Journal of Clinical Laboratory Analysis 16:1–4 (2002)

Urinary Levels of Monocyte Chemoattractant Protein-1 (MCP-1) and Interleukin-8 (IL-8), and Renal Injuries in Patients With Type 2 Diabetic Nephropathy

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We examined the correlation among the levels of urinary monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8), hyperglycemia, and renal injuries in patients with type 2 diabetic nephropathy. The levels of urinary MCP-1, IL-8, protein excretion, blood urea nitrogen (BUN), serum creatinine (s-Cr), glycohemoglobin A1c (HbA1c), and fasting plasma glucose (FPG) were measured in 24 patients with type 2 diabetic nephropathy and 14 healthy adults as controls. Diabetic nephropathy was classified into three stages: stage 1 = normoalbuminuric, stage 2 = microalbuminuric, and stage 3 = macroalbuminuric. All of the patients showed normal ranges in renal function tests. Levels of urinary MCP-1 in all patients with diabetic nephropathy were significantly higher than those in healthy adults ($P < 0.05$). The levels of urinary MCP-1 in patients with diabetic nephropathy increased gradually according to the clinical stage of this disease. In contrast, the levels of urinary IL-8 in patients with diabetic nephropathy increased in stages 2 and 3. There was a significant correlation between the levels of urinary IL-8 and those of HbA1c. High glucose may stimulate MCP-1 and/or IL-8 production and their excretion into the urine independently of the phases or pathological lesions of this disease. It appears that IL-8 increased in the early stage of diabetic nephropathy, and MCP-1 increased in the advanced stage of this disease. It was concluded that measurement of urinary MCP-1 and IL-8 may be useful for evaluating the degree of renal injuries in patients with type 2 diabetic nephropathy. J. Clin. Lab. Anal. 16:1–4, 2002.

Key words: urinary MCP-1; IL-8; type 2 diabetic nephropathy

INTRODUCTION

Recent findings have shown that monocyte chemoattractant protein-1 (MCP-1), a chemotactic cytokine with a high degree of specificity for monocytes, may play an important role in the progression of glomerular and tubulointerstitial injuries in experimental and human glomerulonephritides, including IgA nephropathy (1–3). A variety of cell types, including glomerular endothelial cells, mesangial cells, tubular epithelial cells, and monocytes, may produce MCP-1 in response to inflammatory signals, such as cytokines (IL-1, TNFα, and INFγ) and immune-complexes (4–6). Interleukin-8 (IL-8), a chemotactic cytokine with a high degree of specificity for neutrophils, is produced by lipopolysaccharide (LPS)-stimulated human peripheral blood monocytes and macrophages, fibroblasts, and endothelial cells, in response to a wide variety of endogenous and/or exogenous stimuli, such as inflammatory cytokines (7,8). Among renal resident cells, both glomerular mesangial and proximal tubular epithelial cells can produce IL-8 by proinflammatory stimuli, such as LPS, IL-1, and TNFα (9,10).

Glomerular infiltration of monocytes/macrophages was observed in diabetic rats and diabetic nephropathy patients (11,12). An increase of MCP-1 expression in the glomerular mesangium was also observed in streptozotocin (STZ)-induced diabetic rats (13). A recent report demonstrated that urinary MCP-1 levels were significantly elevated in patients with diabetic nephrotic syndrome and its advanced tubulointerstitial lesions. Moreover, MCP-1-positive cells were detected in the interstitium of diabetic nephropathy (14). In vitro, high-glucose and glycated albumin has already been reported

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Received 9 April 2001; Accepted 24 August 2001

DOI 10.1002/jcla.2057

to facilitate MCP-1 production from human mesangial cells (15,16). IL-8 may also be enhanced by high glucose in human endothelial cells and by glycated human serum albumin in human retinal pigment epithelial cells (17,18).

The objective of the present study was to determine the correlation among the levels of urinary MCP-1 and IL-8, hyperglycemia, and renal injuries in patients with type 2 diabetic nephropathy.

MATERIALS AND METHODS

Patients

Twenty-four patients with type 2 diabetic nephropathy were examined. Thirteen healthy adults were used as controls. Patients with diabetic nephropathy were classified into three stages according to the Japanese Ministry of Health and Welfare (19) as follows: stage 1 = normoalbuminuric (patients with urinary albumin levels of <29 mg/g · creatinine (Cr) (seven patients)); stage 2 = microalbuminuric (patients with microalbuminuria of 30–299 mg/g · Cr (eight patients)); and stage 3 = macroalbuminuric (patients with macroalbuminuria of >300 mg/g · Cr (nine patients)). None of the stages showed any renal dysfunction.

Measurement of Urinary MCP-1 and IL-8 Concentration

Urinary MCP-1 levels were measured by a human MCP-1 immunoassay (quantitative sandwich enzyme-linked immunosorbent assay (ELISA)) (Quantikine™, R and D Systems, Minneapolis, MN). Urinary IL-8 levels were measured by a human IL-8 ELISA kit (Endogen, Inc., Woburn, MA) using the two-step sandwich method. Urinary MCP-1 and IL-8 levels were expressed as values corrected by the urinary creatinine concentration (milligrams of creatinine/deciliter).

Laboratory Data of Patients and Statistical Analysis

The levels of urinary protein, blood urea nitrogen (BUN), serum creatinine (s-Cr), fasting plasma glucose (FPG), and glycohemoglobin A1c (HbA1c) were measured by routine laboratory methods.

Statistical analysis was performed by analysis of variance (ANOVA). Values were expressed as mean ± SE. P values of <0.05 were regarded as significant.

RESULTS

Levels of urinary MCP-1 and IL-8, and laboratory findings in patients with type 2 diabetic nephropathy, and healthy adults are shown in Table 1. Mean levels of urinary MCP-1 in all patients with diabetic nephropathy were significantly higher than those in healthy adults ($P < 0.05$) (Table 1). The levels of urinary MCP-1 in stage 3 were significantly higher than those in stage 1 or healthy adults ($P < 0.01$). The levels of urinary MCP-1 in patients with diabetic nephropathy increased gradually in accordance with the clinical stage of the disease (Fig. 1). The mean levels of urinary IL-8 in all patients with diabetic nephropathy were also higher than those in healthy adults, but the difference was not statistically significant (Table 1). The levels of urinary IL-8 in patients with diabetic nephropathy increased in stage 2, but the levels in stage 3 were slightly lower than those in stage 2 (Fig. 2).

Levels of urinary MCP-1 tended to be correlated with HbA1c in these patients, but the difference was not statistically significant. The levels of urinary IL-8 were significantly correlated with those of HbA1c in all diabetic nephropathy patients ($r = 0.604$, $P < 0.05$) (Fig. 3). The levels of urinary MCP-1 were correlated with urinary protein excretion in stage 2 and 3 patients with diabetic nephropathy (Fig. 4), but the levels of urinary IL-8 were not correlated with urinary protein excretion (Fig. 5).

DISCUSSION

Levels of urinary MCP-1 were increased in experimental models and patients with inflammatory renal diseases and diabetic nephropathy, while serum MCP-1 levels were normal (2,16). These results suggested local production of MCP-1 in the kidneys. Wada et al. (20–22) reported that urinary MCP-1 levels were significantly increased in patients with progressive inflammatory renal diseases, such as IgA nephropathy (especially in the advanced stage), lupus nephritis, and crescentic glomerulonephritis. They (20–22) also reported that MCP-1 is produced mainly in the interstitial lesions, and may be involved in the pathogenesis of disease progression. Local production of IL-8 as well as MCP-1 in

TABLE 1. Levels of urinary MCP-1 and IL-8, and laboratory findings in patients with type 2 diabetic nephropathy

	Patients	MCP-1/Cr (pg/mg·Cr)	IL-8/Cr (pg/mg·Cr)	BUN (mg/dl)	s-Cr (mg/dl)	FPG (mg/dl)	HbA1c (%)
Type 2 diabetic nephropathy							
Stage 1 (normalbuminuria)	7	221.7 ± 28.9	40.3 ± 15.2	15.3 ± 1.7	0.76 ± 0.08	125.6 ± 7.1	6.6 ± 0.4
Stage 2 (normalbuminuria)	8	281.1 ± 71.2	78.8 ± 40.3	13.5 ± 1.6	0.68 ± 0.04	124.6 ± 10.4	6.6 ± 0.3
Stage 3 (normalbuminuria)	9	476.1 ± 82.2	70.3 ± 37.9	16.3 ± 1.9	0.93 ± 0.02	155.2 ± 14.0	7.7 ± 0.6
Healthy adults	13	203.5 ± 27.1	18.0 ± 2.0	8–20	0.8–1.2	60–100	5.2–0.6

BUN, blood urea nitrogen; s-Cr, serum creatinine; FPG, fasting plasma glucose; HbA1c, glycohemoglobin A1c. Mean ± SE.

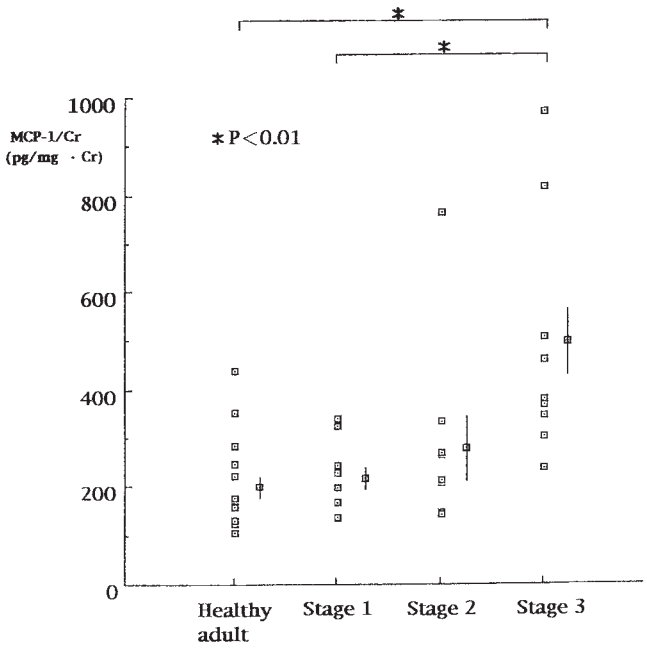

Fig. 1. Levels of urinary MCP-1 in each stage of diabetic nephropathy.

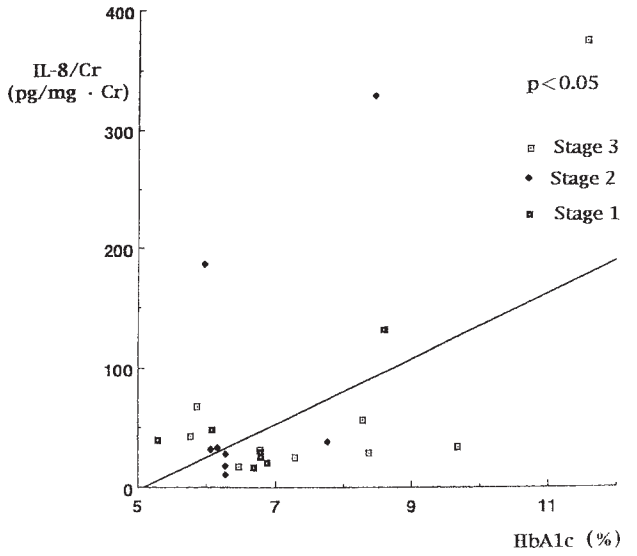

Fig. 3. Correlation between levels of urinary IL-8 and HbA1c in diabetic patients.

human glomerulonephritides has also been suggested (23). However, IL-8 is produced mainly by diseased glomeruli and infiltrating cells, and may be involved in the pathogenesis and acute exacerbation of disease. These findings suggest that the roles of these two chemokines may differ in different disease phases and activities in patients with diabetic nephropathy, as well as other renal diseases. It was recently shown that increased MCP-1 expression in the glomerular mesangium was associated with an increased number of monocytes in streptozotocin (STZ)-induced diabetes (13). Recent reports demonstrated that urinary MCP-1 levels were significantly elevated in patients with diabetic nephrotic syndrome and advanced tubulointerstitial lesions. Moreover, MCP-1-positive cells were detected in the interstitium of diabetic nephropathy patients (14).

In this study, we determined the correlation among levels of urinary MCP-1 and IL-8, hyperglycemia, and renal injuries in patients with type 2 diabetic nephropathy. The levels of urinary MCP-1 and IL-8 in all patients with diabetic nephropathy were significantly higher than those in healthy adults. Moreover, the levels of urinary MCP-1 in patients with diabetic nephropathy increased gradually in accordance with the

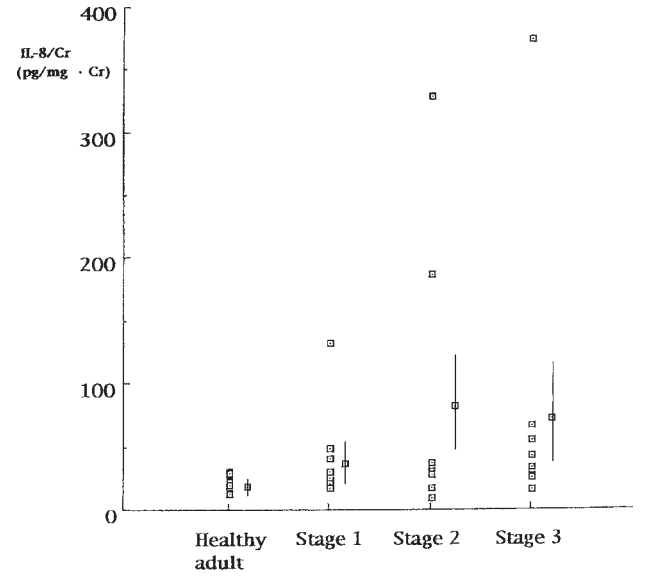

Fig. 2. Levels of urinary IL-8 in each stage of diabetic nephropathy.

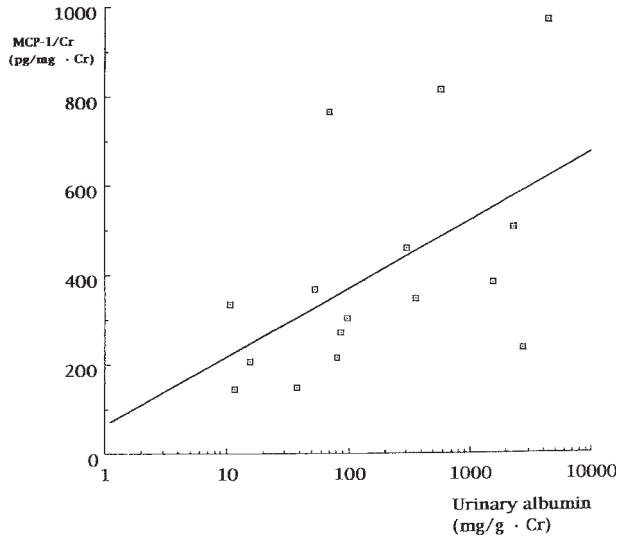

Fig. 4. Correlation between levels of urinary MCP-1 and urinary protein excretion in diabetic patients.

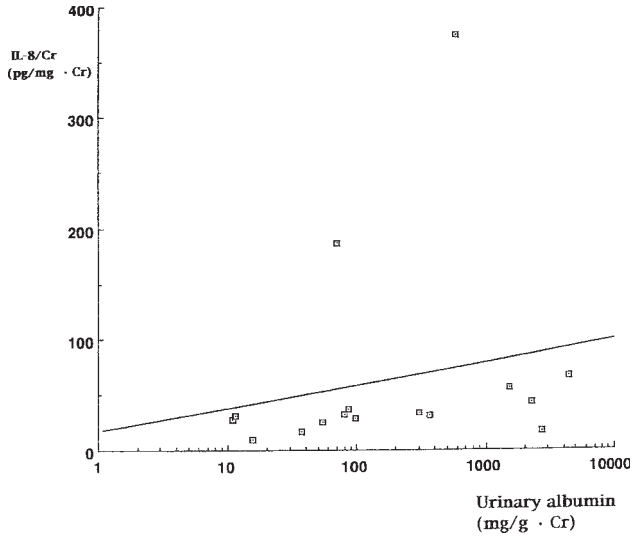

Fig. 5. Correlation between levels of urinary IL-8 and urinary protein excretion in diabetic patients.

clinical stage of the disease. The levels of IL-8 in patients with diabetic nephropathy were increased in stage 2, but not in stage 3. It appears that IL-8 increased in the early stage of diabetic nephropathy and MCP-1 increased in the advanced stage of this disease.

In vitro, high glucose and glycoalbumin have been reported to facilitate MCP-1 production in human mesangial cells (15,16). IL-8 was also enhanced by high glucose in human endothelial cells and by glycated human serum albumin in human retinal pigment epithelial cells (18,19). In vivo, there was a significant correlation between high glucose and serum glycoalbumin, and urinary MCP-1 in diabetic patients (16). The levels of urinary MCP-1 and/or IL-8 were correlated with HbA1c in all diabetic patients in this study. It is postulated that the high levels of glucose, serum glycoalbumin, and/or some cytokines (such as IL-6 (24)) may stimulate MCP-1 and IL-8 production from glomerular mesangial cells and/or tubular epithelial cells.

It was concluded from these results that measurement of urinary MCP-1 and IL-8 may be useful in evaluating the degree of renal injuries in patients with type 2 diabetic nephropathy.

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