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NEPHROLOGY - ORIGINAL PAPER

Kidney injury molecule-1, a sensitive and specific marker for identifying acute proximal tubular injury, can be used to predict renal functional recovery in native renal biopsies

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Abstract
Kidney injury molecule-1 (KIM-1) staining has been shown to be very useful in identifying acute proximal tubular injury, but its sensitivity, specificity and predicting values for the recovery of renal function after injury in renal biopsies have not been well established. In the first study, we randomly selected 184 renal biopsies from a wide age range of patients (children to elderly) with various renal diseases. KIM-1 staining scores were significantly correlated with serum creatinine (sCr) levels ($P < 0.05$) in all age groups. Receiver-operating characteristic curve (ROC) was generated to evaluate true-positive rate (sensitivity) and true-negative rate (1-specificity). The area under the curve (AUC) in pediatric cases was 0.74, which demonstrated KIM-1 was a fair index in correlating with sCr. In adults, the AUC was 0.87, indicating that KIM-1 was an even better index in the adult population in correlating to sCr. The second study was to determine whether KIM-1 could be a potential predictor of the recovery of acute kidney injury (AKI), and 51 indicated native biopsies with acute tubular injury were randomly selected for KIM-1 staining and sCr follow-up over a 6-month period. A higher KIM-1/sCr ratio (0.57 ± 0.06) was significantly and positively associated with a better reduction in sCr over 6 months. In summary, our data demonstrated that KIM-1 staining in renal biopsies is a sensitive and specific marker to identify acute tubular injury and KIM-1/sCr ratio is useful for predicting the recovery of renal function after injury, although some patients' sCr levels cannot return to their baseline levels.

Keywords Kidney injury molecule-1 · Acute kidney injury · Renal biopsy

Introduction

It has been well known that proximal tubules are vulnerable to ischemic and toxic injury, due to their high metabolic activity to carry out active transportation of electrolytes and small molecules. Since proximal tubules are located at the middle portion of the nephron, injury at the upstream glomerular level and/or obstruction at distal nephron level all can result in damage to the proximal tubules. Conventionally, periodic acid-Schiff (PAS) staining has been used to detect the loss of brush borders on proximal tubules as an index for assessing acute tubular injury. Using PAS staining to evaluate acute tubular injury has been reported in human biopsies or animal models [1–4]. Based on the preservation of brush borders on proximal tubules, we previously established a PAS score system to evaluate proximal tubule injury, in which we found a significant correlation between PAS scores and serum creatinine levels (sCr) in native renal biopsies [5].

Kidney injury molecule-1 (KIM-1) is a transmembranous protein on the surface of proximal tubular epithelial cells, which is not expressed in normal kidney. We found that there is an up-regulation of KIM-1 in some native and transplant kidney biopsies with acute tubular injury [6, 7]. Subsequently, it has been shown that KIM-1 mediates phagocytosis of apoptotic cell debris to protect the kidney

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from acute injury by down-regulation of innate immunity and inflammation [8–10]. The sensitivity and specificity of KIM-1 staining were 79% and 53%, respectively, in the autopsy kidneys with acute tubular injury based on our recent study [11]. However, its sensitivity and specificity for evaluating native renal biopsies and how to use KIM-1 staining to evaluate renal functional recovery still remain unresolved. In this investigation, we found that KIM-1 staining was significantly associated with increased sCr levels and this correlation was categorized as "fair" to "good" when plotted on a receiver-operating characteristic (ROC) curve in both pediatric and adult patients. In addition, our previous renal transplant study demonstrated that cases with stronger KIM-1 staining but relatively low sCr had a better recovery in renal function, implying that a higher KIM-1/sCr ratio might be a good index for renal repairing capacity [7]. But this concept using KIM-1/sCr ratio has not been tested in the native kidney biopsies previously. In the current study, we found that higher PAS/sCr ratio and KIM-1/sCr ratio were both significantly and positively associated with a better renal functional recovery in native kidneys, implying that acute injury in proximal tubules would carry a better chance for renal functional improvement.

Materials and methods

The protocol for this retrospective study in archival renal biopsies was approved by the Institutional Research Board of Beaumont Health System, Royal Oak, MI. All renal biopsies were randomly selected and the only exclusion criterion was advanced kidney diseases with severe interstitial fibrosis and tubular atrophy, as the goal of study was to evaluate acute proximal tubular injury.

The sensitivity and specificity of KIM-1 staining scores according to sCr levels (study #1)

Pediatric patients

In total, 63 renal biopsies from a pediatric population (defined as age younger than 20 years as we received this population biopsies sent most by pediatric nephrologists) were included, since no previous study has tested the KIM-1 expression in renal biopsies from pediatric patients. Their renal biopsy samples were stained for human KIM-1 and their sCr were identified from clinical charts.

Adult patients

Adult renal biopsies were further divided into two groups based on patients' age. Patients with ages ranging from 20 to 55 years were arbitrarily included in the adult group, while the patients older than 55 years were arbitrarily listed in the senior adult group, to balance the percentage of patient population along with reasonable age stratifications. KIM-1 staining was performed and sCr before the biopsies were obtained from their medical records.

The PAS/sCr ratio and KIM-1/sCr ratio predicting renal functional recovery (study #2)

To determine if KIM-1 staining can be used to predict renal functional outcome, we evaluated some more recent renal biopsies with acute kidney injury and their sCr over time. In the second study, we also used the conventional marker, PAS, to access acute tubules injury (ATI) together with KIM-1. A total of 81 renal specimens including a normal control group (30 cases normal renal parenchyma away from renal tumors following nephrectomy) and a study group with 51 renal biopsies from various acute renal diseases were randomly selected. Samples with severe interstitial fibrosis or tubular atrophy were excluded from this study.

When injured proximal tubules receive repetitive challenges over time, unrepairable proximal tubules become atrophic, characterized by shrunken tubules to less than 1/3 size of normal proximal tubules with no more brush borders, closed lumina and thickened tubular basement membranes. Interstitial fibrosis then replaces the original space of intact proximal tubules. Facing the process of chronic changes, we observed some positive KIM-1 staining in the atrophic proximal tubules. In these cases, KIM-1 was trapped in these atrophic tubules with persistent positive staining, which was more a marker of chronic changes instead of presenting acute kidney injury. Thus, it will be inaccurate to count KIM-1 staining in these atrophic tubules into acute injury phase. In human biopsies, particularly from senior patients, it was very common to see acute proximal tubular injury on the top of some chronic kidney disease, where microscopically non-atrophic proximal tubules are being intermingled with atrophic proximal tubules. Thus, KIM-1 expression on non-atrophic proximal tubules was best correlated with acutely damaged proximal tubules and elevated sCr. Therefore, evaluating the staining intensity of KIM-1 in non-atrophic proximal tubules was more accurate than calculating the total KIM-1 expression in areas with both non-atrophic and atrophic proximal tubules. In current study, all biopsies were stained for KIM-1 and its intensity in human renal biopsies along the luminal surface of non-atrophic proximal tubular epithelial cells was evaluated, using similar evaluation method to our previous studies [7, 11].

Kidney histology and immunohistochemical assessment of KIM-1 expression

Human kidney biopsy cores were fixed in formalin and paraffin embedded. Four-micron sections were dried for 60 min

at 60 °C. Slides were then de-waxed in 3 xylene baths for 3 min each, 3 100% alcohol baths 3 min each followed by 30 s of running water. Antigen retrieval was carried out in a Tris EDTA Buffer at pH 8.0 for 20 min at 99 °C, followed by a 20 min cool down at room temperature, and then a quick water rinse. Slides were then placed in 3% H_2O_2 for 15 min followed by a quick water rinse and then placed in Tris buffer pH 7.6. Slides were then stained in a programmed Dako Autostainer (DakoCytomation, Carpinteria, CA) using a Thermo Scientific UltraVision LP Detection System (Kalamazoo, MI). The program consisted of 5 min Ultra V block, 30 min incubation with a mouse monoclonal antibody (AKG7 at 1:8 dilution) directed against the ectodomain of human KIM-1 (from Dr. Joseph V. Bonventre's lab, Brigham and Women's Hospital, Boston, MA) [7], with 8 min Primary Antibody Enhancer, 10 min HRP polymer (equivalent to secondary antibody) and 5 min of the chromagen DAB to achieve a brown KIM-1 stain in the proximal tubules.

PAS staining and dual staining of PAS on top of KIM-1 staining

De-paraffined sections were stained for PAS using Dako Artisan PAS Staining Kit (DakoCytomation, Carpinteria, CA). The steps included 12 min in periodic acid solution, twice washes, 12 min in Schiff's reagent, 5 washes, 12 min of washes, 12 min in Meyer's hematoxylin solution, 5 washes and 12 min in bluing agent before coverslipping. Dual-PAS staining was achieved by following above PAS staining procedures on top of the KIM-1-stained sections.

Quantitation of immunohistochemical staining of KIM-1 and PAS staining

KIM-1 staining intensity scores in non-atrophic proximal tubules were graded as from 0 to 3+ (0, no staining; ±[0.5], focal weak fine granular staining; 1+, weak fine granular staining along the complete luminal surface; 2+, moderate granular staining; and 3+, strong large granular staining) [7, 11]. The degree of renal tubular brush border based on PAS staining was as follows: (0) for intact brush borders (no injury), (1+) for scattered loss and minimally diminished brush borders (mild injury), (2+) for diffuse loss with moderately diminished brush borders (moderate injury), and (3+) for total loss of brush borders (severe injury) as reported before [5]. PAS scores, KIM-1 scores, and sCr levels were statistically evaluated by linear regression analysis.

Statistics

Results were expressed as the mean ± SEM. Data of three groups were compared using one-way analysis of variants (ANOVA) (StatView program). Receiver-operating characteristic (ROC) curve was generated using SAS 9.4 (Cary, NC, USA) to compare sensitivity and specificity across a range of sCr levels for the ability of sCr values to predict KIM-1 score. Pearson correlation between PAS/sCr and KIM-1/sCr to changes in sCr over 6 months was assessed (StatView program). *P* value less than 0.05 is considered significantly different.

Results

To evaluate the sensitivity and specificity of KIM-1 staining in association with sCr levels (study #1)

In renal biopsies, KIM-1 expression was absent in proximal tubules when no acute tubular injury occurred (Fig. 1a). In response to various injuries such as inflammation, nephrotoxic or ischemic, the proximal tubules showed KIM-1 expression at the luminal surface when an injury was mild to moderate (Fig. 1b, c), while an injury became severe, the KIM-1 expression extended to the lateral aspects of proximal tubules (Fig. 1d). Table 1 lists clinical and pathologic indices of all pediatric and adult cases, such as acute tubular necrosis (ATN), minimal change disease/focal segmental glomerulosclerosis (MCD/FSGS), C1q nephropathy, lupus nephritis, membranous glomerulopathy (MGN), membranoproliferative glomerulonephritis (MPGN), IgA nephropathy, IgM nephropathy, crescentic glomerulopathy (CGN), thrombotic microangiopathy (TMA), oxalate calcium nephropathy and monoclonal light-chain nephropathy. The diversity of clinical presentation and pathologic indices of pediatric, adult and senior adult cases was shown in Fig. 2a. There was a higher percentage of minimal change disease and focal segmental glomerulosclerosis in pediatric kidney biopsies (Fig. 2b), while a higher percentage of lupus nephritis and HIV/collapsing was noted in adult cases (Fig. 2c), and a higher percentage of primary crescentic glomerulonephritis and monoclonal light-chain nephropathy cases was seen in senior adult kidney biopsies (Fig. 2d). Compared to adult and senior adult groups, both sCr and the expression of KIM-1 were lower in the pediatric group. Only a few cases among the pediatric (9%) and adult (17%) groups had negative KIM-1 expression with elevated sCr (Table 1). All five cases from the old adult group with negative KIM-1 staining were found to have elevated sCr. We speculate that the patients with negative KIM-1 expression and elevated sCr levels may be explained by other causes such as either pre-renal or post-renal injuries. Interestingly, the pediatric group had a high KIM-1/sCr ratio (1.272 ± 0.138), while both adult group and senior adult group had relatively low KIM-1/sCr ratio (0.649 ± 0.095 and 0.551 ± 0.058, respectively) (Table 1).

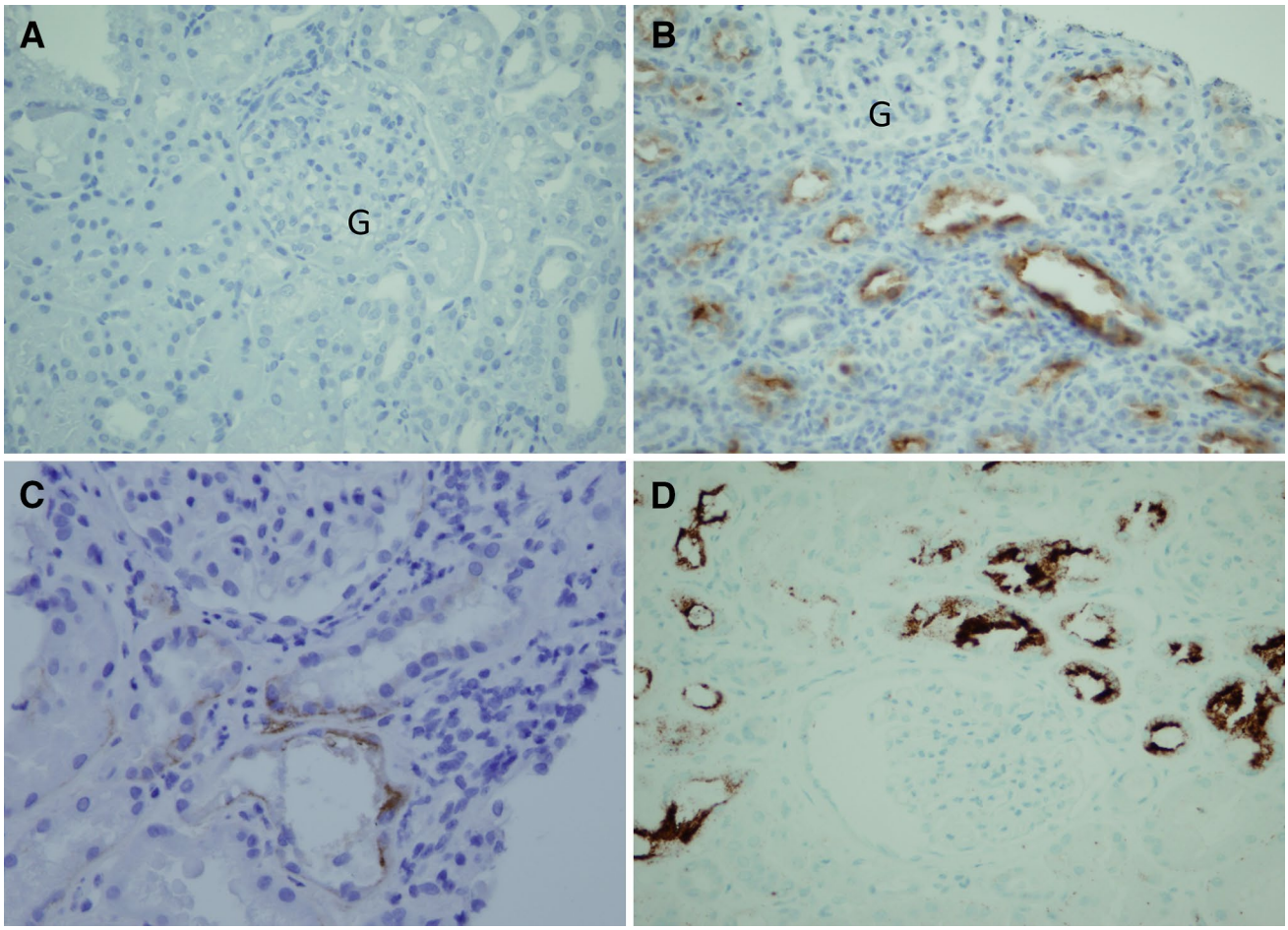

Fig. 1 KIM-1 in pediatric renal biopsies. **a** No expression of KIM-1 in an 8-year-old girl with C1q nephropathy and serum creatinine level at 0.5 mg/dL. **b** KIM-1 was specifically expressed on the injured proximal tubules (brown). A strong expression of KIM-1 (2+) in proximal tubules from a 12-year-old boy who had interstitial nephritis and serum creatinine level at 2.4 mg/dL. **c** KIM-1 was 1+ positive in proximal tubule containing calcium oxalate in 60-year-old man with calcium oxalate nephropathy with sCr at 2.4 mg/dl. **d** KIM-1 showed 3+ positive staining in proximal tubules in a 57-year-old woman with thrombotic microangiopathy with sCr at 1.9 mg/dl. (magnifications ×400 in **a**–**d**)

When combining the adult group and senior adult groups together, the sensitivity was 83% when the concurrent specificity was at 53%. Figure 3 depicts the ROC curve for adults, with area under curve (AUC) of 0.87, which falls into the "good" category. In pediatric group, when the specificity of KIM-1 increased from 43% to 57%, its sensitivity dropped from 82 to 71%. As sCr levels in pediatric group may not as stable as in adults, the AUC of the ROC curve for pediatric group was lower at 0.74, which was considered as a "fair" category. There was no significant difference between the pediatric and adult ROC curves ($P = 0.99$). When linear aggression analysis was applied, all three groups demonstrated significantly linear association between KIM-1 expression and sCr (Table 2).

To evaluate whether higher PAS/sCr ratio and KIM-1/sCr ratio were associated with better renal functional recovery over time (study #2)

Mild acute tubular injury showed subtle morphology in proximal tubules, including mildly distorted tubular structures and mildly diminished brush borders (1+) on periodic acid-Schiff (PAS)-stained sections. Moderately acute proximal tubular injury was easy to identify microscopically, characterized by dilated lumina, flattened epithelial cells with obviously diminished brush borders (2+), and some luminal epithelial cell casts. Severe acute tubular injury revealed features of significantly flattened epithelial cells with scattered drop out of epithelial nuclei and

Table 1 Clinical and pathologic indices of pediatric, adult and senior adult cases

	Pediatric (< 20 years)	Adults (21–54 years)	Senior adults (> 55 years)
Total number	63	64	57
Primary ATN	3	7	8
Interstitial nephritis	2	0	1
Oxalate Ca^{2+} nephropathy	0	1	3
C1q nephropathy	4	0	0
Lupus nephritis	2	12	2
MCD/FSGS	34	7	7
MGN	0	6	2
MPGN	4	3	3
IgM nephropathy	2	0	1
IgA nephropathy	7	5	3
CGN	1	6	13
TMA	2	5	6
HIV/collapsing	2	9	0
LC nephropathy	0	3	8
Age (years)	12.6 ± 0.5	38.3 ± 1.3*	69.5 ± 1.0*#
N of KIM-1 positive	82% (52/63)	80% (51/64)	91% (52/57)
N of KIM-1 negative	17% (11/63)	20% (23/64)	9% (5/57)
Mean KIM-1 levels (AU)	1.37 ± 0.129	1.44 ± 0.138	1.83 ± 0.143*#
Mean serum creatinine (mg/dl)	1.37 ± 0.193	3.54 ± 0.446*	4.08 ± 0.363*
KIM-1/sCr ratio	1.27 ± 0.138	0.65 ± 0.095	0.55 ± 0.058*
Number of negative KIM-1 biopsies with elevated sCr	1/11 (9%)	4/23 (17%)	5/5 (100%)
	IgA nephropathy	1 "AKI"	1 "AKI"
		2 MPGN	3 MCD/FSGS
		1 IgA nephropathy	1 IgM nephropathy

ATN acute tubular necrosis, *MCD/FSGS* minimal change disease/focal segmental sclerosis, *MGN* membranous glomerulopathy, *MPGN* membranoproliferative glomerulonephritis, *CGN* crescentic glomerulopathy, *TMA* thrombotic microangiopathy, *LC nephropathy* monoclonal light-chain nephropathy

*$P < 0.05$ vs pediatric biopsies and #< 0.05 vs adult biopsies

disappearance of brush borders in proximal tubules (3 +) on PAS-stained sections. Mild granular up-regulation of KIM-1 (1 +) along luminal surface was seen in the mildly injured proximal tubules. During moderate acute tubular injury, the staining of KIM-1 became stronger and granular up-regulation of this protein became diffuse along the surface of proximal tubules (2 +). In cases of a severe acute tubular injury, up-regulation of KIM-1 was intense and extended from the luminal surface to the lateral aspect of the tubular epithelial junctions (3 +). While uninjured proximal tubules showed intact brush borders on routinely PAS-stained section, KIM-1 staining positivity reversely correlated with diminished brush borders on the PAS-stained sections. Figure 4a showed a non-injured control biopsy with intact brush borders on PAS-stained section, while Fig. 4b–d revealed renal biopsies with acute tubular injury characterized by opposite changes between PAS (diminished brush borders in some proximal tubules) and KIM-1 (graduate increase in KIM-1 intensity along PAS absent areas from mild to severe extents). It was noted that positive KIM-1 staining could be patchy in the injured proximal tubules.

In this study, the patients in the control group and in the study group had a similar range of ages (Table 3). Significantly, higher scores of PAS and KIM-1, and higher level of sCr were found in the study group than the control groups as expected (Table 3). When control and study cases were calculated together, both PAS and KIM-1 were significantly and positively correlated with sCr levels. In addition, there was a significant correlation between KIM-1 and PAS scores ($r = 0.780$, $P = 0.0001$). The study group data are listed in Table 4. Many cases (21/51) were diagnosed with acute tubular injury (ATN) while remaining diagnoses ranged from immune complex/complement-mediated renal diseases, thrombotic microangiopathy, variants of monoclonal light-chain-associated renal injury to interstitial nephritis. The data indicate that acute injury in proximal tubules may result from either primary acute tubular injury (so called acute tubular necrosis) or from other renal diseases as listed in Table 4. During the follow-up, 6 patients out of 51

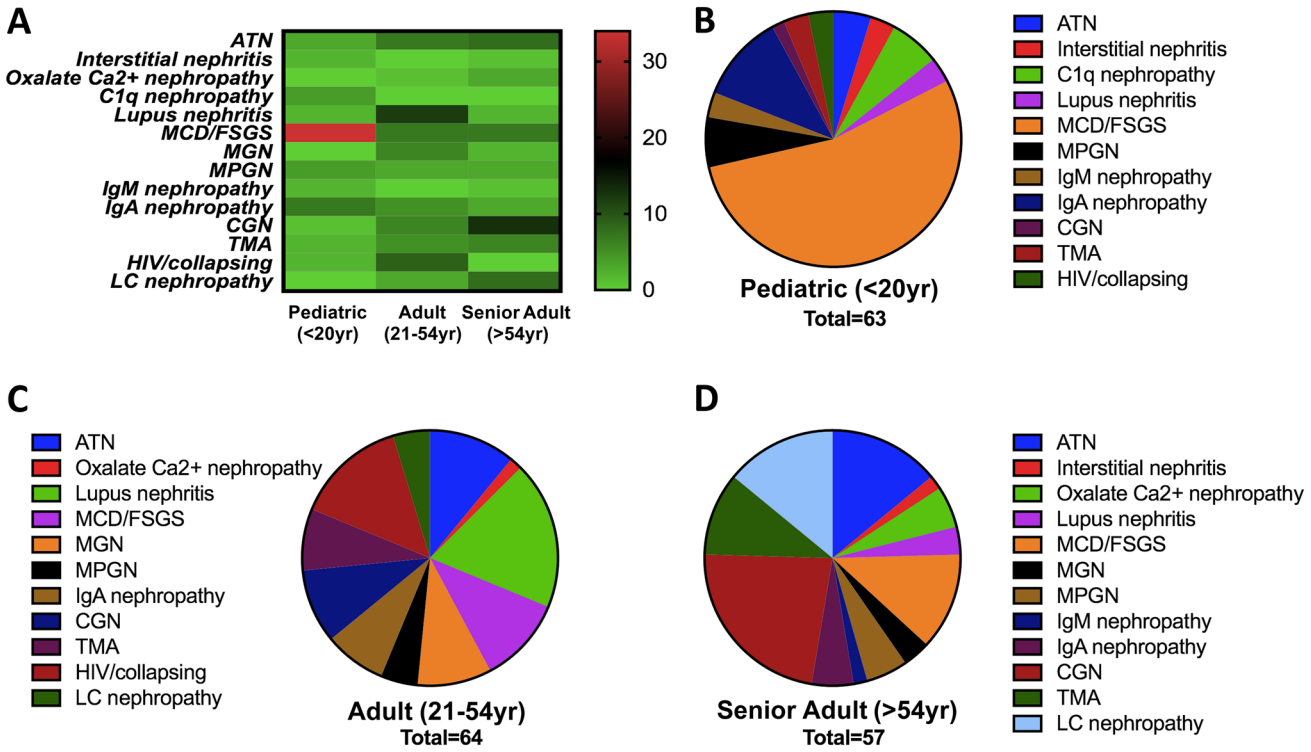

Fig. 2 The diversity of clinic presentation and pathologic indices of pediatric, adult and senior adult cases. **a** Renal biopsy shows the various renal diseases in pediatric, adult and senior adult. **b** A higher percentage of minimal change disease and focal segmental glomerulosclerosis was present in pediatric kidney biopsies. **c** A higher percentage of lupus nephritis and HIV/collapsing was shown in adult cases. **d** A higher percentage of primary crescentic glomerulonephritis and monoclonal light-chain nephropathy cases were seen in senior adult kidney biopsies

patients were found diseased. Over the next 6 months after the initial biopsies, mean sCr levels gradually declined. For the study cases, both higher PAS/sCr ratio and KIM-1/sCr ratio were significantly and positively correlated with the reduction of sCr over 1 month and 6 months (Table 5).

Discussion

Although several studies report that the KIM-1 is upregulated in acutely injured renal tubules of human biopsies [6, 12], the correlation of the sensitivity or specificity of KIM-1 staining with renal dysfunction has not been substantially established in native renal biopsies using ROC curve. The AUC of ROC curve from adult groups in the current study was 0.87, which indicated that KIM-1 staining is a "good" predictor of acute renal tubes injury in correlating with sCr. According to our knowledge, this is the first time that KIM-1 immuno-staining has been effectively used to detect acute tubular injury in pediatric population. Pediatric muscle mass is not stable as adults, therefore, their sCr levels may be difficult to determine at one number across younger and "older" pediatric patients. In addition, the total number of pediatric group in this study was lower than adult cases in the first cohort. The pediatric AUC of ROC curve was found to be 0.74, which was still categorized as "fair" level. The sensitivity of KIM-1 in correlating with sCr was 83% in the adult renal biopsies, when the specificity of KIM-1 in adult renal biopsies was matched to the 53% in our recent autopsy study (the sensitivity at 79% in the autopsy study) [11]. Based on the current ROC curve for adults, the KIM-1 sensitivity still could achieve 57% with a given specificity of 95%. Although the proximal tubules appear to be a common site leading to renal failure [13, 14], other factors such as pre-renal cause and injury causing increase in sCr at non-proximal tubular level (vessels, glomeruli, and distal tubules) [15, 16] may all affect the KIM-1 specificity in correlating with sCr. Alternatively, some mild primary glomerular diseases including the IgA nephropathy, and minimal change disease/focal segmental glomerulosclerosis (MCD/FSGS) could not present with the manifestation of increase on creatinine.

Another major finding in the study is that there was close correlation between conventionally diminished brush borders in the injured proximal tubules on PAS-stained sections and upregulated KIM-1 staining, which both are significantly associated with sCr levels. The villi of proximal tubules play roles in increasing surface of

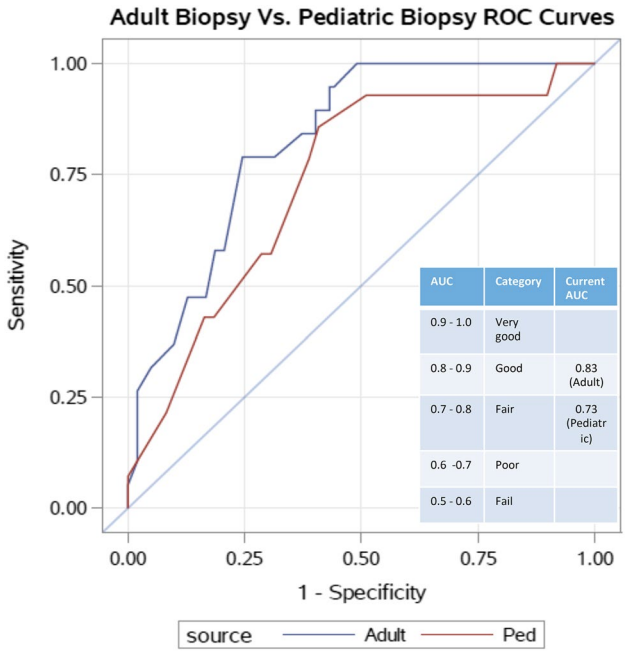

Fig. 3 Receiver-operative characteristics (ROC) curves with analysis of area under the curve (AUC) for the performance of KIM-1 staining based on a range of serum creatinine levels in both pediatric and adult groups. The adult group had an AUC at 0.83 (good category) and the pediatric group had an AUC at 0.73 (fair category)

reabsorption and serve as the first line of physical defense of luminal surface. During acute tubular injury, brush borders are usually damaged and characterized with shortening [5], while the transmembranous glycoprotein KIM-1 is upregulated along the luminal surface line with remarkable loss of villi (Fig. 4). From mild to severe acute tubular injury, paradoxical changes between reduced brush borders on PAS-stained sections and upregulated KIM-1 in the injured proximal tubules depicts the damage of proximal tubular structure have being repaired by KIM-1, as KIM-1 carries a phagocytic capacity of cleaning epithelial debris to reduce the inflammatory reactions caused by antigen exposure [10, 17]. As PAS staining is a conventional method to detect proximal tubular injury [5] and KIM-1 is more specific for proximal tubular injury [7], the significant correlation between the two markers further mutually validates the role of both markers in identifying acute tubular injury at the proximal tubular site.

The third important finding in the current study is that both higher PAS/sCr and KIM-1/sCr ratios were significantly and positively linked to renal functional recovery over a 6-month period in native kidneys with various renal diseases. We speculate that a higher either KIM-1/sCr ratio or PAS/sCr ratio imply a stronger capacity of proximal tubular repair during acute injury, possibly partially related to the phagocytosis function of KIM-1 during the reparative processes. These current findings were consistent with our previous finding in transplant recipients that graft functional recovery was better in the biopsies with higher KIM-1 scores and lower sCr levels over 1½-year period [7]. This implied that acutely injured proximal tubules are capable to generate a robust KIM-1 response as an index for active repairing response [7]. In the first cohort, the pediatric group had significantly higher KIM-1/sCr ratio than adults groups (Table 1), implying that pediatric kidneys might have a general overall better capacity for recovery when compared to adult kidneys following an acute kidney injury. Thus, a high KIM-1/sCr ratio could be a potential reparative and

Table 2 Linear regression analysis between serum creatinine (sCr, *Y* axis) and kidney injury molecule-1 (KIM-1, *X* axis) in adult and pediatric patients with renal biopsies

	Children	Adults	Senior adults
n	63	64	57
R	0.48	0.48	0.27
R^2	0.23	0.23	0.07
Intercept and slope (beta coefficient)	0.37 and 0.73	1.28 and 1.57	2.84 and 0.68
Standard error (beta coefficient)	0.16	0.36	0.33
Odds ratio	1.39	3.52	4.22
95% confidence interval	1.03–1.72	2.75–4.32	3.37–4.79
P	0.0001*	0.0001*	0.0444*

n number using pediatric cases as an example; R, the correlation coefficient indicated 0.48 linear association between KIM-1 and sCr; R^2 the coefficient of determination (0.23 or 23.4%) implied that 23.4% of the variability in KIM-1 can be accounted for by sCr; intercept and slope—mathematical constants, formulated an equation between KIM-1 (X) and sCR (Y) as for rodent model: Y (sCr) = 0.37 + 0.73 X (KIM-1); standard error, this estimated precision of the coefficient for KIM-1; odds ratio, for every unit increase in KIM-1, the odds of high sCr increased by 1.39 in pediatric group as an example; 95% confidence interval, the "true" odds ratio was likely to fall between lower 95% and upper 95%

*Statistic significance = $P < 0.05$—KIM-1 was a statistical significant predictor of high sCr in four scenarios

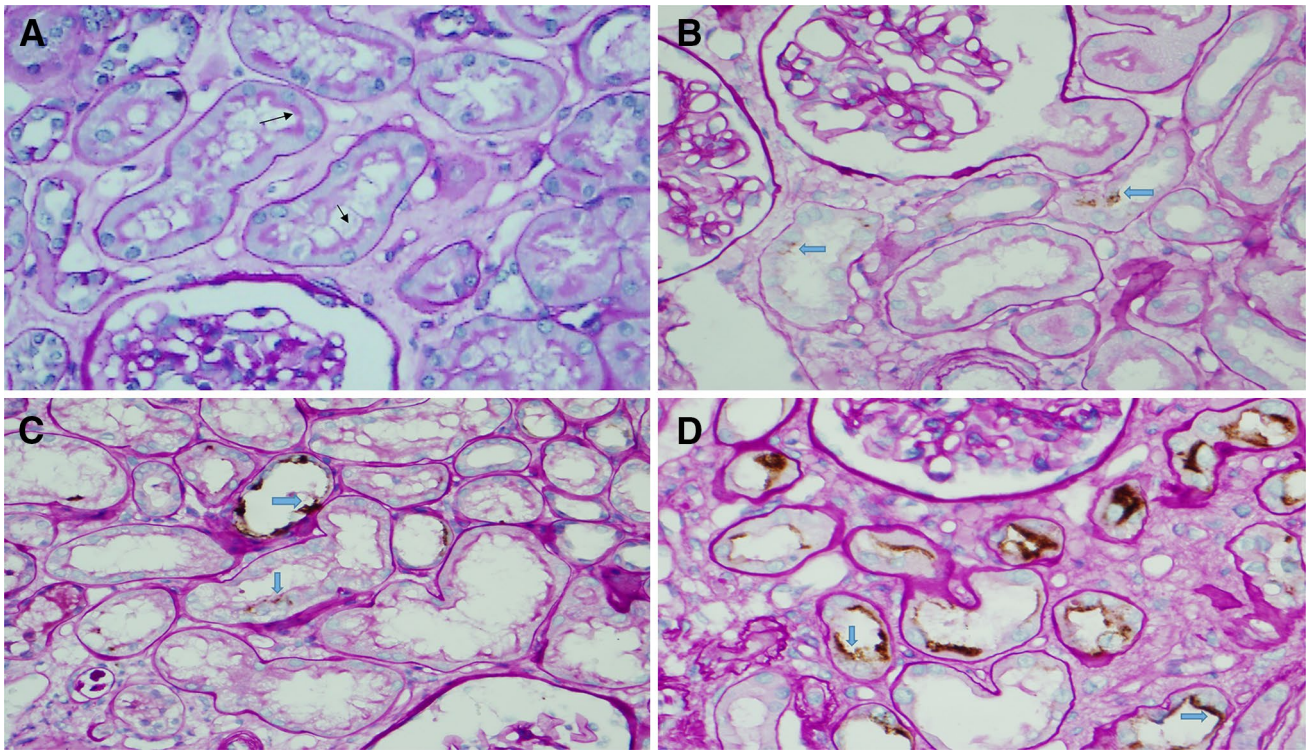

Fig. 4 The reversed expressions of PAS and KIM-1 in adult renal biopsies. **a** Intact pink brush borders (black arrows) were present in non-injured proximal tubules with PAS staining, in the absence of KIM-1 staining, and **b–d** a diminished PAS pink staining with defective brush borders was shown in proximal tubules from 1+ in (**b**), 2+ in (**c**) and 3+ in (**d**), accompanied with increased positive KIM-1 staining (brown color indicated by blue arrows) from 1+ in (**b**), 2+ and 3+ in (**d**). (Magnifications × 600 in **a**–**d**)

Table 3 Baseline levels of PAS, KIM-1, and serum creatinine (sCr)

	Control (*n*)	AKI group (*n*)
Age (years)	61 ± 3 (30)	62 ± 2 (51) NS
PAS scores (AU)	0.01 ± 0.06 (30)	2.22 ± 0.09 (51)*
KIM-1 scores (AU)	0.01 ± 0.04 (30)	1.97 ± 0.11 (51)*
Pre-biopsy sCr (mg/dl)	0.99 ± 0.06 (30)	5.12 ± 0.50 (51)*

*$p < 0.05$ versus control values

PAS periodic acid-Schiff, *KIM-1* kidney injury molecule-1, *sCr* serum creatinine levels

regeneration predictor in the early phase of AKI for accelerating the recovery of renal function after injury.

In addition to primary acute tubular injury (also called acute tubular necrosis) in proximal tubules, secondary acute tubular injury seen in other glomerular, vascular and tubulointerstitial diseases appears to be a "known" phenomenon in the nephropathology field. In this study, we took advantage of KIM-1 being a specific injury marker for proximal tubules and evaluated potential injury mechanisms for the proximal tubules. KIM-1 has been shown to be upregulated in response to many various insults, such as acute cellular rejection, type 1 acute antibody-mediated rejection, Tacrolimus or by direct deposits of toxic substances, such as iron from broken sickled red blood cells in renal grafts [7, 18–20]. In this study, we also demonstrated that acute insults could result in up-regulation of KIM-1 in proximal tubules from native kidney biopsies in pediatric and adult populations with a large range of pathologic entities. Most of the cases with glomerulonephritis, located upstream of proximal tubules, were associated with more or less injury in the proximal tubules, characterized by up-regulation of KIM-1. We speculate that proliferative glomeruli lesions may not only result in ischemia directly to the proximal tubules but also damage proximal tubules by dumping large amounts of proteins and products of red blood cells or white cells debris. In cases of thrombotic microangiopathy, a direct ischemic insult to the glomeruli may damage to proximal tubules, confirmed by upregulated KIM-1 staining. It is intriguing how the distal tubular injury could cause the damage of proximal tubules. One of our previous studies shows that in monoclonal cast nephropathies, the direct obstruction by monoclonal casts in monoclonal cast nephropathy had concurrent monoclonal proximal tubulopathy, leading to acute renal failure [5]. Certainly, the obstruction of distal tubules by either calcium or uric acid precipitations at the distal end may be enough to cause the damage of proximal

Table 4 Expression of PAS and KIM-1 and serum creatinine (sCr) over 6 months

Study group	Age/G	Diagnosis	PAS score	KIM-1 scores	Biopsy sCr (mg/dL)	1 month sCr (mg/dL)	6 months sCr (mg/dL)
1	20 M	C3GN	1	0.5	1.5	n/a	1.16
2	64 M	ATN	1	1.0	1.55	1.27	1.46
3	45 F	Lupus nephritis, type 4	1	3	1.34	0.89	0.96
4	45 M	Infectious GN	1	2	0.78	0.81	1.25
5	59 F	Lupus nephritis, type 5	2	1	1.9	1.09	n/a
6	80 M	ATN	2	2	2.0	1.32	1.27
7	69 M	ATN	3	2	2.7	3.44	Died
8	7 0 M	ATN	2	2	2.5	1.59	n/a
9	83 F	ATN	1	0.5	2.9	3.35	8.52
10	84 F	ATN	3	2	3.79	1.92	n/a
11	55 F	TMA	2	1	3.76	3.30	1.47
12	48 F	Malignant hypertension	2	2	3.53	1.29	1.03
13	79 F	Crescentic GN	3	3	2.2	2.65	1.05
14	82 M	Crescentic GN	2	2	3.8	1.81	1.19
15	57 M	LCDD, kappa type	2	3	3.8	3.6	2.64
16	80 M	LCDD, kappa type	2	2	2.5	2.58	2.22
17	65 F	ATN	1	0	5.0	1.62	3.06
18	65 M	C3GN	3	2	9.09	3.37	n/a
19	59 M	ATN, Ca^{2+} oxalate	3	2	6.4	4.94	4.12
20	67 M	ATN	2	2	5.6	3.47	0.9
21	73 F	ATN	2	2	5.3	2.54	0.75
22	60 M	ATN	2	1	15	4.47	n/a
23	59 M	Cast nephropathy	3	3	9.03	9.05	4.14
24	64 M	MPGN	3	3	6.83	4.81	Died
25	65 M	Proximal tubulopathy	2	3	4.9	5.41	2.56
26	58 F	ATN	2	2	6.7	1.28	0.78
27	79 M	Cast nephropathy	3	2	8.14	7.63	Died
28	63 F	Cast nephropathy	3	3	4.79	5.09	3.35
29	35 M	Malignant hypertension	2	2	4.19	4.18	5.16
30	48 M	ATN	2	3	12.5	9.04	7.12
31	57 F	TMA	3	2	1.9	1.33	1.47
32	50 F	TMA	3	2	7.3	4.16	4.47
33	78 F	TMA	3	2	2.83	3.16	2.18
34	70 M	TMA	2	2	4.0	n/a	n/a
35	38 M	MCD	2	1	2.35	0.89	0.76
36	69 M	ATN	3	2	2.7	Died	n/a
37	80 M	ATN	2	2	2	1.32	1.27
38	65 M	ATN	1	0.5	5	Died	n/a
39	61 M	ATN	3	2	11.3	n/a	n/a
40	65 M	ATN, Ca^{2+} oxalate	3	3	6.4	4.66	4.20
41	67 M	Interstitial nephritis	2	2	5.6	1.26	0.74
42	73 F	ATN	2	2	5.3	0.78	1.65
43	60 M	ATN	2	1	15	11.4	1.34
44	64 F	C3GN	2	2	10	Died	n/a
45	68 F	ATN	3	3	3	Died	n/a
46	43 F	Interstitial nephritis	3	3	1.35	0.87	0.67
47	26 M	ATN	2	1	13.2	1.31	1.17
48	51 M	ATN	2	1	2.4	1.38	1.03
49	48 M	ATN	2	3	6.2	2.30	1.35
50	73 F	ATN	2	2	6.12	2.51	0.92
51	86 M	ATN	3	2	3.15	n/a	n/a

G gender, *F* female, *M* male, *n/a* not available, *ATN* acute tubular necrosis, *GN* glomerulopathy, *Ca^{2+} oxalate* calcium oxalate nephropathy, *C3GN* C3 dominant glomerulopathy, *MPGN* membranoproliferative glomerulonephritis, *LCDD* kappa, light-chain deposition disease, kappa type

Table 5 Correlation between either PAS/sCr ratio or KIM-1/sCr ratio and serum creatinine recovery over 1 or 6 months

	N = 43	*N* = 36
Baseline sCr (mg/dl) (*N* = 44 with follow-up sCr) 5.05 ± 0.54	1 month sCr (mg/dl)	6 months sCr (mg/dl)
	3.15 ± 0.37	2.20 ± 0.31
	$P = 0.0049^{\#}$	$P = 0.0001^{\#}$
	Delta of 1 m sCr (mg/dl)	Delta of 6 m sCr (mg/dl)
	1.98 ± 0.41	2.58 ± 0.57
Correlation between PAS/sCr (0.632 ± 0.064) and change in sCr over time	*R* = 0.52	*R* = 0.47
	P = 0.0004*	*P* = 0.0036*
Correlation between KIM-1/sCr (0.617 ± 0.084) and change in sCr over time	*R* = 0.44	*R* = 0.40
	P = 0.0029*	*P* = 0.015*

PAS periodic acid-Schiff, *KIM-1* kidney injury molecule-1, *sCr* serum creatinine

*$P < 0.05$ for correlation

#$P < 0.05$ vs baseline level of sCr

tubules [21]. KIM-1, also named T cell immunoglobulin and mucin-domain-containing molecule-1 (TIM-1), has been reported to be present in T cells (particularly Th2 cells) and B cells, potentially involved in autoimmune disorders [22, 23]. However, antibodies against KIM-1 we used do not stain T lymphocytes, other types of inflammatory cells, or any other normal tissues in humans [7]. The ectodomain of KIM-1 is shed into the urine where it can be detected easily in adult and pediatric patients with acute kidney injury [7, 12, 24–26]. In the urine and kidney tissue, KIM-1 is a sensitive and specific marker for kidney injury [6, 26–28]. Recent studies indicate that when proximal tubules fail to be repaired, persistent presence of KIM-1 may be involved in the processes of interstitial fibrosis [29, 30], implying a different role of KIM-1 in chronic kidney disease.

In clinic, serum creatinine has been used widely for the definition of acute kidney injury. It is been well known that the sensitivity and specificity of serum creatinine were not satisfied. Some primary glomerular disease and renal tubular disease may not present with increasing serum creatinine at the early stage. KIM-1 staining in renal biopsy could be a sensitive and specific biomarker for the acute tubular injury (primary or secondary to glomerular diseases), as well as a predictor of the capacity of renal functional recovery. However, the limitations of the studies may include twofold. The monoclonal antibody against KIM-1 we used is not available on commercial market. Therefore, only limited groups can verify the role of KIM-1 in AKI [6, 12]. Second, there is lack of other biomarkers compatible to KIM-1 for immunohistochemical staining for identifying acute tubular injury, which limits our understanding of acute tubular injury beyond KIM-1 capability. Therefore, the conventional PAS as a traditional marker used in the study was taken as a great advantage to show similar results to that of KIM-1.

In summary, based on the ROC curves, KIM-1 staining can be used as a sensitive marker in identifying acute tubular injury in proximal tubules. Higher PAS/sCr and KIM-1/sCr ratios were both positively associated with better renal function recovery over a 6-month period, indicating the powerful predictive value of using these markers to evaluate acutely injured proximal tubules. Pediatric KIM-1/sCr ratio was significantly higher than that in adults, implying a stronger recovery capacity in young kidneys.

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Compliance with ethical standards

Conflict of interest None of the authors has financial disclosure to claim and there is no conflict of interest among the authors.

Ethical approval All the procedures performed in studies involving human participants (approved by Institutional Research Board of Beaumont Health System, Michigan) were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Human and animal rights No animal work is involved in the study.

Informed consent Not applicable for the retrospective study.

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