Urinary kidney injury molecule-1 in patients with IgA nephropathy is closely associated with disease severity

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Abstract

Background. The pathological characteristics of IgA nephropathy (IgAN) are highly variable. Urinary kidney injury molecule-1 (KIM-1) is a sensitive biomarker for proximal tubule injury. The aim of the study is to investigate the value of KIM-1 as a biomarker for assessing the renal injury in IgAN.

Methods. The levels of urinary KIM-1 in 202 patients with IgAN, 46 patients with other renal diseases as disease controls and 60 healthy blood donors as normal controls were measured. Correlations with clinical and histopathological features of patients with IgAN were evaluated.

Results. The levels of urinary KIM-1 were significantly higher in patients with IgAN than in normal controls (P < 0.001) and in patients with non-IgAN (P = 0.011). Urinary levels of KIM-1 in IgAN positively correlated with levels of serum creatinine and proteinuria and negatively with creatinine clearance. The more severe the tubulointerstitial injury was, the higher the levels of urinary KIM-1. Patients with severe mesangial proliferation, crescents formation or endocapillary proliferation had higher levels of urinary KIM-1 than those without. The levels of tubular KIM-1 expression in immunohistochemistry closely correlated with the levels of urinary KIM-1 (r = 0.553, P = 0.032). Renal survival was significantly worse in patients with elevated urinary KIM-1 (P = 0.020).

Conclusion. Urinary KIM-1 may be a useful biomarker to evaluate kidney injury in IgAN.

Keywords: IgA nephropathy; KIM-1; tubulointerstitial lesions

Introduction

IgA nephropathy (IgAN) is the most common glomerulonephritis in the world, constituting 20–45% in primary glomerular diseases [1–3]. Over one-third of patients would progress to end-stage renal disease (ESRD) in 20–25 years [4]. The pathological characteristics of IgAN are highly variable. Certain clinical and pathological
parameters including hypertension, massive proteinuria, renal insufficiency, glomerular sclerosis and interstitial fibrosis have been identified as predictors of poor renal outcome in IgAN [5, 6]. It has been suggested that tubulointerstitial injury is the final common pathway to ESRD. It has even been suggested that in patients with IgAN, tubulointerstitial lesions correlated more closely with progression of renal functions than glomerular lesions did [7, 8].

Kidney injury molecule-1 (KIM-1) is a recently discovered protein expressed on dedifferentiated renal proximal tubule epithelial cells undergoing regeneration after toxic or ischemic injury. It is a Type I transmembrane glycoprotein, with an ectodomain containing an Ig-like domain and a mucin domain [9]. The KIM-1 ectodomain can be cleaved and then detected in urine. Urinary KIM-1 is proved to be a sensitive urinary biomarker for proximal tubule injury [10–12]. It was reported that urinary KIM-1 was upregulated not only in patients with acute ischemia, toxic renal injury, polycystic kidney disease and renal cell carcinoma [13–15] but also in patients with chronic renal disease [11, 16]. In addition, KIM-1 is associated with renal interstitial fibrosis and inflammation in certain kinds of renal diseases [14, 16, 17].

However, whether urinary KIM-1 could be used as a noninvasive biomarker for early assessment of tubulointerstitial injury in IgAN is still not clear. In the current study, we first detected the levels of urinary KIM-1 in patients with IgAN with different extents of glomerular and tubulointerstitial lesions and then we further analyzed the relationship between the levels of urinary KIM-1 and clinicopathological parameters.

**Materials and methods**

**Patients and controls**

Two hundred and two patients with renal-biopsy-proven IgAN, from Renal Division, Peking University First Hospital between September 2000 and October 2006, were recruited. Clinical and laboratory data of the patients were collected at the time of renal biopsy. Indications for renal biopsy were described previously [18]. Sixty gender- and age-matched healthy blood donors with normal urinalysis were used as normal controls.

The following disease control groups were included: idiopathic membranous nephropathy (IMN; n = 17), lupus nephritis (LN; n = 16) and antineutrophil cytoplasmic autoantibody (ANCA)-associated glomerulonephritis (ANCA-GN; n = 13, all of whom were serum peri-nuclear ANCA and myeloperoxidase-ANCA positive). The study was in compliance with the declaration of Helsinki and approved by the ethics committee of our hospital. Informed consent was obtained from each participant.

**Evaluation of renal histopathology**

For histology, sections were stained for hematoxylin and eosin, periodic acid Schiff and/or periodic acid-silver methenamine. Two pathologists who were blinded to patients’ data examined the slides separately. Differences in scoring between the two pathologists were resolved by re-reviewing the biopsies to achieve consensus. The minimum number of glomeruli in a renal biopsy in order for inclusion into the study was 10. For glomerular lesions, mesangial proliferation was graded as 1 for mild (four to six cells per mesangial area) and 2 for moderate to intense (more than six cells per mesangial area). Global and segmental sclerosis, total crescents, cellular crescents and fibrous crescents were calculated as percentages of the total number of glomeruli, respectively. Tubulointerstitial lesions, tubular atrophy, interstitial fibrosis and interstitial infiltration were graded 0 for absent, 1 for mild (involving <25% of the interstitium and tubules), 2 for moderate (involving 25–50% of the interstitium and tubules) and 3 for intense (involving >50% of the interstitium and tubules).

The severity of renal tubular lesions was graded according to the grade of tubular atrophy and interstitial fibrosis. Among the 202 patients, 32 were graded as 0, 98 were graded as 1, 35 patients were graded as 2, and 37 patients were graded as 3. The severity of renal interstitial inflammatory cell infiltration was graded similarly as 0, 1, 2 and 3.

**Urinary samples**

Spot morning urine samples were collected on the day of renal biopsy. Samples were centrifuged at 2000 r.p.m. for 10 min to remove cellular components, and the supernatant was frozen in aliquots at −70°C until use. Spot morning urine samples from normal individuals were collected as controls.

**Detection of urinary KIM-1 by sandwich enzyme-linked immunosorbent assay**

Goat anti-human KIM-1 (R&D System, Inc.) at a concentration of 1.6 μg/mL in 0.05 M bicarbonate buffer (pH 9.6) was coated to the wells of one-half of a polystyrene microtiter plate (Costar, Mankato, MN). The wells were also coated with bicarbonate buffer alone as antigen-free wells to exclude nonspecific binding. The protein was dissolved in a total volume of 100 μL for coating and in each well for this step and for subsequent steps, the same volume was used during the assay. All incubations were carried out at 37°C for 1 h and the plates were washed three times with 0.01 M phosphate-buffered saline (PBS) containing 0.1% Tween-20 (PBST). The plates were then blocked with PBST containing 1% bovine serum albumin (PBST/BSA). The undiluted urine samples of patients or controls were added in duplicate to both antigen-coated and antigen-free wells. After incubation and washing, biotinylated goat anti-human KIM-1 (R&D System, Inc.) diluted to 0.4 μg/mL in PBST/BSA was added. Then, the wells were incubated with 1:20 000 diluted avidin-horseradish peroxidase (Sigma). The reaction was developed using 0.04% 3,3′-diaminobenzidine and 0.1% H2O2, prepared in 0.1 M citrate phosphate buffer (pH 5.0). At appropriate color, the reaction was stopped with 100 μL of 1 M H2SO4. The absorbance at 490 nm was recorded using an ELISA reader (Bio-Rad 550; Tokyo, Japan). Recombinant human KIM-1 (R&D System, Inc.) was used to establish the standard curve. The level of KIM-1 of each sample was calculated using linear equation according to the standard curve.

**Development of standard curve for KIM-1**

Serial concentrations of recombinant human KIM-1 from 0.19–50 ng/mL were used to develop the standard curve. The assay was repeated multiple times to determine the inter-assay variance. A maximum of 10% variance was observed in multiple repeats. The linear portion of the curve was subsequently used for the measurement of KIM-1. Intra-assay and interassay variations were also measured to validate the assay.

**Detection of urinary creatinine in patients with IgAN and controls**

To adjust urinary KIM-1 levels, urinary creatinine was measured (by Beckman Coulter, SYNCHRON LX20, clinical system) in the same urine specimens of patients and controls. The adjusted urinary KIM-1 was expressed as concentration of KIM-1/concentration of creatinine (ng/mg Cr).

**Immunohistochemistry of patients with IgAN**

The method was adapted from the procedure described by van Tinteren et al. [16] with some minor modifications. Renal biopsy specimens from 15 patients with IgAN were randomly selected. Unaffected parts of kidneys from patients with renal cell carcinoma (n = 5) were used as normal controls. Tissue was fixed in 4% paraformaldehyde and embedded in paraffin. Deparaffinized sections (4 μm) were subjected to heat-induced antigen retrieval by overnight incubation in 0.1 M Tris/1% buffer (pH 9.0) at 80°C. Endogenous peroxidase was blocked with 0.075% H2O2 in PBS for 30 min. Monoclonal Mouse anti-human KIM-1 (R&D System, Inc.), diluted at 25 μg/mL in 1% BSA/PBS, were incubated for 60 min at room temperature. Binding was detected with appropriate peroxidase (PO)-labeled secondary antibodies (DakoCytomation, Glostrup, Denmark), diluted in PBS with 1% BSA and 1%
normal human serum. Peroxidase activity was developed using 3,3′-diaminobenzidine tetrachloride for 3 min. KIM-1 staining was scored semi-quantitatively by estimating the percentage of cortical tubules expressing KIM-1 per field (the complete biopsy area was scored, with a minimum of five fields in controls, 30 fields were scored); 0 accounts for no staining, 1/2 for 0–12.5%, 1 for 12.5–25%, 2 for 25–50%, 3 for 50–75% and 4 for 75–100%.

**Statistical analysis**

The quantitative data were expressed as mean ± SD or median range as appropriate. Differences of quantitative parameters between groups were assessed using the one-way analysis of variance or Kruskal–Wallis test. Differences of qualitative data were compared using the chi-square test. The Spearman coefficient correlation was used to analyze correlation among various parameters. Kaplan–Meier curves were used to analyze the renal survival with the use of a log-rank test. A P-value of < 0.05 was considered to be statistically significant. Analysis was performed with SPSS statistical software package (version 11.0; Chicago, IL).

**Results**

**Clinical and histopathological characteristics of patients with IgAN**

The clinical parameters of patients with IgAN with various glomerular and tubulointerstitial lesions were shown in Table 1. Compared with patients with tubular atrophy and interstitial fibrosis Grade 0, the level of mean arterial pressure, serum creatinine (Scr) and proteinuria were significantly higher in patients with Grades 2 and 3; the level of creatinine clearance rate (Ccr) was significantly lower in patients with Grades 1, 2 and 3.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1, N = 32</th>
<th>Group 2, N = 98</th>
<th>Group 3, N = 35</th>
<th>Group 4, N = 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>19/13</td>
<td>50/48</td>
<td>21/14</td>
<td>25/12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.00 ± 8.94</td>
<td>33.41 ± 10.64*</td>
<td>37.1 ± 9.31**</td>
<td>32.08 ± 10.84</td>
</tr>
<tr>
<td>Duration (months)</td>
<td>3 (0–120)</td>
<td>5.5 (0.25–396)</td>
<td>12 (0.25–108)</td>
<td>4.5 (0.3–48)</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>87.86 ± 21.73</td>
<td>92.53 ± 15.91</td>
<td>103.96 ± 14.25**</td>
<td>103.11 ± 24.21**</td>
</tr>
<tr>
<td>Scr (μmol/L)</td>
<td>81.08 ± 16.84</td>
<td>88.15 ± 31.16</td>
<td>136.71 ± 85.84*</td>
<td>306.39 ± 209.96**</td>
</tr>
<tr>
<td>Ccr (mL/min)</td>
<td>106.39 ± 31.75</td>
<td>93.62 ± 25.83*</td>
<td>68.82 ± 21.46**</td>
<td>31.26 ± 18.79**</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>1.61 ± 2.14</td>
<td>1.28 ± 1.17</td>
<td>2.00 ± 1.52*</td>
<td>3.55 ± 2.05**</td>
</tr>
</tbody>
</table>

*aGroups 1, 2, 3 and 4 represented patients with tubular atrophy and interstitial fibrosis graded as 0, 1, 2 and 3, respectively. MAP, mean arterial pressure. Compared with Group 1: *P < 0.05; **P < 0.001.

**Table 2.** Histopathological characteristics of patients with IgAN with various tubulointerstitial lesions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IgAN group (n = 202)</th>
<th>Disease control group (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesangial proliferation (Grade 1/Grade 2)</td>
<td>30/2</td>
<td>16/1</td>
</tr>
<tr>
<td>Patients with endocapillary proliferation</td>
<td>8/32</td>
<td>9/37</td>
</tr>
<tr>
<td>Proportion of segmental sclerosis</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Proportion of global sclerosis</td>
<td>0 (0–16.7)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Proportion of total crescents</td>
<td>0 (0–10.7)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Proportion of cellular crescents</td>
<td>0 (0–7.14)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Proportion of fibrous crescents</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Intestinal infiltration (Grade 0/1/2/3)</td>
<td>27/5/0/0</td>
<td>4/11/2/0</td>
</tr>
</tbody>
</table>

*aGroups 1, 2, 3 and 4 represented patients with tubular atrophy and interstitial fibrosis Grades 0, 1, 2 and 3, respectively. Global sclerosis: affecting the entire glomerulus; segmental sclerosis: affecting a part of each glomerulus. *P < 0.05; **P < 0.001, compared with Group 1.

The histopathological parameters, including extent of mesangial proliferation, proportion of segmental sclerosis, proportion of global sclerosis, proportion of crescents, endocapillary proliferation and infiltration of interstitial mononuclear cells, are listed in Table 2.

**Levels of urinary KIM-1 in patients with IgAN and controls**

Recombinant human KIM-1 could be detected by the sandwich ELISA at a range of 0.19–50 ng/mL (Figure 1).

Urine samples of patients and controls were analyzed for cleaved KIM-1 ectodomain levels. In 53 of the 60 normal controls, 4 of the 46 disease controls and 43 of the 202 patients with IgAN, urinary KIM-1 was undetectable and these subjects were assigned a value of 0. The urinary KIM-1 level was adjusted by urinary creatinine level.

The urinary level of KIM-1 was significantly higher in patients with IgAN than that in normal controls [1.44 (0–44.0) versus 0 (0–9.57) mg/mg urinary Cr, P < 0.001] (Figure 2). The urinary level of KIM-1 was 0.53 (0–5.3) ng/mg urinary Cr for patients with IMN, 0.66 (0–2.92) ng/mg urinary Cr for patients with LN and 0.40 (0–5.30) ng/mg urinary Cr for patients with ANCA-GN, respectively. The urinary level of KIM-1 was significantly higher in patients with non-IgAN than in those with IgAN [1.44 (0–44.0) versus 0.73 (0–5.3) ng/mg urinary Cr, P = 0.011] (Figure 2).
Association between urinary KIM-1 and clinical parameters

In patients with IgAN, the level of urinary KIM-1 positively correlated with the level of SCr and proteinuria and negatively with Ccr. In patients without cellular crescents, the urinary KIM-1 did not correlate with SCr ($r = -0.030$, $P = 0.773$) or Ccr ($r = -0.033$, $P = 0.768$) but still correlated with the level of proteinuria ($r = 0.228$, $P = 0.03$). In disease control groups, the levels of urinary KIM-1 correlated with the level of proteinuria ($r = 0.336$, $P = 0.024$) (Table 3).

Association between urinary KIM-1 and histopathological parameters of patients with IgAN

The levels of urinary KIM-1 in patients with tubular atrophy and interstitial fibrosis Grades 0, 1, 2 and 3 were significantly higher than that of normal controls. Furthermore, in patients with tubular atrophy and interstitial fibrosis Grade 3, the levels of urinary KIM-1 were significantly higher than that in those with Grades 0, 1 and 2 (Figure 3A). Similar results were found among patients with different grades of interstitial infiltration (Figure 3B).

The level of urinary KIM-1 was significantly higher in patients with mesangial proliferation Grade 2 than that in those with mesangial proliferation Grade 1 [3.17 (0–41.7) versus 1.15 (0–44.0) ng/mg urinary Cr, $P < 0.001$] (Figure 4A). The levels of urinary KIM-1 were significantly higher in patients with crescents or endocapillary proliferation than that in those without [2.59 (0–41.7) versus 0.73 (0–44.0) ng/mg urinary Cr, $P < 0.001$; 3.70 (0–44.0) versus 1.29 (0–28.62) ng/mg urinary Cr, $P = 0.01$, respectively] (Figure 4B and C).

In the univariate correlation analysis, the urinary KIM-1 positively correlated with the mesangial proliferation, proportions of global sclerosis, total crescents, cellular crescents, fibrous crescents and interstitial infiltration in renal specimens (Table 3). However, in the multivariable analysis that included all the above-mentioned parameters, the urinary KIM-1 did not significantly correlate with any histopathological parameters (Table 4).

Clinical and histopathological parameters of patients with IgAN with and without elevated urinary KIM-1

Since the level of urinary KIM-1 in normal controls was not normally distributed, the value of the 95th percentile (4.17 ng/mg urinary Cr) was considered as a cutoff point. Patients were therefore divided into two subgroups according to levels of urinary KIM-1, which are elevated (>4.17 ng/mg urinary Cr) or not elevated (≤4.17 ng/mg urinary Cr).

Table 3. Associations between the levels of urinary KIM-1 and the clinico-histopathological parameters of patients with IgAN

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IgAN ($n = 202$)</th>
<th>IgAN without cellular crescents ($n = 90$)</th>
<th>Disease control ($n = 46$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$-value</td>
<td>P-value</td>
<td>$r$-value</td>
</tr>
<tr>
<td>Age</td>
<td>-0.052</td>
<td>0.465</td>
<td>-0.042</td>
</tr>
<tr>
<td>Duration (months)</td>
<td>0.086</td>
<td>0.225</td>
<td>-0.171</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>0.064</td>
<td>0.364</td>
<td>-0.035</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>0.225</td>
<td>0.001*</td>
<td>-0.030</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>0.374</td>
<td>0.001*</td>
<td>0.228</td>
</tr>
</tbody>
</table>
The level of SCR and proteinuria were significantly higher in patients with elevated urinary KIM-1 than that in patients without. The levels of Ccr were significantly lower in patients with elevated urinary KIM-1 than in those without. In the renal specimens, scores of mesangial proliferation, proportion of patients with endocapillary proliferation, proportion of global sclerosis, proportion of crescents as well as scores of tubular atrophy and interstitial fibrosis, and scores of interstitial infiltration were also significantly higher in patients with elevated urinary KIM-1 than in those without (Table 5).
**Association between the expression of tubular KIM-1 and the levels of urinary KIM-1 of patients with IgAN**

KIM-1 expression could be detected in the renal specimen of patients with IgAN. KIM-1 was localized to the apical side of dilated tubules in fibrotic areas. However, KIM-1 was not expressed completely on atrophic tubules. The median score of tubular KIM-1 expression was 2 (0.5–4). In normal controls, KIM-1 expression was virtually undetectable in the renal specimen (Figure 5). The scores of tubular KIM-1 expression in immunohistochemistry were positively correlated with the levels of urinary KIM-1 of patients with IgAN ($r = 0.553$, $P = 0.032$).

**Renal outcomes and urinary KIM-1 level in IgAN**

One hundred and seven patients with IgAN, who were followed up for >12 months were evaluated in survival analysis. The mean duration was $47.65 \pm 23.97$ months.
Urinary KIM-1 in patients with IgAN

(12–120 months). Patients were divided into two subgroups according to the levels of urinary KIM-1 (>4.17 ng/mg urinary Cr and ≤4.17 ng/mg urinary Cr) as above. Renal survival was significantly worse in patients with higher urinary KIM-1 (P = 0.020; Figure 6).

Discussion

To the best of our knowledge, the present study investigated, for the first time, urinary KIM-1 in a large cohort of patients with IgAN with various glomerular and tubulointerstitial lesions. Compared with normal controls, the levels of urinary KIM-1 were markedly elevated in patients with different extents of tubular atrophy, interstitial fibrosis and inflammatory cell infiltration, even in patients with mild tubulointerstitial lesions. The more severe the tubulointerstitial lesion was, the higher the KIM-1 levels. These findings suggest that urinary KIM-1 was closely associated with tubulointerstitial lesions in IgAN and might be an early biomarker for tubulointerstitial lesions. Previous studies had demonstrated that KIM-1 expression in proximal tubules was associated with interstitial fibrosis and inflammation in polycystic kidney disease, protein-overload nephropathy and other renal diseases [14, 16, 17]. These findings indicated that there might be some interactions between KIM-1 and interstitial lesions. It was speculated that KIM-1 expression in tubular epithelial cells might be a primary event leading to interstitial fibrosis or might be a consequence of epithelial injury caused by tubulointerstitial lesions. KIM-1 is a protein with Ig and mucin domains and has some similarity to adhesion molecules, which might function in cell–cell or cell–matrix interactions. The KIM-1 ectodomain might act as a proinflammatory molecule and recruit interstitial macrophages, contributing to the development of interstitial inflammation and fibrosis. It was also presumed that shed KIM-1 could reach the interstitium and play a role in the proliferative and fibrogenic response [14]. Furthermore, other evidence suggested that molecules of the KIM-1 family are involved in T-cell differentiation and macrophage activation [19, 20]. However, the exact role of KIM-1 still remains unclear. It had been proved that KIM-1 was dramatically upregulated in the regenerative proximal tubule epithelial cells in the posts ischemic rat kidney, which suggested that KIM-1 may play an important role in the restoration of the morphological integrity and function to post ischemic kidney [9]. Until now, there is no direct evidence showing whether KIM-1 functions as a positive or negative regulator for the process of tubulointerstitial injury.

More interestingly, the current study found that in patients with severe glomerular lesions, such as intense mesangial proliferation, endocapillary proliferation and crescent formation, the levels of urinary KIM-1 were significantly higher than those without. More importantly, among the 32 patients without evident tubulointerstitial lesions (tubular atrophy and interstitial fibrosis Score 0, interstitial infiltration Score 0), the levels of urinary KIM-1 were also significantly elevated in 5 patients with glomerular endocapillary proliferation and 3 patients with crescent formation. These findings indicate that urinary KIM-1 is not only a biomarker for well-established tubulointerstitial lesions but also a potential biomarker for active glomerular lesions. These findings support the study from van Timmeren et al. [16], who found, that in various renal diseases, KIM-1 expression was associated with glomerular histopathology such as mesangial matrix expansion. However, it is well known that tubulointerstitial lesions are parallel with glomerular lesions in patients with IgA nephropathy; some even suggest that the tubulointerstitial lesions are secondary to glomerular lesions. Therefore, the elevated urinary KIM-1, in patients with active glomerular lesions but without evident tubulointerstitial lesions, might just reflect early tubulointerstitial lesions. A study from Zhang et al. [21] found that in kidney tissues from allograft biopsies, KIM-1 expression could be detected in proximal tubules with normal morphology, which suggests that KIM-1 is more sensitive than histopathology for detecting early tubular lesions. It was proved that KIM-1 is a specific biomarker for ischemic tubule injury. Its expression was dramatically upregulated in the posts ischemic rat kidney, and urinary KIM-1 levels were significantly elevated in patients with ischemic acute tubular necrosis [11, 12]. The glomerular lesions, such as intense renal mesangial proliferation, endocapillary proliferation and crescent formation, may alter the normal structure of glomerular capillaries and thus influence the blood supply to the glomeruli, then to the tubules and interstitium even in those with normal morphology. This might be the reason why the levels of KIM-1 were elevated in the above patients without evident tubulointerstitial injury.

Our study also found that the levels of urinary KIM-1 were closely associated with parameters of renal function of patients with IgAN and patients with elevated urinary KIM-1 presented with more severe kidney injury than those without. This again demonstrates that urinary KIM-1 is closely associated with renal lesions in patients with IgAN. Furthermore, we observed a positive association of urinary KIM-1 with 24 h proteinuria. It had been demonstrated that
KIM-1 expression in damaged tubules and urinary KIM-1 levels were significantly elevated in rat with proteinuria-induced renal injury [17]. It has been postulated that many mechanisms might be involved in inducing KIM-1 production, and one of them is proteinuria. A previous study demonstrated that, when exposed to heavy proteins in vitro, presented in the ultrafiltrate of nephrotic patients, the proximal tubular cells could synthesize increased cytokines/chemokines [22]. Therefore, KIM-1 might also be induced from activated proximal tubular cells caused by exposure to heavy proteinuria. This might explain the association of KIM-1 and proteinuria in patients with IgAN.

The limitation of the current study is the limited sensitivity of ELISA method used in our study. From Figure 1, it was shown that the lowest detectable level of KIM-1 was just 0.19 ng/mL. However, this sensitivity is enough for our current study to investigate the association between urinary KIM-1 and clinical and histopathological parameters in patients with IgAN.

In conclusion, urinary KIM-1 is elevated in patients with IgA nephropathy and is closely associated with disease severity. Urinary KIM-1 is not only an early biomarker for tubulointerstitial lesions but also a potential biomarker for glomerular lesions.

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Conflict of interest statement. None declared.

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