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Urinary KIM-1 and AQP-1 in patients with clear renal cell carcinoma: Potential noninvasive biomarkers

Molekul oštećenja bubrega-1 (KIM-1) i akvaporin-1 (AQP-1) u urinu kod bolesnika sa karcinomom svetlih ćelija bubrega: potencijalni neiznvazivni biomarkeri

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Abstract

Background/Aim. Kidney injury molecule-1 (KIM-1) and aquaporin-1 (AQP-1) are potential early urinary biomarkers of clear renal cell carcinoma (cRCC). The aim of this study was to ascertain relationship between the urine concentrations KIM-1 and AQP-1 with tumor size, grade, pT stage and type of operation (radical or partial nephrectomy) in patients with cRCC. **Methods.** Urinary concentrations of urinary KIM-1 (uKIM-1) and urinary AQP-1 (uAQP-1) were determined by commercially available ELISA kits. The analysis included 40 patients undergoing partial or radical nephrectomy for cRCC and 40 age- and sex-matched healthy adult volunteers. **Results.** The median preoperative concentrations of KIM-1 in the cRCC group [0.724 ± 1.120 ng/mg urinary creatinine (Ucr)] were significantly greater compared with controls (healthy volunteers) (0.210 ± 0.082 ng/mgUcr) ($p = 0.0227$). Postoperatively, uKIM-1 concentration decreased significantly to control values (0.177 ± 0.099 ng/mgUcr *vs* 0.210 ± 0.082 ng/mgUcr, respectively). The size, grade and stage of tumor were correlated positively with preoperative uKIM-1 concentrations. Contrary to these results, concentrations of uAQP-1 in the cRCC group were significantly lower (0.111 ± 0.092 ng/mgUcr) compared with the control group (0.202 ± 0.078 ng/mgUcr) ($p = 0.0014$). Postoperatively, the concentrations of uAQP-1 increased progressively up to control values, approximately. We find no significant correlation between preoperative uAQP-1 concentrations and tumor size, grade and stage. **Conclusion.** uKIM-1 was found to be a reliable diagnostic marker of cRCC, based on its significantly increased values before and decreased values after the nephrectomy.

Key words:
kidney neoplasms; diagnosis; biological markers; urine; nephrectomy.

Apstrakt

Uvod/Cilj. Molekul oštećenja bubrega-1 (KIM-1) i akvaporin-1 (AQP-1) su potencijalni rani biomarkeri karcinoma svetlih ćelija (cRCC). Cilj ove studije bio je da se utvrdi povezanost između koncentracija KIM-1 i AQP-1 u urinu i veličine, gradusa, stadijuma i vrste operacije (radikalna ili parcijalna nefrektomija) kod bolesnika sa cRCC. **Metode.** Urinarne koncentracije KIM-1 i AQP-1 određene su primenom komercijalnih ELISA kitova. Analizom je bilo obuhvaćeno 40 bolesnika koji su bili podvrgnuti parcijalnoj ili radikalnoj nefrektomiji zbog tumora bubrega i 40 zdravih odraslih ispitanika. Grupe su bile komparabilne po polu i godinama života. **Rezultati.** Srednja preoperativna koncentracija urinarnog KIM-1 (uKIM-1) u cRCC grupi [0,724 ± 1,120 ng/mg kreatinia u urinu (Ucr)] bila je statistički značajno viša u poređenju sa koncentracijom u kontrolnoj grupi (0,210 ± 0,082 ng/mgUcr) ($p = 0,0227$). Postoperativno, koncentracija uKIM-1 značajno je padala i približavala se vrednosti u kontrolnoj grupi (0,177 ± 0,099 ng/mgUcr

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nasuprot 0,210 ± 0,082 ng/mgUcr). Veličina, gradus i stadijum tumora bili su u pozitivnoj korelaciji sa preoperativnim koncentracijama uKIM-1. Nasuprot ovim rezultatima, koncentracija urinarnog AQP-1 (uAQP-1) u cRCC grupi bila je značajno niža (0,111 ± 0,092 ng/mgUcr) u poređenju sa kontrolnom grupom zdravih osoba (0,202 ± 0,078 ng/mgUcr) ($p = 0,0014$). Postoperativno, koncentracija uAQP-1 progresivno se povećavala, približno do vrednosti u kontrolnoj grupi. Nismo našli značajnu korelaciju između preoperativnih koncentracija uAQP-1 i veličine, gradusa i stadijuma tumora. **Zaključak.** uKIM-1 bi mogao biti dodatni pouzdani dijagnostički marker za cRRC na osnovu njegove značajno više preoperativne koncentracije i sniženja vrednosti nakon nefrektomije.

Ključne reči:
bubreg, neoplazme; dijagnoza; biološki pokazatelji; mokraća; nefrektomija.

Introduction

Renal cell carcinoma (RCC) represents approximately 3.8% of all malignancies in adults, increasing permanently over the past 30 years by 2−4% *per* year [1]. The most common and also the most aggressive form of RCC is clear renal cell carcinoma (cRCC), often characterized by the lack of early symptoms, signs and laboratory abnormalities. For these reasons cRCC is most often diagnosed accidentally during abdominal imaging performed for unrelated diagnostic reasons. Consequently, at the time of diagnosis, one-third of patients are already at metastatic stage of the disease [2]. Thus, reliable marker for cRCC should be powerful tool in screening patients for this particular type of the tumor and additionally, it could be used for monitoring of response to therapy and for the post-treatment surveillance, as well.

Recently, elevated urine concentrations of kidney injury molecule-1 (uKIM-1) and aquaporin-1 (uAQP-1) in patients with cRCC were recognized as specific and sensitive early markers of the disease [3]. KIM-1 is a type I transmembrane protein, whose ectodomain is secreted into urine in a response to the damage of the proximal tubule [4]. It has been shown that up regulation of KIM-1 in tumor cells is caused by de-differentiation of cells of the proximal tubules, such as cRCC and papillar renal cell carcinoma (pRCC). Patients with histologically confirmed cRCC have significantly higher concentration of uKIM-1 in pre-surgery phase, as compared with healthy individuals [5]. Current studies are focused on potential usage of levels of uKIM-1 for the early diagnosis of cRCC and possibility of preoperative determination of the RCC type [6, 7]. Aquaporin-1 (AQP-1) is a water channel expressed in many epithelial tissues and endothelium, including the proximal tubule of the kidney [8]. More recently, the diagnostic and prognostic usefulness of AQP-1 has been tested in cRCC tissues by using microarray techniques showing a reduction of AQP-1 expression in cRCC, as compared to normal renal tissue. A general decrease of AQP-1 protein can be related to the loss of differentiation, which is more pronounced in higher grade tumor [9]. Contrary to the above histopathological findings, recent studies have shown elevated uAQP-1 concentrations, determined by specific Western blot analysis in patients with cRCC [10–12].

The aim of this study was to determine if uKIM-1 and uAQP-1 concentrations in cRCC diagnosed patients undergoing partial or radical nephrectomy are related to tumor size, grade, and pT stage.

Methods

Patients

KIM-1 and AQP-1 were measured in urine samples of 40 patients submitted to radical or partial nephrectomy of renal tumor and in urine samples of 40 healthy adult volunteers. The study was conducted by our Institution between September 2012 and August 2013 and it was approved by the Ethical Committee of the Military Medical Academy, Belgrade, Serbia. Written and informed consent was obtained from each participant. All patients were preoperatively staged by thoracoabdominal multislice computed tomography (MSCT) imaging. The preoperative estimated glomerular filtration rates (GFR) − eGFR were calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [13]. The patients with a GFR less than 60 mL/min/1.73 m^2 and/or the history of previous renal and malignant diseases were not included. For each patient/healthy volunteer, the following variables were obtained from the medical records: gender, age, comorbidities that could influence the baseline level of markers, baseline serum creatinine, eGFR and the type of surgery (partial or radical nephrectomy). A radical or partial nephrectomy was performed by open approach. The postoperative pathology reports provided tumor type, size according to the largest dimension, tumor-node-metastasis (TNM) stage and Fuhrman grade. The study included only patients with the diagnosis of cRCC categorized according to the American Joint Committee on Cancer 2010 TNM staging system [14].

Sample collection

Voided urine samples were aseptically collected preoperatively and on the day 7 and 30 after the operation. All fresh samples were immediately processed within four hours of collection to ensure optimal protein stability. Urine was centrifuged (1,800 g, 10 min) to remove debris, dividing into 1.5 mL aliquots and frozen at -80°C until analysis. Preoperative blood samples were also collected to measure serum creatinine. Patients' venous blood was drawn by trained, qualified flebotomists. The levels of uKIM-1 and uAQP-1 were determined by commercially available ELISA kits (ELISA, TIM-1/KIM-1/HAVCR, R&D Systems Inc, Minneapolis, MN, USA and AQP1 (Human) ELISA Kit, Abnova, Heidelberg, German), respectively. The absolute values (pg/mL) of uKIM-1 and uAQP-1 were calculated *per* norma-

lized urinary creatinine (Ucr) to avoid variability in urine flow and the results were expressed in ng/mgUcr. The minimum detectable dose for uKIM-1 and uAQP was 0.009 ng/mL and 0.04 ng/mL, respectively.

Creatinine determination

Serum and urine creatinine concentrations were measured by the modified Jaffe method using Siemense Dimension Rxl Max chemistry analyzer.

Statistics

Statistical software GraphPad Prism 5.0 was used for statistical analysis. After determining basic parameters of descriptive statistical analysis [mean value ($\bar{x}$), standard deviaton (SD), median (med), standard error of the mean (SEM)] we have analyzed Gaussian distribution of data with Kolmogorov Smirnov test. Data from multiple groups were compared with one way analysis of variance with Bonferroni multiple post-testing comparison. Nonparamteric Mann Whitney (MW) test was used to compare the differences between the two groups, where needed, while serial data from each patient in different time points were compared as paired data with Wilcoxon test.

Results

The clinical and pathological characteristics of the studied participants are shown in Table 1. In the cRCC group (n = 40) we measured uKIM-1 and uAQP-1 before surgery in all participants, in 37 (92.5%) patients on the postoperative day 7 and in 23 (57.5%) patients on the postoperative day 30. Urine samples on the day 30 after the operation were not available from 14 patients with cRRC because they did not come to follow-up visit. The control group included 40 healthy volunteers (n = 40). The cRCC group and the control group did not differ significantly according to age, sex and eGFR. The pathologic tumor size for the cRCC group was 5.50 ± 3.05 cm ($\bar{x}$ ± SD). The distribution of the pathologic stage was as follows: pT1a in 13 (32.5%), pT1b in 10 (25%), pT2a in 3 (7.5%), pT3a in 14 (35%) and there was no patients in pT4. The postoperative TNM stage was as follows: stage I in 22, stage III in 13 and only 3 and 2 patients in stage II and IV, respectively. The classification according to the Fuhrman grade was: 30 (75%) patients with grade 2; 9 (22.5%) patients with grade 3; and 1 (2.5%) patient with grade 4. None of the patients had tumor grade 1 and positive lymph nodes. Two patients had metastases in the lung preoperatively.

The median pre- and postoperative uKIM-1 concentrations are listed in Table 2.

The median preoperative concentration of uKIM-1 in the cRCC group (0.724 ± 1.120 ng/mgUcr) was significantly greater compared with that in the control group (0.210 ± 0.082 ng/mgUcr, $p = 0.0227$) (Figure 1). The postoperative uKIM-1 concentration decreased significantly on the first postoperative control visit (0.327 ± 0.225 ng/mgUcr) compared with the preoperative concentration. On the second control visit the concentration of uKIM-1 in the cRCC group was statistically indistinguishable from the control group (0.177 ± 0.099 ng/mgUcr *vs* 0.210 ± 0.082 ng/mgUcr, respectively) (Table 2).

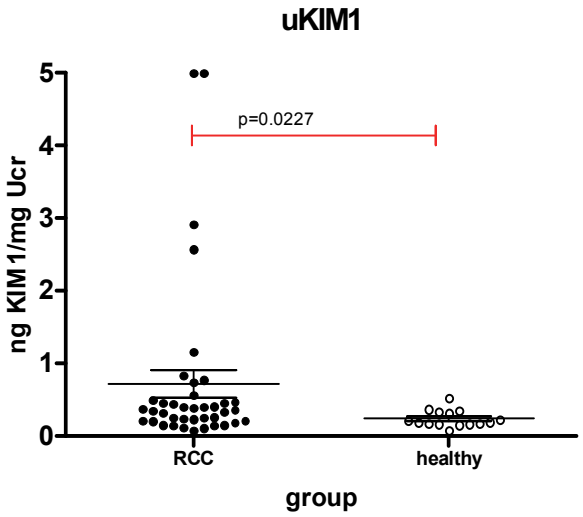

Fig. 1 – uKIM-1 in urine samples of the cRCC patients and the healthy volunteers.
For abbreviations see Table 2.

Table 1

Clinical and pathological characteristics of the examined participants

Parameter	cRCC group (n = 40)	Control group (healthy volunteers) (n = 40)
Gender, (male/female), n	25 / 15	22 / 18
Age (years), $\bar{x}$ ± SD	56.24 ± 11.73	60.45 ± 18.12
GFR (mL/min/1.73m^2), $\bar{x}$ ± SD	87.49 ± 16.46	88.82 ± 18.39
Tumor dimension (cm), $\bar{x}$ ± SD	5.50 ± 3.05	/
Grade, n	G1 = 0 G2 = 30 G3 = 9 G4 = 1	/
TNM stage, n	I = 22 II = 3 III = 13 IV = 2	/

cRCC – clear renal cll carcinoma; GFR – glomerular filtration rate; TNM – tumor-node-metastasis.

Table 2

Urinary kidney injury molecule-1 (uKIM-1) and urinary aquaporin-1 (uAQP-1) in the examined patients with clear renal cell carcinoma (cRCC)

Parameter	uAQP-1 (ng /mg creatine), x̄ ± SD			uKIM-1 (ng /mg creatine), x̄ ± SD		
	before operation	I control visit	II control visit	before operation	I control visit	II control visit
	0.111 ± 0.092	0.153 ± 0.163	0.212 ± 0.201	0.724 ± 1.120	0.327 ± 0.225	0.177 ± 0.099
Fuhrman gradus						
G1	none	none	none	none	none	none
G2	0.110 ± 0.097	0.113 ± 0.068	0.202 ± 0.172	0.409 ± 0.503	0.326 ± 0.225	0.170 ± 0.096
G3	0.113 ± 0.080	0.287 ± 0.293	0.237 ± 0.283	1.852 ± 2.146	0.339 ± 0.248	0.190 ± 0.112
Tumor size, cm						
≤ 4	0.141 ± 0.115	0.119 ± 0.072	0.267 ± 0.298	0.293 ± 0.169	0.357 ± 0.206	0.246 ± 0.124
4.1−7	0.073 ± 0.035	0.110 ± 0.069	0.205 ± 0.064	0.278 ± 0.139	0.307 ± 0.209	0.133 ± 0.033
>7	0.099 ± 0.075	0.261 ± 0.284	0.123 ± 0.105	2.092 ± 2.116	0.342 ± 0.300	0.153 ± 0.096
TNM stage						
I	0.117 ± 0.109	0.117 ± 0.072	0.210 ± 0.205	0.259 ± 0.133	0.319 ± 0.217	0.189 ± 0.113
II	0.129 ± 0.108	0.131 ± 0.058	0.000 ± 0.000	0.383 ± 0.075	0.380 ± 0.308	0.095 ± 0.029
III	0.099 ± 0.059	0.212 ± 0.248	0.224 ± 0.215	1.623 ± 1.924	0.347 ± 0.249	0.181 ± 0.100
Surgery type						
partial nephrectomy	0.126 ± 0.137	0.074 ± 0.047	0.160 ± 0.101	0.202 ± 0.118	0.327 ± 0.314	0.225 ± 0.164
radical nephrectomy	0.107 ± 0.078	0.173 ± 0.176	0.226 ± 0.222	0.917 ± 1.456	0.204 ± 0.226	0.138 ± 0.087

Note: The concentration uAQP-1 in the healthy volunteers before operation were 0.202 ± 0.079 μ/mg urinary creatinine (Ucr) and 0.210 ± 0.082 ng/mg Ucr, respectively.

TNM – tumor-node-metastasis.

There was a positive correlation between preoperative uKIM-1 concentration and tumor size, grade and stage (Table 3). The preoperative uKIM-1 concentration in the patients with cRCC correlated positively with tumor size, based on maximum tumor dimension. The mean uKIM-1 concentration in the group of patients with tumors larger than 7 cm was significantly higher (p = 0.0008) compared with the mean concentration of uKIM-1 in the patients with the tumor size of 4.1−7 cm and/or less and equal to 4 cm (p = 0.0004) (Figure 2). In addition, a positive correlation was obtained between the concentration of uKIM-1 and tumor grade (p = 0.00075).

The patients with high-grade tumors had a higher uKIM-1 level compared with those who had low-grade lesions (1.852 ± 2.146 ng/mgUcr *vs* 0.409 ± 0.503 ng/mgUcr), respectively (Table 2) and these differences were statistically significant (p = 0.0003) (Figure 3).

The preoperative mean values of uKIM-1 were significantly higher in patients with TNM stage III than with stage I (p < 0.0001, MW test) (Figure 4). The elevated baseline uKIM-1 (1.623 ± 1.924 ng/mgUcr) was significantly reduced to 0.347 ± 0.249 ng/mgUcr on the first control visit after the operation in patients with stage TNM III. In the patients with stage TNM I and TNM II, a significant reduction of uKIM-1 occurred only at the second control after the surgery.

It was shown that the type of surgery, partial or radical, had an impact on the concentration of uKIM-1, based at the first postoperative control visit. Before surgical intervention, the median uKIM-1 value was significantly higher in the group that later had radical resection compared to the partial resection group (p = 0.0044) (Figure 5). Contrary, on the first control examination uKIM-1 concentration was higher in the partial resection group than in the those who had radical resection. On the second control examination the average uKIM-1 value did not differ significantly regardless of the type of surgery.

Interestingly, analysis of uAQP-1 levels after adjustment to urinary creatinine (Ucr) concentration showed completely different dynamics compared to uKIM-1. In pati-

Table 3

Correlation analysis between uKIM-1 and uAQP-1 and tumor size, Fuhrman gradus, tumor pathological stage and TNM grade

Analyse	*vs* parameter	R coefficient	*p*
uKIM1 (0)	Tu (cm)	+0.564	0.00004
uKIM1 (0)	Tu (pT std)	+0.512	0.00085
uKIM1 (0)	G (Fuhrman)	+0.490	0.00064
uKIM1 (0)	TNM	+0.580	0.00005
uAQP-1 (0)	Tu (cm)	-0.112	> 0.05
uAQP-1 (0)	Tu (pT std)	-0.151	> 0.05
uAQP-1 (0)	G (Fuhrman)	+0.024	> 0.05
uAQP-1 (0)	TNM	+0.042	> 0.05

For abbreviations see Table 2.

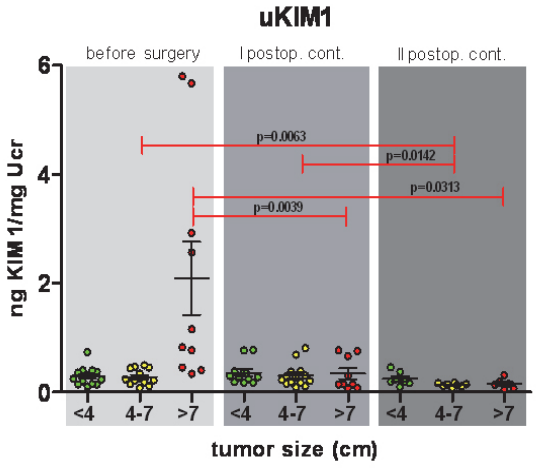

Fig. 2 − uKIM-1 according to tumor size. For abbreviations see Table 2.

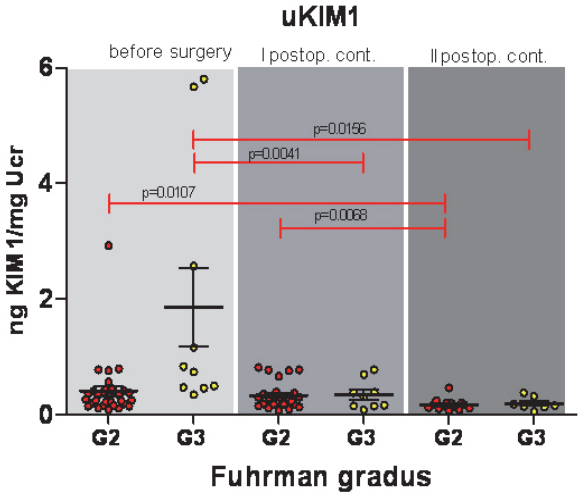

Fig. 3 – uKIM-1 according to Fuhrman grade. For abbreviations see Table 2.

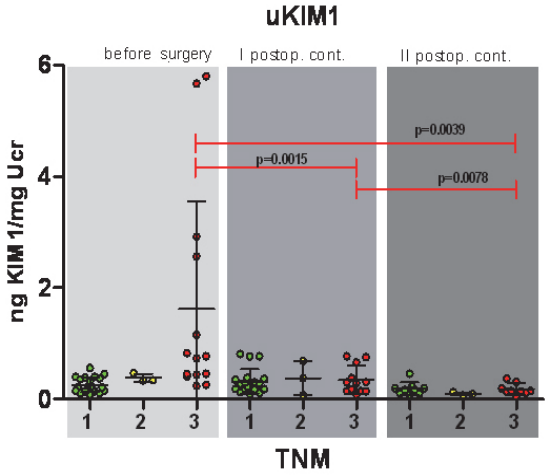

Fig. 4 – uKIM-1 according to TNM stage. For abbreviations see Table 2.

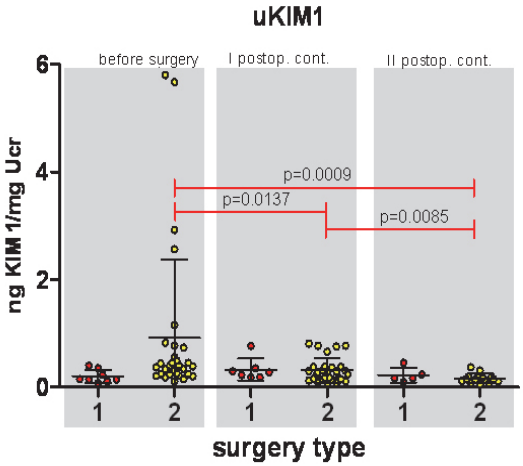

Fig. 5 − uKIM-1 according to the applied surgery type (partial nephroctomy and 2- radical nephroctomy). For abbreviations see Table 2.

ents with cRCC, uAQP-1 was significantly lower (0.111 ± 0.092 ng/mgUcr) than in the control group (0.202 ± 0.078 ng/mgUcr) ($p = 0.0014$). Postoperatively, the concentration of uAQP-1was progressively increasing and on the second control achieved approximately the same value as the control group (Table 2). We find no significant correlation between the preoperative uAQP-1 concentration and the tumor size, grade and stage (Table 3).

Discussion

The widespread use of modern radiological techniques such as ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) performed for unrelated diagnostic reasons has increased the diagnosis of renal tumors, especially tumors of smaller size detected incidentally [15, 16]. Although, preoperative needle biopsy of small lesion (≤ 3 cm) is followed by a relatively high sensitivity and specificity for the diagnosis of RCC, it carries a risk of false negative results and complications such as hematuria, subcapsular/perinephric hematoma and spread of malignant cells [17]. Sensitive and specific marker of the tumor needs to be able to predict certain type of cancer and also to monitor disease after treatment.

In order to determine the nature of preoperative tumor changes in the kidney and optimal treatment, we investigated urinary concentrations of KIM-1 and AQP-1 as a potential diagnostic marker for CRCC.

The results of our study showed that the preoperative concentrations of uKIM-1 in the patients with cRCC were statistically significantly higher than the value of postoperative uKIM-1 and than in the control group of healthy volun-

teers. After resection of the renal tumor using either partial or radical nephrectomy, uKIM-1 concentrations decreased to the levels essentially equivalent to that of the controls. Similar findings were reported by Han et al. [5]. Morrissey et al. [3] and Zhang et al. [7] indicating significantly higher preoperative uKIM-1 concentration in patients with cRCC compared with the patients with non-cRCC. Accordingly to Han et al. [5] and Morrissey et al. [11] we also found a positive correlation between tumor volume and uKIM-1 level. A different observation was reported by Shalabi et al. [18] who found no correlation between the concentration uKIM-1 and the size of the tumor, which might be explained by the fact that the most of the patients had tumor of small size (4.57 ± 0.37 cm). Zhang et al. [7] also found no correlation between uKIM-1 and the size of the tumor.

Tumor size is associated with malignant potential in RCC. A study of Thompson et al. [19] shows that the risk of malignancy of tumor and the percentage of high nuclear grade increases with the size of the tumor. Our study shows that the prevalence of high nuclear grade tumors increases with the tumor size. Thus, in the group with tumor ≤ 4 cm in diameter the prevalence of high grade tumor (G3 and G4) was 7.14%, in the group with tumor of 4.1−7 cm in diameter it was 17.64%, while in the group with tumor of > 7 cm in diameter the prevalence was 60%. The data we obtained showed a positive correlation between the size of the tumor and the nuclear grade.

In our study, the highest percentage of patients had G2 (75.61%) and G3 (21.95%) tumor grade, whereas only one patient had grade G4 (2.44%). None of the patients had G1 tumor grade. A similar distribution of Furman grade was found by other authors [20, 21]. In our study, a positive correlation was obtained between concentration of uKIM-1 and tumor grade. Different results were observed by Shalabi et al. [18] who found no correlation between uKIM-1 and tumor grade. Statistical analysis showed a different time course profile of uKIM-1 depending on the grade of the tumor. In the group of G3 grade operations induced a significant decrease of uKIM-1 on the first control examination, which later retained virtually the same level. In the group with G2 grade there was a significant decrease in uKIM-1 only between the first and the second control examination. A possible explanation for these results lies in the fact that the secretion of KIM-1 was independent of the tumor. Specifically, all eight patients who were submitted to a partial nephrectomy had G2 grade of the tumor. Obviously, after the surgery the recovery process resulted in elevated uKIM-1 because of renal ischemic injury during partial nephrectomy. The same result was obtained by Morrissey et al. [3] who confirmed a significantly lower reduction in postoperative uKIM-1 in patients with partial nephrectomy than in patients submitted to radical nephrectomy.

Partial nephrectomy is the optimal therapeutic modality of treatment in most patients with organ-confined RCC, achieving good disease control with preservation of renal function. Chronic Kidney Disease Prognosis Consortium Matsushita et al. [22] showed that the reduction of GFR below 60 mL/min/1.73m^2 is an independent predictor of total and cardiovascular mortality. To our knowledge, a study of Abassi et al. [23] is the only one comparing uKIM-1 before and after partial nephrectomy at different time intervals in 27 patients with RCC. A significant increase of uKIM-1 was registered from 3 to 24 hours after ischemic injury. In our study average concentrations of uKIM-1 at the first control examination on the postoperative day 7 was almost twice as high in the group of patients with partial nephrectomy compared to the group with a radical nephrectomy, but it did not reach statistical significance. This result was expected because the kidney still has been in the process of recovering at the time of the first control. Average uKIM-1 values did not differ significantly on the second control examination on the postoperative day 30, regardless of the applied intervention type, partial nephrectomy or radical nephrectomy, confirming acute ischemic renal damage. In addition, the patients subjected to radical nephrectomy had statistically significant higher uKIM-1 before the operation than the group subjected to partial nephrectomy. This result is a consequence of the larger size and higher-nuclear grade and stage of the tumor in the radical nephrectomy group. We showed that the uKIM-1 directly correlates with pathological characteristics of tumor.

Interestingly, in contrary to uKIM-1, analysis of uAQP-1 levels after adjustment to Ucr concentration showed a completely different dynamics during examination period. At first, the concentration uAQP-1 was significantly lower in the cRCC group than in the group of healthy subjects. Although a nonstatistically significant correlation was found between uAQP-1 and the size, stage or grade of the tumor, we observed slightly lower but not significant levels of this biomarker in patients with more aggressive grade (III–IV) and larger (> 7 cm) tumors.

In contrast to our results, Morrissey et al. [11] showed a direct correlation between the concentration uAQP-1 (determined by Western blot analysis) and the size and stage, but not the grade of the tumor. Similarly, Morrissey et al. [11] Sreedharan et al. [12] found a strong positive correlation between uAQP-1 and cRCC in 11 patients before operation. But, due to the small number of patients in their study correlation between the concentration uAQP-1 and pathological characteristics of the tumor was not performed [12]. Unlike previous studies in which the concentration of uAQP-1 was determined by Western blot analysis, we used commercial ELISA test to quantify the concentration uAQP-1, for the first time, to our knowledge.

Our results are complementary to the results of the histopathological study of the expression of AQP-1 in cRCC tissue. Ticozzi-Valerio et al. [24] showed a reduction of AQP-1 expression in tissue of cRCC from moderate to almost complete, depending on tumor grade. Tumor with high grade had a higher reduction of tissue AQP-1 expression compared to normal kidney tissue. Mazal et al. [25] proved a significant reduction or loss of AQP-1 expression in higher nuclear grade cRCC compared with those of lower grade, while there was no significant correlation accomplished between pT stage and expression of AQP-1. Unfortunately, we were not able to determine the expression of AQP-1 in the tumor tissue.

Conclusion

In summary, uKIM-1 but not uAQP-1 was statistically and clinically significantly increased in the patients with cRCC compared to the group of healthy subjects. Taking into the consideration the type of sample used for KIM-1 measurement (urine is a sample of choice what implies noninvasive sampling, simplicity and commitment of uKIM-1 measurements by using a commercial ELISA kit, mainly literature supported), we concluded that uKIM-1 might be used as a valuable and reliable biomarker of cRCC diagnosis and for monitoring patients with cRCC after operation, in routine clinical practice.

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