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Plasma Fibronectin: An Early Marker of Diabetic Microangiopathy

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Abstract

The study included 40 diabetic patients (22 IDDM and 18 MIDDM) and 10 controls. Patients had diabetes for 10 years or more, with no overt proteinuria. Patients were subjected to clinical evaluation, fundus examination, urine analysis, estimation of fasting and postprandial blood glucose, serum creatinine, plasma fibronectin, urinary albumin excretion rate and glycosylated hemoglobin levels. Plasma fibronectin (pFN) levels were significantly higher in diabetic patients with evidence of microangiopathy in the form of microalbuminuria and/or retinopathy than among those without these complications. The extent of pFN elevation significantly correlated with albumin excretion rate in patients with microalbuminuria. There was no correlation between plasma fibronectin level and blood pressure. A high plasma fibronectin level can stand as a marker of endothelial injury in diabetic patients with microangiopathy.

Introduction

DIABETIC nephropathy is now recognized as the most common worldwide cause of end stage renal disease. Microalbuminuria almost always precedes and predicts overt diabetic nephropathy and is re-

gard as a reliable and strong marker of widespread endothelial injury in diabetes [1].

Other plasma markers of endothelial damage may accompany or even precede microalbuminuria. Fibronectin; a major extracellular glycoprotein, may accompany microalbuminuria and proved to be a reliable marker of endothelial damage in diabetes mellitus. In diabetes, plasma fibronectin levels were studied by many investigators, with controversial results [2].

The aim of this work is to find a possible relation between plasma fibronectin level (pFN) and microangiopathy in the form of microalbuminuria and/or retinopathy in an attempt to use pFN as an early marker of microangiopathic lesions in diabetes mellitus.

Material and Methods

The study was conducted on 40 diabetic patients attending the kasr El-Aini outpatient diabetic clinic and suffering from the disease for ten or more years. All patients were selected to fulfill the following criteria:

1. Normal dipstick urine analysis.
2. Absence of severe uncontrolled hypertension.
3. A serum creatinine of less than 1.4 mg/dl.
4. Absence of clinically evident other nephrourologic problems, liver disease, heart failure or neoplasms.

The diabetic patients were categorized into two groups:

A) Insulin dependendt diabetic patients (IDDM) with the onset of diabetes before the age of 30 years and controlled by insulin injections from the onset of the disease.

B) Non insulin dependent diabetic patients (NIDDM) with the onset of diabetes after the age of 30 years and controlled satisfactorily with oral hypoglycemic agents.

Ten normal, age and sex matched controls were included in the study.

All subjects were subjected to the following:

1. Clinical examination.
2. Fundus examination.
3. electrocardiogram and renal ultrasonography.
4. Complete urine analysis examined chemically by Cambur 9 strips (Boehringer Mannheim Gmbt) and microscopically.
5. Fasting and postprandial blood glucose estimation using the enzymatic glucose oxidase method (Stanbio Lab. Inc., San Antonio, Texas, U. S. A).
6. Estimation of serum and 24 hour urine creatinine using the Jaffe method with deproteinization [3].
7. Plasma fibronectin (pEN) estimation:

By single radial immunodiffusion

technique [4] using the already prepared fibronectin immunodiffusion plates (Behringwerke AG, Marburg, Germany).

8. Estimation of microalbuminuria:

By the single radial immunodiffusion technique [4] using VLC-Patrigen Albumin immunodiffusion plates with a quantitative range of 6-89 mg/l (Behringwerke AG, Marburg, Germany).

9. Estimation of glycosylated hemoglobin (HbA1):

The method was based on charge dependent separation of HbA1 from total hemoglobin by cation-exchange resin, with the elimination of the labile fraction and then colorimetric determination of HbA1 [5].

Applied Statistical Methods:

The significance of differences for normally distributed data was assessed by the standard unpaired Student's t-test. The correlation between independent variables was assessed by linear regression analyses and the strength of correlation was calculated using Pearson's product correlation coefficient (r).

Results

Table (1) shows the important clinical and laboratory data of group I (IDDM), group II (NIDDM) and normal controls. In table (2) data of group I and group II were further categorized into two main subgroups according to the urine albumin excretion rate (UAER), whether in the normoalbuminuric (N) range (group IN & IIN) or in the microalbuminuric (M) range (group IM & IIM).

The pFN was significantly higher in the IM group than the in group ($p < 0.05$) (Fig 1) and was also significantly higher in group IIM than group IIN ($p < 0.05$) (Fig. 2).

The diastolic and the mean blood pressure were significantly higher in group IIM than in group IIN ($p < 0.01$ and $p < 0.02$ respectively).

The MAP and pFN were significantly higher in group IM than in the control group ($p < 0.05$, and $p < 0.01$; respectively) (Fig. 1). In group IMM, the MAP and pFN were significantly higher than those of the control group ($p < 0.05$; both). Group IIN showed only a MAP that was significantly higher than that of the control group ($p < 0.05$) (Fig. 2).

In table (3), all patients of groups I and II were put into two main subgroups according to the UAER disregarding the type of diabetes mellitus. In the microalbuminuric group, the MAP and pFN were significantly higher than the control group ($p < 0.01$; both). The MAP and pFN of normoalbuminuric group showed no significant difference from those of the control group (Fig. 3).

The studied diabetic patients were categorized into 4 groups: all patients as a whole including both types of diabetes mellitus, microalbuminuric patients, group I

Table (1): General Features of Control Subjects, IDDM (Group I) and NIDDM (Group II) (Mean ± S.D).

	Controls Normals		Group I IDDM		Group II NIDDM	
	Mean	± S.D	Mean	± S.D	Mean	± S.D
FBS			132.0	51.5	141.0	39.8
PPBS			172.1	51.0	194.7	52.6
HBAI%	5.7	0.5	9.2	1.5	8.9	2.2
DBP	75.0	4.7	79.5	9.0	87.1	8.8
MABP	89.4	5.3	96.0	10.3	105.7	10.3
S.CR	0.8	0.1	0.8	0.3	1.0	0.2
pFN	30.7	4.3	39.5	11.9	41.5	9.5
			Med	Range	Med	Range
UAER			47.0	7-164	47.5	12-192

Table (2): Important Data of IDDM and NIDDM Subjects Classified According to UAER (Mean ± S.D).

	IDDM Group I		NIDDM Group II	
	Microalb.	Normoalb.	Microalb.	Normoalb.
N (M / F)	12 (3 / 9)	10 (4 / 6)	12 (5 / 7)	6 (1 / 5)
HBAI	9.2 ± 1.1	9.3 ± 1.9	9.5 ± 2.4	7.8 ± 0.7
DBP	82.0 ± 10.8	76.5 ± 5.3	90.8 ± 6.4	79.7 ± 8.3
MAP	98.6 ± 12.2	92.7 ± 6.5	109 ± 8.2	97.5 ± 9.6
pFN	44.5 ± 12.1	33.5 ± 8.8	44.9 ± 9.6	34.7 ± 4.2

and group II patients. In all these groups there was significant correlation between pFN and UAER ($p < 0.01$, $p < 0.02$, $p < 0.01$, and $p < 0.01$ respectively).

Plasma FN was found to be significantly higher in patients with retinopathy when compared to either the control group ($p < 0.001$) or to patients withour retinopathy $p < 0.02$). No significant difference was found in pFN level between patients without retinopathy and the control subjects.

Table (3): Important Data of Micro- and Normoalbuminuric Diabetic Subjects (Mean ± S.D.).

	Microalbuminuria	Normoalbuminuria
MAP	104.2 ± 11.6	94.5 ± 7.9
p FN	44.7 ± 10.7	33.9 ± 7.3

FBS: Fasting blood sugar in mg / dl. PPBS : Postprandial blood sugar. HBAl % : Glycohemoglobin. DBP : Diastolic blood pressure. MABP: Mean arterial blood pressure. S.CK: serum creatinine mg / dl. PFN: Plasma fibronectin. UAER: Urine albumin excretion rate. Microalb : UAER > 20, < 200 µgm / min. Normoalb : UAER < 20 µgm / min.

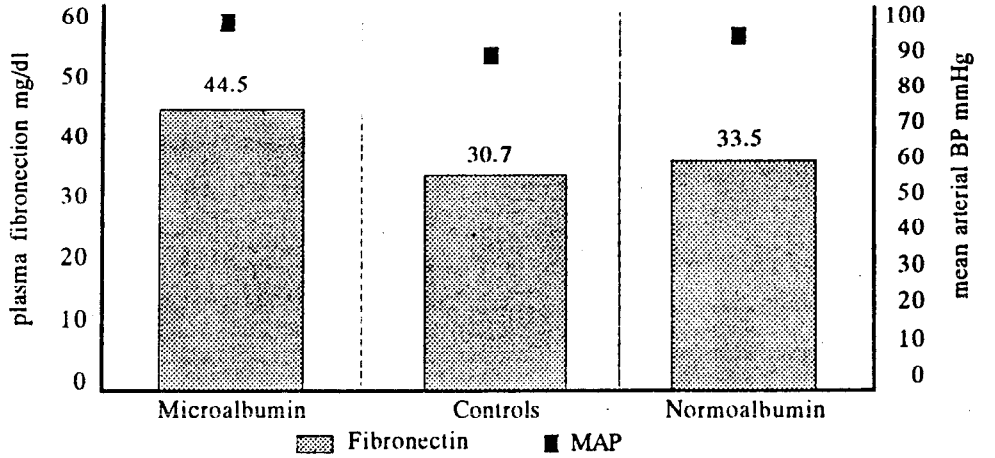

Fig. (1): FPN and MAP in IDDM and control subjects.

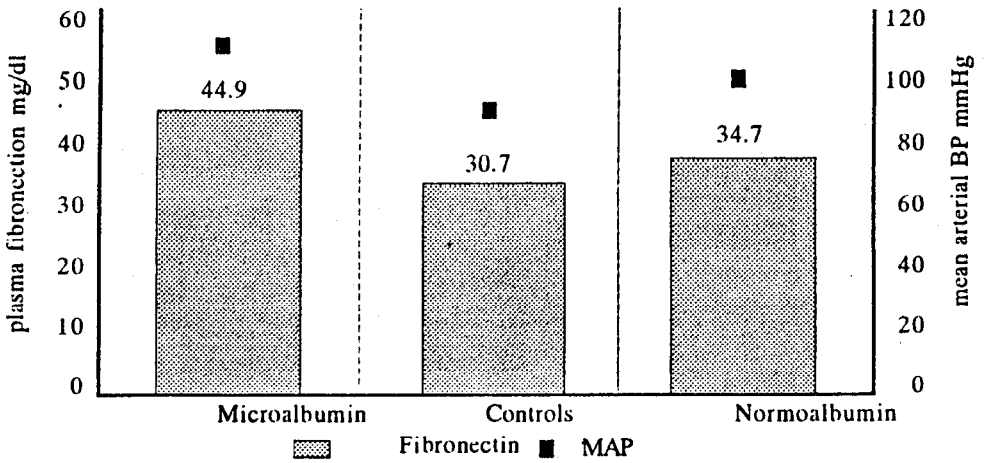

Fig. 2: Plasma fibronectin and MAP in NIDDM patients and control subjects.

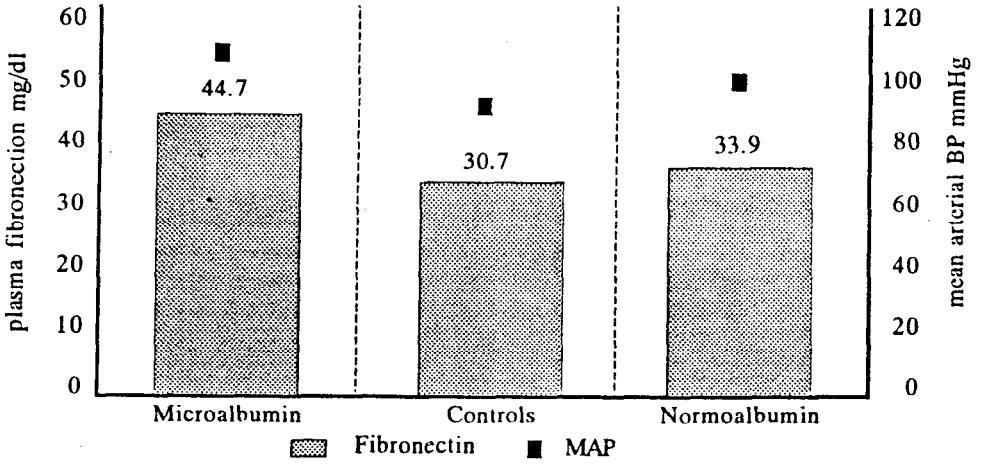

Fig. 3: Significant variables in micro & normoalbuminuric patients & controls.

Discussion

The mean plasma fibronectin (pFN) was significantly higher in diabetic patients (IDDM and NIDDM) with microalbuminuria than in those with normoalbuminuria (Figs. 1&2). Patients with retinopathy showed significantly raised pFN compared to those without retinopathy. These findings agree with the results of Seghiere et al. [6] and De giorgio et al. [7].

There was a statistically significant positive correlation between pFN and the degree of UAER in different patient groups: all patients, the 24 microalbuminuric patients, the 22 IDDM and the 18 NIDDM patients, separately. Similar positive correlation between pFN and UAER was reported [8].

There was no significant difference in pFN level between normoalbuminuric patients or patients without retinopathy and control subjects. This agrees with the results of Seghieri et al. [6] who found that diabetics lacking microalbuminuria and retinopathy had normal pFN levels.

There was no significant correlation between pFN and blood pressure recording in the studied patients. This agrees with others [9] who reported that hypertension associated vascular disease and diabetic macroangiopthy were not found to raise pFN to abnormal values.

Microalbuminuria, the forerunner of clinical diabetic nephropathy, is strongly prognostic of cardiovascular disease and death in diabetes mellitus [10]. It is also the most reliable marker of widespread endothelial injury in diabetes [1]. Fibronectin in diabetes, represents a key factor in transducing and organizing the neovascularization process in vitro and in vivo

[11]. It was found to increase in relation to glomerular cell proliferation in human diabetic kidneys [12] and has been strongly suggested to be associated with endothelial injury [13].

Under physiological conditions, pFN is mainly synthesized by the liver; endothelial cells and macrophages share in its production to a lesser extent [14]. The synthesis of pFN by endothelial cells was found to increase significantly in response to injury or angioproliferative processes [11]. The main source of this increase is the endothelium of the microvessels not macrovessels [13].

It is believed that the higher pFN level observed in the patients with microalbuminuria and retinopathy than those without these lesions is due to the widespread microangiopathic injury in these patients leading to increased FN synthesis and/or release. The lack of correlation between BP recordings and pFN in the studied patients is in favor of the belief that FN is mainly released from the microvasculature and not macrovasculature in complicated diabetes mellitus.

The lack of relation between macroangiopathy and pFN could be explained by the difference in the biochemical and physiologic properties of endothelia of small and large vessels; the latter being devoid of any angioproliferative property. Large vessel endothelial damage is not followed by widespread replication of fibronectin producing cells [15].

The results of this work point out that pFN is significantly raised in conditions of widespread small vessel endothelial damage and or proliferation typically exemplified by diabetes mellitus with microangiopathic complications. Accordingly, a high pFN encountered in a diabetic subject is highly suggestive of the presence of microangiopathic disease.

Estimation of pFN level, being a marker of endothelial damage, can be used to detect early diabetic microvascular complications.

Conclusions:

1. pFN is higher in diabetic patients with evidence of microvascular disease than those without.
2. There is no significant difference in pFN between diabetics without microvascular complications and normal controls.
3. pFN can stand as a useful marker for detection of endothelial microvascular damage.

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