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Kidney Injury Molecule-1 (KIM-1): A novel biomarker for human renal proximal tubule injury

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Kidney Injury Molecule-1 (KIM-1): A novel biomarker for human renal proximal tubule injury.

Background. Traditional blood and urine markers for the diagnosis of various renal diseases are insensitive and nonspecific. Kidney Injury Molecule-1 (KIM-1) is a type 1 transmembrane protein, with an immunoglobulin and mucin domain, whose expression is markedly up-regulated in the proximal tubule in the post-ischemic rat kidney. The ectodomain of KIM-1 is shed from cells. The current studies were carried out to evaluate whether KIM-1 is present in human acute renal failure and might serve as a urinary marker of acute renal tubular injury.

Methods. Kidney tissue samples from six patients with biopsy-proven acute tubular necrosis (ATN) were evaluated by immunohistochemistry for expression of KIM-1. Urine samples were collected from an additional thirty-two patients with various acute and chronic renal diseases, as well as from eight normal controls. Urinary KIM-1 protein was detected by immunoassay and was quantified by ELISA.

Results. There was extensive expression of KIM-1 in proximal tubule cells in biopsies from 6 of 6 patients with confirmed ATN. The normalized urinary KIM-1 levels were significantly higher in patients with ischemic ATN (2.92 ± 0.61; $N = 7$) compared to levels in patients with other forms of acute renal failure (0.63 ± 0.17, $P < 0.01$; $N = 16$) or chronic renal disease (0.72 ± 0.37, $P < 0.01$; $N = 9$). Adjusted for age, gender, length of time delay between the initial insult and sampling of the urine, a one-unit increase in normalized KIM-1 was associated with a greater than 12-fold (OR 12.4, 95% CI 1.2 to 119) risk for the presence of ATN. Concentrations of other urinary biomarkers, including total protein, γ-glutamyltransferase, and alkaline phosphatase, did not correlate with clinical diagnostic groupings.

Conclusions. A soluble form of human KIM-1 can be detected in the urine of patients with ATN and may serve as a useful biomarker for renal proximal tubule injury facilitating the early diagnosis of the disease and serving as a diagnostic discriminator.

Key words: acute renal failure, acute tubular necrosis, contrast nephropathy, urinary biomarker, ischemia, dialysis, nephrotoxicity.

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Acute renal failure (ARF) is an important cause of morbidity and mortality in hospitalized patients. The mortality rate of patients with severe ARF requiring dialysis has not decreased significantly over the last 50 years despite advances in supportive care [1]. Mortality of patients with postoperative renal failure ranges from 24 to 100%, and 50 to 70% among patients in intensive care units who require dialysis [2–5]. Mortality is directly proportional to the magnitude of rise in serum creatinine [3, 6, 7]. It has been postulated that introduction of therapy early in the disease process will reduce the mortality rate associated with ARF. The identification of reliable biomarkers for tubule injury would be useful to facilitate early intervention, evaluate the effectiveness of therapeutic interventions, and guide pharmaceutical development by providing an indicator for nephrotoxicity.

The most frequently used serologic indicators for the diagnosis of renal disease are serum creatinine, serum urea nitrogen, and creatinine clearance, all of which are insensitive and nonspecific for detection of renal injury [8]. Many urinary proteins and biochemical markers have been evaluated as noninvasive indicators of renal injury [9–14]. However, attempts to use them as general markers to screen patients for renal injury and to identify the site of injury within the kidney have been disappointing.

The proximal tubule is particularly sensitive to ischemic injury in animal models [15]. Surviving cells in this segment undergo an active process of dedifferentiation and proliferation after ischemia ultimately resulting in the reconstitution of a well differentiated polarized morphology [16–18]. We have cloned a novel molecule, Kidney Injury Molecule-1 (KIM-1), from rats, mice and humans. KIM-1 is markedly up-regulated in postischemic rat kidney [19]. The KIM-1 ectodomain is shed into the extracellular milieu of human 769-P (human kidney adenocarcinoma cells) and HK-2 (human kidney proximal tubular cells) cell lines which express KIM-1 under normal culture conditions (unpublished data). To test the utility of KIM-1 as a marker of acute tubular damage,

kidney biopsy sections were examined for expression of the KIM-1 protein and urine samples were collected from other patients with various acute and chronic renal diseases. Urinary KIM-1 protein levels were compared with levels of urinary γ-glutamyltransferase (γGT), alkaline phosphatase, and total protein as potential biomarkers for tubule injury in various renal diseases.

METHODS

Renal biopsy samples

Human kidney biopsy sections from six patients with a confirmed pathological diagnosis of acute tubular necrosis (ATN) were randomly obtained and evaluated for expression of the KIM-1 protein by immunohistochemistry. All these biopsy sections were collected previously for other medical indications. Urine samples from these patients were not available.

Patient selection for urine collections

Forty patients were studied for the presence of KIM-1 protein in the urine: 23 with ARF; 9 with chronic renal diseases (CRD); and 8 normal individuals with no evidence of renal disease. The 23 patients with various causes of ARF were randomly selected from patients seen for renal consultation at Massachusetts General Hospital from June 1998 to February 2000 (8 females, 15 males; aged 17 to 85 years, mean 61 years old). An individual was considered to have ARF if the patient's serum creatinine value either (*1*) increased by at least 0.5 mg/dL when the baseline serum creatinine level was less than 2.0 mg/dL; (*2*) increased by at least 1.5 mg/dL when the baseline serum creatinine value was greater than or equal to 2.0 mg/dL; or (*3*) increased by at least 0.5 mg/dL, regardless of the baseline serum creatinine, as a consequence of exposure to radiographic agents. The urine sediment was classified as abnormal if muddy-brown casts, coarse granular casts, multiple fine granular casts, white cell casts, red cell casts or renal tubular cells were present. Nine patients with various CRD were randomly selected from the renal clinic at Massachusetts General Hospital during the same interval (7 females, 2 males; aged 30 to 74 years, mean 54 years old). Patients included in this group (*1*) had an established renal disease which was diagnosed by renal biopsy and/or by serological study; and (*2*) had a relatively stable (±0.5 mg/dL) serum creatinine level for at least six months. Chronic renal diseases represented in this study include Wegener's granulomatosis, focal segmental glomerulosclerosis, systemic lupus erythematosus (SLE) nephropathy, diabetic nephropathy and chronic renal allograft dysfunction. Urine samples were also collected from 8 individuals with no known renal disease. Gender, diagnoses, comorbid conditions, time of onset of ARF, serum creatinine, and serum urea nitrogen concentrations were obtained from review of patients' records.

Immunohistochemistry

Human kidney biopsy sections from six kidneys were deparaffinized, and endogenous peroxidase activity was ablated by incubation in 2% hydrogen peroxide in methanol for 20 minutes. The sections were heated with a microwave oven in 0.1 mol/L citrate buffer, pH 6.0 for 10 minutes, and were blocked with 1.5% horse serum in PBS at room temperature for one hour. AKG7 anti-human KIM-1 monoclonal antibody was then added to the sections and incubated overnight at 4°C with 50 μL of antibody at 5 μg/mL in blocking solution. After application of the primary antibody, KIM-1 was detected using a commercially available Vectastain Elite ABC kit (for mouse IgG) with DAB Substrate (Vector Labs, Burlingame, CA, USA) kit for peroxidase staining. The sections were counterstained with hematoxylin.

Urine samples

Collected fresh urine samples were centrifuged to remove cellular components. Protease inhibitor cocktail tablets (Boehringer Mannheim, Mannheim, Germany) were added to the transferred supernatant. Urine analysis included urinary dipstick (Multistix 8 SG; Bayer Corporation, Tarrytown, NY, USA) and microscopic examinations of sediment (Olympus microscope), which were performed prior to storage at −80°C.

Monoclonal antibodies against human KIM-1

Highly specific murine monoclonal antibodies were raised against the extracellular domain of human KIM-1 and were purified from ascites fluid or hybridoma conditioned medium (a more complete description of these antibiotics is submitted for publication by Bailly V et al). Monoclonal antibodies designated ARD5, ACA12 and AKG7 were used for this study. ARD5, which recognizes the native protein and binds to the Ig-like domain, was used as a trapping antibody in the immunoextraction and the enzyme-linked immunosorbent assay (ELISA), while AKG7 and ACA12 were used as detection antibodies for Western blots and ELISA read out. AKG7 antibody was labeled with biotin using the sulfo-NHS-biotin (Pierce Chemical Co., Rockford, IL, USA) and was used as detection antibody for immunohistochemistry.

Immunoextraction, Western blot analysis, and ELISA immunoassay

Urinary soluble KIM-1 protein was detected by immunoextraction followed by Western blot analysis and was quantified by ELISA. Briefly, the wells of an ELISA plate (MaxiSorp; Nunc, Naperville, IL, USA) were coated with ARD5 anti-human KIM-1 monoclonal antibody [over-

Table 1. Expression of KIM-1 in human kidney biopsy sections with acute tubular necrosis

Cause of acute tubular necrosis	KIM-1 expression in proximal tubules	KIM-1 expression in glomeruli
Ischemia with minimal change disease	Yes	No
Early allograft dysfunction without rejection	Yes	No
Ischemia with allergic interstitial nephritis	Yes	No
Ischemia in setting of NSAID use and membraneous nephropathy	Yes	No
Early allograft dysfunction without rejection	Yes	No
Ischemia with vasculitis	Yes	No

NSAID is nonsteroidal anti-inflammatory drug.

night incubation at 4°C with 300 μL of antibody at 25 μg/mL in phosphate buffered saline (PBS)]. The wells were blocked with a bovine serum albumin (BSA) solution (1% in PBS) and were washed four times with PBST (PBS with 0.05% Tween 20). For immunoextraction followed by Western blot analysis, the wells were incubated with 250 μL urine samples at room temperature for three hours. After four washes with PBST, samples were mixed with 25 μL Laemli loading buffer containing 5% β-mercaptoethanol, heated five minutes at 95°C. Twenty microliters were loaded on a 4 to 20% polyacrylamide gel. After electrophoresis, the proteins were electrotransferred onto a nitrocellulose sheet. The blot was blocked with a 5% nonfat dry milk solution in PBST and probed with hybridoma supernatant (ACA12 or AKG 7) in the blocking solution, followed by horseradish peroxide (HRP)-conjugated goat anti-mouse IgG antibodies diluted in the blocking solution. The reactive bands were revealed by electrochemiluminescence. For ELISA, the wells were incubated with 100 μL urine samples at room temperature for three hours. After four washes with PBST, biotinylated AKG7 antibody was added, followed by HRP-conjugated streptavidin. The urinary KIM-1 content was expressed in absolute terms and also normalized to the urinary creatinine concentration.

Analytical tests

Urinary total protein, creatinine, alkaline phosphatase, and γ-glutamyltransferase (γGT) were measured by Ani-Lytics Inc. (Gaithersburg, MD, USA) with a Hitachi 717 Analyzer. Total protein was measured with Pyrogallol-Red reagent (Biotrol Diagnostic, Chennevièié-res les-Louvres, France). Different system Packs (Boehringer Mannheim Diagnostics) were used to measure the creatinine (Jaffe System Pack), alkaline phosphatase (Aminomethyl-propanol System Pack) and γGT (Glycyl-glycone System Pack). These values were normalized to the urinary creatinine concentration. Serum creatinine was measured by the Jaffé creatinine assay using a Roche/Hitachi 917 system. (Roche Diagnostics, Indianapolis, IN, USA).

Statistical analysis

Categorical variables were analyzed using chi square tests, and continuous variables using Mann Whitney-U tests. Logistic regression was used for multivariate analysis, and all *P* values are two tailed. Statistical analysis was performed using SAS (SAS Institute, Cary, NC, USA). Data are expressed as means ± SE.

RESULTS

Expression of KIM-1 in the proximal tubules with acute tubular necrosis.

The expression of KIM-1 was evaluated in six randomly selected biopsies in which a pathological diagnosis of ATN had been assigned. The underlining cause of ATN in these patients is presented in Table 1. KIM-1 is expressed on the apical aspect of proximal tubule epithelial cells in all six patients. A typical staining pattern is demonstrated in Figure 1. KIM-1 was not present in glomeruli. KIM-1 was not detectable in normal kidney tissue derived from a patient with renal cell carcinoma (Fig. 1). Urine was not available from these patients.

Characteristics of patients from whom urine was examined for the presence of KIM-1

Clinical characteristics of all patients from whom urine was evaluated for the presence of KIM-1 are reported in Table 2. Patients with ischemic ATN ($N = 7$) were diagnosed based on evidence for tubular cell injury including muddy-brown or granular casts in urine sediments, clinical history, and elevation of serum creatinine and blood urea nitrogen. Table 3 summarizes the causes of ischemic ATN, the baseline and peak level to which serum creatinine increased, the serum creatinine at the time of urine collection, the day of urine collection and the day that the serum creatinine reached its peak. Ischemia, either to the native kidney (6/7), or a renal cadaveric allograft (1/7) was the cause of ATN in each of the seven patients. In four patients the ischemia was associated with sepsis and in two other patients a cardiac arrest and cardiogenic shock accompanied a myocardium infarction. One patient had delayed allograft function after cadaveric renal transplant. In six of seven patients urine was collected prior to the day when serum creatinine reached its peak. In the seventh patient urine was collected on the same day that serum creatinine reached

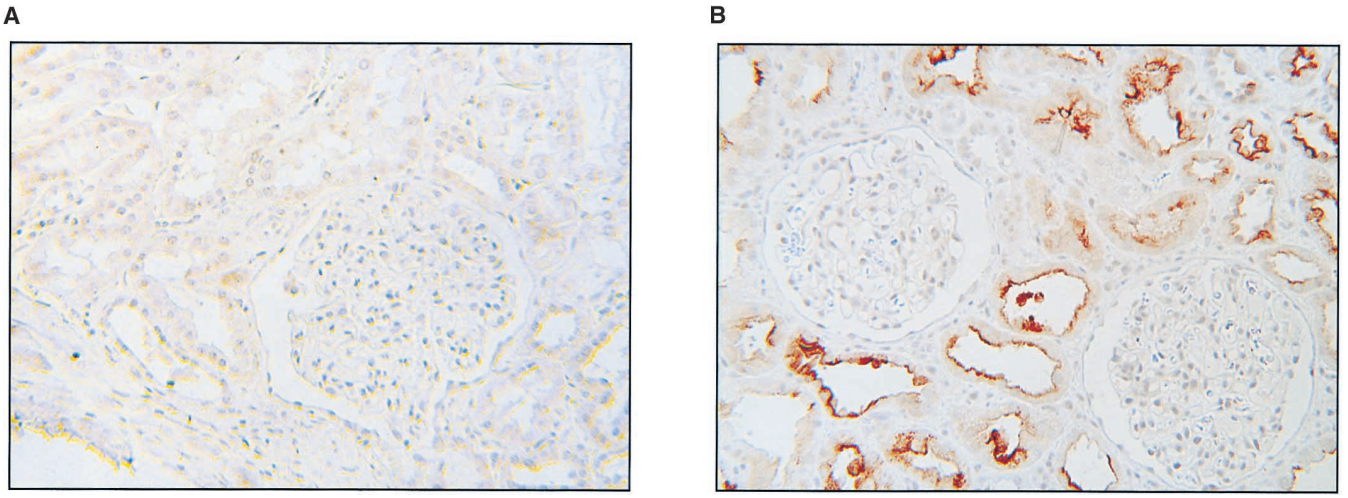

Fig. 1. KIM-1 expression in a normal human kidney (*A*) and one with acute tubular necrosis; (*B*) human kidney paraffin sections are stained for KIM-1 using the AKG7 anti-human KIM-1 monoclonal antibody, and an ABC peroxidase detection system with DAB substrate (Vector). Normal kidney tissue was taken from a patient with renal cell carcinoma.

Table 2. Baseline characteristics of patients with renal diseases and control

Characteristic	Ischemic ATN ($N = 7$)	Contrast nephropathy ($N = 7$)	Other ARF ($N = 9$)	CRD ($N = 9$)	Normal ($N = 8$)
Age *years*	63.0 ± 4.7	69.3 ± 2.6	52.3 ± 8.3	53.6 ± 5.5	38.9 ± 1.3
Sex *M/F*	4/3	4/3	7/2	2/7	4/4
Baseline serum creatinine *mg/dL*	1.6 ± 0.6	1.9 ± 0.1	1.8 ± 0.2	2.6 ± 0.6	
Peak serum creatinine *mg/dL*	4.9 ± 0.9	4.5 ± 0.8	4.6 ± 0.9		
Serum creatinine at time of urine collection *mg/dL*	3.8 ± 0.7	3.8 ± 0.8	3.8 ± 0.8	2.6 ± 0.6	

Abbreviations are: ATN, acute tubular necrosis; other ARF, acute renal failure not associated with ischemia and contrast nephropathy; CRD, chronic renal diseases. Values are means ± SE.

Table 3. Summary of patients with Ischemic ATN

Patient	Sex	Age	Etiology	Baseline S_{Cr} *mg/dL*	Peak S_{Cr} *mg/dL*	S_{Cr} at time of urine collection *mg/dL*	KIM-1 *ng/mL*	Normalized KIM-1	Time to reach peak S_{Cr} *days*	Time of urine collection *day*
1	M	75	Ischemia: cardiogenic shock	1.3	3.4	3.4	2.95	5.01	18	18
2	F	56	Ischemia: delayed graft function	5.1	8.7	7.0	2.99	2.46	5	2
3	M	51	Ischemia: sepsis	0.7	1.9	1.2	1.34	1.90	6	3
4	M	63	Ischemia: sepsis	1.0	5.5	4.3	1.81	1.28	12	11
5[a]	F	47	Ischemia: sepsis	0.9	4.3	2.1	1.50	5.30	7	4
6	F	68	Ischemia: sepsis	1.2	7.6	5.3	2.00	2.80	11	3
7	M	81	Ischemia: cardiac arrest	2.2	3.1	2.3	1.40	1.70	5	1

Abbreviations are: M, male; F, female; S_{Cr}, serum creatinine.
[a] Patient required dialytic support

its peak level. One patient (# 5) required dialytic support. In patients with contrast nephropathy (7/7 patients), urine granular casts and/or renal tubular cells were present. One patient whose contrast ARF was complicated by rhabdomyolysis had muddy brown casts. Each of these patients with contrast-induced ARF had (*1*) at baseline, chronic renal insufficiency prior to the exposure to contrast media; (*2*) exposure to contrast dye with a well documented elevation of serum creatinine and blood urea nitrogen temporally related to the contrast dye exposure; and (*3*) rapidly reversible ARF. Patients with other forms of ARF (9/9 patients) had no urine casts. This group included five patients with pre-renal azotemia, two with late allograft rejection in the setting of chronic cyclosporine administration, one with postobstructive nephropathy, and one with acute interstitial nephritis. Patients with acute interstitial nephritis ($N = 1$), or late allograft rejection ($N = 2$) were diagnosed by

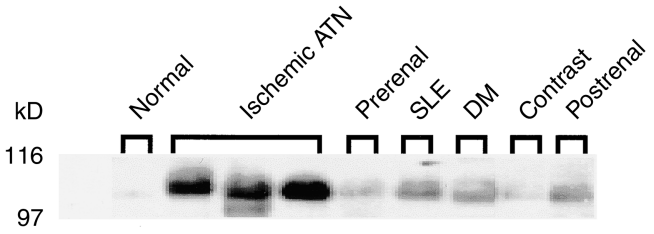

Fig. 2. Western blot analysis of urine collected from patients with different forms of renal failure. Each lane represents protein immunoextracted from urines of patients without renal disease, with ischemic ATN, other forms of ARF (prerenal azotemia, contrast nephropathy, post-renal), or chronic diseases (systemic lupus erythematosus nephropathy, SLE; diabetic nephropathy, DM). After immunoextraction and PAGE, Western blot analysis was carried out with the AKG7 anti-KIM-1 antibody.

renal biopsies. A patient with postobstructive nephropathy ($N = 1$) was diagnosed by renal ultrasound, which disclosed the presence of bilateral hydronephrosis due to obstruction of the ureters by prostate carcinoma. Patients with prerenal azotemia ($N = 5$) were assigned this diagnosis after intrarenal and postrenal etiologies were excluded; benign urine sediments were documented and renal function returned to baseline levels with improved renal perfusion.

Detection of a soluble released form of human urinary KIM-1 protein

Western blot results, presented in Figure 2, reveal low levels of urinary KIM-1 in samples from patients without acute or chronic renal disease. Although urine samples from patients with either (*1*) non-ischemic ATN etiologies or (*2*) various chronic renal diseases contained greater amounts of KIM-1 than were found in urines from individuals without known renal diseases, the amount of KIM-1 present was much greater in urines from patients with ischemic ATN. KIM-1 Western blot analysis correlated well with the quantitation of KIM-1 in the urine by ELISA. Both absolute urinary human KIM-1 concentration and KIM-1 concentration normalized to urine creatinine concentration were much higher in patients with ischemic ATN than in patients with other forms of renal injury (Table 4).

We determined whether potential confounding variables explained the association between KIM-1 concentration and ischemic ATN. After adjusting for age, gender, length of time delay between the initial insult and sampling of the urine, a one-unit increase in normalized KIM-1 was strongly associated with the diagnosis of ATN (OR 12.4, 95% CI, 1.2 to 119). The absolute KIM-1 concentration showed similar results with this end-point. In Figure 3, the urinary KIM-1 values in each of the patients are presented. One patient in the CRD group with SLE nephropathy had a high KIM-1 level when compared to the other eight patients with chronic renal disease. There was no clinical sign of active SLE nephropathy at the time of urine collection. The urine sediment was benign other than the presence of trace protein. When normalized to urinary creatinine concentration, however, the KIM-1/creatinine value was 0.87, a value close to the mean value of the other patients with CRD.

Our studies do not permit us to identify how early in the course of disease urinary KIM-1 can be detected. We do know that levels are detectable within 12 hours of the insult since one of our patients who had high levels of urinary KIM-1 had a well-documented ischemic episode 12 hours prior to collection of the urine (patient 7, Table 3). This finding is consistent with the observed time course of KIM-1 expression in the urine of a patient who underwent an elective repair of an abdominal aortic

Table 4. Comparison of KIM-1 concentration and KIM-1 levels normalized to urine creatinine concentration for various forms of renal diseases

	KIM-1 *ng/mL*	Normalized KIM-1
Ischemic ATN	2.00 ± 0.26	2.92 ± 0.61
Contrast nephropathy	0.34 ± 0.13^a	0.85 ± 0.30^a
Other ARF	0.13 ± 0.02^a	0.48 ± 0.19^a
Contrast nephropathy & other ARF	0.22 ± 0.06^a	0.64 ± 0.17^a
CRD	0.69 ± 0.41^b	0.72 ± 0.37^a

Abbreviations are: ATN, acute tubular necrosis: other ARF, acute renal failure not associated with ischemia and contrast nephropathy; CRD, chronic renal diseases. Values are means ± SE.

[a] $P < 0.01$, [b] $P = 0.02$, vs. ATN-ARF

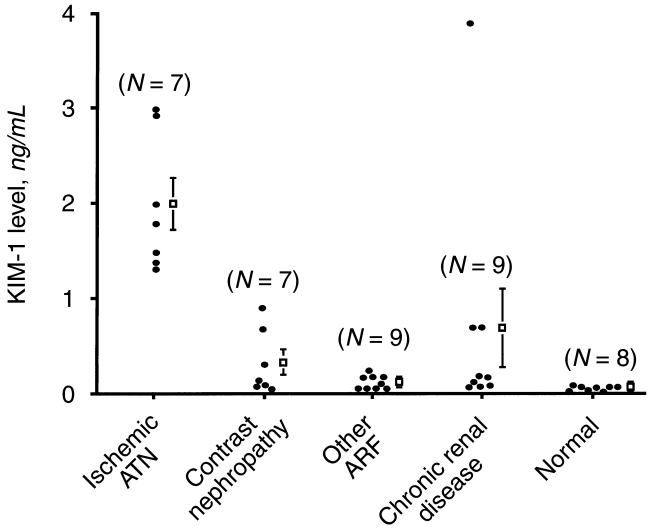

Fig. 3. Comparison of urinary KIM-1 concentration in various forms of renal diseases. Scatterogram of absolute KIM-1 concentration is shown to discriminate the value of KIM-1 among the different groups of patients. Number of patients in each group is indicated above the scatterogram. Values shown are means ±(SE). Abbreviations are: ATN, acute tubular necrosis; Other ARF, acute renal failure with causes other than ischemia and contrast nephropathy.

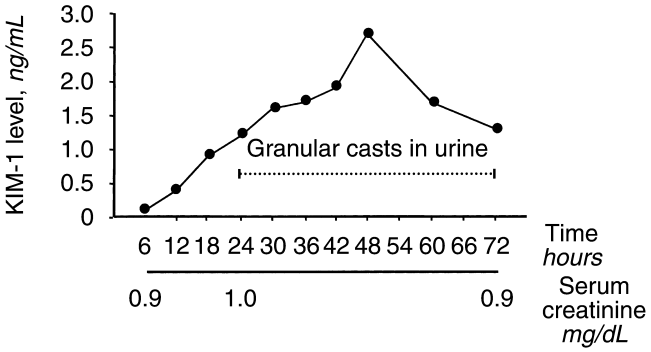

Fig. 4. KIM-1 expression in urine of patient after abdominal aortic aneurysm repair. Time course of KIM-1 expression in urine of a patient who underwent an elective repair of an abdominal aortic aneurysm. Sequential urine samples were collected every 6 hours from the onset of operation. Urine sediment was checked at every time point KIM-1 was measured.

aneurysm (Fig. 4). In this patient, KIM-1 was present in the urine within 12 hours after clamping of aorta. It is of interest that urinary KIM-1 could be detected before any urinary casts were noted on microscopic examination of the urine.

Other urinary biomarkers

Urinary γGT, alkaline phosphatase and total protein were measured for comparison with urinary KIM-1 as markers for ATN (Fig. 5). When either non-normalized or normalized to urine creatinine concentration, concentrations of these biomarkers, other than KIM-1, did not discriminate ATN from other causes of renal failure.

DISSCUSION

This study demonstrates that urinary KIM-1 protein concentration is significantly higher in urine samples from patients with ischemic ATN compared to urine samples from patients with other forms of acute and chronic renal failure. We have detected urinary KIM-1 levels with murine monoclonal antibodies that we developed and have characterized to be highly specific to human KIM-1. The traditional laboratory approach for detection of renal disease involves determination of the serum creatinine, blood urea nitrogen, creatinine clearance, urinary electrolytes, microscopic examination of the urine sediment, and radiological studies. These indicators are not only insensitive and nonspecific, but do not allow for early detection of the disease. In this initial study of the presence of KIM-1 in human urine, we report that KIM-1 is a more specific marker for ischemic tubule injury than a number of other markers that have been previously examined.

Surface membrane components of proximal tubule epithelia have been previously used as urinary markers of injury [9, 11, 20–23]. Brush-border enzyme activities in urine, including leucine aminopeptidase, alkaline phosphatase, α-glucosidase, and γGT, have been used to detect acute and chronic kidney injury induced by drugs and pollutants [11, 20]. Unfortunately, these markers are neither universal urinary biomarkers nor do they reflect a final common pathway of renal expression of injury [24]. In addition, there are characteristics of each of these markers that prevent their ready usage in general clinical settings. The detection of urinary enzyme activity also is complicated by the frequent loss of activity associated with the physicochemical characteristics of the urine or the presence of pyuria [25]. In the case of low-molecular-weight proteinuria, timing of urine collection is important because protein excretion follows a circadian rhythm with lowest levels observed during sleep [26]. Tubular brush border antigens have been reported to be sensitive indicators of proximal tubular injury [9, 23]; however, standard immunoassays for these antigens have not been developed. In our study, quantitative levels of urinary γGT, total protein, and alkaline phosphatase did not correlate with the extent of functional renal injury nor did these markers distinguish ATN from other causes of renal insufficiency.

KIM-1 expression was found in proximal tubule epithelial cells in human kidney biopsy sections from patients with ATN. KIM-1, a type 1 transmembrane glycoprotein, is expressed only minimally in a normal adult rat kidney and is dramatically up-regulated in the S3 segment of the proximal tubule in post-ischemic rat kidneys, under conditions where the S3 segment is highly susceptible to an ischemic insult [19]. In our study ischemic ATN appears to be the most common predisposing factor for elevation of urinary KIM-1 protein in cases of ARF. Low levels of KIM-1 expression in random individuals without known renal diseases likely represent background of the ELISA assay since no urinary KIM-1 protein was detected by Western blot in normal volunteers. Urine samples from patients with diabetic nephropathy and SLE nephropathy, who had significant proteinuria (>3 g/day), but were without evidence for tubule necrosis on urine sediments, had no elevation of urinary KIM-1 protein. Thus, high grade proteinuria does not alter the excretion profile of urinary KIM-1. The normalization of urinary KIM-1 to urine creatinine concentration poses a limitation since patients with ARF are not in a steady state of creatinine balance. The unsteady creatinine volume output will influence the data considerably. In order to minimize this uncertainly, we used both non-normalized urinary KIM-1 and normalized KIM-1 in our study, and the results did not markedly differ. Although our sample size was small, adjustment for potential confounders did not diminish the association between KIM-1 and ATN.

Other glycoproteins such as intercellular adhesion molecule-1 and selectins have been shown to be involved

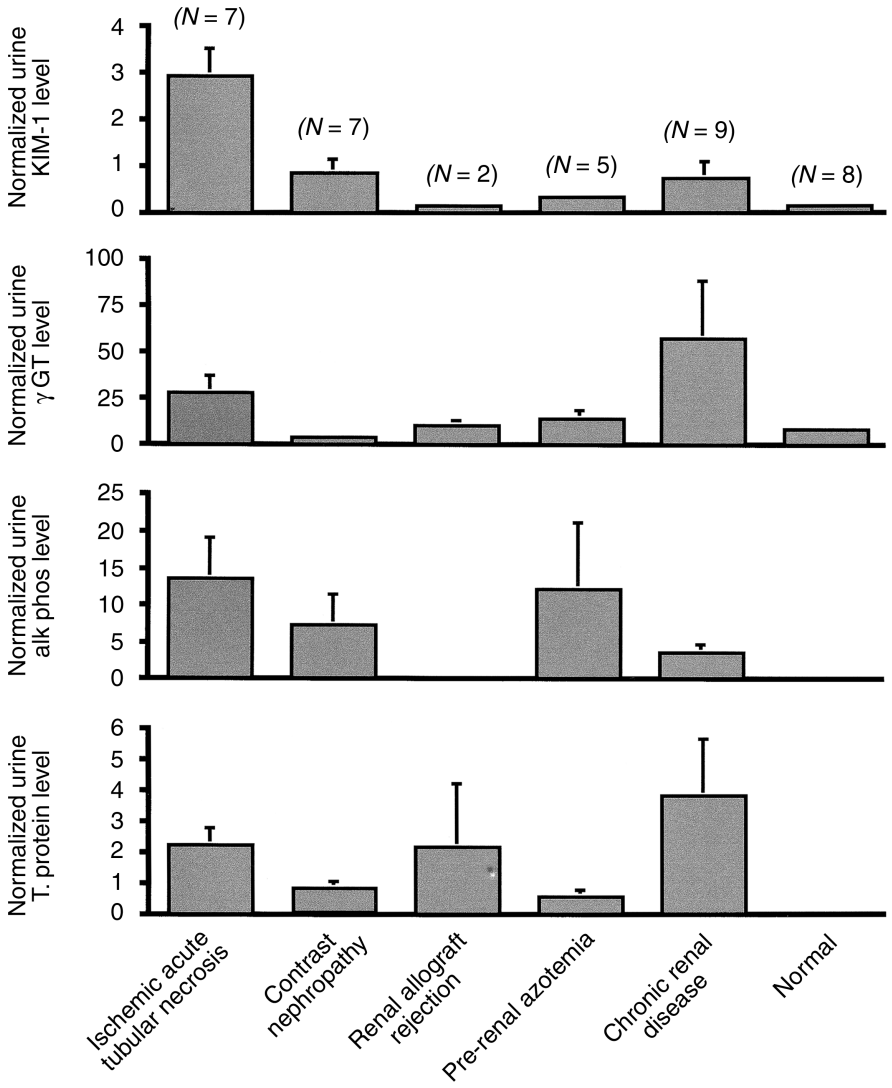

Fig. 5. Comparison of KIM-1 and other urinary proteins as biomarkers for tubule injury in various renal diseases. Urinary alkaline phosphatase, γ-glutamyltransferase, (γGT) and total protein were measured for comparison with urine KIM-1 as markers for ATN. Number of patients in each group is indicated above the top bars. Urinary biomarker concentrations are normalized to urine creatinine concentration.

in the pathogenesis of ischemic ARF [1]. KIM-1 expression has been co-localized with bromodeoxyuridine (a marker for proliferation) and vimentin (a marker for dedifferentiation) staining proximal tubular epithelial cells in the post-ischemic rat kidney [19]. During the repair process the proximal tubule epithelium undergoes a complex series of events, including proliferation of surviving epithelial cells and formation of a poorly differentiated regenerative epithelium over the denuded basement membrane [1]. Our study shows that KIM-1 can be expressed in urine within 12 hours after the initial ischemic renal insult, prior to regeneration of the epithelium, and persists over time. In animals, it has been shown that KIM-1 is present coincident with the appearance of markers of epithelial cell dedifferentiation and proliferation. Further studies are necessary to determine the functional role of KIM-1 in the post-ischemic kidney.

The level of urinary KIM-1 may be a useful tool in diagnosing the etiology of ARF when this is uncertain and may obviate the need for renal biopsy. The presence of urinary KIM-1 also might be useful as a marker in clinical trials so that therapy can be initiated at an early stage in the course of the disease. A sensitive urine test for proximal tubule injury also will be useful for the evaluation of nephrotoxicity of pharmaceutical agents in development.

In conclusion, we have demonstrated that a soluble released form of human KIM-1 can be detected in the urine of patients with ischemic ARF and may serve as a novel biomarker for renal proximal tubule injury. A larger prospective study is needed to further validate the utility of this biomarker in the clinical setting.

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