Urinary Human Kidney Injury Molecule1- (hKIM1-) is not Increased in Patients with Renal Cell Carcinoma

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Purpose: Human Kidney Injury Molecule-1 (hKIM-1) was proposed as urinary biomarker of renal cell carcinoma (RCC). The aim of the study was to validate urinary hKIM-1 as a biomarker of RCC.

Material and methods: Forty-six participants were enrolled into the study, including 30 patients with clear-cell or papillary RCC and 16 matched patients in the comparison group. Preoperative urinary hKIM-1 levels were measured using commercially available ELISA kit and normalized to urinary creatinine levels.

Results: The concentrations of urinary hKIM-1 normalized to urinary creatinine in patients with RCC and comparison group did not differ significantly (1.35 vs. 1.32 ng/mg creatinine, $p = .25$). There was also no difference in urinary hKIM-1 concentration regarding stage or grade of renal cancer. Additional analysis of patients without chronic kidney disease (defined as eGFR $\geq$ 60mL/min/1.73m^2) also did not reveal significant difference in urinary hKIM-1 concentrations between the groups (1.54 vs. 1.37; $p = .47$).

Conclusion: Results of our study do not confirm recent suggestions that urinary hKIM-1 may be a biomarker of RCC.

Keywords: hKIM-1; biomarker; kidney cancer; diagnosis; urine

INTRODUCTION

Renal Cell Carcinoma (RCC) is the 13th most common malignancy worldwide[(1)]. Incidence rates of RCC continue to increase steadily with age, with a peak of incidence at the age of 75 years[(2)]. Despite intensive technological development in imaging diagnostics and the increasing availability of various imaging techniques, biomarkers that could indicate the presence of kidney cancer are still awaited.

Recently, human Kidney Injury Molecule-1 (hKIM-1) also known as TIM-1 (T cell immunoglobulin domain and mucin domain protein 1) or HAVCR1 (hepatitis A virus cellular receptor 1) was identified as a sensitive and specific biomarker for renal proximal tubules injury[(3)]. Moreover, several studies confirmed that hKIM-1 may be a useful immunohistochemical marker for clear cell and papillary RCC diagnosis[(4-6)]. Furthermore hKIM-1 has been introduced as a promising urinary biomarker of RCC[(5-8)]. For example, Mijuskovic et al.[(5)] performed a study in which they noticed that expression of tissue hKIM-1 was documented in all cases of patients who underwent radical nephrectomy. Moreover, preoperative urinary hKIM-1 was significantly higher in patients with kidney cancer than in controls, and its values decreased after the surgery. Similar results has been previously published by Han et al.[(6)]. However, it is worth to notice that control groups in all of those studies consisted mainly of healthy participants. Recently published study by Kushlinskii et al.[(9)] shows that hKIM-1 concentration is also increased in blood plasma in patients with kidney cancer with sensitivity of 81% for stage I kidney cancer and 97% for stage II-IV.

The aim of our study was to assess diagnostic performance of urinary hKIM-1 as a biomarker of RCC in patients with clear cell or papillary RCC and patients suffering from benign urological conditions.

MATERIALS AND METHODS

Participants

The study was designed as a prospective cohort study and was approved by the local ethic committee.

Recruited patients were divided into two study groups. One group consisted of 32 consecutive patients qualified for partial or radical nephrectomy due to primary renal tumor. Out of them 30 patients had histologically confirmed clear-cell or papillary RCC and were included in further analyses (RCC group). The second group consisted of 16 patients with benign urological conditions and no suspicion of cancer (7 patients with benign prostate hyperplasia, 4 with uretero-pelvic junction stricture, 3 with overactive bladder, 1 with detrusor underactivity, 1 with interstitial cystitis). They were

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Table 1. Patient characteristics.

	RCC group	Comparison group	*p*-value
number	30	16	
age	64.1 ± 11.4	67.8 ± 9.4	.28*
sex	10F / 20M	5F / 11M	.85**
serum creatinine (mg/dl)	0.97 (0.77; 1.29)	1.08 (0.76; 1.26)	.97***
eGFR (ml/min/1.73m²)	77.2 ± 33.5	74.1 ± 21.9	.74*
Stage of renal cancer	pT1a – 21 (70%) pT1b – 5 (16.7%) pT2 – 2 (6.7%) pT3 - 2 (6.7%)	pT0 – 16 (100%)	-

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

matched according to gender, age and smoking history. Urolithiasis and urinary tract infection were the exclusion criteria. Basic patient characteristics are summarized in Table 1. All participants voluntarily gave written informed consent to participate in the study and were asked to void first morning urine before the surgery for hKIM-1 and creatinine levels assessment.

Urine analysis

Both serum and urine creatinine levels, as well as urinalysis were performed as clinical samples. eGFR was calculated using MDRD equation (eGFR = 186 x Serum Cr-1.154 x age-0.203 (x 0.742 if female)). Urine hKIM-1 concentration was measured using Human Urinary TIM-1/KIM-1/HAVCR Quantikine ELISA kit (R&D Systems BioTechne) for direct 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 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 the groups and genders, t-test was used to assess the difference between age and eGFRs among the groups, Mann-Whitney *U* test was used to evaluate the difference in serum creatinine and urinary hKIM-1 concentrations between the groups, Kruskal-Wallis test was used to evaluate the difference in urinary hKIM-1 concentrations regarding stage and grade. The results are presented as means ± SD or medians (1st,3rd quartiles). Statistical analysis was performed using Statistica 13.1 software, StatSoft, USA.

RESULTS

The concentrations of urinary hKIM-1 normalized to urinary creatinine in patients with RCC and comparison group did not differ significantly (1.35 (1.08; 2.1) vs. 1.32 (0.72; 1.59) ng/mg creatinine (median, 1st; 3rd quartile), p=.25). There was also no difference in the urinary hKIM-1 concentration with respect to renal cancer stage (p = .92) or grade (p = .54) (**Table 2**).

As increased levels of hKIM-1 may reflect different types of kidney injuries (10), we have also performed a secondary analysis including only patients with or without chronic kidney disease (eGFR of less or more than 60 mL/min/1.73m²). However, this again showed no difference in hKIM-1 concentration between study groups (1.54 ± 0.71 vs. 1.37 ± 0.36; p = .47 - for patients without kidney injury and 1.39 (0.86; 2.83) ± vs. 0.48 (0.35; 2.31); p = .76 - for patients with kidney injury).

DISCUSSION

The incidence of RCC in the UK increased by 3.1% annually from 1993 and achieved 21 newly diagnosed cases per 100000 in 2014[11]. However population screening is not recommended, because of the risk of overdiagnosis, costs and radiation exposure[11]. Major barriers to population screening include also the relatively low prevalence of the disease and a substantial risk of detecting benign renal tumor or slow-growing RCC, which does not need specific management[11, 12]. Usage of urinary biomarker may be an interesting alternative but so far no test has been clinically validated with proper sensitivity and specificity.

In the previous studies matrix metalloproteinases (MMPs), aquaporin-1 (AQP1), perilipin-2 (PLIN2) and neutrophil gelatinase-associated lipocalin (NGAL) concentrations were tested as potential biomarkers for RCC but did not achieve satisfactory results[13,14]. Recently hKIM-1 was discovered as not only the early biomarker for renal proximal tubule injury but also as a potential renal cancer biomarker[6,15].

Contrary to the previously published data[5-8], the results of our study showed no effect of renal cancer on urinary

Table 2. Urinary hKIM-1 concentrations in groups.

	Number of patients in the group	urinary hKIM-1	concentration (ng/mg creatinine)
RCC	30	1.35	(1.08 ; 2.10)
pT1a	21	1.32	(0.93 ; 2.10)
pT1b	5	1.40	(1.34 ; 1.66)
pT2	2	2.16	(1.11 ; 3.22)
pT3	2	1.46	(1.26 ; 1.66)
G1	9	1,36	(1.07 ; 2.44)
G2	17	1,40	(1.11 ; 2.10)
G3	4	1,16	(1.02 ; 1.44)
Comparison group	16	1.32	(0.72 ; 1.59)

hKIM-1 concentration. Partially this can be explained by the construction of a comparison group. In previous studies, it consisted of orthopedic patients[7] or healthy participants[5]. Only in the study by Han et al. the control group consisted partially of patients with urological disorders, namely prostate cancer[6]. The fact that the comparison group in our study consisted of patients with urogenital disorders may be, at least in part, the answer why our results differ from the data available in the literature. However, this does not limit the significance of our results.

The main limitation of our study is small number of recruited patients. Comparison group patients suffered from various urological disorders, what also suggests heterogeneity of the group. However, it is worth to notice that none of the patients in the comparison group suffered nor had the suspicion of malignancy or urolithiasis. Another limitation is the fact that the urinary creatinine levels were not measured as scientific but clinical samples, which may partially be a source of a bias. Despite previous studies showing higher hKIM-1 concentrations in acute kidney diseases compared to RCC [4,5], in our study we noticed that hKIM-1 not only cannot differentiate RCC patients from controls, but also hKIM-1 values in both groups were similar to those previously reported for acute kidney diseases[9,10]. This brings into question the real clinical role of hKIM-1, while the reason for these phenomena remains unclear.

CONCLUSIONS

Results of our study did not confirm recent suggestions that urinary hKIM-1 may be a urinary biomarker of RCC. The reason for high urinary hKIM-1 concentration among patients with benign urological conditions is unclear and needs further assessment.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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