mu\text{g}/\text{ml}$ versus 299.5 ± 105.5 $\mu\text{g}/\text{ml}$, $P>0.05$, respectively).

In the whole group of diabetic patients, there was a weak positive correlation between PF and AER ($r=0.35$, $P<0.05$, Fig. 1).

There were no correlations between the PF concentrations and age, duration of diabetes, blood pressure, BMI, HbA1 and fructosamine. AER correlated only with systolic blood pressure (Table 2).

Table 1. Characteristics of patients

	Normoalbuminuric group	Microalbuminuric group	Control group	F	P
n (m/f)	17 (14m/3f)	20 (17m/3f)	20 (17m/3f)		
Age (yr)	23.4 ± 4.4	23.3 ± 3.6	22.6 ± 4.1	0.23	P>0.05
Duration of diabetes (yr)	4.6 ± 4.4	4.5 ± 3.9	—	—	—
Body mass index (kg/m ²)	21.6 ± 2.3 ^c	21.4 ± 3.6 ^c	24.7 ± 3.3 ^d	6.69	P<0.05
Albumin excretion rate (μg/min)	7.8 ± 2.1 ^h	47.1 ± 39.5 ^s	6.7 ± 2.1 ^h	18.75	P<0.001
Systolic blood pressure (mmHg)	115.0 ± 7.7 ^f	121.2 ± 10.5 ^e	113.5 ± 7.4 ^f	4.40	P<0.05
Diastolic blood pressure (mmHg)	76.5 ± 9.3	77.8 ± 7.3	70.0 ± 8.5	4.82	P<0.05
Retinopathy (No. of patients)	1	2			
Neuropathy (No. of patients)	3	7			
Fasting blood glucose (mg%)	184.1 ± 71.2 ⁺	231.9 ± 100.0 ⁺	78.4 ± 8.5 ⁺⁺	24.35	P<0.001
Fructosamine (mmol/L)	3.3 ± 0.7 ⁺	3.5 ± 0.9 ⁺	1.6 ± 0.3 ⁺⁺	41.07	P<0.001
HbA1 (%)	10.3 ± 2.0 ⁺	10.4 ± 2.0 ⁺	7.0 ± 0.7 ⁺⁺	24.89	P<0.001
Plasma fibronectin (μg/ml)	295.6 ± 96.9 ^a	406.5 ± 122.9 [*]	299.5 ± 105.5 ^a	6.40	P<0.01

Values are means ± SD. P<0.001 for ⁺ vs. ⁺⁺, P<0.001 for ^{*} vs. ^a; P<0.05 for ^d vs. ^c; P<0.01 for ^e vs. ^f; P<0.001 for ^g vs. ^h, Duncan's test after ANOVA.

Fig. 1. Correlation of albumin excretion rate (AER) with plasma fibronectin (PF) in the whole group of diabetic patients. $Y=318.6+1.27x$; $r=0.35$, $P<0.05$.

Discussion

Our results showed that a high PF concentration is associated with microalbuminuria independent of the other variables considered, but there is a weak positive correlation between microalbuminuria and the PF concentration. We therefore conclude that the PF concentration could be a weak marker for early diabetic nephropathy. Our results also suggest that we cannot use the PF concentration instead of microalbuminuria for detecting early diabetic nephropathy because of this weak correlation.

In agreement with our study, DeGiorgia *et al.* [12] found a significant correlation between the PF concentration and microalbuminuria in patients

Table 2. Correlation of plasma fibronectin and albumin excretion rate with other variables

Variable	Plasma fibronectin		Albumin excretion rate	
	r	P	r	P
Age	0.2	NS	0.1	NS
Disease duration	0.17	NS	0.05	NS
Body mass index	0.24	NS	0.2	NS
Fasting blood glucose	0.17	NS	0.1	NS
Fructosamine	0.02	NS	0.1	NS
HbA1	0.1	NS	0.14	NS
Systolic blood pressure	0.2	NS	0.46	<0.01
Diastolic blood pressure	0.13	NS	0.14	NS

NS: Not Significant.

with non-insulin dependent diabetes mellitus. Although Skrha *et al.* [13] did not evaluate the intact plasma fibronectin, they found a positive correlation between the plasma free N-terminal fibronectin 30-kDa domain and albuminuria. On the other hand, Takahashi *et al.* [21] found increased urinary fibronectin excretion in diabetic patients with microalbuminuria.

We did not find any relationship between the PF concentration and the control of diabetes evaluated by HbA1 and the serum fructosamine concentration. This is in close agreement with the observation of other authors [12–14, 22–24]. On the other hand, Schwartz and Scherthaner [25] found that metabolic long-term control had an effect on the PF concentration. Similarly Borkenstein *et al.* [26] reported a higher fibronectin concentration in diabetic children who had poorly controlled diabetes. But Davis *et al.* [27] reported that short-term improved glucose control did not effect the PF concentration but the presence of diabetic microangiopathy rather than short- or long-term control of diabetes appears to be the cause of the increased plasma fibronectin concentration [3, 22, 24]. This may be due to the dysfunction of endothelial cells in patients with diabetic micro-

angiopathy [24, 28]. Increased synthesis of fibronectin in endothelial cells and fibroblast was reported in diabetes [5, 6, 29]. In our study a high fibronectin concentration may be the effect of widespread endothelial injury to small vessels. Although a recent study showed no modulation of PF function in diabetic patients receiving insulin treatment [30], some studies showed that glycated fibronectin is characterized by altered properties, especially by decreased binding to collagen and endothelial cells [9, 11]. This may contribute to disturbed vessel wall integrity. It is therefore possible to propose that a specific role of fibronectin, originating in its binding properties, occurs during neovascularization [31]. As a result of this process, an increased plasma fibronectin could be seen as a marker of injured small vessels and angiogenesis. This is consistent with the findings of Dixon *et al.* [32] who found significant fibronectin deposition within the glomerular microvessel lesion of diabetic kidneys.

We conclude that the PF is a weak marker for early diabetic nephropathy, but we cannot use it instead of microalbuminuria in clinical practice because there is only a weak correlation between the two.

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