Kidney injury molecule-1 expression is closely associated with renal allograft damage

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Abstract

The aim of our study was to investigate the expression of kidney injury molecule-1 (KIM-1) in renal allograft biopsy samples and assess the clinical significance of its use as a biomarker for tissue damage. A total of 69 renal allograft biopsy samples from 17 patients with normal serum creatinine and 52 cases of increased serum creatinine were collected. They were divided into different groups according to the Banff 2007 diagnostic criteria. KIM-1 expression was detected by immunohistochemical methods and the association of KIM-1 and blood biochemical indexes was analyzed. KIM-1 expression increased as Banff 2007 classification grade increased and was positively correlated with tubular inflammation severity in the acute T-cell rejection group. Moreover, KIM-1 expression was strongly positive in the chronic active antibody-mediated rejection group. Interestingly, KIM-1 was weakly positive in the normal group without obvious acute rejection and injury of immunosuppressant toxicity. In this group, 27.3% (3/11) of the cases with normal serum creatinine level showed weakly positive KIM-1 expression in their renal tissues. KIM-1 expression level is positively correlated with renal allograft damage and tubular cell injury. KIM-1 is expressed in tubular epithelial cells before blood biochemical indexes become elevated and morphological changes occur. KIM-1 expression is an early, sensitive, and specific biomarker to determine renal tubular epithelial cell injury in renal allograft tissue.

KEY WORDS: kidney injury molecule-1, renal allograft, biomarker.

INTRODUCTION

Kidney injury molecule-1 (KIM-1) is a type I transmembrane protein that was first identified in renal cells after injury in 1998 [1]. KIM-1 mRNA and protein are expressed at low levels in normal kidney but are specifically up regulated in dedifferentiated proximal tubular cells after ischemic or nephrotoxic acute kidney injury (AKI). An extracellular domain of KIM-1 is detectable in the urine soon after AKI [2]. KIM-1 represents a promising biomarker for the early diagnosis of AKI and its clinical outcomes [3-5]. In hospitalized patients with established AKI, urinary KIM-1 levels predicted adverse clinical outcomes, such as dialysis requirement and mortality [3]. Preliminary studies have reported the potential utility of KIM-1 as a chronic kidney disease (CKD) biomarker. In a kidney biopsy study of 74 patients with CKD from various aetiologies, KIM-1 was primarily expressed at the luminal side of dedifferentiated proximal tubules, in areas with fibrosis and inflammation [6]. KIM-1 staining in the kidney correlated positively with morphological damage and renal function decline. It appears to be a highly sensitive and specific biomarker for the diagnosis of acute or chronic renal injury [2, 7]. In recent years, much attention has been paid to the role of KIM-1 in renal transplantation, although no consensus has been reached. Most researchers believe KIM-1 may become an early marker of kidney transplant rejection injury and an early biomarker of graft survival and prognosis. Our study was designed to explore KIM-1 expression in renal allograft biopsy and assess the clinical significance of its use as a potential biomarker of renal tissue injury.

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MATERIALS AND METHODS

Patients

A total of 69 patients (19 women, 50 men) who underwent kidney transplantation between May, 2009 and

TABLE 1. Basic clinical characteristics of patients and KIM-1 expression in different groups.

Group	A	B	C	D	E	F
Age (years)	27.2 ± 3.6	45.0 ± 3.5	48.2 ± 2.1	37.6 ± 1.1	36.4 ± 2.3	31.1 ± 4.6
Female / Male	7/20	4/5	2/3	3/10	2/9	1/3
Duration from transplantation to biopsy (weeks)	24.8 ± 12.6	231 ± 101	333 ± 157	24.2 ± 16.8	34.6 ± 13.4	221 ± 111
KIM-1 expression						
0	0 (0)	0 (0)	0 (0)	2 (15.4)	0 (0)	0 (0)
1+	1 (3.7)	1 (11.1)	0 (0)	2 (15.4)	1 (9.0)	0 (0)
2+	8 (29.6)	0 (0)	1 (20.0)	5 (38.5)	5 (45.5)	2 (50.0)
3+	12 (44.4)	2 (22.2)	3 (60.0)	4 (30.7)	3 (27.3)	2 (50.0)
4+	6 (22.2)	6 (66.7)	1 (20.0)	0 (0)	2 (18.2)	0 (0)

Legend: group A, acute T cell-mediated rejection group (27 cases); group B, chronic active antibody-mediated rejection group (9 cases); group C, chronic active T cells mediated rejection group (5 cases); group D, borderline lesions group (13 cases); group E, normal group without obvious acute rejection or injury of immunosuppressant toxicity (11 cases); group F, recurrence of allograft nephropathy group (4 cases).
The numbers in parentheses are the percentage of cases with positive KIM-1 expression in each group.

June, 2011 in the Urology and Nephrology Center of the First Hospital, Jilin University were enrolled in this study. The renal allograft biopsy tissues were collected, fixed in formalin and embedded with paraffin. All patients were given basiliximab before the transplantation and prednisone, mycophenolate mofetil, and calcineurin inhibitors after the operation. The clinical characteristics of the patients are listed in Table 1. The study was approved by the Ethics Committee of the First Hospital, Jilin University and informed consent was obtained from each patient. The renal allograft biopsy tissue samples were divided into six groups according to the Banff 2007 standards [8]. The groups were acute T cell-mediated rejection group (group A, 27 cases), chronic active antibody-mediated rejection group (group B, 9 cases), chronic active T cells mediated rejection group (group C, 5 cases), borderline lesions group (group D, 13 cases), normal group without obvious acute rejection or injury of immunosuppressant toxicity (group E, 11 cases), and recurrence of allograft nephropathy group (group F, 4 cases).

Renal function

Serum creatinine was tested using the creatinine acid oxidase method and blood urea nitrogen (BUN) was tested by the ultraviolet glutamic acid deoxidizing enzymatic method (Hitachi 7600-210 instrument).

Immunohistochemistry

Paraffin-embedded tissue samples were observed by hematoxylin and eosin staining, PAS staining, and Masson staining. Samples were deparaffinized and rehydrated. After antigen retrieval, primary antibody against KIM-1 mAb (MAB1750; R&D; Minneapolis, MN, USA) was used at a dilution of 1:100, and incubated overnight at 4°C. IgG conjugated horseradish peroxidase (HRP) and 3,3-diaminobenzidine tetrahydrochloride (DAB) (Vector Laboratories; Burlingame, CA, USA) were used to visualize antibody binding. KIM-1 staining in renal tissues was evaluated by three pathologists who had no knowledge of patient clinicopathologic factors or outcomes. Immunoreactivity of samples was further graded as follows [9]: 0, no staining; 1+, weak fine granular staining focally present along the luminal surface of non-atrophic proximal tubules; 2 +, weak fine granular staining completely surrounding the luminal surface of non-atrophic proximal tubules; 3+, moderate granular staining completely surrounding the luminal surface of non-atrophic proximal tubules and extending into intercellular junctions; and 4 +, strong large granular staining completely surrounding the luminal surface of non-atrophic proximal tubules and extending into intercellular junctions.

Statistical analysis

Data are presented as mean ± SEM unless indicated otherwise. SPSS software package (versions 18.0, SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. Correlations of KIM-1 expression with clinicopathologic factors were examined by Chi-square tests. Correlation between KIM-1 staining scores and renal function indexes was assessed using simple regression analysis. Differences were considered significant when the p values were less than 0.05.

RESULTS

KIM-1 expression in renal allograft tissue

KIM-1 was expressed in proximal tubular epithelial cells at various levels in the different pathological diagnosis groups. As shown in Table 1, the total positive KIM-1 expression rate was 97.1% (67/69). In the acute T cell mediated rejection group, 66.7% of the cases showed strongly positive expression, and the total positive expression rate was 100%. Furthermore, 88.9% of the cases in the chronic active anti-

body mediated rejection group showed strongly positive expression and the total positive expression rate was 100% as well. A preponderance of the cases (80%) in the chronic active T cells mediated rejection group showed strongly positive expression and the total positive expression rate was 100%. In contrast, only 30.7% of the cases in the borderline lesions group showed strongly positive expression and the positive expression rate was 84.6%. In the normal group without obvious acute rejection and injury of immunosuppressant toxicity, 54.6% of the cases also showed weakly positive expression and 45.5% of the cases showed strongly positive expression. All four cases in the recurrence of allograft nephropathy group had strongly positive expression.

KIM-1 expression correlated with declines in renal function in renal transplant patients

The association of KIM-1 expression with creatinine and BUN was analyzed. KIM-1 expression was significantly correlated with creatinine level ($p = 0.0028$, Table 2). BUN level is easily affected by many factors and analysis did not show KIM-1 expression was correlated with BUN level. The results indicated that KIM-1 expression in renal allograft tissue was positively correlated with declines in kidney function.

KIM-1 expression contributed to acute T cells mediated rejection

Acute T cells mediated rejection was graded as IA, IB, IIA, IIB, and III [10]. KIM-1 expression was detected in 12 (63.2%) grade I tissues and in 5 (71.4%) grade II tissues (Table 3). KIM-1 expression increased as the Banff 2007 classification grade increased and was positively correlated with the acute T-cell rejection group.

KIM-1 expression is related to severity of tubular inflammation

In the acute T cell mediated rejection group, four cases that showed strongly positive expression of KIM-1 although no tubular inflammation was observed. This indicated that renal tubular epithelial cells had been damaged before the morphological change of tubular inflammation had occurred. The number of cases of KIM-1 expression increased as tubular inflammation grade rose; KIM-1 expression was strongly positive when tubular inflammation grade was 3 + (Table 4).

KIM-1 expression in cases with normal serum creatinine levels

In the present study we also found that 47.1% (8/17) of the cases showed weakly positive KIM-1 expression and 47.1% (8/17) of the cases showed relatively strongly positive expression although they had normal levels of serum creatinine (data not shown). In the normal group without obvious acute rejection and injury of immunosuppressant toxicity, 27.3% (3/11) of the cases had normal serum creatinine levels, but they showed weakly positive KIM-1 expression in their renal tissues.

TABLE 2. KIM-1 expression correlation with decreased renal function in renal transplant patients.

Groups	KIM-1 Expression				
	0	1+	2+	3+	4+
Case numbers	2	5	21	26	15
Serum Creatinine (μmol/l)	95.9±3.0	142.3±56.6	200±138	200±174	375±285
BUN (mmol/l)	8.00 ± 0.14	9.36 ± 1.57	13.9 ± 8.5	12.2 ± 6.5	12.2 ± 6.5
Age at menopause	48.85	6.238	42.2	15.48	<0.05

TABLE 3. Correlation between KIM-1 expression and acute T-cell mediated rejection.

Grade	KIM-1 expression				
	0	1+	2+	3+	4+
IA (11)	0	0	6	5	0
IB (8)	0	1	0	3	4
IIA (7)	0	0	2	4	1
IIB- III (1)	0	0	0	0	1

TABLE 4. Correlation between KIM-1 expression and tubular inflammation.

Grade (case number)	KIM-1 expression (case number)				
	0 (0)	1+ (1)	2+ (7)	3+ (12)	4+ (7)
0 (4)	0	0	0	3	1
1+ (9)	0	0	5	4	0
2+ (11)	0	1	2	5	3
3+ (3)	0	0	0	0	3

DISCUSSION

Renal tubular epithelial cells are very sensitive to toxic injury and anoxic damage, and a series of changes occur after injury. Tubular cells lose polarity and integrity of the cytoskeleton, apoptosis, and necrosis occur, and surviving cells start to proliferate and differentiate. KIM-1 is expressed in the damaged renal tubular epithelial cells after toxic and anoxic injury [1]. It is a marker with high sensitivity and specificity in the diagnosis of renal tubular damage. In our study, KIM-1 expression was assessed in proximal tubular epithelial cells from several groups representing patients with varying degrees of renal damage. KIM-1 expression was detected in 66.7% of renal tissues in the acute T cell mediated rejection group, 88.9% of renal tissues in the chronic rejection group, and 30.8% of renal tissues in the borderline lesions group. In the normal group without obvious acute rejection and injury of immunosuppressant toxicity, 57.1% of renal tissues showed had weakly positive KIM-1 expression, while 42.9% of renal tissues showed strongly positive KIM-1 expression. KIM-1 was highly expressed in the recurrence of allograft nephropathy group.

Sensitive biological indexes are needed to identify renal tubular epithelial injury and to evaluate renal tubular damage when morphological changes have not yet occurred in the allograft. In this study, we detected KIM-1 expression by immunohistological methods in renal allograft tissue to judge renal tubular epithelial injury and acute cellular rejection damage, and found that KIM-1 expression was positively related with kidney function decline [9]. The results show that KIM-1 expression in renal allograft tissue is more sensitive than rises in serum creatinine or morphological changes in the assessment of renal tubular epithelial injury. Chronic renal allograft function loss is the main cause of graft failure. Therefore, the early evaluation of renal allograft function is beneficial for early anti-rejection treatment. In a prospective study of 145 kidney transplant patients followed for an average of four years, elevated urinary KIM-1 levels were associated with a 5.1-fold increased risk of graft loss [12]. Prediction of graft loss by KIM-1 was independent of donor age, creatinine clearance, and proteinuria. In a retrospective study of non-diabetic proteinuric subjects, anti-proteinuric therapies reduced the urinary excretion of KIM-1, suggesting its use as an efficacy marker [13]. KIM-1 has proven to be an excellent marker of nephrotoxicity in preclinical studies [14]. In our study, 63.2% (12/19) of cases showed strongly positive KIM-1 expression in IA and IB acute T cells mediated rejection subgroup; in the grade II subgroup, 71.4% (5/7) of the cases showed strongly positive KIM-1 expression. KIM-1 expression gradually increased as the Banff 2007 classification level rose in acute cellular rejection. In the 27 cases of acute T cell mediated rejection, KIM-1 was highly expressed in four cases with no tubular inflammation, which suggests that damage to renal tubular epithelial cells occurred before the morphological change appeared. The number of cases of strongly positive KIM-1 expression increased when the classification grade of tubular inflammation rose. All renal tissues showed strongly positive KIM-1 expression in grade 3+, which suggests that renal tubular epithelium is significantly damaged. In the chronic active antibody mediated rejection group, 88.9% of the cases showed strongly positive expression and total positive expression rate was 100%. In the chronic active T cell mediated rejection group, 80% of the cases exhibited strongly positive expression. All these data strongly suggest that active rejection caused kidney injury and KIM-1 positive expression helps to identify early renal tubular epithelial injury. We also found KIM-1 positive expression in patients with normal biochemical indexes, including 47.1% (8/17) of cases with weak expression and 47.1% (8/17) with strong expression. Therefore, increased KIM-1 expression in kidney tissue occurs earlier than changes in blood biochemical indexes and better reflects renal tubular epithelial cell damage. There was weak KIM-1 expression in three cases when there was no rejection and immunosuppressant toxic injury confirmed by morphology, which suggests increases in KIM-1 expression, can be an earlier marker than the visible morphological change in identifying renal tubular damage. KIM-1 expression was weakly positive in 27.3% of cases (3/11) with normal serum creatinine, which is consistent with the result that KIM-1 expression was detected in 28% of the cases with procedural renal biopsy [9]. Our study also had some limitations. First, 75.4% (52/69) of patients in our study had abnormal renal function, and renal biopsy indicated that KIM-1 expression was positive in most of the total patient pool. Second, we did not compare other related clinical data and did not follow up patients. Third, we did not investigate the effect of treatment on the relationship between KIM-1 and renal function. We also did not assess the effect on renal function after blocking KIM-1 expression. Finally, the sample number may limit the reliability of the research results and increased sample numbers in future studies may be helpful to further define the relationship between KIM-1 expression and renal function. The molecular biological function of KIM-1 is not fully understood, although evidence indicates a role for its involvement in renal tubular cells at early stages of damage and repair, in adhesion and immune reaction processes, and in renal fibrosis [6, 11, 12, 16]. Most patients had varying levels of renal dysfunction, and a renal biopsy was performed. Most renal tissues exhibited high levels of KIM-1 expression and rejection injury was the primary cause. Thus, KIM-1 may be involved in the renal allograft rejection process. The role of KIM-1 in graft immune injury or downstream molecular events needs further research. It has been shown that KIM-1 is mainly expressed and increased in tubular interstitial inflammation and fibrosis induced atrophy. The extracellular domain of KIM-1 is short, which includes a conserved tyrosine phosphorylation site used for signal transduction mediated by receptor and ligand interactions. The extracellular domain of KIM-1 can be cleaved into soluble portions by matrix metalloproteases and released into the urine [15]. Additionally, urinary KIM-1 expression is also related to declines in allograft function [9, 16]. Therefore, while our study supports the conclusion that urinary KIM-1 amount may be an early, non-invasive, specific, sensitive biochemical indicator to identify renal tubular epithelial injury, further investigation is needed to better understand the function of KIM-1 and more completely validate its use as a biomarker.

CONCLUSION

KIM-1 expression level is positively correlated with renal allograft damage and tubular cell injury.

KIM-1 expression in renal allograft tissue is more sensitive than rises in serum creatinine or morphological changes in the assessment of renal tubular epithelial injury. Increased KIM-1 expression in allograft tissue occurs earlier than changes in blood biochemical indexes and better reflects renal tubular epithelial cell damage. KIM-1 expression is an early, sensitive, and specific biomarker to determine renal tubular epithelial cell injury in renal allograft tissue.

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DECLARATION OF INTEREST

The authors report no conflicts of interest.

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