1. Introduction

In urological practice, there are many case scenarios that present with partial ureteral obstruction. In such cases, early detection of renal damage caused by obstruction is of paramount importance. Children who are on a follow-up schedule and suffer from renal damage caused by obstruction is of paramount importance. Therefore it has been used in drug development and exposure research, because it is more sensitive to renal damage than serum creatinine. KIM-1 has been proven to be a useful biomarker for the detection of chronic renal failure in clinical and sub-clinical kidney injury. KIM-1 is significantly elevated in obstructive uropathy. Such an elevation might be advantageous in the early diagnosis and subsequent early intervention of cases with partial ureteral obstruction.

Conclusions: Urinary KIM-1 is significantly elevated in obstructive uropathy. Such an elevation might be advantageous in the early diagnosis and subsequent early intervention of cases with partial ureteral obstruction.

Keywords: Biomarker; Injury; Kidney; Kidney injury molecule; Nephropathy; Obstruction; Uropathy

1. Introduction

In urological practice, there are many case scenarios that present with partial ureteral obstruction. In such cases, early detection of renal damage caused by obstruction is of paramount importance. Children who are on a follow-up schedule and suffer from vesicoureteral reflux, posterior urethral valves, and those with partial ureteral obstruction are typical examples. In such cases, progressive deterioration of serum creatinine and radio isotope scanning are late alarming signs of parenchymal damage. Therefore, there is an urgent need for a biomarker which can detect early parenchymal changes in cases with acute kidney injury (AKI).

The kidney injury molecule-1 (KIM-1) is a recently discovered transmembrane protein. It is expressed in dedifferentiated proximal renal tubular epithelial cells and plays a central role in removal of apoptotic debris from the tubular lumen.\(^1\) Furthermore, KIM-1 has been implicated in immune responses that regulate the development of autoimmune and allergic diseases.\(^2\) KIM-1 has been proven to be a useful biomarker for the detection of chronic renal failure in clinical and sub-clinical kidney injury. Therefore it has been used in drug development and exposure research, because it is more sensitive to renal damage than serum creatinine.\(^3\) Recent studies showed that KIM-1 in urine is a very promising biomarker for the detection of AKI, because urinary KIM-1 levels are elevated within hours after the onset of AKI.\(^4,5\)

Reviews of the literature have shown growing evidence that urinary KIM-1 is a reliable marker in toxic and anoxic nephropathy.\(^6-7\) Moreover, it has a potential role in the prediction of long-term renal outcomes.\(^8\) However, data about its value in the early identification of renal damage due to obstructive nephropathy is still lacking both in clinical and experimental trials. In a recent prospective study, urinary KIM-1 and other markers were investigated as noninvasive biomarkers in children with congenital hydronephrosis.\(^9\) However, the small sample size, lack of statistically significant correlations between urinary KIM-1 levels and the degree of obstruction, were potential limitations. Furthermore, there was no histopathological evidence of renal damage and its impact on urinary KIM-1 levels.
The aims of this work were to evaluate urinary KIM-1 as a predictor for early detection of AKI in cases with obstructive nephropathy in an animal model and to correlate urinary KIM-1 with the progress of obstructive nephropathy on a histopathological basis.

2. Materials and methods

2.1. Study population

The current study was conducted on 90 wild-type 10-week-old male Sprague–Dawley rats weighting 250–300 g. They were housed at 4 rats per polycarbonate cage and were placed in a controlled environment, maintained under a 12 hour light-dark cycle, air conditioned at 24°C ± 2°C, and 50%–70% humidity. Food and water were available ad libitum throughout the period of the experiment. Rats were anesthetized with a mixture of ketamine 7.5 mg/kg and diazepam 5 mg/kg. All steps in the experiments were conducted in accordance with guidelines for proper animal-involved studies.

2.2. Study design

Three models that underwent partial ureteral obstruction were induced and subdivided into 3 groups: unilateral obstruction with a normal contralateral kidney as Group 1, with nephrectomy of the contralateral kidney (solitary kidney) as Group 2, and bilateral partial ureteral obstruction as Group 3. Each group was further divided into 2 subgroups: the sham group (10 rats) and the disease group (20 rats). Partial ureteral obstruction was conducted in accordance with a previously published technique.[9] After anesthesia, the animal was fixed in the supine position on the operating table followed by shaving of the abdominal skin and abdominal incision was made to permit access to the right kidney, ureter, and psoas muscle. Then, a 15 mm groove was created in the psoas muscle. Without any dissection of the kidney, the ureter was fi
ced in the muscle groove and thus laid in a tunnel with psoas muscle. Then, a 15 mm groove was created in the right kidney. Then, a 15mm groove was created in the right kidney and psoas muscle. Without any dissection of the kidney, the ureter was

2.3. Measurable outcomes

Serum urea and creatinine concentrations were measured by the Architect c4000 Clinical Chemistry System (Abbott Laboratories Diagnostics, IL). The urinary KIM-1 assay was conducted by ELISA[1,10] using a commercially available kit (Boster Biological Technology Co., Ltd, Rat KIM-1 ELISA, Catalog No. EK0882).

Rats were sacrificed by cervical dislocation after blood and urine sample collections. Harvested kidneys were cut in half and fixed in 10% neutral buffered formalin for staining and then embedded into paraffin blocks. For histological evaluation, embedded tissues were cut into 5 μm sections, rehydrated, rehydrated, and stained with hematoxylin and eosin (H&E). The sections were evaluated under a light microscope by a pathologist (K.A.).

2.4. Statistical analyses

All data are expressed as mean ± standard deviation (SD) unless otherwise specified. Intrgroup and intergroup difference variables were assessed by Descriptive test, Wilcoxon test, Kruskal-Wallis test, and Mann-Whitney test. Differences in parameters between groups were evaluated for multiple comparisons. All p values quoted are two-tailed and the significance is defined as p < 0.05. Statistical analysis was done by SPSS version 20.

3. Results

3.1. Changes of serum creatinine, blood urea nitrogen, and urinary KIM-1

By the end of the first week, there was a significant rise of all biomarker levels in all groups when compared with the basal level. Similarly, biomarker levels at the 14th day were significantly higher than those obtained at the 7th day (Table 1).

![Table 1](image-url)
The urinary KIM-1 level was not detected at the baseline condition. Expression of urinary KIM-1 was highest in Group 3 at the end of the 1st week followed by Group 2, and Group 1 was the lowest. By the end of the 2nd week, expression of urinary KIM-1 in Group 1 increased by 3.3 times that found in the 1st week. In Group 2, urinary KIM-1 was expressed in urine samples and it increased by 2.3 times that found in the 1st week at the end of the 2nd week. Finally, in Group 3 urinary KIM-1 increased by 1.6-fold of that found in the 1st week at the end of the 2nd week (Fig. 1).

The levels of serum creatinine, blood urea nitrogen, and urinary KIM-1 were comparable at the basal level. Significant differences were detected when the 3 groups at 1st and 2nd week after the obstruction were compared using the Kruskal–Wallis test (Table 2).

In the sham group, urinary KIM-1 remained undetected at the basal level and throughout the study period in all groups (Table 3).

3.2. Evaluation of tubulointerstitial injury

Sections from kidneys harvested from the sham and Groups 1-3 at the 1st and 2nd weeks after obstruction, were examined for assessment of tubulointerstitial injury by H&E stain. In sham-operated rats, there was no evidence of tubulointerstitial injury. Group 1 showed mild cellular infiltration at the 1st week, formed mainly of macrophages and lymphocytes. Focal areas of infiltration by lymphoid aggregates were seen at the 2nd week. Group 2 showed scattered macrophages beneath the transitional epithelium of the renal pelvis at the 1st week and multiple focal areas of tubular injury at the 2nd week. These focal areas of

![Figure 1. Trends of urinary KIM-1 levels over time after obstruction. KIM = Kidney injury molecule.](image-url)
tubular injury included loss of the proximal tubule brush border, patchy loss of tubule cells, focal areas of proximal tubular dilation, and distal tubular casts. Also, focal collapses of the glomerular tuft were seen in association with the tubular injury. Group 3 showed focal areas of mixed inflammatory cells at the 1st week that were rich in polymorph leucocytes, and multifocal areas of mostly regenerative changes in the tubular epithelial lining following acute tubular injury at the 2nd week (Fig. 2).

4. Discussion

There is no doubt that in some circumstances the diagnosis of partial obstruction is equivocal and there may be no cutoff measures to confirm the presence or absence of obstruction. Unfortunately, serum creatinine is an unreliable indicator during acute changes in kidney function. In addition, the serum creatinine level can widely vary with many variables such as age, gender, muscle bulk, medications, and hydration status. Moreover, one of its main limitations is the inevitable delay in the presence of a problem not only in the kidneys, but also in obstructed kidneys is very important from a clinical point of view. So, if urinary KIM-1 is detected at any level, this indicates the potential ability to estimate the degree of renal damage.

In the current study, the serum creatinine level was considered as the gold standard reference tool to test the newly introduced biomarker “urinary KIM-1” in cases with obstructive nephropathy. The mean basal creatinine level was around 0.25mg/dL and reached 0.58mg/dL at its maximum calculated value. Despite a statistically significant rise of the serum creatinine level in the 3 animal models of partial obstruction, this variation was still within the normal range. So, such a rise would be insignificant from a practical point of view. In addition, serial estimation should be done to confirm a progressive rise of the serum creatinine level.

On the other hand, the role of urinary KIM-1 in the early detection of AKI due to nonobstructive pathology has been proven. In a prospective case–control study in children undergoing cardiopulmonary bypass, Han et al. reported a significantly higher KIM-1 concentration in urine samples from patients with AKI when compared with urine samples from normal controls. Moreover, they reported an elevated urinary KIM-1 before an increase in serum creatinine. An earlier study demonstrated that the KIM-1 level, was markedly elevated in the urine of patients presenting with AKI, within 12 hours after initial ischemic renal insult prior to the appearance of casts in the urine. Moreover, consistent with the current results, van Timmeren et al. reported that urinary KIM-1 was increased in patients with renal impairment versus controls. They reported that urinary KIM-1 might be associated with inflammation, and reflected tissue KIM-1, indicating that it could be used as a noninvasive biomarker in renal disease. Furthermore, Nejat et al. revealed that the median concentration of KIM-1 was significantly greater in patients with prerenal AKI compared with those without AKI.

Absence of expression of urinary KIM-1 in normal nonobstructed kidneys is very important from a clinical point of view. So, if urinary KIM-1 is detected at any level, this indicates the presence of a problem not only in the kidneys, but also in proximal convoluted tubules. In the current study a significant difference was detected in the urinary KIM-1 level at all-time intervals, the 1st, and 2nd weeks after induction of the partial obstruction. This difference was shown for all case scenarios of partial obstruction. An interesting finding was that the rise of urinary KIM-1 varies from 22 to 85 folds which may reflect its potential ability to estimate the degree of renal damage.

Because of lack of conclusive evidence about the role of urinary KIM-1 as a biomarker in cases with obstructive uropathy, we confirmed the deteriorative effect of the obstruction on renal tissue by histopathological examination. The findings were multifaceted and varied from mild cellular infiltration at the 1st week to focal areas of lymphoid infiltration after the 2nd week. Other structural response of tubule cells to injury included loss of cell polarity and brush borders, cell apoptosis, dedifferentiation of viable cells, proliferation, and regeneration of a normal epithelium with the presence of KIM-1.

The current study design was chosen to be an experimental trial on an animal model with obstructive nephropathy despite an earlier published trial on humans by Wasilewska et al. and Mohammadjafari et al. for 2 reasons. First was to test different case scenarios of obstruction. The second was to harvest kidneys after obstruction and investigate the histopathological evidence.

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal</th>
<th>Day 7</th>
<th>Day 15</th>
</tr>
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<tr>
<td>Serum creatine (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>PBUO sham</td>
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<td>0.2833</td>
<td>0.2833</td>
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<td>PBUO sham</td>
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<table>
<thead>
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<th>Group</th>
<th>Basal</th>
<th>Day 7</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary KIM-1 (pg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBUO sham</td>
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<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>PSUO sham</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>PBUO sham</td>
<td>0.0000</td>
<td>0.0000</td>
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</table>

**Table 3**

Results of the sham group.

*BUN = blood urea nitrogen, KIM = kidney injury molecule, PBUO = partial bilateral ureteral obstruction, PSUO = partial solitary kidney ureteral obstruction, PUUO = partial unilateral ureteral obstruction.*
of renal deterioration. Nevertheless, further trials are needed to correlate levels of urinary KIM-1 with grades of renal damage on an immunohistopathological basis and to test the sensitivity of urinary KIM-1 in the recovery of kidneys after relief of the obstruction. Finally, there are many case scenarios that represent true challenges and should be considered as limitation for KIM-1 application. It cannot differentiate between acute or chronic obstruction unless basal and follow-up levels are detected. In addition, it cannot detect the cause of the obstruction as in vesicoureteral reflux of pelviureteric junction obstruction patients. Likewise, in case of chronic obstruction of one kidney, it cannot detect acute onset of obstruction of the other kidney. However, it still has its potential advantage as an early detector of renal injury which might influence decision making.

5. Conclusions
From the present study, urinary KIM-1 was proven to be significantly elevated in obstructive nephropathy. Such an elevation might be advantageous in the early diagnosis and subsequently early intervention of cases with partial ureteral obstruction.

Acknowledgments
None.

Statement of ethics
The study gained ethical approval from local ethical committee in UNC, IRB Faculty of Medicine, Mansoura University. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest statement
The authors declare that they have no financial conflict of interest.

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Author contributions
Ahmed S El Hefnawy: Study design, Manuscript writing, Data analysis; Mona El Hussiny: Study design, Chemical analysis, Data analysis; Ahmed Ali Ibrahim: Chemical analysis, Data collection; Khadija Ali: Histopathology analysis; Mohammed Attwa: Supervision, Monitoring of data collection; Nashaw Barakat: Animal model surgeries; Ahmed Shokeir: Supervision, Reviewing final manuscript.

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