Human kidney injury molecule-1 as a urine biomarker differentiating urothelial and renal cell carcinoma

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Introduction Urine concentration of Human Kidney Injury Molecule-1 (KIM-1) is suggested to be increased in patients with renal cell carcinoma (RCC). However, it has never been tested in patients with urothelial tumors, while preoperative differentiation between RCC and upper tract urothelial carcinoma (UTUC) plays an essential role in therapeutic decisions.
The aim of the study was to evaluate the role of urinary KIM-1 expression in preoperative differentiation between RCC and urothelial carcinoma (UC).
Material and methods Sixty-four participants were enrolled in the study, including 30 patients with RCC and 27 with UC (16 with UTUC and 11 with bladder tumor). Preoperative urinary KIM-1 levels were measured using a commercially available ELISA kit and normalized to urinary creatinine levels.
Results The median concentration of urinary KIM-1 normalized to urinary creatinine was lower in patients with RCC compared to UC (1.35 vs 1.86 ng/mg creatinine, p = 0.04). The comparison between RCC and UTUC shows even more significant difference (1.33 vs 2.23 ng/mg creatinine, p = 0.02). Urinary KIM-1 concentration did not correlate with tumor stage nor grade in any of the groups. ROC analysis to identify UC revealed AUC of 0.657 with sensitivity 33.3% and specificity 96.7% at the cut-off value of 3.226 ng/mg creatinine. Among patients with eGFR ≥60 mL/min/1.73 m², ROC analysis to detect UC achieved AUC of 0.727 with sensitivity 69.5% and specificity 70.2%.
Conclusions Urine KIM-1 can potentially differentiate UC from RCC. However, a wide range of observed results and limited sensitivity and specificity requires caution in making clinical decisions before confirmatory studies.

Key Words: urothelial cancer ‹› kidney cancer ‹› biomarker ‹› hKIM-1

INTRODUCTION

Renal cell carcinoma (RCC) and urothelial carcinoma (UC) are common urological malignancies. Among UC, most of the patients suffer from bladder cancer; only about 5% of them develop tumors originating from the upper urinary tract (upper tract urothelial carcinoma – UTUC) [1]. Preoperative differentiation between RCC and UTUC is a significant step in diagnostics, as the primary surgical treatment is different with radical or partial nephrectomy reserved for RCC cases and radical nephroureterectomy or endoscopic management for UTUC cases [2, 3].
Human kidney injury molecule-1 (KIM-1, also named T-cell immunoglobulin and mucin domain 1 (TIM-1) and hepatitis A virus cellular receptor 1 (HAVCR-1) is a type I transmembrane glycoprotein. Its ectodomain is released into the lumen of the

renal tubule. Since its discovery in 1998 [4], many studies suggested that it may be a sensitive and specific biomarker of proximal renal tubule injury [5]. Few research groups indicated that urinary KIM-1 is also increased in patients with clear cell and papillary RCC [6–9]. To the best of our knowledge, there are no studies evaluating urinary KIM-1 as a potential diagnostic tool in patients with UC. However, regarding the origin of this molecule we hypothesized, that urinary KIM-1 in patients with UC will not be increased, and thus KIM-1 may help preoperatively differentiate patients with RCC and UC.
The aim of our study was to assess urinary KIM-1 concentration in patients with RCC and UC and to evaluate its potential in preoperative differentiation of these two malignancies.

MATERIAL AND METHODS

Participants

The study was designed as a prospective cohort study and was approved by the local ethics committee.
All participants were patients qualified for the surgery due to primary RCC or UC. Out of 57 participants, 30 underwent radical or partial nephrectomy for primary solitary RCC, 16 underwent radical nephroureterectomy or endoscopic ablation for UTUC, and 11 underwent transurethral resection of the bladder tumor for primary urothelial bladder cancer. On the day of surgery, all patients were asked to void first-morning urine for the assessment of KIM-1 and creatinine levels. The pathological evaluation of the surgical specimen was performed in the standard manner. All participants gave written informed and voluntary consent to participate in the study. The patient-flow diagram is presented in Figure 1.

Urine analysis

Both serum and urine creatinine levels, as well as a urinalysis, were performed as clinical samples. Estimated glomerular filtration rate (eGFR) was calculated using the Modificiation of Diet for Renal Disease (MDRD) equation (eGFR = 186 x Serum $Cr^{-1.154}$ x $age^{-0.203}$ (x 0.742 if female)). Urinary KIM-1 concentration was measured using Human Urinary TIM-1/KIM-1/HAVCR Quantikine ELISA (R&D Systems BioTechne, Minneapolis, MN, the United States) kit for direct enzyme-linked immunosorbent assay (ELISA) in accordance to manufacturer protocol. Measured concentrations (ng/ml) were normalized to urinary creatinine to compensate for the differences in relative amounts of water removed along the nephrons. Urinary KIM-1 was expressed as an ng/mg creatinine.

Statistical analysis

To test the normality of variables, the Shapiro–Wilk test was used. Chi-square test was used to examine the association between diagnosis and sex; t-test was used to assess the difference between age and eGFRs, Mann-Whitney U test was used to evaluate the difference in serum creatinine and urinary KIM-1 concentrations. Spearman correlation coefficients were calculated between KIM-1 concentration and tumor stage and grade. Receiver-operating characteristic (ROC) curves were determined to assess the sensitivity, specificity, and optimum cut-off point to identify patients with urothelial cancer. The results are presented as means ±SD or medians (1^{st}, 3^{rd} quartiles). Statistical analysis was performed using Statistica 13.1 software (Dell, Round Rock, Texas, the United States).

RESULTS

Patient and tumor characteristics are summarized in Table 1. The concentration of urinary KIM-1 normalized to urinary creatinine was lower in patients with RCC compared to UC (median 1.35 vs 1.86 ng/mg

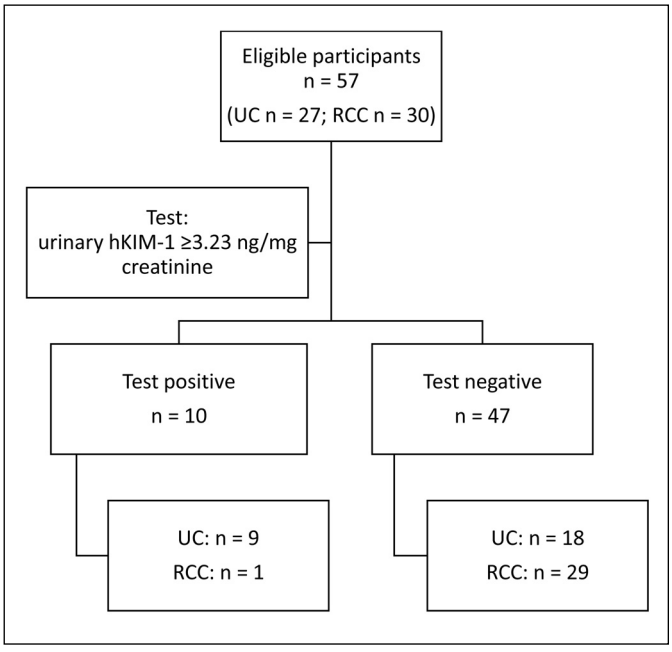

Figure 1. *STARD diagram to report flow of participants through the study.*

STARD – standards for the reporting of diagnostic accuracy studies; n – number; UC – urothelial carcinoma; RCC – renal cell carcinoma; hKIM-1 – human kidney injury molecule-1

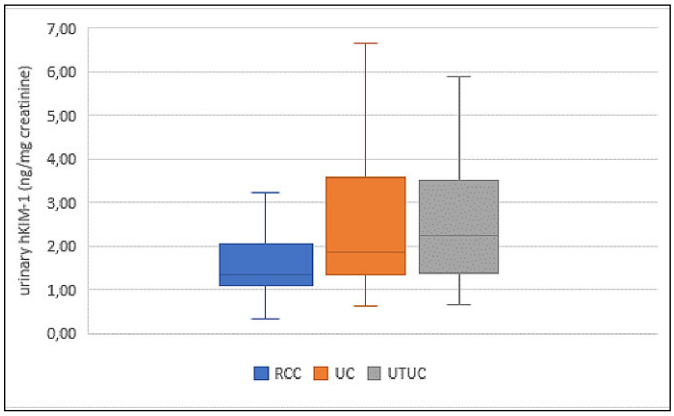

Figure 2. *Urinary hKIM-1 concentrations in RCC, UC and UTUC groups.*

hKIM-1 – human kidney injury molecule-1; RCC – renal cell carcinoma; UC – urothelial carcinoma; UTUC – upper tract urothelial carcinoma

creatinine, p = 0.04). The comparison between RCC and UTUC shows even more significant difference (1.33 vs 2.23 ng/mg creatinine, p = 0.02) (Figure 2). However, the range of KIM-1 concentrations among patients was wide. Urinary KIM-1 concentration did not correlate with neither tumor stage nor grade in any of the groups. ROC curve analysis revealed that the optimal cut-off point for the detection of urothelial cancer is 3.23 ng/mg creatinine, with sensitivity 33.3% and specificity 96.7% (AUC = 0.657, p = 0.03) (Figure 3A). ROC analysis performed among patients with RCC and UTUC revealed a cut-off point to detect UTUC at 1.86 ng/mg creatinine, with sensitivity 62.6% and specificity 73.3% (AUC = 0.688, p = 0.03) (Figure 3B).

Secondary analyses

It is well known that increased levels of KIM-1 may reflect different types of kidney injuries [10]. Thus, we performed a secondary analysis among patients with eGFR of ≥60 mL/min/1.73 m². Based on this criterion, we identified 20 patients in the RCC group and 13 patients in the UC group. Median KIM-1 concentrations (1st ;3rd quartiles) in RCC and UC groups were 1.35 (1.16; 1.79) vs 1.86 (1.39; 3.72) ng/mg creatinine respectively (p = 0.03). ROC analysis revealed a cut-off point for the detection of urothelial cancer at 1.69 ng/mg creatinine, with sensitivity 69.5% and specificity 70.2% (AUC = 0.727, p = 0.02) (Figure 2C).

Secondary analyses concerning abnormalities in urinalysis (such as erythrocyturia, leukocyturia, etc.) did not reveal any substantial change in the results.

DISCUSSION

KIM-1 is a promising biomarker of renal injury. It was shown to be overexpressed and excreted in increased concentration in urine in patients with IgA nephropathy, nephrotoxins, diabetes, proximal tubule injuries, and others [5, 11, 12]. Several studies also indicated increased immunohistochemical expression of KIM-1 in clear cell and papillary carcinoma [8, 13] and increased excretion of urinary

Table 1. *Patient and tumor characteristics*

	RCC	UC	p-value (RCC vs UC)	UTUC	p-value (RCC vs UTUC)
Number	30	27		16	
Age	64.1 ±11.4	68.4 ±7.4	0.11*	69.9 ±8.8	0.08**
Sex	10F / 20M	10F / 17M	0.79**	7F / 9M	0.53**
Serum creatinine	0.97 (0.77; 1.29)	1.07 (0.95; 1.43)	0.19***	1.20 (1.00; 1.64)	0.08***
eGFR	77.3 ±33.5	59.8 ±21.2	0.05*	55.6 ±20.2	0.02*
Histologic type	Clear-cell – 24 papillary – 6	Urothelial – 27		Urothelial – 16	
Stage	pT1a – 21 pT1b – 5 pT2 – 2 pT3 – 2	pTa – 16 pT1 – 4 pT2 – 3 pT3 – 2 pT4 – 2		pTa – 9 pT1 – 1 pT2 – 2 pT3 – 2 pT4 – 2	
Grade	G1 – 9 G2 – 17 G3 – 4	LG – 13 HG – 14		LG – 7 HG – 9	

* t-test; ** chi-square test; *** Mann-Whitney U test

RCC – renal cell carcinoma; UC – urothelial carcinoma; UTUC – upper tract urothelial carcinoma; F – female; M – male; eGFR – estimated glomerular filtration rate; pT – pathological stage; LG – low-grade; HG – high-grade

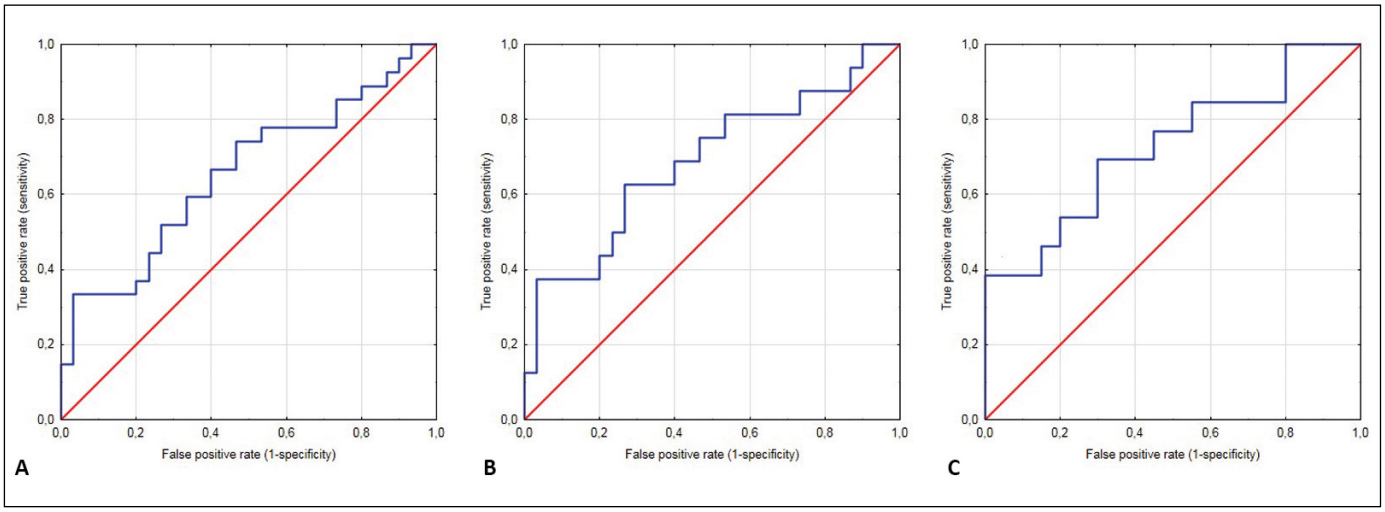

Figure 2. A – *ROC curve analysis of urinary hKIM-1 sensitivity and specificity to detect urothelial carcinoma. Analysis performed comparing the urinary hKIM-1 concentrations of 30 patients with RCC and 27 patients with UC. AUC = 0.657; 95%CI 0.513–0.801; p = 0.0326.* **B** – *ROC curve analysis of urinary hKIM-1 sensitivity and specificity to detect upper tract urothelial carcinoma. Analysis performed comparing the urinary hKIM-1 concentrations of 30 patients with RCC and 16 patients with UTUC. AUC = 0.688; 95% CI 0.518–0.857; p = 0.03.* **C** – *ROC curve analysis of urinary hKIM-1 sensitivity and specificity to detect urothelial carcinoma among patients with eGFR >60 mL/min/1.73 m². Analysis performed comparing the urinary hKIM-1 concentrations of 20 patients with RCC and 13 patients with UC. AUC = 0.727; 95% CI 0,544–0.910; p = 0.015.*

KIM-1 among patients with renal cancer in comparison with other surgical controls [6–9].

KIM-1 is a signaling molecule and may be a functional protein in RCC [6]. Findings from Bonventre's group indicate that KIM-1 is a signal to phagocytosis and removing apoptotic bodies in injured proximal tubules what causes reduced antigen exposure to inflammatory cells and prevents reaction of the immune system [14, 15, 16]. Therefore, it is reasonable to assume that RCC cells may adapt the phagocytotic function of KIM-1 to clear tumor apoptotic bodies and in this way to prevent the activation of the immune system [17].

In our study, we evaluated urinary excretion of KIM-1 in patients with RCC and UC. Clinical preoperative differentiation of these two malignancies may be challenging in some cases, while these two clinical scenarios require different therapeutic approaches (radical or partial nephrectomy vs endoscopic treatment or chemotherapy and nephroureterectomy). Thus, the appropriate preoperative diagnosis of the type of cancer seems to be crucial in proper patient management. Surprisingly we noticed, that the urinary KIM-1 concentrations tend to be higher in patients with UC. However, the specificity and sensitivity of KIM-1 in the differentiation of RCC and UC was limited, which requires caution when interpreting the results of our study. Urinary tract obstruction or concomitant benign renal disease can potentially affect measured KIM-1 concentration [8]. For this reason, we have also performed a secondary analysis in patients with eGFR of ≥60 mL/min/1.73m², showing that in these patients, KIM-1 testing is even more sensitive.

Our results bring into question the clinical value of KIM-1 as a biomarker of renal diseases. First, we noticed higher KIM-1 values in UC patients than in RCC patients. Second, the range of results in our study was similar to previous reports on acute kidney diseases [11, 12], while previous studies by Morrissey et al. and Zhang et al. showed lower KIM-1 concentrations in patients with RCC as compared to those with acute kidney disease [6, 7]. Based on these facts, one can assume that KIM-1 protein may not be as specific for renal diseases as it was previously suggested.

The phenomenon of high KIM-1 urine expression in UC patients also needs discussion. Besides RCC, urinary KIM-1 may be overexpressed in 33–93% of clear cell carcinomas of either endometrial or ovarian primary origin [13]. There is also weak and minimal KIM-1 expression in colorectal carcinoma (possibly due to a mucinous component in the KIM-1 protein) [17]. In the study by Lin et al., immunohistochemical expression of KIM-1 was tested in many different non-renal tumors, including urothelial carcinoma of the renal pelvis with a negative result [13]. It seems possible that the source of high KIM-1 results is the cross-reaction of the antibody used in the ELISA with another protein produced by urothelial cancer cells. However, we lack the data to prove this

hypothesis. To the best of our knowledge, there was no study focused on urinary KIM-1 in patients with urothelial carcinoma.

Our study is not free from limitations, including a wide range of observed results, a limited number of enrolled patients, and the inclusion of both upper urinary tract and bladder urothelial tumors. However, we believe these limitations do not substantially influence conclusions made from the study. At the same time, this is the first study on the role of KIM-1 in urothelial cancers, and the first study showing significant doubts regarding KIM-1 testing.

CONCLUSIONS

Urinary KIM-1 can potentially differentiate UC from RCC and may potentially be implemented to preoperative decision strategies as an accessory finding in a specific group of patients. However, higher urinary KIM-1 concentration among patients with UC is surprising, thus needs confirmatory studies. Moreover, a wide range of observed results and limited sensitivity and specificity requires caution in making clinical decisions and supports the need for further studies on this topic.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

CLINICAL SIGNIFICANCE

Preoperative differentiation between RCC and UTUC is a significant step in diagnostics, as the primary surgical treatment is different. We present the results of our study which showed that patients with urothelial tumors, especially UTUC, tend to have higher KIM-1 urinary concentration. This may suggest that urinary KIM-1 may be a promising biomarker differentiating RCC and UTUC.

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