EJMA

DOI: 10.14744/ejma.2024.93063
EJMA 2024;4(1):28–33

Research Article

The Predictive Value of Urinary HMGB1 Level in the Diagnosis and Prognosis of Renal Cell Carcinoma

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Abstract

Objectives: To investigate the diagnostic and prognostic value of HMGB1 when used as a urinary biomarker in patients with renal cell carcinoma.

Methods: The study was conducted in a total of 99 participants including 34 patients diagnosed with Renal cell carcinoma [RCC], 34 patients with an acute urinary tract infection [UTI] and 31 healthy controls.Urinary HMGB1 levels of the study groups were evaluated. Urinary HMGB1 levels and tumor diameter among patients with different subtypes of renal cell carcinoma were comparison.

Results: Urinary HMGB1 levels differed significantly among the three groups [p<0.001]. Pairwise comparisons revealed statistically significant differences between RCC and UTI groups [p<0.001] and between RCC and control groups [p<0.001]. No significant difference was detected between acute UT In and control groups [p=0.078]. Urinary HMGB1 levels were significantly different when compared among RCC subgroups, [p=0.035], with a much higher median value [499,9 pg/mL] in the sarcomatoid type in particular. The tumor diameter also significantly differed among patients with different types of RCC [p=0.002]. Specifically, a much greater tumor diameter was found in patients with the sarcomatoid type. The cut-off values derived from the ROC analysis were 104.85 pg/mL for distinguishing RCC from a UTI, 35.15 pg/mL for acute UTI versus controls, and 87.55 pg/mL for RCC versus. Sensitivity 79%, specificity was found 85%.

Conclusion: Urinary HMGB1 levels as measured by a non-invasive method in the present study were higher in renal cell carcinoma and closely associated with high-grade tumor.

Keywords: Renal cell carcinoma, subtypes of renal cell carcinoma, tumor marker in urine, urinary HMGB1

Cite This Article: *Solakhan M, Benlier N, Barut O, Çiçek H, Sever ÖN, Yıldırım M. The Predictive Value of Urinary HMGB1 Level in the Diagnosis and Prognosis of Renal Cell Carcinoma. EJMA 2024;4(1):28–33.*

Kidney tumors constitute approximately 3% of all tumors occurring in adults. They are predominantly diagnosed in the 5th and 6th decades of life and the male/female ratio is 3/2.[1,2] Renal cell carcinoma (RCC) accounts for 90% of kidney tumors and clear cell, papillary and chromophobe subtypes represent 76%, 17% and 6% of the tumors respectively.[3] Sarcomatoid RCC is characterized by a biphasic lesion with both mesenchymal and epithelial elements. Clinically, sarcomatoid differentiation can be observed across all histological subtypes of RCC. Sarcomatoid differentiation has

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Submitted Date: April 24, 2024 **Revision Date:** April 24, 2024 **Accepted Date:** June 25, 2024 **Available Online Date:** August 29, 2024

an aggressive behavior and is associated with poor prognosis.[4] These malignant lesions often have a very high mortality rate and one-third of the patients have metastatic disease at the time of diagnosis. Additionally, other one-third of the patients are expected to develop metastasis despite treatment.[5] RCC is an invasive and chemoresistant disease which is most commonly treated with surgical resection because it responds poorly to radiotherapy.[6] Currently, targeted therapies for metastatic renal cell carcinoma are routinely used in clinical practice, but predictive biomarkers are lacking to guide the selection of such treatments in the clinical setting.

Therefore, reliable biomarkers of renal cell carcinoma are urgently needed which could allow monitoring of prognosis, early diagnosis and therapeutic efficacy and potential recurrence of the disease.

High mobility group box1 (HMGB1) or amphoterin is a chromatin-associated, non-histone chromosomal protein found in eukaryotic cells.[7] HMGB1 has many biological activities in normal and cancerous cells and regulates many events such as transcription, replication, recombination, DNA repair, genomic stabilization and Toll-like receptors (TLR) 2,4 activation. It also plays an important role in the migration of healthy and cancerous cells. It is also secreted from monocytes, macrophages, natural killer cells and acts as an extracellular pro-inflammatory cytokine. In addition, HMGB1 can be secreted in cancer cells under the influence of growth factors, cytokines and cellular stress impulses, causing its overproduction.[8] Moreover, HMGB1 is an agonist for receptor for advanced glycation end products (RAGE) and TLR4, and these two receptors are found in both cancer cells and immune cells.[9,10] HMGB1 also has extracellular functions that promote cancer development. HMGB1 has been shown to be released into the extracellular space during tumor growth, angiogenesis, and metastasis.[11] Previous studies suggested that HMGB-1 may have an important role in the development of cancer.

In light of this information, we aimed to investigate the diagnostic and prognostic value of HMGB1 when used as a urinary biomarker in patients with renal cell carcinoma.

Methods

The study included 34 patients who had been diagnosed with kidney cancer and had not started treatment, and 34 patients with urinary tract infection (UTI) in the same age group who had applied to the Urology Clinic, and 30 volunteers (for the control group). The control group was formed from healty volunteers who applied to the same clinic and no urological diseases were detected during the examination. Urine samples collected from the patients were centrifuged (2000rpm-20min) and stored at -80 degrees. Urine samples were transferred to the refrigerator and stored at +4ºC for one night before the testing. Then, they were kept at 250C for 2 hours before performing ELISA test. Lastly, the assay procedure was initiated after vortexing the samples.

Urinary HMGB1 Measurement

The Human HMGB1 ELISA Kit (SEA399Hu) used the sandwich enzyme immunoassay technique for the quantitative measurement of human HMGB1 in urine.

All data calculations for urine HMGB1 testing were obtained using integrated software (BioTek ELx808, USA). The sensitivity of the test was determined to be 27.1 pg/mL. The detection range was 61.7 to 4100 pg/mL. Analysis coefficients of variation were 8.1% and 9.2%, respectively.

Local ethics committee approval was received for the study. All participants were given detailed information about the nature and scope of the study and their written consent was obtained.

Statistical Analysis

Evaluation of the data obtained from all samples was made with Graph Pad Instat statistical software. Mann-Whitney U, Student's unpaired t, Chi-square, Kruskal-Wallis tests, One-Way ANOVA and correlation analysis were used to compare the data. In cases where the distribution was not normal, pairwise comparisons were made with the Mann-Whitney U test and evaluated using the Bonferroni correction ($p<0.017$). A p value below 0.05 was considered statistically significant. The equation sensitivity+specificity-1 was used. Cutoff values to maximize the sum of sensitivity and specificity were determined using the Youden Index.

Results

Age and BMI data of the three study groups showed a normal distribution (One-way ANOVA) with no statistically significant inter-group difference ($p=0.964$ and $p=0.862$, respectively) (Table 1). Urinary HMGB1 levels showed significant differences between the three groups ($p<0.001$). In pairwise group comparisons, statistically significant differences were detected between the RCC and UTI groups and between the RCC and control groups (Bonferroni correction $p<0.017$, $p<0.001$). No significant difference was detected between UTI and control groups ($p=0.078$; Bonferroni correction $p<0.017$). Urinary HMGB1 levels were significantly different when compared among renal cell carcinoma subgroups, ($p=0.035$), with a much higher median value (499,900 pg/mL) in the sarcomatoid type in particular (Table 2). The tumor diameter also significantly differed among patients with different types of renal cell carcinoma ($p=0.002$) (Table 2). Specifically, a much greater tumor diameter was found in patients with the sarcomatoid type.

Table 1. Demographic characteristics and urinary HMGB1 levels of study groups

	Renal Cell Carcinoma (n=34)	Urinary Tract Infection (n=34)	Control Group (n=31)	p
Gender (Male/Female) n (%)	20 (58.8)/14(41.2)	19(55.9)/15(44.1)	21(67.7)/10(32.3)	0.599*
Age (years) mean±standard deviation	63.029±9.84	63.352±10.73	63.677±7.83	0.964**
BMI mean±standard deviation	29.588±5.48	29.470±3.35	30±2.81	0.862**
HMGB1 (pg/mL) Median (min-max)	147.150 (14.27-758.90)	39.450 (10.3-178.5)	24.8(11.5-141.7)	p<0.001***

n: Number of subjects; *Chi-square test; **One-way ANOVA; *** Kruskal-Wallis test

Table 2. Comparison of urinary HMGB1 levels and tumor diameter among patients with different subtypes of renal cell carcinoma

Renal Cell Carcinoma	HMGB1 (pg/mL) Median (Min-Max)	Tumor Diameter Mean±SD
Clear cell (n=20)	124.250 (14.27-758.9)*	6.34±2.26**
Chromophobe (n=5)	131.9 (113.3-164.8)*	5.98±1.08**
Papillary (n=4)	87.3(20.2-715.8)*	5.95±1.85**
Sarcomatoid (n=5)	499.9 (347.7-735.2)*	10.30±1.2**
p	0.035	0.002

n: Number of subjects; *Kruskal-Wallis test; **One-way ANOVA; SD: Standard Deviation.

Additionally, the correlation analysis showed a strong positive correlation between the tumor diameter and urinary HMGB1 levels, i.e. higher urinary HMGB1 levels were observed as the tumor diameter increased (r=0.874, p<0.001).

The cut-off values were 104.85 pg/mL for distinguishing renal cell carcinoma from a UTI (Fig. 1), 35.15 pg/mL for UTI versus controls (Fig. 2), and 87.55 pg/mL for renal cell carcinoma versus (Fig. 3) (Table 3).

Discussion

In our study, significantly higher urinary HMGB1 levels were shown in patients with renal cell carcinoma than in controls. This suggests that HMGB1 expression is increased in RCC and can be detected by urinary analysis.

It was previously reported that HMGB1 has pro-tumorigenic activity via inflammatory mechanisms and while HMGB1 expression is upregulated in cancer, it is downregulated during aging.[12] HMGB1 was also found to induce apoptosis in studies focusing on destruction of cancer tissue through apoptotic processes.[13,14] Increased HMGB1 expression was

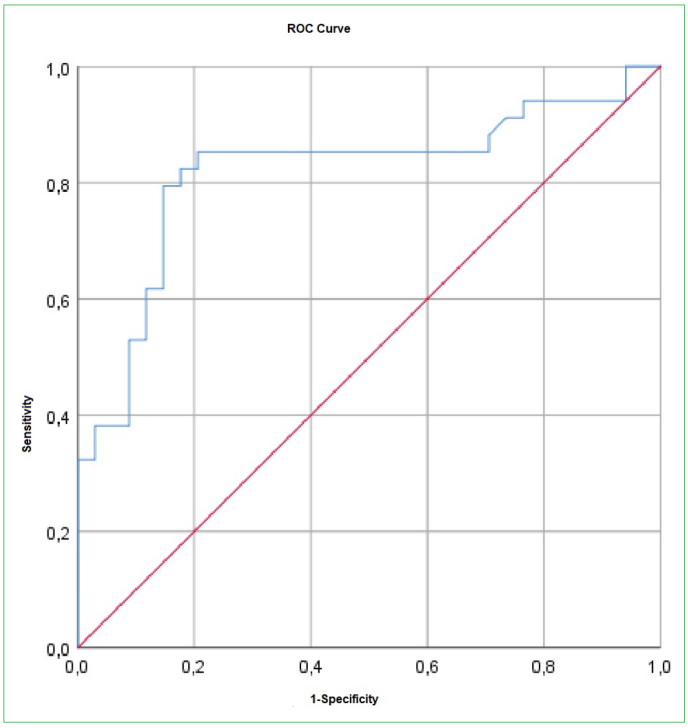

Figure 1. ROC curve analysis between renal cell carcinoma and urinary tract infection. The area under the curve (AUC) was 0.818 (95% CI 0.710-0.926) for HMGB1 (p<0.001).

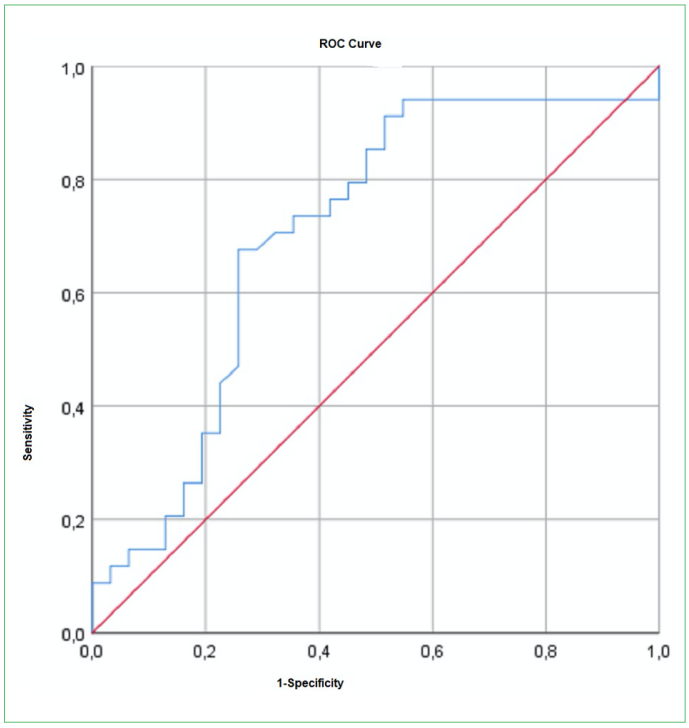

Figure 2. ROC curve analysis between urinary tract infection and controls. The area under the curve (AUC) was 0.704 (95% CI 0.571-0.837) for HMGB1 (p=0.005).

Table 3. Cut-off values of the groups determined by sensitivity and specificity

	Cut-Off Value	Sensitivity	Specificity
Cut-off value of HMGB1 for distinguishing renal cell carcinoma from urinary tract infection	104.85 pg/mL	0.794	0.85
Cut-off value of HMGB1 for urinary tract infection versus control group	35.15 pg/mL	0.676	0.74
Cut-off value of HMGB1 for renal cell carcinoma versus control group	87.55 pg/mL	0.824	0.87

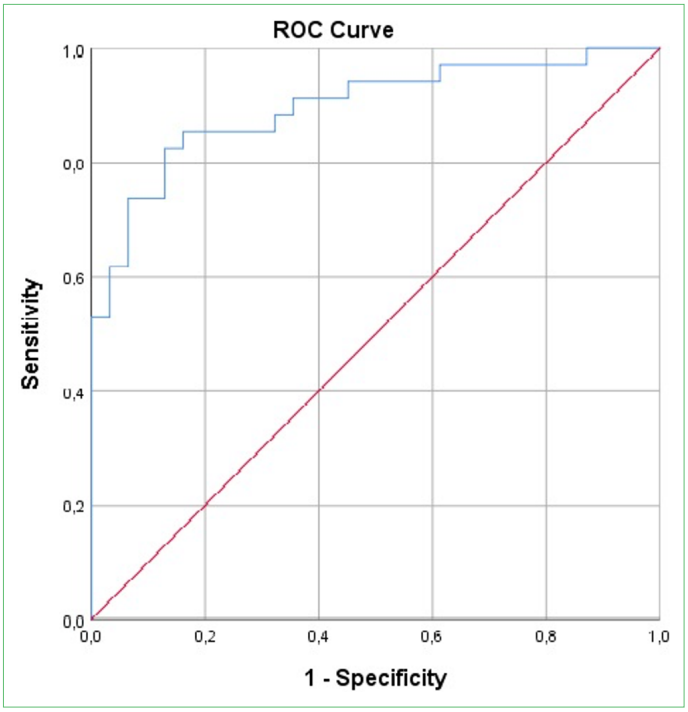

Figure 3. ROC curve analysis between renal cell carcinoma and controls. The area under the curve (AUC) was 0.897 (95% CI 0.819-0.974) for HMGB1 (p<0.001).

demonstrated both during the early and late stages of cancer. Lin et al.[15] found that HMGB1 is involved in the development and progression of clear cell RCC through activation of ERK 1/2 signaling.

Currently, there is no validated biomarker for renal cell carcinoma. The agents targeting the VEGF pathway have become a major part of targeted therapeutics in the treatment of RCC. Thus, researchers sought to determine whether baseline values of serum VEGF levels can be used as a prognostic or predictive biomarker. Significant improvements in progression-free survival and overall survival were observed in relation to low VEGF levels in a study involving 903 patients.[16] Tran et al.[17] showed better treatment outcomes in patients with low concentrations of IL-6. In a separate study, high levels of LDH have been associated with poor prognosis.[18] Krazinski et al.[19] argued that increased expression of nuclear factor kappa B kinase subunit B inhibitor was correlated with higher nuclear grade and lower rate of survival in clear cell RCC. Another study showed that overexpression of low density lipoprotein receptor related protein was associated with decreased survival in patients with clear cell RCC.[20] To date, several molecules have been studied as candidate serum biomarkers. Some molecules were identified in higher concentrations in RCC compared to control group including Tumor Necrosis Factor Receptor-Associated Factor-1, Hsp27, serum Amyloid A, C-reactive protein, Gamma-Glutamyltransferase, M-65, carbonic anhydrase-9, pyruvate kinase type 2, thymidine kinase and osteopontin.[21]

A limited number of studies are available in literature on urinary markers in RCC. Kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) have been studied for their potential use as urinary biomarkers. However, they were found to have low sensitivity and specificity as they can also be elevated in benign conditions.[22] Morrissey et al.[23] detected significantly increased urinary concentrations of exosomal proteins aquaporin-1 and perilipin-2 molecules in patients with RCC. More than 80% reduction was detected in these molecules post-nephrectomy. Also, Massimo et al.[24] demonstrated a significant increase in the urinary levels of Raf Kinase Inhibitor Protein in patients with RCC.

Consistently, we found a significant difference in urinary HMGB1 levels among three groups (p<0.001). In addition, we observed a direct correlation between HMGB1 levels and aggressiveness and size of the tumor. Urinary HMGB1 levels were significantly different when compared among renal cell carcinoma subgroups, (p=0.035), with a much higher urinary HMGB1 level (499,900 pg/mL) detected in the sarcomatoid type. Sarcomatoid RCC is not a distinct histologic entity. It represents the transformation of different histological types of RCC into high-grade tumors. These tumors may originate from any of the RCC subtypes. Many studies reported that the presence of sarcomatoid component was associated with a higher risk of metastasis and poor prognosis.[25] In the current study, we found much higher urinary HMGB1 levels in this subgroup than in other subgroups in direct proportion to cancer progression.

There are some limitations in our study. Firstly, HMGB1 levels were not measured in serum and tissue and secondly, comparison of the difference (post-treatment versus pre-treatment) in urinary HMGB1 levels was not performed for the study sample.

Conclusion

In summary, urinary HMGB1 levels as measured by a non-invasive method in the present study were higher in renal cell carcinoma and closely associated with high-grade tumor. The originality of our study involves quantification of urinary HMGB1 levels using a non-invasive tool in patients with renal cell carcinoma. Our findings showed greater release of HMGB1 from tumor cells in comparison to control group as well as urinary tract infection. In RCC, the utility of urinary HMGB1 measurement as a novel diagnostic tool and as a biomarker to predict high-grade tumors can be demonstrated by further studies in larger patient series.

Disclosures

Ethics Committee Approval: Sanko University School of Medicine Ethic Report 2019/12-03 - 19.09.2019.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – M.S., N.B., O.B., H.Ç., Ö.N.S., M.Y.; Design – M.S., N.B.; Supervision – H.Ç., Ö.N.S., M.Y.; Materials – M.S., N.B., O.B., H.Ç., Ö.N.S., M.Y.; Data collection and processing – M.S., N.B., H.Ç., M.Y.; Analysis and interpretation –H.Ç., N.B., M.Y.; Literature search – M.S., N.B. ; Writing – M.S., N.B., H.Ç., Ö.N.S., M.Y.; Critical Review – M.Y., H.Ç.

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