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Urinary Levels of Interleukin-8 (IL-8) and Disease Activity in Patients With IgA Nephropathy

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Using quantitative sandwich ELISA, we studied 27 patients with IgA nephropathy to determine whether the levels of urinary IL-8 might reflect the disease activity. The levels of urinary IL-8 in patients with advanced stage IgA nephropathy were significantly higher than those in the patients with the mild stage of this disease, or in the healthy controls. The results showed a positive significant correlation between the levels of IL-8 and disease activity, i.e., between levels of urinary protein and urinary casts. A significant correlation between levels of urinary IL-8 and tubular function damage was also found. It was thus suggested that measurement of urinary IL-8 might be useful in evaluating the degree of renal injuries and/or prognosis in patients with IgA nephropathy. J. Clin. Lab. Anal. 15:30–34, 2001. © 2001 Wiley-Liss, Inc.

Key words: IL-8; ELISA; urine; renal histology; IgA nephropathy

INTRODUCTION

IgA nephropathy is an immune complex-mediated glomerulonephritis that has been recognized as the most common primary glomerulonephritis worldwide and is characterized by mesangial deposition of IgA in renal specimens (1). Since it was first described by Berger (2) in 1968, several investigators have reported the developmental and/or exacerbating factors for patients with IgA nephropathy although the etiology and pathogenesis remain enigmatic (1). It is not clear if only IgA deposits in glomeruli are responsible for the glomerular inflammatory changes characteristic of the advanced stage of IgA nephropathy. The precise mediators that signal lymphocytes and monocytes to migrate and colonize the kidney are not known. It has been postulated that altered T-cell function may play a major role in the pathogenesis of IgA nephropathy. Accumulating evidence indicates that various cytokines and growth factors may be involved in the progression of IgA nephropathy (1,3,4). Our previous studies indicated that neutrophils may contribute to the inflammatory process (5) and showed a significant correlation between the levels of serum interleukin-2 receptor (IL-2R) and the disease activity (6). We also reported that high levels of urinary IL-6 or monocyte chemoattractant protein-1 (MCP-1) reflected the glomerular inflammatory changes in patients with IgA nephropathy (7,8).

Interleukin-8 (IL-8) was originally described as a product secreted from lipopolysaccharide (LPS)-stimulated human peripheral blood monocytes (9). Like most of the superfamily of proinflammatory cytokines, IL-8 is produced by a large number of cell types such as fibroblasts, endothelial cells, monocytes, and macrophages, in response to a wide variety of endogenous and exogenous stimuli with inflammatory cytokines as the most common and potent (10). Among renal cells, both glomerular mesangial cells and proximal tubular epithelial cells can produce IL-8 by proinflammatory stimuli such as LPS, IL-1 and/or INF-α (11,12). IL-8 is a chemoattractant for lymphocytes as well as neutrophils (13), and it may play a pathogenic role in the inflammatory processes in patients with IgA nephropathy (14). Recently, Wada et al. (15) indicated that the urinary excretion of IL-8 reflected its local production, either by cells resident in the glomeruli or by infiltrating cells in various glomerular diseases, and suggested that measurement of IL-8 in the urine may be useful for monitoring glomerular diseases.

The objective of this study was to determine whether the levels of urinary IL-8 reflect renal injures and could serve as a useful indicator in evaluating the prognosis of patients with IgA nephropathy.

MATERIALS AND METHODS

Patients

Twenty-seven patients with IgA nephropathy (Berger's disease) were examined. Patients with IgA nephropathy whose

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biopsy specimens stained predominantly for IgA in the glomerular mesangial areas were included in this study after exclusion of patients with SLE, Henoch-Schönlein purpura (HSP) nephritis, liver cirrhosis, or other systemic diseases. None of the patients was treated with any corticosteroid or immunosuppressive agent, and they did not have any infectious disease at the time of the study. Fifteen age-matched adults were used as healthy controls. The histopathological changes of IgA nephropathy were divided into two stages according to our classification as described previously (16). In brief, the mild stage (grades I and II) was characterized by minimal or slight mesangial thickening with an increase in the homogeneous PAS-positive mesangial matrix. Mild mesangial cell proliferation was observed. The advanced stage (grades III and IV) was characterized by diffuse mesangial thickening, mesangial cell proliferation with or without capsular adhesion, fibrocellular crescents, and/or glomerular sclerosis. Glomerular capillary walls were thickened by extension of the mesangial matrices. More widespread interstitial cell infiltration and fibrosis, and tubular atrophy were also observed.

ELISA for Quantitative Detection of IL-8 Concentration in Urine

Urinary IL-8 was measured with a human interleukin-8 enzyme-linked immunosorbent assay kit (Endogen, Inc. Woburn, MA) by the 2-step sandwich method. In brief, on a 96-well microplate was coated with affinity purified antibody specific for human IL-8, i.e., primary antibody was immobilized on the wells. Then 50 µl of standard or urine sample was pipetted into the wells, and the samples were incubated at room temperature for 1 hour. During this incubation, the IL-8 in the standard or urine was bound to the solid phase. After the wells were washed, 100 µl of biotinylated antibody for IL-8 was added and bound to the captured IL-8 during 1 hour of incubation. Unbound material was removed by aspiration and washing. After washing, 100 µl of prepared streptavidin-HRP solution was added and adhered to the biotin in the immune complex on the plate during 30 minutes of incubation. Following incubation, the wells were washed and 100 µl of TMB substrate solution was added to the wells, producing a blue color in the presence of horseradish peroxidase during 30 minutes of incubation in the dark. The color reaction was stopped by the addition of 100 µl of stop solution (acid) which changed the blue color to yellow. Finally, the absorbance at dual wavelengths, 450 minus 550 nm, was measured by the ELISA plate reader. Duplicate tests were performed in this study. The sensitivity of the kit was less than 2 pg/ml. All urine specimens and reagents were kept at room temperature prior to use. The levels of urinary IL-8 were expressed as values corrected by the urinary creatinine concentration (per milligram of creatinine; pg/mg·Cr).

Parameters of Disease Activity

In order to measure urinary IL-8 and the parameters of disease activity, early morning fasting urine specimens were obtained. Urinalysis including proteinuria, microscopic examination of sediment, specific gravity (S.G.), detection of urinary creatinine concentration, β2-microglobulin (β2MG), and N-acetyl-β-D-glucosaminidase (NAG) were performed. The remaining urine specimens were stored at –80°C for detection of IL-8. Urinary protein levels were expressed as values corrected by the urinary creatinine concentration. Renal tubular functional damage (TFD) was defined as less than 1.017 of S.G., and increased levels of urinary NAG and/or β2MG compared with normal levels. The levels of serum creatinine (sCr) were measured at the same time.

Statistical Analysis

The Mann-Whitney U test and Spearman rank correction analysis were used for statistical analysis of the data. Values were expressed as mean ± SE. P values of < 0.05 were regarded as significant.

RESULTS

Urinary IL-8 levels are shown in Fig. 1, and in Tables 1 and 2. The level of urinary IL-8 in the healthy controls was 5.65 ± 1.38 pg/mg·Cr. The levels of urinary IL-8 in patients with the advanced stage IgA nephropathy was significantly higher than that in the patients with the mild stage of the disease (35.50 ± 9.47 vs. 8.98 ± 2.04 pg/mg·Cr, $P < 0.01$). The level of urinary IL-8 in patients with the advanced stage was also significantly higher than that in healthy controls (35.50 ± 9.47 vs. 5.65 ± 1.38 pg/mg·Cr, $P < 0.001$). Nevertheless, the difference in the levels of urinary IL-8 between the mild stage of IgA nephropathy and healthy controls was not significant ($P > 0.1$, Fig. 1).

Levels of urinary protein in patients with the advanced stage

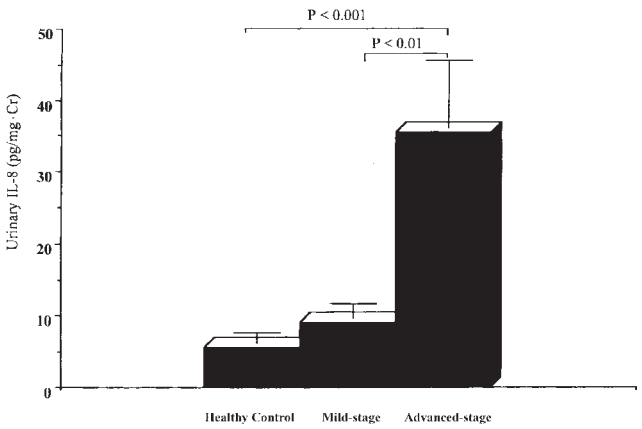

Fig. 1. Levels of urinary IL-8 in patients with IgA nephropathy and healthy controls. Mann-Whitney U test was used for statistical analysis.

TABLE 1. Level of urinary IL-8 and laboratory data in patients with advanced-stage IgA nephropathy[a]

Patient no.	Age/sex	U-IL-8 pg/mg·Cr	Urinalysis				sCr[b] mg/dl	TFD
			Protein mg/g·Cr	RBC/HPF	WBC/HPF	Cast		
1	31/M	12.6	226	5	5	–	0.84	+
2	52/M	15.2	701	10	5	+	1.34	+
3	55/F	21.4	333	Numerous	5	+	0.94	+
4	19/M	25.0	1095	5	5	+	0.70	+
5	53/M	22.2	1036	20	10	+	0.68	–
6	41/M	11.4	2807	Numerous	5	+	1.37	+
7	54/F	15.4	4000	Numerous	10	+	2.08	+
8	33/M	33.1	1219	20	5	+	0.85	+
9	42/M	85.5	3980	5	Numerous	+	2.69	+
10	46/M	5.2	642	5	5	–	1.18	–
11	41/M	120.8	1532	5	5	+	0.92	+
12	29/F	64.5	420	15	10	+	0.58	+
13	43/M	29.2	4039	5	Numerous	+	3.45	+
Mean ± SD		35.5 ± 9.47	1695 ± 409				1.36 ± 0.24	

[a]U-IL-8 is significantly co-related with urinary protein ($P < 0.001$) and leukocyturia ($P < 0.05$).
[b]sCr, serum creatinine; TFD, tubular functional damage.

of IgA nephropathy were significantly higher than those in the mild stage ($P < 0.001$, Table 1). There was a significant correlation between the levels of urinary IL-8 and those of urinary protein in patients in both stages of IgA nephropathy ($r = 0.695$, $P < 0.001$). The grade of microscopic hematuria in both stages IgA nephropathy was not significantly different, and was not correlated with the levels of urinary IL-8 ($P > 0.1$ in both cases). In contrast, the grade of microscopic leukocyturia in the advanced stage of IgA nephropathy was higher than that in the mild stage ($P < 0.05$). There was a significant correlation between the levels of urinary IL-8 and grades of microscopic leukocyturia in patients with both stages of IgA nephropathy ($r = 0.482$, $P < 0.05$). Urinary casts were more frequently observed in patients with the advanced stage of IgA nephropathy than in patients with the mild stage of the disease. The levels of urinary IL-8 in patients in both stages of IgA nephropathy with casts were significantly higher than those in patients without casts (32.65 ± 8.39 vs. 8.13 ± 2.31 pg/mg·Cr, $P < 0.001$, Fig. 2). Furthermore, there was a significant correlation between the levels of urinary IL-8 and the grades of urinary casts in both stages of the disease ($r = 0.504$, $P < 0.05$).

The levels of sCr in the advanced stage were significantly higher than those in the mild stage (1.36 ± 0.24 vs. 0.75 ± 0.04 mg/dl, $P < 0.05$). In addition, tubular functional damage (TFD) was more common in the advanced stage than in the mild stage ($P < 0.05$). The levels of urinary IL-8 in patients in both stages with TFD were significantly higher than those without TFD (30.8 ± 8.0 vs. 8.5 ± 2.8 pg/mg·Cr, $P < 0.01$). There was a significant correlation between the levels of urinary IL-8 and the presence of TFD in both stages ($r = 0.619$, $P < 0.01$) (Tables 1 and 2).

TABLE 2. Level of urinary IL-8 and laboratory data in patients with mild-stage IgA nephropathy[a]

Patient no.	Age/sex	U-IL-8 pg/mg·Cr	Urinalysis				sCr[b] mg/dl	TFD
			Protein mg/g·Cr	RBC/HPF	WBC/HPF	Cast		
1	55/F	11.8	309	5	5	+	0.62	+
2	29/F	2.8	532	25	5	–	0.65	–
3	32/M	3.8	110	15	5	–	0.81	–
4	51/M	7.0	260	15	5	+	1.00	–
5	52/F	5.2	120	numerous	5	–	0.70	–
6	51/M	30.5	490	10	5	–	0.65	–
7	35/F	13.3	446	5	10	–	0.68	+
8	34/M	10.7	210	5	5	+	0.98	+
9	25/F	2.9	100	5	5	–	0.77	–
10	43/M	16.5	537	numerous	5	+	0.92	+
11	34/F	6.9	120	10	5	–	0.76	+
12	22/M	3.3	120	5	5	–	0.80	–
13	51/M	9.1	490	5	5	–	0.65	–
14	21/M	1.9	100	25	5		0.55	–
Mean ± SD		8.98 ± 2.04	282 ± 48				0.75 ± 0.04	

[a]U-IL-8 is significantly co-related with urinary protein ($P < 0.001$) and leukocyturia ($P < 0.05$).
[b]sCr, serum creatinine; TFD, tubular functional damage.

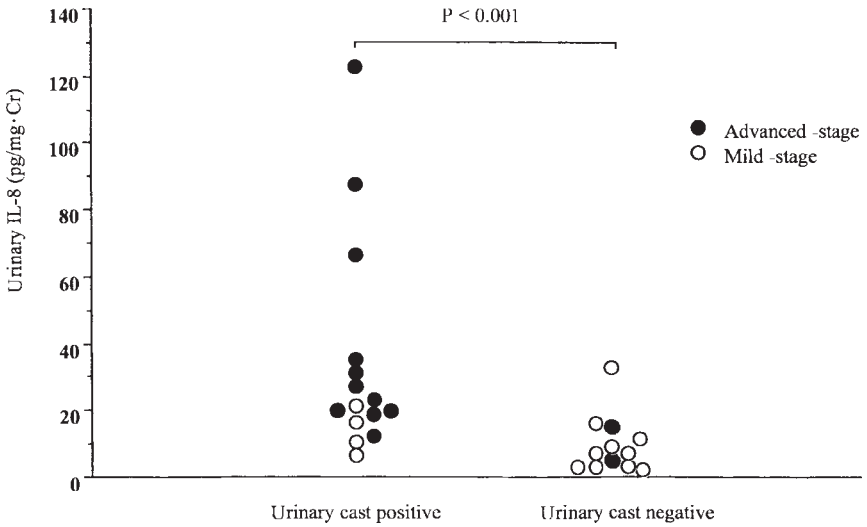

Fig. 2. Levels of urinary IL-8 in patients with IgA nephropathy with or without urinary casts. Mann-Whitney U test was used for statistical analysis.

DISCUSSION

IL-8 is a host defense cytokine secreted as part of the response to noxious or infectious agents (17), and it is detected at inflammatory sites characterized by neutrophil infiltration (18). It may play an important role in the infiltration of neutrophils and T cells, which occurs in patients with IgA nephropathy (14,19,20). We also reported that infiltration of lymphocytes and monocytes was observed in both the glomeruli and interstitium in patients with the advanced stage of IgA nephropathy (21). Recently, Kruger et al. (22) suggested that the production of IL-8 by renal tubular epithelial cells after stimulation with IL-1β may play an important role in the initiation of inflammatory cell influx into the renal parenchyma. Sekikawa et al. (23) examined renal biopsy specimens obtained from patients with IgA nephropathy and lupus nephritis. They found a significant correlation between the expression of IL-8 mRNA and the number of neutrophils in glomeruli. They also showed a negative correlation between the expression of IL-8 mRNA and creatinine clearance (Ccr.). Therefore, they suggested that IL-8 might be involved in the pathophysiology of proliferative glomerulonephritis. Yoshioka et al. (24) reported that cells expressing IL-8 were observed in both the glomeruli and interstitium in renal tissues biopsied from patients with IgA nephropathy. The correlation between IL-8 positive cells infiltrating in the glomeruli and the magnitude of proteinuria was significant. The population of IL-8 positive interstitial cells was associated with both the histological grading of tubulo-interstitial changes and proteinuria.

Herein, we have demonstrated that levels of urinary IL-8 were markedly increased in patients with the advanced stage but not with the mild stage of IgA nephropathy. High levels of urinary IL-8 were observed in patients with IgA nephropathy associated with urinary casts and urinary WBC. There was a significant positive correlation between the levels of urinary IL-8 and those of urinary protein in such patients. There was also a significant correlation between the levels of urinary IL-8 and tubular dysfunction in both stages. Because histopathological changes in the advanced stage of IgA nephropathy are characterized by diffuse mesangial cell proliferation and tubulointerstitial injury, measurement of urinary IL-8 may be useful for evaluating the degree of renal changes in patients with IgA nephropathy. In fact, high levels of urinary IL-8 might reflect glomerular inflammatory changes such as mesangial cell proliferation, glomerular adhesion to Bowman's capsules, crescent formation, glomerular sclerosis, and tubulointerstitial injury in patients with IgA nephropathy. Even more important is the fact that because we usually undertake the urinalysis (i.e., urinary protein, sediment RBC, WBC, and casts) at outpatient visits to evaluate disease activity and therapeutic effects, measurement of urinary IL-8 at that time can serve as an additional predictor and indicator for more accurate clinical decisions. It may also be worth measuring urinary IL-8 when one can not proceed renal biopsy or can not obtain enough material to accurate evaluation by biopsy. But repeated measurement of urinary IL-8 and its comparison with other traditional predictors is needed to evaluate if IL-8 would be a more valuable parameter for following patients. In summary, it appears that the measurement of urinary IL-8 is useful in evaluating the degree of renal injuries and/or prognosis in patients with IgA nephropathy.

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