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Abstract

To investigate the role of vitronectin in the progression of diabetic nephropathy, plasma concentrations of vitronectin were measured by enzyme-linked immunosorbent assay in patients with diabetes mellitus and compared with normal control subjects. In diabetic patients with normoalbuminuria and microalbuminuria, plasma concentrations of vitronectin were significantly higher than those of control subjects. Plasma concentrations of vitronectin in diabetic patients with chronic renal failure were significantly lower than those with normal renal function. There was a significant positive correlation between plasma concentration of vitronectin and blood platelet counts. In the early stage of diabetic nephropathy, vitronectin may be increased caused by synthesis from activated platelets. With progression of diabetic nephropathy, plasma vitronectin may be decreased because of accumulation in sclerotic glomeruli and arteriosclerotic lesions. In conclusion, the plasma concentration of vitronectin appears to be an important marker for the progression of diabetic nephropathy.

KEYWORDS: vitronectin(S-protein), diabetic nephropathy, hypertension, chronic renal failure, enzyme-linked immunosorbent assay (ELISA)

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To investigate the role of vitronectin in the progression of diabetic nephropathy, plasma concentrations of vitronectin were measured by enzyme-linked immunosorbent assay in patients with diabetes mellitus and compared with normal control subjects. In diabetic patients with normoalbuminuria and microalbuminuria, plasma concentrations of vitronectin were significantly higher than those of control subjects. Plasma concentrations of vitronectin in diabetic patients with chronic renal failure were significantly lower than those with normal renal function. There was a significant positive correlation between plasma concentration of vitronectin and blood platelet counts. In the early stage of diabetic nephropathy, vitronectin may be increased caused by synthesis from activated platelets. With progression of diabetic nephropathy, plasma vitronectin may be decreased because of accumulation in sclerotic glomeruli and arteriosclerotic lesions. In conclusion, the plasma concentration of vitronectin appears to be an important marker for the progression of diabetic nephropathy.

Key words: vitronectin (S-protein), diabetic nephropathy, hypertension, chronic renal failure, enzyme-linked immunosorbent assay (ELISA)

Vitronectin (S-protein) is a 75-kD glycoprotein with many functions. Vitronectin acts as an adhesion molecule for the interaction with cell surfaces and components of the extracellular matrix (1-3). On the blood coagulation and fibrinolytic system, vitronectin neutralizes the anticoagulation activity of heparin by forming a thrombin-antithrombinIII-vitronectin complex and regulates the fibrinolytic system by binding to plasminogen activator inhibitor-1 (4-9). The functions of this glycoprotein also includes inhibition of the membrane attack complex (MAC) formation (10-12).

Diabetic nephropathy is morphologically associated with expansion of the mesangial matrix, and thickening of the glomerular basement membrane, which result in the development of glomerulosclerosis (13, 14). These changes, may account for the increase in proteinuria from normoalbuminuria to microalbuminuria commonly seen in diabetic nephropathy and resulting in persistent proteinuria which finally develops to renal failure (15).

We previously performed an immunohistochemical study showing extensive accumulation of vitronectin in the extracellular matrix of sclerotic glomeruli and tubulointerstitial lesions around sclerotic glomeruli (16). Fibrotic lesions in many other organs have also been reported to be positive for vitronectin by immunohistochemical staining (17). In this study, to investigate the possibility that vitronectin may be a marker for the progression of diabetic nephropathy, the plasma concentration of vitronectin was measured by enzyme-linked immunosorbent assay (ELISA) in patients with non-insulin dependent diabetes mellitus (NIDDM).

Subjects and Methods

Patients. We studied 59 patients with NIDDM, and 16 healthy volunteers as normal controls. The patients' clinical characteristics are shown in Table 1. None of the patients had concurrent diseases such as liver diseases, infectious diseases, or malignant diseases.

Patients with blood pressure of higher than 160/95 mmHg were classified as the hypertensive group, while those lower than that were classified as the non-hypertensive group".

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Table I Clinical findings of the patients with diabetic nephropathy

Stage	N	Sex (male/female)	Age (mean ± SD)	Duration of DM (mean ± SD)	Presence of hypertesion
DN-0	18	11/7	55.8 ± 9.2	6.7 ± 3.8	7
DN-1	19	6/13	63.9 ± 10.2	8.5 ± 6.0	11
DN-2	12	8/4	58.1 ± 8.1	11.8 ± 7.8	5
DN-3	5	4/1	55.6 ± 3.7	20.0 ± 8.6	6
DN-4	4	3/1	68.3 ± 4.2	25.3 ± 7.6	4

DN-0: normoalbuminuria group, urinary albumin index ≦ 15 mg/gCr
DN-1: microalbuminuria group, 15 mg/gCr < urinary albumin index ≦ 200 mg/gCr
DN-2: persistent proteinuria group, persistent proteinuria proven by test paper
DN-3: chronic renal failure group, serum creatine level ≧ 1.5 mg/dl
DN-4: undergoing hemodialysis group
N: number of patients, DM: diabetes mellitus, DN: diabetic nephropathy

Methods.

Blood and urine samples. Venous blood samples were collected into evacuated tubes containing EDTA. Plasma was obtained by centrifugation at 3,000 × *g* for 15 min. Fresh urine samples were collected in the morning. Plasma and urine samples were frozen at −70℃ until use.

Measurement of concentration of vitronectin. The concentration of vitronectin in plasma and urine was determined using VN test kit (Iwaki Glass Co. Ltd., Funabashi, Japan) (18, 19). Briefly, 96 well ELISA plates were coated with monoclonal antibody against human vitronectin M1 and then blocked with phosphate buffered saline containing 1% bovine serum albumin (PBS-BSA) to prevent non-specific binding. After the plates were rinsed three times with PBS containing 0.05 % Tween 20 (PBS-Tween), a mixture (50 μl) of plasma sample or urine sample and horseradish peroxidase-conjugated Fab' (M4) solution which were mixed at a 1:1 ratio just before being added to each well. The plates were incubated for 1 h at room temperature and rinsed three times with PBS-Tween. The bound enzymatic activity was measured using o-phenylenediamine and H_2O_2 as substrates. The absorbance at 492 nm was measured in a microplate reader.

Other parameters. Proteinuria was checked by the test paper (Multistics SGL, Miles-Sankyo, Tokyo, Japan). Urinary albumin concentration was measured by radioimmunoassay (20) and the urinary albumin: creatinine ratio (urinary albumin index; UAI: mg/gCr) was calculated from the urinary concentration divided by the urinary creatinine concentration.

We measured blood glucose by Glu-DH method (21), glycosylated hemoglobin (HbA1c) by high-performance liquid chromatography (HPLC) (22), serum creatinine level (23), blood platelet count, and serum complement level (CH 50) by the CH 50 method (24), and examined the correlations between the plasma concentrations of vitronectin and these parameters.

Grading of diabetic nephropathy. The degree of diabetic nephropathy was rated as DN-0–DN-4 for each patient: DN-0, normoalbuminuria group, UAI ≦ 15 mg/gCr; DN-1, microalbuminuria group (15 mg/gCr < UAI ≦ 200 mg/gCr); DN-2, persistent proteinuria group (persistent proteinuria proven by test paper); DN-3, chronic renal failure (CRF) group (serum creatinine level ≧ 1.5 mg/dl); and DN-4, hemodialysis group.

Statistical analysis. Statistical analysis was performed by Wilcoxon's test to test for the significance of the difference between the values of the each of the experimental groups and the control group. Spearman's rank correlation test was used to calculate the correlation coefficient.

Results

Plasma vitronectin concentrations of healthy volunteers and patients with various stages of diabetic nephropathy were compared. The concentrations in DN-0 group (mean ± SE; 279.6 ± 17.7 μg/ml, n = 18), and DN-1 group (295.3 ± 12.3 μg/ml, n = 19) were significantly higher than the control values (227.1 ± 12.4 μg/ml, n = 16) ($P < 0.05$, and $P < 0.01$). However, in DN-3 group (195.2 ± 31.6 μg/ml, n = 6), the plasma concentration of vitronectin was significantly lower than those of

DN-0, DN-1, and DN-2 ($302.0 \pm 27.9 \mu g/ml$, $n = 12$) ($P < 0.05$). Moreover, in the DN-4 group plasma concentrations of vitronectin were significantly decreased compared with normal subjects ($154.5 \pm 13.4 \mu g/ml$, $n = 4$; $P < 0.05$) (Fig. 1).

There was a significant negative correlation between the plasma concentrations of vitronectin and serum creatinine levels ($y = -0.06x + 18.1$, $r = -0.53$, $P < 0.002$) (Fig. 2).

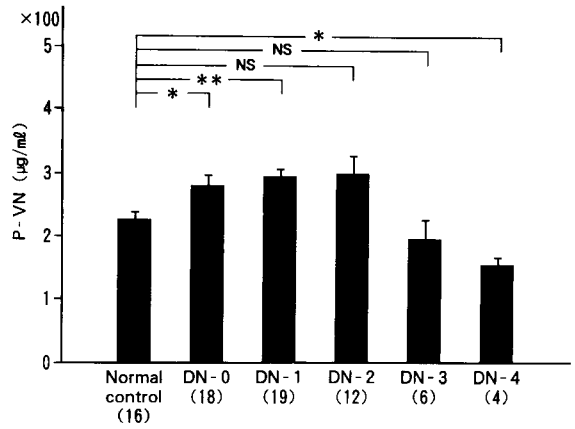

Fig. 1 Plasma concentrations of vitronectin in various stages of diabetic nephropathy.
Mean ± SE, *: $P < 0.05$, **: $P < 0.01$
DN: diabetic nephropathy, ( ): number of patients

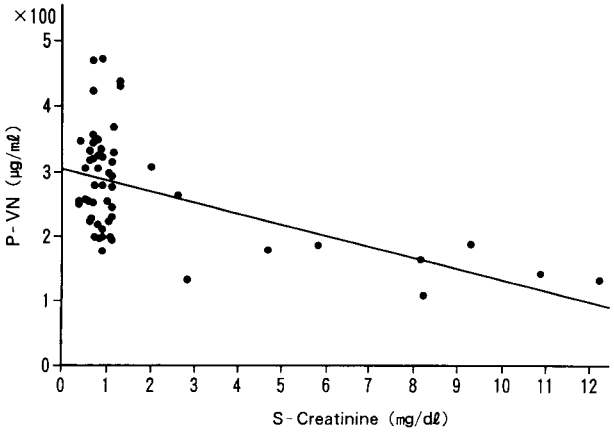

Fig. 2 Correlation between plasma concentrations of vitronectin and serum creatinine levels.
P-VN: plasma vitronectin concentration, S-Creatinine: serum creatinine

These results showed that, in the patients with diabetes mellitus, plasma vitronectin concentration was increased in the early stage and decreased with the progression of diabetic nephropathy.

Hypertension is an important risk factor for the progression of diabetic nephropathy (25, 26). Fig. 3 shows the influence of hypertension on the plasma concentrations of vitronectin in the various stages of diabetic nephropathy. All DN-3 patients also had hypertension. In the hypertensive patients, the plasma concentrations of vitronectin decreased with the progression of diabetic nephropathy. The plasma concentrations of vitronectin in the DN-3 group ($195.2 \pm 31.6 \mu g/ml$, $n = 6$) were significantly lower than those of DN-1 with hypertension ($P < 0.05$) (Fig. 3).

Platelets are one of the source of plasma vitronectin (27). Plasma concentrations of vitronectin showed a significant positive correlation with the blood platelet count ($y = 0.17x - 23.9$, $r = 0.41$, $P < 0.05$) (Fig. 4). Additionally, a significant negative correlation was found between plasma concentrations of vitronectin and durations of diabetes mellitus ($y = -0.15x + 52.7$, $r = -0.61$, $P < 0.002$) (Fig. 5).

In patients with diabetes mellitus, there were no

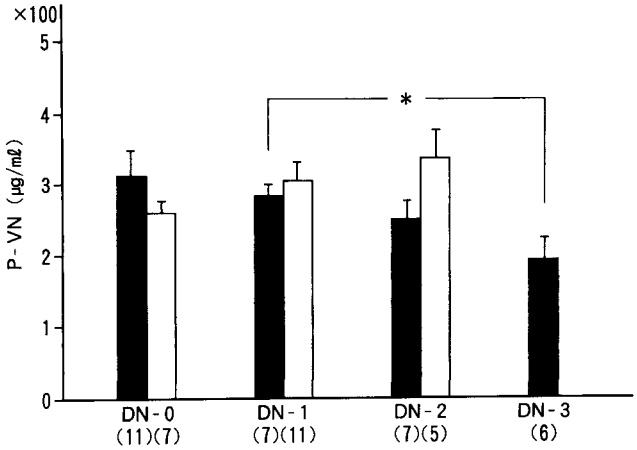

Fig. 3 The influence of hypertension on plasma concentrations of vitronectin in the various stages of diabetic nephropathy.
Mean ± SE, * $P < 0.05$
Open bars: non-hypertension group, closed bars: associated hypertension group, HT: hypertension, P-VN: plasma vitronectin concentration, DN: diabetic nephropathy

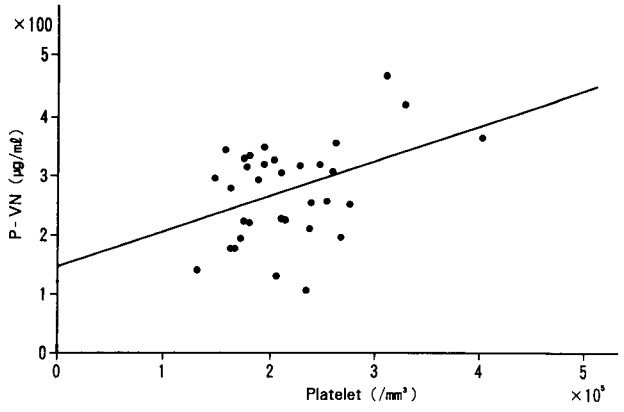

Fig. 4 Correlation between plasma concentrations of vitronectin and blood platelet counts,
P-VN: plasma vitronectin concentration

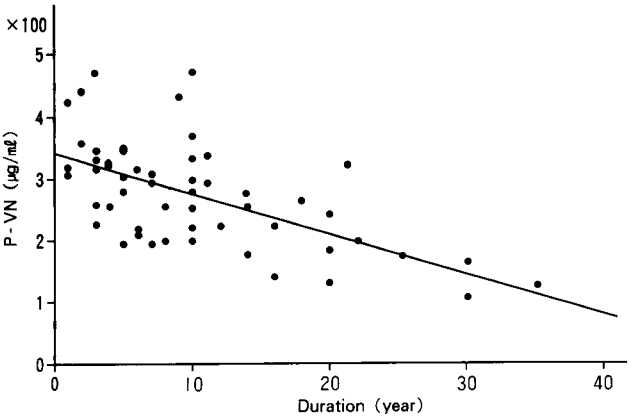

Fig. 5 Correlation between plasma concentrations of vitronectin and durations of diabetes melltitus.
P-VN: plasma vitronectin concentration

significant correlations between HbA 1 c, blood glucose levels, serum complement levels, or urinary albumin index and plasma concentrations of vitronectin.

Discussion

Vitronectin synthesis by hepatocytes accounts for the majority of this protein in plasma (28, 29). In addition, platelets (27), megakaryocytes (30), and monocytes/macrophages (31) have also been reported to produce vitronectin. Plasma concentrations of vitronectin have been reported to decrease in cases of severe liver dysfunction such as liver cirrhosis (28). In the present study, because none of the patients had concomitant liver dysfunction, increased plasma vitronectin was probably mainly produced by the hepatocytes. However, the positive correlation between plasma vitronectin concentrations and blood platelet counts was found in the present study. Platelets were reported to be activated in the patients with diabetes mellitus (32, 33). Thus, a part of increased plasma vitronectin may be released from activated platelets.

Mohri and Ohkubo pointed out that vitronectin inhibited thrombin-mediated platelet aggregation (34), and Preissner suggested that vitronectin might participate in localized regulatory functions of blood coagulation and fibrinolysis in platelet-matrix interactions and the protection of matrix against proteolysis (27). Thus, increased plasma vitronectin concentrations may inhibit the progression to diabetic nephropathy.

Activation of serum complement has been reported in patients with diabetes mellitus (35), but in our study we could prove neither elevated serum complement titers nor a significant correlation with the plasma vitronectin concentration.

The decreased plasma concentrations of vitronectin associated with deterioration in renal function may be due to its consumption in damaged glomeruli and fibrotic tissue around the sclerotic glomeruli. Another possible cause of the decreased plasma concentration of vitronectin is the denaturation of plasma vitronectin by uremic toxins such as blood urea nitrogen (36).

In the patients with hypertension, plasma concentrations of vitronectin decreased with the progression of diabetic nephropathy from an early stage. Hypertension associated with diabetes mellitus is considered to be caused by systemic arteriosclerosis. Recently, the accumulation of vitronectin in atherosclerotic lesions has received much attention (37). In the atherosclerotic lesions, vitronectin acts as an anchoring site for smooth muscle cells to migrate. The sources of vitronectin involved in the atherosclerotic lesions are considered to be derived from plasma, secreted from vessel wall cells, or released from activated platelets (37). Thus, decreased plasma concentrations of vitronectin in the patients with hypertension are considered to be caused by consumption in arteriosclerotic lesions.

From these results, we conclude the following: a) the plasma concentrations of vitronectin increase in early

stages of diabetes mellitus, and decrease with the progression of diabetic nephropathy: b) increased plasma vitronectin might be released from activated platelets; c) arteriosclerosis, renal injury, and uremic toxins may also contribute to the decreased plasma vitronectin concentration; d) vitronectin contributes to the progression of diabetic nephropathy in a process of protection and repair; and e) measurement of plasma concentration of vitronectin is a useful marker of the progression of diabetic nephropathy.

Immunohistochemical study of diabetic nephropathy is required to clarify the change of plasma vitronectin concentration in diabetic nephropathy.

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