Journal of Clinical Laboratory Analysis 12:1–5 (1998)

Urinary Levels of Monocyte Chemoattractant Protein (MCP)-1 and Disease Activity in Patients With IgA Nephropathy

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Using a quantitative sandwich ELISA, we studied 17 patients with IgA nephropathy to determine if levels of urinary monocyte chemoattractant protein-1 (MCP-1) might reflect the disease activity. The levels of urinary MCP-1 in patients with the advanced stage were significantly higher than those in patients with the mild stage of the disease, or in healthy controls. The results showed a significant correlation between the levels of urinary MCP-1 and the disease activity, i.e., levels of urinary casts and urinary protein. It was thus suggested that the measurement of urinary MCP-1 is useful in evaluating the degree of renal injuries and/or prognosis in patients with IgA nephropathy. J. Clin. Lab. Anal. 12:1–5,1998.

Key words: MCP-1; ELISA; urine; renal histology; IgA nephropathy

INTRODUCTION: MCP-1 AND RENAL INJURIES

IgA nephropathy is well recognized worldwide as one of the most common primary glomerulonephritides and is characterized by mesangial deposition of IgA in renal specimens (1). Since the original description by Berger (1) in 1968, several investigators have indicated the developmental and/or exacerbating factors for patients with IgA nephropathy (2). 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, and the precise mediators signaling lymphocytes and monocytes to migrate and colonize the kidney are not known, but it is clear that immunoglobulin deposition and/or complement activation have a determinant role (3). It has been postulated that altered T-cell function may play a major role in the pathogenesis of IgA nephropathy. The authors showed a significant correlation between the levels of serum interleukin (IL)-2 receptor and disease activities, i.e., levels of urinary protein, blood urea nitrogen (BUN), and uric acid (4). We also reported that the high levels of urinary IL-6 reflected the glomerular inflammatory changes in patients with IgA nephropathy (5).

Recent findings that monocyte chemoattractant protein-1 (MCP-1), a chemotactic cytokine with a high degree of specificity for lymphocytes and monocytes, is overexpressed in glomeruli from rats with immune-complex glomerulonephritis prompted us to explore the possibility that MCP-1 might be implicated in the renal inflammatory response (6). A variety of cell types, including glomerular endothelial cells, mesangial cells, and monocytes, produce MCP-1 in response to inflammatory signals, including cytokines (IL-1, TNFα, and INFγ) and immune-complexes (7–9). MCP-1 is chemotactic for monocytes and T lymphocytes (7,10,11). Recently, Noris et al. (12) reported that urinary MCP-1 in patients with active lupus nephritis was significantly higher than in lupus patients studied in the inactive phase of the disease or in healthy volunteers.

The purpose of this study was to determine if high levels of urinary MCP-1 might reflect the renal injuries in patients with IgA nephropathy.

MATERIALS AND METHODS

Patients

Seventeen patients with primary IgA nephropathy (Berger's disease) were examined. Patients with IgA nephropathy whose biopsy specimens stained predominantly for IgA in the glomerular mesangial areas were included in this study after exclusion of patients with SLE, Henoch-Schoenlein purpura (HSP) nephritis, liver cirrhosis, or other systemic disease. Thirty-three age-matched healthy adults were used as controls. The first urinary samples in the early morning were collected from these patients and healthy controls.

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Received 16 April 1997; Accepted 1 May 1997

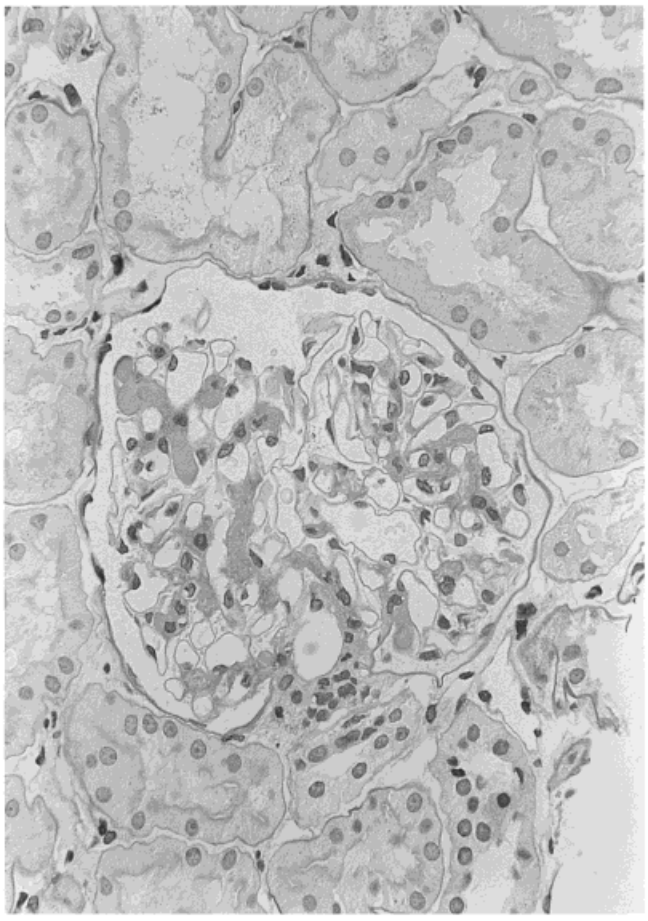
The histopathological changes of IgA nephropathy were divided into two stages according to our classification as described previously (13). In brief, the mild stage (grades I and II) was characterized by minimal or slight mesangial thickening with an increase in the homogeneous PAS-positive mesangial 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 matrix. More widespread interstitial cell infiltration and fibrosis, and tubular atrophy were also observed (Fig. 1a,b) (13).

ELISA for Quantitative Detection of MCP-1 Concentration in Urine

Urinary MCP-1 levels were measured by human MCP-1 immunoassay (quantitative sandwich ELISA) (Quantikine™, R&D Systems, Minneapolis, MN) (14). Procedures of the assay are summarized in Figure 2. In brief, a murine monoclonal antibody specific for MCP-1 (part 890223) was precoated onto a microtiter plate, 200 μl of MCP-1 standard (part 890225) or urinary sample was pipetted into the wells, and any MCP-1 present was bound by the immobilized antibody. The samples were incubated at room temperature for 2 hr. After washing away any unbound substances, 200 μl of polyclonal antibody against MCP-1 conjugated to horseradish peroxidase (part 890224) was added to the wells that were then incubated at room temperature for 1 hr. After aspirating each well and washing with wash buffer 3 times, 200 μl of substrate solution was added and incubated at room temperature for 20 min. The enzyme reaction was stopped by the addition of 50 μl of 2N sulfuric acid. Finally, the absorbance at 450 nm was measured by the ELISA plate reader. Duplicate tests were performed in this study. Optimum dilution of antibodies and incubation periods were determined by preliminary experiments. Urinary MCP-1 levels were expressed as values corrected by the urinary creatinine concentration (milligrams of creatinine).

Parameters of Disease Activity

The following clinical tests were performed at the time of measurement of urinary MCP-1: levels of proteinuria (mg/dl), hematuria (/HPF), BUN, and serum creatinine (s-Cr). Urinary protein levels were expressed as values corrected by the urinary creatinine concentration.



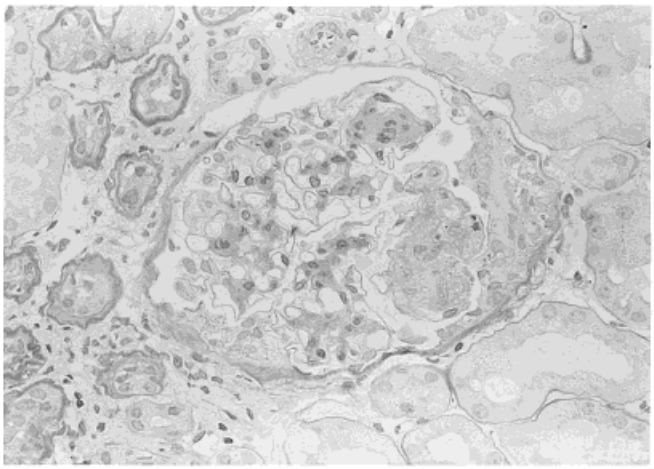


Fig. 1. **(a)** Histopathological findings of a glomerulus in a patient with mild stage IgA nephropathy. PAS. ×400. **(b)** Histopathological findings of a glomerulus in a patient with advanced stage IgA nephropathy. PAS ×400.

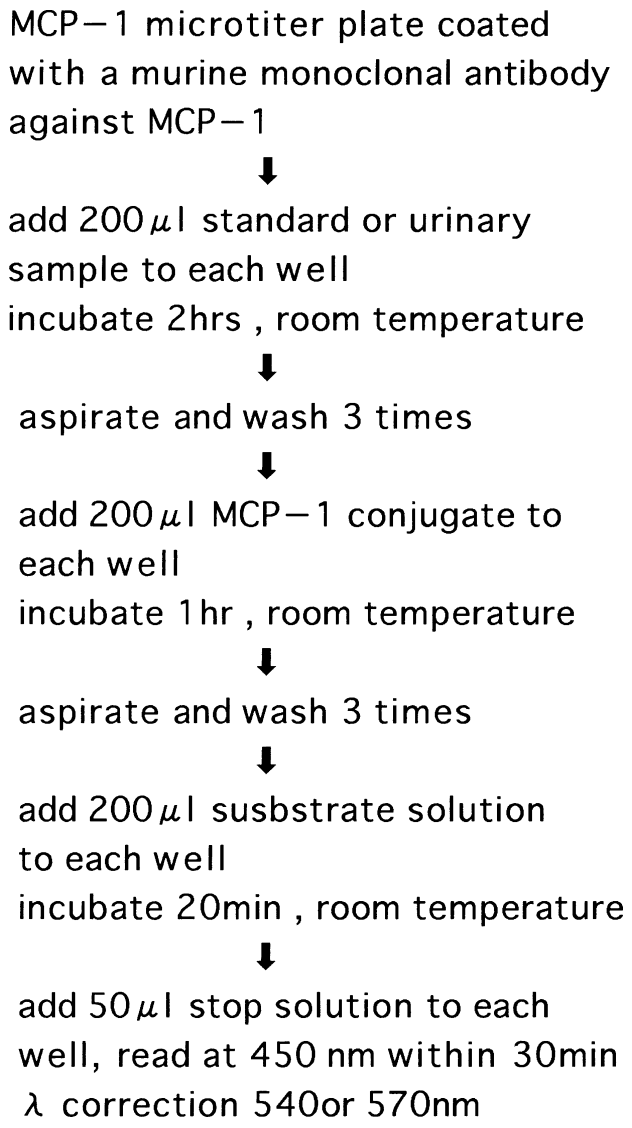


Fig. 2. Procedure of the assay for measurement of urinary MCP-1.

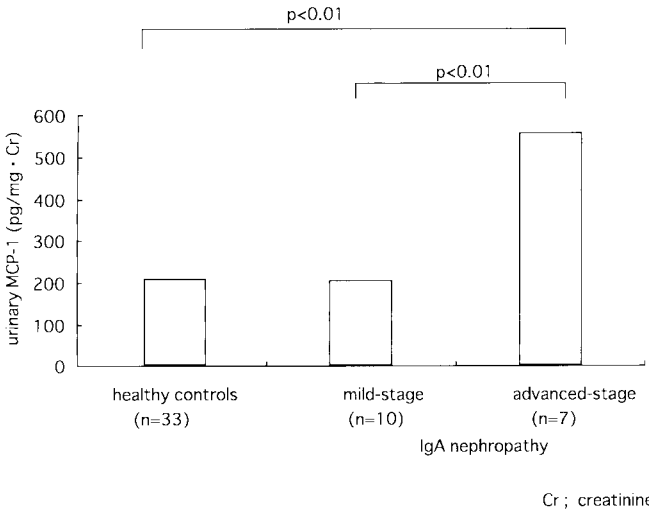


Fig. 3. Levels of urinary MCP-1 in patients with IgA nephropathy and healthy controls. Mann-Whitney U test was used for statistical analysis.

Statistical Analysis

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

RESULTS

Results of measurement of urinary MCP-1 are shown in Figure 3 and Tables 1 and 2. Levels of urinary MCP-1 in healthy controls were < 200 pg/mg · Cr. The levels of urinary MCP-1 in patients with advanced stage IgA nephropathy were significantly higher than those in patients with the mild stage of this disease or healthy controls ($P < 0.01$, respectively). However, there was no significant change in the levels of urinary MCP-1 between the mild stage and the healthy controls.

The levels of urinary protein in patients with advanced stage IgA nephropathy were significantly higher than those in the mild stage ($P < 0.05$). There was a significant correlation between the levels of urinary MCP-1 and those of urinary protein in patients in both stages of IgA nephropathy ($P < 0.01$). The grade of microscopic hematuria in the advanced stage IgA nephropathy was higher than that in the mild stage. Uri-

TABLE 1. Levels of Urinary MCP-1 and Laboratory Data in Patients With Advanced Stage IgA Nephropathy

Patient no.	Age/sex	Urinary MCP-1 pg/mg·Cr	Urinalysis			BUN[a]	s-Cr[b]
			protein mg/g·Cr	RBC/HPF	cast	mg/dl	mg/dl
1	29/M	524.9	3,563.0	5–10	+	12	1.31
2	51/M	399.1	629.1	5–10	+	18	0.94
3	27/F	844.1	1,606.1	numerous	+	12	0.63
4	30/F	628.8	1,062.6	20–25	+	18	0.71
5	40/M	362.6	1,205.0	1–5	+	24	1.73
6	37/M	463.5	898.4	5–10	+	15	0.99
7	52/F	695.9	2,290.7	numerous	+	14	1.19
Mean±SE		559.8 ± 65.3	1,607.8 ± 384.5			16.1 ± 1.6	1.07 ± 0.14

[a]Blood urea nitrogen.
[b]Serum creatinine.

TABLE 2. Levels of Urinary MCP-1 and Laboratory Data in Patients With Mild Stage IgA Nephropathy

Patient no.	Age/sex	Urinary MCP-1 pg/mg·Cr	Urinalysis			BUN[a]	s-Cr[b]
			protein mg/g·Cr	RBC/HPF	cast	mg/dl	mg/dl
1	21/M	183.3	997.1	numerous	+	6	0.68
2	31/M	41.9	236.8	numerous	+	12	0.75
3	29/M	338.3	816.5	5–10	+	13	0.80
4	53/F	128.6	409.0	1–5	–	22	0.89
5	32/F	201.7	344.8	numerous	+	12	0.75
6	32/M	185.1	260.8	1–5	–	11	0.76
7	60/F	174.4	264.2	1–5	–	16	0.56
8	49/M	133.6	259.9	1–5	–	13	0.97
9	31/F	348.9	1,097.0	1–5	–	10	0.69
10	32/M	333.3	1,826.1	1–5	+	19	0.82
Mean±SE		206.9 ± 32.4	651.2 ± 166.9			13.4 ± 1.5	0.77 ± 0.04

[a]Blood urea nitrogen.
[b]Serum creatinine.

nary casts were observed in all patients with the advanced stage of this disease, although that was observed in five out of 10 patients with the mild stage. The levels of urinary MCP-1 in both stages of IgA nephropathy with urinary casts were significantly higher than in those without urinary casts ($P < 0.05$, Fig. 4). The levels of s-Cr and BUN in the advanced stage was slightly higher than those in the mild stage. However, there were no statistically significant differences in the levels of s-Cr or BUN between the stages of IgA nephropathy (Tables 1 and 2).

DISCUSSION

A group of inflammatory cytokines (chemokines) with potent chemoattracting properties for leukocytes may play a relevant role in the accumulation of inflammatory cells in the kidneys (7). MCP-1 possesses monocyte-specific chemoattractant properties and activates monocytes by up-regulating cell surface expression of adhesion molecules (15). We reported that the expression of intercellular adhesion molecule (ICAM)-1 in the glomerular capillary walls is closely linked to glomerular cell proliferation, and infiltration of lymphocytes and monocytes in patients with advanced stage IgA nephropathy (16). Interstitial infiltration of lymphocytes and monocytes was also observed in advanced stage IgA nephropathy (16). In experimental and human glomerulonephritis, glomerular MCP-1 was found to be predominant in a focal, granular, mesangial distribution; however, no glomerular MCP-1 immunoreactivity was detected in patients with renal diseases lacking significant inflammatory components (17). Prodjosudjadi et al. (18) reported an association between the intensity of MCP-1 staining in tubular epithelial cells and interstitial infiltration of macrophages in patients with membranous nephropathy and IgA nephropathy. Grandaliano et al. (19) suggested that production of MCP-1 in the tubulointerstitial compartment may play a key role in modulating monocytes influx and, consequently, tubulointerstitial damage.

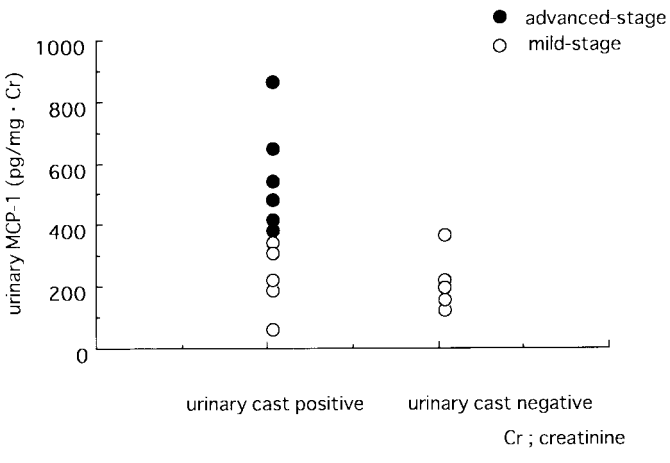


Fig. 4. Levels of urinary MCP-1 in patients with IgA nephropathy with or without urinary casts. Mann-Whitney U test was used for statistical analysis.

In the present study, we found that levels of urinary MCP-1 were marked in patients with the advanced stage but not the mild stage IgA nephropathy. High levels of urinary MCP-1 were observed in the patients with IgA nephropathy associated with urinary casts. There was a significant correlation between the levels of urinary MCP-1 and the amounts of urinary protein in the patients. Since the histopathological changes in the advanced stage of this disease were characterized by diffuse mesangial cell proliferation and tubulointerstitial injury, the measurement of urinary MCP-1 may be of value in evaluating the degree of renal lesions in patients with IgA nephropathy. In fact, the high levels of urinary MCP-1 reflected the glomerular inflammatory changes, such as mesangial cell proliferation, glomerular adhesion to Bowman's capsules, crescent and glomerular sclerosis, and tubulointerstitial injury in patients with IgA nephropathy. All these findings taken collectively show that the measurement of urinary MCP-1 is useful in evaluating the degree of renal injuries and/or prognosis in patients with IgA nephropathy.

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