Levels of Urinary Matrix Metalloproteinase-9 (MMP-9) and Renal Injuries in Patients With Type 2 Diabetic Nephropathy

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To determine correlations among the levels of urinary MMP-9 and type-IV collagen, hyperglycemia, urinary protein excretion, and renal injuries in patients with type 2 diabetic nephropathy, we measured levels of urinary MMP-9 and protein, blood urea nitrogen (BUN), serum creatinine (s-Cr), fasting plasma glucose (FPG), and glycohemoglobin A1c (HbA1c) in 47 diabetic patients and 14 healthy adults. Urinary type-IV collagen was also measured in 28 diabetic patients and seven healthy adults. Patients with diabetic nephropathy were divided into two groups: 1) patients with normoalbuminuria or microalbuminuria (0–299 mg/g·Cr; n=27), and 2) patients with macroalbuminuria (>300 mg/g·Cr; n=20). The mean level of urinary MMP-9 in group 2 was significantly higher than those in healthy adults ($P<0.05$), and the levels of urinary MMP-9 in patients with

diabetic nephropathy increased in accordance with the clinical stage of the disease. The levels of urinary MMP-9 tended to be correlated with HbA1c in these patients, but the correlation was not statistically significant. The mean level of urinary type-IV collagen in group 2 of patients with diabetic nephropathy was significantly higher than that in group 1 and healthy adults. Levels of urinary type-IV collagen in patients with diabetic nephropathy also increased in accordance with the clinical stage of the disease. The results suggest that measurements of urinary MMP-9, as well as urinary type-IV collagen, may be useful for evaluating the degree of renal injuries in patients with type 2 diabetic nephropathy, especially in the early stage. *J. Clin. Lab. Anal.* 18:206–210, 2004. © 2004 Wiley-Liss, Inc.

Key words: MMP-9; type-IV collagen; urine; diabetic nephropathy

INTRODUCTION

Diabetic nephropathy is one of the most important causes of end-stage renal failure (ESRF); however, the exact underlying mechanisms of the pathogenesis and progression of diabetic nephropathy are not completely understood. The histological changes in diabetic nephropathy, including glomerular hypertrophy, glomerular basement membrane (GBM) thickening, and mesangial expansion, collectively involve the remodeling of the extracellular matrix (ECM). Type-IV collagen is the major collagenous component of the ECM. We previously reported that the level of urinary type-IV collagen in diabetic patients with microalbuminuria is significantly higher than that in patients with normoalbuminuria (1–3). It is speculated that urinary type-IV collagen may increase prior to an increase in urinary albumin excretion in diabetic patients. The major physiologic regulators of glomerular ECM degeneration are matrix metalloproteinases (MMPs), a family of

zinc-dependent matrix-degrading enzymes. MMP-9 is considered to be one of the main enzymes that degrade type-IV collagen, which constitutes the architectural structure of the GBM. In a previous study, we demonstrated increased mRNA expression of MMP-9 and levels of urinary MMP-9 in patients with IgA nephropathy (4). Ebihara et al. (5) reported that plasma elevation of MMP-9 was found in type 2 diabetic patients prior to the onset of microalbuminuria. Diamant et al. (6) reported that in type 1 diabetic patients, increased urinary MMP-2 and MMP-9 activities, but not serum MMP antigen levels, may be

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sensitive markers of the extent of renal disease. Other investigators (7) reported that MMP-2 and MMP-9 activity was decreased in kidney biopsies from diabetic nephropathy patients. These results suggest that matrix accumulation in diabetic nephropathy is due to decreased degradation of type-IV collagen caused by a decrease in these MMPs. The objective of the present study was to determine correlations among the levels of urinary MMP-9 and type-IV collagen, hyperglycemia, urinary protein excretion, and renal injuries in patients with type 2 diabetic nephropathy.

MATERIALS AND METHODS

Patients

Forty-seven patients (29 males and 18 females, 49–79 years old) with type 2 diabetic nephropathy were examined. Fourteen healthy adults were used as controls. On the basis of clinical findings, patients with diabetic nephropathy are classified by the Ministry of Health, Labor and Welfare of Japan (8) into five stages: stage I shows normoalbuminuria. Stage II shows microalbuminuria (30–299 mg/g·Cr). Stage III is divided into two substages: A and B. Stage IIIA shows macroalbuminuria (>300 mg/g·Cr) without renal dysfunction, and stage IIIB shows macroalbuminuria with renal dysfunction (<60 mL/min of GFR). Stage IV is the renal failure stage (<30 mL/min of GFR). Stage V is the dialysis stage. In this study, patients with diabetic nephropathy were divided into two groups: 1) 27 patients with normoalbuminuria or microalbuminuria (stages I and II), and 2) 20 patients with macroalbuminuria (stage IIIA). The mean level of urinary protein in group 2 was 1806.2 ± 401.7 mg/g·Cr (mean $\pm$ SE).

Measurement of Urinary MMP-9 and Type-IV Collagen Concentrations

Urinary MMP-9 levels were measured in 14 healthy adults and 47 patients with type 2 diabetic nephropathy

by means of a one-step sandwich enzyme immunoassay (EIA) system (Fuji Chemical Industries, Ltd., Takaoka, Japan), using two kinds of monoclonal antibodies against human MMP-9, as described previously (9). Urinary type-IV collagen levels were measured in seven healthy adults and 28 patients with type 2 diabetic nephropathy, with the use of a highly sensitive one-step enzyme immunoassay (EIA) (10). Urinary MMP-9 and type-IV collagen levels were expressed as values corrected by the urinary creatinine concentration.

Laboratory Data From Patients, and Statistical Analysis

Levels of urinary protein, blood urea nitrogen (BUN), serum creatinine (s-Cr), and fasting plasma glucose (FPG) were measured by routine laboratory methods in our hospital. Glycohemoglobin A1c (HbA1c) in sera was measured by high-performance liquid chromatography (HPLC). Urinary albumin excretion was measured by a turbidimetric immunoassay. Statistical analysis was performed by analysis of variance (ANOVA). Values were expressed as mean $\pm$ SE. *P*-values <0.05 were regarded as significant.

RESULTS

The laboratory findings from the healthy adults and the patients with type 2 diabetic nephropathy are shown in Table 1. The levels of urinary MMP-9 and type-IV collagen in the patients and healthy adults are shown in Figs. 1 and 2. The mean level of urinary MMP-9 in group 2 of patients with diabetic nephropathy was significantly higher than that in healthy adults ($P < 0.05$) (Fig. 1). The levels of urinary MMP-9 in patients with diabetic nephropathy increased in accordance with the clinical stage of the disease. The mean level of urinary type-IV collagen in group 2 of patients with diabetic nephropathy was significantly higher than that in group 1 and healthy adults ($P < 0.05$ and $P < 0.01$, respectively) (Fig. 2). Levels of urinary type-IV collagen in patients

Table 1 Laboratory findings in patients with type 2 diabetic nephropathy and healthy adults

	Patients	BUN (mg/dl)	s-Cr (mg/dl)	FPG (mg/dl)	HbA1c (%)
Type 2 diabetic nephropathy	47	16.0 ± 0.7	0.88 ± 0.06	135.7 ± 4.3	7.0 ± 0.2
Stage 1 (Normoalbuminuria or microalbuminuria)	27	14.3 ± 0.7	0.70 ± 0.02	130.1 ± 4.8	6.8 ± 0.2
Stage 2 (Microalbuminuria)	20	18.3 ± 1.3	1.11 ± 0.11	143.3 ± 7.4	7.3 ± 0.4
Healthy adults	14	14 ± 6	1.0 ± 0.2	80.0 ± 20.0	5.2 ± 0.6

BUN, blood urea nitrogen.

s-Cr, serum creatinine.

FPG, fasting plasma glucose.

HbA1c, glycohemoglobin A1c.

Mean $\pm$ SE.

Fig. 1. Levels of urinary MMP-9 in healthy adults and patients with diabetic nephropathy. Group 1: Diabetic patients with normoalbuminuria or microalbuminuria. Group 2: Diabetic patients with macroalbuminuria. The bars indicate means, and the error bars are SE.

with diabetic nephropathy also increased in accordance with the clinical stage of the disease. There tended to be a correlation between the levels of urinary MMP-9 and those of urinary type-IV collagen, but it was not statistically significant.

The levels of BUN and s-Cr in both stages did not show any elevation. The levels of urinary MMP-9 tended to be correlated with HbA1c in these patients, but the correlation was not statistically significant (Fig. 3). However, the levels of urinary MMP-9 were not correlated with those of FPG in these patients. The levels of urinary type-IV collagen were also not correlated with HbA1c and FPG levels in these patients (data not shown). The levels of urinary MMP-9 were not correlated with those of urinary protein excretion (Fig. 4) or urinary albumin excretion in these patients. The levels of urinary type-IV collagen were also not correlated with those of urinary protein excretion or urinary albumin excretion in these patients.

DISCUSSION

Diabetic nephropathy is an important factor in morbidity and mortality resulting from diabetes. In

Fig. 2. Levels of urinary type-IV collagen in healthy adults and patients with diabetic nephropathy. Group 1: Diabetic patients with normoalbuminuria or microalbuminuria. Group 2: Diabetic patients with macroalbuminuria. The bars indicate means, and the error bars are SE.

Japan, diabetic nephropathy is a common disease, and there are an increasing number of new cases of ESRF every year (11). Diabetic nephropathy is characterized by a profound shift in the ECM turnover toward increased matrix accumulation, leading to mesangial matrix expansion and a decrease in filtration areas, and ultimately ESRF. Type-IV collagen is the major collagenous component of the ECM. An increase in the intensity of immunohistochemical staining of type-IV collagen was observed in renal tissues from patients with diabetic nephropathy. The increase of urinary type-IV collagen concentration in diabetic patients may be reflected by the increase of its production and/or degradation. We previously reported that urinary type-IV collagen may be increased prior to an increase of urinary albumin in diabetic patients (1–3). The urinary type-IV collagen measured in the current study is considered to be the intact form, with a molecular weight of about 540 kDa. It is speculated that type-IV collagen detected in the urine is released from the renal tissues, but not from blood samples. The major

Fig. 3. Correlation between the levels of urinary MMP-9 and HbA1c in diabetic patients.

Fig. 4. Correlation between the log of urinary protein excretion and the levels of urinary MMP-9 in diabetic patients.

physiologic regulators of ECM degeneration in glomeruli are MMPs. Data from human studies have shown an elevation of MMP levels in serum samples (12,13), and positive staining of MMP in glomeruli (14) in various glomerulonephritides. In a previous study, we demonstrated increased mRNA expression of MMP-9 and levels of urinary MMP-9 in patients with IgA nephropathy (4). These results suggest that glomerular MMP expression and levels of MMP in serum and urine are associated with disease activity and glomerular damage in inflammatory glomerular diseases. In noninflammatory glomerular diseases (including diabetic nephropathy), the glomerular MMP expression in vitro or in vivo, and levels of MMP in serum or urine were as reported previously (5–7). It was suggested that glomerular MMP expression may decrease, and MMP in urine may increase in patients with diabetic nephropathy, and that the matrix accumulation in diabetic nephropathy may

be due to a decreased degradation of type-IV collagen by these decreased MMPs (5–7). In the current study, the levels of urinary MMP-9 and urinary type-IV collagen in patients with diabetic nephropathy increased in accordance with the clinical stage of the disease. It appears that the overproduction and urinary excretion of type-IV collagen, one of the ECM components, and the increase of urinary excretion of MMP-9, a degradation factor, may occur in the early (normoalbuminuric or microalbuminuric) stage of type 2 diabetic nephropathy.

Glucose levels may indirectly influence matrix turnover, since it has been shown that glycated fetal bovine serum decreases matrix turnover by glomerular epithelial cells (15). In the present study, the levels of urinary MMP-9 tended to be correlated with HbA1c in patients with diabetic nephropathy, although the correlation was not statistically significant. High glucose may stimulate MMP-9 production and excretion into the urine independently of the phase or pathological lesions of this disease. On the other hand, the levels of urinary type-IV collagen were not correlated with HbA1c and FPG in these patients.

In conclusion, we indicate that MMP-9 may be involved in the pathogenesis and progression of type 2 diabetic nephropathy. It appears that measurements of urinary MMP-9, as well as urinary type-IV collagen, may be useful for evaluating the degree of renal injuries in patients with type 2 diabetic nephropathy, especially in the early stage.

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