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Plasma Type IV Collagen and Fibronectin Concentrations in Diabetic Patients with Microangiopathy

In-Kyu Lee, M.D., Keun-Yong Park, M.D., Hyun-Kyung Oh, M.D.,*
Rang-Woon Park, M.D.,* and Joon-Seung Jo, M.D.*

Department of Internal Medicine, School of Medicine, Keimyung University and Department of Biochemistry, School of Medicine, Kyungbuk National University, Taegu, Korea*

In diabetes mellitus, thickening of basement membrane in capillaries and small vessels is a well-known finding and important in the progression of diabetic microangiopathy. We evaluated whether the plasma levels of type IV collagen and fibronectin, which are important factors of basement membrane, are related with the presence of diabetic microangiopathy. Plasma type IV collagen and fibronectin levels were measured in 40 healthy controls(Mean±SD, age ; 50.3±5.5 yr) and 94 diabetic patients(age ; 52.4± 13.5 yr) with and without microvascular complications. The mean plasma levels of type IV collagen(5.3±2.9 ng/ml)and fibronectin(474.4±119.4 ug/ml) in diabetic patients were significantly higher($p<0.01$) than in healthy controls(3.7±1.3 ng/ml and 319±50.9 ug/ml). The mean plasma level of type IV collagen in diabetic patients with complications(6.6±3.7 ng/ml) was significantly higher($p<0.01$) than in those without complications(4.3±1.7 ng/ml) and became higher in more complicated patients. Furthermore, the severity of retinopathy and several indicators of nephropathy such as serum BUN, creatinine and proteinuria were closely associated with plasma type IV collagen level and a significant correlation was found between plasma type IV collagen and creatinine clearance($r=-0.31$, $p<0.001$). There was no significant difference in plasma fibronectin concentrations, however, between the diabetic patients with complications and those without complications. The results of this study suggest that plasma type IV collagen is a sensitive guide for the occurrence and progression of diabetic microangiopathy but plasma fibronectin is not related with diabetic microangiopathy though it is increased in diabetic patients.

Key Words : *Type IV collagen, Fibronectin, Diabetic microangiopathy.*

INTRODUCTION

Vascular disease is a common and clinically significant complication of diabetes mellitus. Especially, the increased thickness of capillary basement membranes is one of the important detectable manifestations in patients with diabetic retinopathy and nephropathy(Williamson and Kilo, 1976 ; Timple, 1983). Basement membranes are composed of several macromolecules including type IV collagen, laminin, heparan sulfate, proteoglycan and

Address for correspondence : *In-Kyu Lee, M.D., Department of Internal Medicine, School of Medicine, Keimyung University, 194, Dongsan-dong, Joong-gu, Taegu, 700-310, Korea. Tel : (053)250-7421.*

nidogen(entactin)(Martin et al., 1983). Of these intrinsic components, type IV collagen is unique to basement membranes and differs from other interstitial collagens, type I, II, and III.

Fibronectin is an alpha-2 glycoprotein present in tissues and in both plasma and other biologic fluids(Davis, 1981). Plasma fibronectin(PF) is believed to be produced mainly by the liver, as well as by endothelial cells(Owens and Cimino, 1982). Increased amounts of fibronectin are found in the thickened glomerular basement membranes and mesangium of the kidney(Solerte et al., 1985).

This study is therefore aimed at whether the plasma levels of fibronectin and type IV collagen are related with the presence of diabetic microvascular complications and might be used as biochemical markers for assessing abnormal basement membrane metabolism in patients with diabetes.

MATERIALS AND METHODS

The study was performed on 40 healthy control subjects(Group I) and on 94 diabetic patients. The diabetic patients were divided into three groups : 54 patients without microvascular complications, 21 patients with retinopathy only, 19 patients with overt nephropathy and retinopathy.

Among the diabetic patients, 12 patients received diet therapy only, 44 received sulfonylureas, 38 received insulin therapy. Diabetic patients with any clinical and/or serological signs of such fibrotic disorders as chronic liver disease, pulmonary fibrosis, and hematologic disorders were excluded from this study.

Diabetes was diagnosed according to the criteria of the National Diabetes Data Group. Microangiopathy was diagnosed by clinical and serological examinations as well as by subjective complaints. The grade of retinopathy was determined by ophthalmoscopy and fluorescent angiography and classified as no change, background or proliferative retinopathy. The diabetic nephropathy was defined as a urinary protein excretion rate above 500 mg/24 hr at resting period.

Blood samples were obtained without venostasis from diabetics and controls at 08 : 00 AM, after a 12 hour fast, plasma was separated and kept at −20℃ until assay. Plasma type IV collagen levels were measured by radioimmunoassay(DPC, Los Angeles, USA), plasma fibronectin levels were measured by Laurell's electroimmunoassay(1966) using the Sigma antisera(St. Louis, USA) and glycosylated hemoglobin(HbA_{1c}) by ion exchange chromatography(Stanbio, St. Antonio Texas).

All results were given as mean±SD. Student's t-test and Pearson's correlation coefficient were used to test statistical significance between groups with $p<0.05$ as significant.

RESULTS

The clinical characteristics of the subjects were summarized in Table 1. There were no significant differences in age and sex distribution among all experimental groups. But diabetic durations were significantly higher($p<0.01$) in diabetic patients with

Table 1. Clinical characteristics of control and diabetic subjects

	Control subjects (n=40)	Diabetic patients without microvascular complications (n=54)	Diabetic patients with retinopathy only (n=21)	Diabetic patients with retinopathy and nephropathy (n=19)
Age (years)	50.3±5.5	49.7±13.7	56.8±15.2	55.3±8.5
Sex (M/F)	25/15	30/24	11/10	11/18
Duration of diabetes (years)		3.8±1.3	9.5±8.8**[b]	11.7±6.4**[b]
Body mass index (kg/m^2)	24.5±1.8	23.9±1.2	24.2±1.9	24.1±0.9
Systolic blood pressure (mmHg)	120.9±10.7	122.4±15.6	124.2±25.5	135.2±22.5*[abc]
Glycosylated hemoglobin (%)	7.4±0.4	11.2±2.7**[a]	11.2±3.3**[a]	11.1±3.1**[a]

Significant differences compared with control[a], diabetic patients without complications[b], or diabetic patients with retinopathy only[c] ; *$p<0.05$, **$p<0.01$.

Table 2. Plasma levels of type Ⅳ collagen and fibronectin in control and diabetic subjects

	Control subjects (n=40)	Diabetic patients Without microvascular complications (n=54)	Diabetic patients with retinopathy only (n=21)	Diabetic patients with retinopathy and nephropathy (n=19)
Type Ⅳ collagen (ng/ml)	3.7±1.3	4.3±1.7**[a]	5.4±2.6**[a,b]	7.9±4.3*[b],**[3,c]
Fibronectin (μg/ml)	319.3±50.9	439.5±114.9**[a]	487.8±128.6**[a]	464.8±125.5**[a]

Significant differences compared with control[a], diabetic patients without complications[b], or diabetic patients with retinopathy only[c] ; *p<0.05, **p<0.01.

complications(Mean±SD ; 9.5±8.8 yr in diabetic patients with retinopathy only and 11.7±6.4 yr in diabetic patients with retinopathy and nephropathy) than in diabetic patients without complications(Mean ±SD ; 3.8±1.3 yr). Systolic blood pressures were significantly higher(p<0.05) in diabetic patients with nephropathy and retinopathy(135.2±22.5 mmHg) than other groups(120.9±10.7, 122.4±15.6, and 124.2±25.5 mmHg).

The plasma level of type IV collagen was shown to be closely associated with the clinical status of diabetes. Mean plasma level of type IV collagen in diabetic patients(5.3±2.9 ng/ml) was significantly higher(P<0.01) than the control group(3.7±1.3 ng/ml). Even among diabetic subgroups the plasma type IV collagen level in diabetic patients with complications(6.6±3.7 ng/ml) was significantly higher (P<0.01) in diabetic patients without complication(4.3±1.7 ng/ml), and it seems likely that plasma type IV collagen levels were much higher when the complications were more severe(5.4±2.6 ng/ml in diabetic patients with retinopathy only, and 7.9±4.3 ng/ml in diabetic patients with retinopathy and nephropathy). However there were no significant differences in plasma fibronectin levels among diabetic subgroups(439.5± 114.9 ug/ml, 487.8±128.6 ug/ml, and 464.8± 125.5 ug/ml in those without complications, with retinopathy only, and with retinopathy and nephropathy, respectively) even though the mean level of diabetic patients(474.4±119.4 ug/ml) was significantly higher(p<0.01) than that of healthy controls(319.3±50.9 ug/ml) (Table 2).

Mean plasma type IV collagen levels were significantly elevated in diabetic patients with nephropathy especially those whose blood urea nitrogen(BUN), serum creatinine and proteinuria exceeded 30 mg/dl, 2.0 mg/dl, and 1.0 g/24 hour, respectively(Fig. 1, Fig. 2, and Fig. 3). A significant degree of correlation was found between plasma type IV collagen level and creatinine clearance(r= −0.31, p<0.001) as shown in Fig. 4. But there was no significant difference in plasma fibronectin concentrations(data not shown).

Moreover, plasma type IV collagen level was closely related to the severity of diabetic retino-

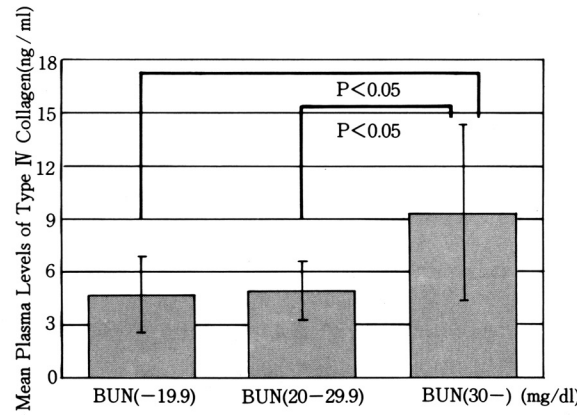

Fig. 1. Plasma type Ⅳ Collagen levels in diabetic patients in relation to serum BUN levels

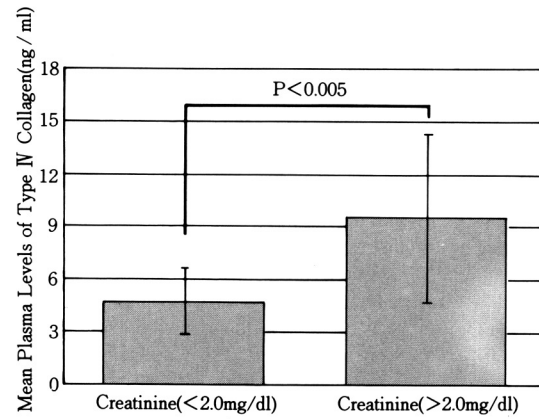

Fig. 2. Plasma type Ⅳ collagen levels in diabetic patients in relation to serum creatinine levels

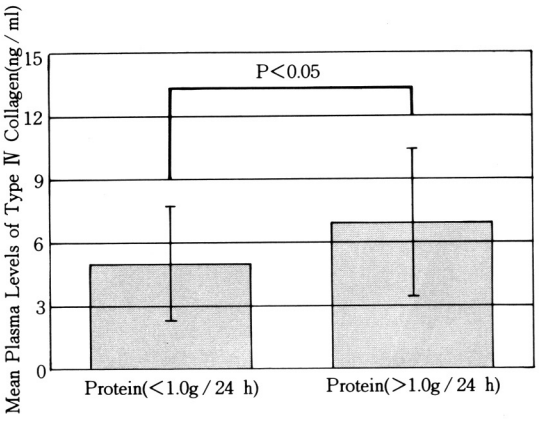

Fig. 3. Plasma type Ⅳ collagen levels in diabetic patients in relation to 24 hour urine protein levels

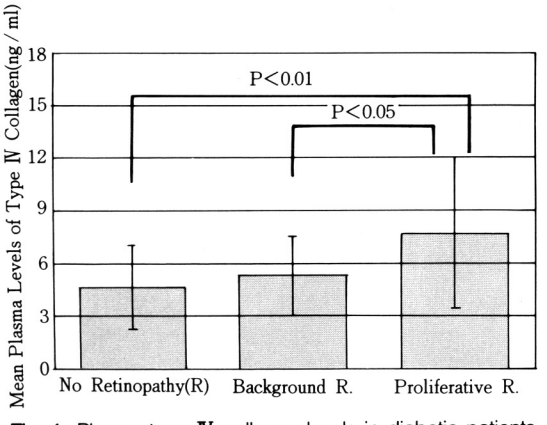

Fig. 4. Plasma type Ⅳ collagen levels in diabetic patients in relation to severity of retinopathy

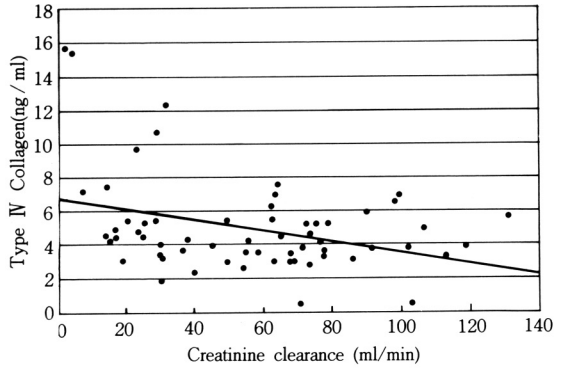

Fig. 5. Correlations between plasma levels of type collagen and creatinine clerance (r=−0.31, P<0.001)

pathy(Fig. 5). Mean plasma type IV collagen level in diabetic patients with proliferative retinopathy(7.7±4.3 ng/ml) was significantly higher(P<0.01) than in those with background retinopathy(5.3±2.2 ng/ml) or in those without retinopathy(4.6±2.4 ng/ml).

DISCUSSION

In the diabetic kidney, type IV collagen increased in the thickened basement membrane of glomerular capillaries, small vessels, and renal tubules. According to immunohistochemical study with the monoclonal antibody, the increase of type IV collagen was especially notable in the mesangial areas and nodular lesions(Matsumoto et al., 1990). Type IV collagen is located primarily in the lamina densa, where it forms a large three dimensional network. Four molecules of type IV collagen are linked via the NH_2-terminal region. This disulfide-rich, cross-linking domain is called the 7S-collagen and is resistant to various proteases, including bacterial collagenase. Two molecules of type IV collagen are linked via the COOH-terminal, noncollagenous domain(NC1 domain) (Davis, 1981 ; Timple et al., 1981).

7S-Collagen can be detected in serum by a radioimmunological method(Risteli et al., 1981). Serum concentration of this antigen is elevated in diabetic animals and can be normalized by insulin treatment(Risteli et al., 1982). Several competitive radioimmunoassay methods with polyclonal monospecific antibodies have been developed to determine the serum concentrations of 7S-collagen or NC1 domain in diabetic humans or experimentally induced diabetic rats(Timple et al., 1981 ; Risteli et al., 1981 ; Risteli et al., 1982 ; Hogemann et al., 1984 ; Hasslacher et al., 1984 ; Brocks et al., 1985 ; Hogemann et al., 1986 ; Hasslacher et al., 1987). Results from these investigations have shown that the elevated synthetic activity or increased turnover rate of type IV collagen might be involved in the progression of diabetic microangiopathy characterized by the thickening of basement membrane. This may account for elevated levels of serum type IV collagen in long-term diabetic patients. Our results also showed that the plasma levels of type IV collagen in diabetic patients with complications were significantly higher than those without complications. Moreover, plasma type IV collagen concentrations gradually increased according to the severity of the clinical signs of retinopathy or nephro-

pathy. But there were no relationships with fasting plasma glucose levels and hemoglobin A_{1C} levels.

These elevations of plasma type IV collagen may be due to its increased synthesis and/or different clearance rates and/or increased degradation of basement membrane collagen. The comparison of clearance rates of 7S-collagen in normal and diabetic animals showed a similar biphasic profile. In the initial phase about 80% of antigen disappeared within 2-4 hr, presumably due to tissue uptake of 7S-collagen. As no difference could be detected between normal and diabetic rats, these experiments indicate that differences in the excretion rate cannot explain the elevated serum concentrations in diabetic animals(Brocks et al., 1985). The type IV collagen antigen in the high molecular weight peak increases during the development of streptozotocin-induced diabetes. This indicates increased biosynthesis of type IV collagen, and is in agreement with the findings of Hasslacher et al.(1984), who showed a positive correlation between the rate of glomerular basement membrane synthesis and 7S-collagen in serum. Brocks et al.(1985) and Matsumoto et al.(1990) reported that increased serum type IV collagen antigen reflected increased synthesis of type IV collagen and no difference was found in the clearance rate of type IV collagen between nondiabetic and diabetic animals. Shuppan et al.(1986) reported that serum NC1 was derived from degradation of basement membrane collagen. They concluded that the concentration of serum type IV collagen peptide indicated a newly synthesized type IV collagen molecule that could not be deposited in the tissue and overflowed into the bloodstream, rather than liberation of type IV collagen already deposited in the tissue. We evaluated only the plasma levels of 7S-collagen and found even in diabetic patients without complication, the levels were significantly higher than in healthy subjects.

We can also find that there is a significant negative correlation between plasma type IV collagen and the creatinine clearance. Matsumoto et al.(1990) reported that there was no obvious relationship between plasma type IV collagen level and serum creatinine level in patients with severe nephropathy, hence it seems that the increased plasma type IV collagen is not due to decreased clearance rate.

Therefore the plasma type IV collagen peptide(7S) concentration is a useful and noninvasive marker for the development of diabetic microangiopathy. It could also be used to predict the development of diabetic complications and to assess therapeutic effects or the prognosis of diabetes.

Plasma fibronectin(PF) is an alpha2-glycoprotein, which is produced mainly by the liver as well as in part by vascular endothelia and platelets both in vivo and in vitro(Mosesson and Amrani, 1980 ; Jaffe and Mosher, 1978 ; Owens and Cimino, 1982 ; Ginsberg et al., 1974). Plasma fibronectin enhances the adhesion of erythrocytes to vascular endothelium in vitro(Wautier et al., 1981). Moreover, in diabetes mellitus, fibronectin might reduce erythrocyte deformability and filtrability(Solerte et al., 1983 ; Schmid-Schonbein and Volger, 1976).

Raised PF levels in diabetics were first observed by Davis(1981) and report of Bartolomei et al.(1984) confirmed this increase only in insulin requiring patients under 40 years of age. These findings might be due to the influence of aging which is directly related to the PF levels rather than diabetes. In the later report, they did not show any significant difference between the PF level of controls and treated groups of diabetics whose age ranged from 35 to 65 years. But in diabetic patients with retinopathy, the PF concentrations were significantly higher than in controls or diabetic patients without retinopathy(De Giorgio et al., 1984).

De Giorgio et al.(1988) also reported that there was a significant correlation between PF increase and the presence of early diabetic nephropathy with microalbuminuria independent of the other considered variables, including clinically evident retinopathy. Other studies have presented conflicting results on the relationship between PF levels and proteinuria in subjects with overt diabetic nephropathy(Solerte et al., 1985). Preston et al.(1984) reported that they did not find a significant difference in the plasma fibronectin level between their diabetic patients as a whole and their control group, we also did not find any significant difference in the plasma fibronectin level among our diabetic subgroups, which suggested that plasma fibronectin did not play a primary role in the genesis of diabetic angiopathy, but this did not exclude the possibility that there was an abnormality in the tissue component of fibronectin in diabetes(Atherton and Hynes, 1981). But in order to confirm this, further and more extended observations would be required.

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