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Research Article

Diagnostic Value of Serum and Urine Endocan Levels in Nonmuscle Invasive Bladder Cancer: A Prospective Comparative Study

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Objective. This prospective study aimed to explore the potential diagnostic value of endocan levels in bladder cancer by investigating a possible association of serum and urine endocan levels with the stage and grade of bladder tumors in patients with nonmuscle-invasive bladder cancer (NMIBC) in terms of risk stratification. *Materials and Methods*. Participants included 66 male patients with NMIBC. Patients with full pathology results, NMIBC stage T1, and healthy controls were categorized as groups 1, 2, and 3, respectively. Patients were further classified into high- and low-grade groups following their pathology results. Risk classification according to the European Association of Urology (EAU) was assigned to patients with NMIBC, and associations of risk groups with serum and urine endocan levels were analyzed. An enzyme-linked immunosorbent assay was used to identify serum and urine endocan concentrations. *Results*. Serum endocan levels according to pathological staging were significantly higher in groups 1 and 2 than in group 3. The urine endocan level was statistically significantly higher in group 2 than in group 3 ($p < 0.001$). The predictive power of the urine endocan level was evaluated for its ability to predict T1 disease, revealing an area under the curve of 0.735 and a threshold of 903. The EAU classification was evaluated according to risk groups, and the urine endpoint was statistically significantly higher in the univariate analysis for the high and very high-risk groups ($p = 0.034$). *Conclusion*. Our results indicate that endocan levels hold significant promise in prognostic feature evaluation in NMIBC, particularly in the context of screening patients with hematuria.

1. Introduction

Bladder cancer is the most prevalent urinary system malignancy and is exceedingly predominant, globally [1, 2], affecting men four times as often as women [3]. Many genetic, carcinogenic, and etiological factors, as well as genetic polymorphism, play roles in bladder cancer development and progression [4].

Bladder cancers can be classified according to the depth of histopathologically confirmed invasion as non-muscle-invasive (pTa, PT1, and CIS) and muscle-invasive (pT2–T4), and within each, the subtype demonstrates clinical relevance for prognosis and therapeutic response [5]. However, the molecular diagnosis and classification of bladder cancer still warrant improvement.

The gold standard for diagnosing bladder cancer is cystoscopic transurethral lesion resection. The gathered tissue samples enable pathological staging, and the tumor grade guides the treatment modalities [6]. However, this procedure is expensive, invasive, and not particularly

comfortable. Hence, simple, low-cost, noninvasive, and lower-morbidity procedures are crucial for both diagnosis and treatment [7, 8].

New molecular studies have improved the ability to evaluate the characteristics and invasiveness potential of tumors. One candidate is endocan, also known as endothelial cell-specific molecule-1 (ESM-1). ESM-1, a novel molecule identified in 1996 by Lassale et al., is coded by a single gene (5q11.2) located on the fifth human chromosome [9]. Endocan is a 50 kDa proteoglycan composed of a mature protein core (20 kDa) of 165 amino acids and a chondroitin/dermatan sulfate chain attached to the serine residue at position 137 [9]. Its primary source is the endothelial cells in the kidney, liver, lung, and gastrointestinal tract [10, 11]. It is significantly involved in tumor angiogenesis, progression, and recurrence, which are signs of an unfavorable prognosis [12].

One study revealed elevated plasma endocan and VEGF-A levels in patients with bladder cancer compared with healthy individuals or those with non-muscle-invasive bladder cancer (NMIBC) [13]. In addition, they proposed endocan as an auxiliary biomarker in evaluating the prognoses of patients with MIBC who have elevated plasma endocan levels [13].

Studies based on serum and urine endocan levels in patients with bladder cancer have been fairly limited [14]. Furthermore, no research has focused on the association of bladder tumor stage with serum and urine endocan levels.

This study aimed to investigate the possible association of serum and urine endocan levels with T-stage and bladder tumor grade in patients with NMIBC and assess the potential clinical implications of this information.

2. Materials and Methods

This prospective study obtained approval from the Atatürk University Faculty of Medicine's Ethics Committee (No.B30.2.ATA.0.01.00/282). The study included 66 male patients who applied to the urology clinic with complaints of macroscopic hematuria from July 2019 to July 2021, whose bladder tumor was detected by radiological imaging methods, and who were diagnosed with bladder cancer for the first time after cystoscopic tissue resection. The study categorized the participants based on pathology results into those with Ta results, T1 findings, and healthy controls as groups 1, 2, and 3, respectively. The control group included participants with a similar age and no known chronic disease, who presented to urology outpatient clinics because of nonspecific symptoms, such as scrotal pain or flank pain, and who were without any medical pathology detected in examinations and analyses. Furthermore, the patients were classified into high- and low-grade groups based on pathology results. The European Association of Urology (EAU) NMIBC Risk calculator, recommended in the latest EAU guideline, was used for risk classification. According to the risk classification, patients were categorized into low-risk, intermediate-risk, high-risk, and very high-risk groups. Tumor diameter and size were identified intraoperatively, as recommended in the EAU guideline, in reference to the 5-mm-long cutting loop utilized during transurethral resection. Growth in the urine culture indicated a urinary infection.

Exclusion criteria were patients with non-malignant pathology results; female; with bladder cancer pathology other than transitional cell carcinoma; with bladder cancer recurrence; undergoing active chemotherapy, immunotherapy, or irradiation; diagnosed with any malignancy other than bladder cancer; suffering from hypertension, diabetes mellitus, or cardiovascular disorders; previous endoscopic procedure or surgical operation involving the urinary system within the past 3 months.

2.1. Blood Samples. Blood samples of patients who were suspected of having bladder cancer and agreed to participate in the study were obtained after overnight fasting at 7 AM, the day before the cystoscopic tissue resection procedure. Vacutainer needles (BD Vacutainer, EclipseTM, USA), preferably accompanied by an adapter, are used to obtain approximately 2 mL of venous blood samples from each patient, in a single tube, from their right or left antecubital fossa. The obtained samples were then transferred to gel serum separator tubes (BD Vacutainer SSTTM II Advance 5 mL, USA). The blood samples were kept in an upright position at room temperature for 30 min to enable the clotting process. The blood samples were centrifuged for 15 min at 4000 rpm at +4°C to determine the endocan levels. The resulting serum was transferred to sterile Eppendorf tubes (Axygen Snaplock microcentrifuge tubes 2 mL, USA). The serum samples were stored in a deep freezer at −80°C until the day of analysis.

2.2. Urine Samples. Spot urine samples of patients suspected of having bladder cancer who agreed to participate in the study were collected into sterile urine collection cups (BD Vacutainer® urine collection cup, Becton Dickinson and Company, Franklin Lakes, NJ, USA), simultaneously with blood samples the day before cystoscopic tissue resection. Urine was then transferred from the containers to the respective urine transfer tubes (BD Vacutainer® Plus urinalysis tubes, Becton Dickinson and Company, Franklin Lakes, NJ, USA). Urine samples were centrifuged at +4°C for 5 min at 2000 rpm. The supernatant of 2 mL that resulted from this process was collected in an Eppendorf tube (Axygen Snaplock Microcentrifuge Tubes 2 mL, USA) using an automated pipette and aliquoted. The samples were stored at −80°C until the day of analysis.

2.3. Analyte Determination Methods. Serum and urine ESM-1 levels were simultaneously evaluated to prevent discrepancies caused by day-to-day variations. Serum and urine ESM-1 levels were measured using the enzyme-linked immunosorbent assay (ELISA) method and the Human ESM-1 ELISA Kit (Elabscience, Cat No: E-EL-H1557, USA), following the manufacturer's protocol. The kit demonstrated a measuring capacity of 15.63–1000 pg/mL. Serum samples had no dilution before testing, but 1/2 wash buffer was added

to urine samples to adjust the pH. This was accomplished by combining a urine sample of 50 L with a washing buffer of 50 L.

Briefly, the measurement procedures were as follows: serum, urine samples, and standard solutions, with decreasing concentrations acquired by successive dilutions, were inserted in 96-well microplates coated with specific monoclonal antibodies produced against human ESM-1.

ESM-1 molecules present in the samples were bound to these coated antibodies. Any unbound molecules were eliminated via a washing process. A second antibody specific for ESM-1 and labeled with biotin was added to the wells. A peroxidase enzyme attached to streptavidin was added after another round of washing. The peroxidase enzyme in this avidin-bound complex generated a color change in direct proportion to the ESM-1 content in the samples by oxidizing the 3,3′ 5,5′-tetra-methyl benzidine supplied to the environment. An acid was subsequently added to each well to stop the reaction. A spectrophotometer at a wavelength of 450 nm was used to measure the absorbance values for each well. ESM-1 concentration was calculated in pg/mL in each sample from the absorbance–concentration graph using standards generated at concentrations of 15.63 pg/mL, 31.25 pg/mL, 62.5 pg/mL, 125 pg/mL, 250 pg/mL, 500 pg/mL, and 1000 pg/mL. The results were multiplied by 2 because urine samples were diluted by half. Obtained urine ESM-1 levels were reported in creatinine at pg/mg by dividing by creatinine values determined in the same urine sample to avoid dilution effects during the day. Commercial kits on the Beckman Coulter AU5811 device (Japan), using the colorimetric "modified Jaffe" method, were utilized to determine urine creatinine levels. The results are reported in mg/dL.

2.4. Statistical Analysis. Categorical data were shown as numbers and percentages and continuous variable data as mean (and standard deviation) and the median (the interquartile range, IQR). The Shapiro–Wilk test was used to identify the normality of the distributions of continuous variables. The mean differences between two independent groups of normally distributed data were compared with an independent *t*-test. The mean differences among more than two independent groups of normally distributed data were compared with an analysis of variance. Bonferroni correction was utilized for pairwise comparison of more than two groups with statistically significant differences. Fisher's exact test was used to compare the frequencies of categorical variables. Receiver operating characteristic (ROC) analysis was performed for urine endocan levels, predicting T1 (Figure 1). Spearman's correlation analysis was performed for serum and urine endocan levels in terms of the T1 stage. Univariable and multivariable logistic regression analyses were conducted to identify T1 predictors. A p value of <0.05 indicated a statistical significance.

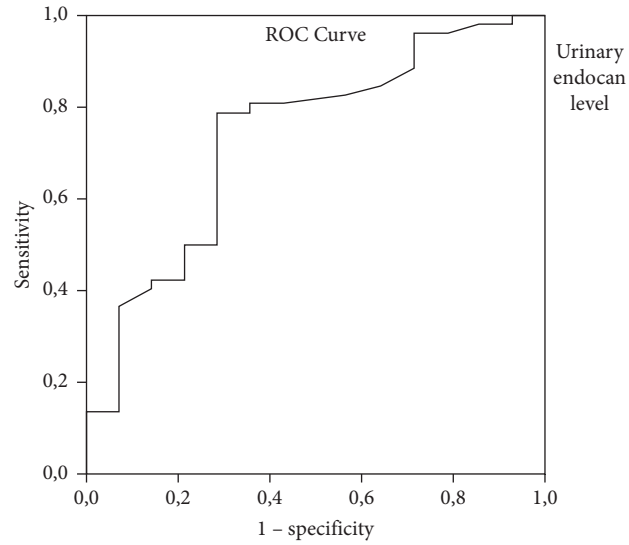

Figure 1: Receiver operating characteristic (ROC) analysis of urinary endocan level predictive value in predicting T1 disease.

3. Results

This study included 66 male patients and 41 healthy male controls. The median ages of groups 1, 2, and 3 were 70.5 (interquartile ranges (IQR): 38–83) years, 65.5 (IQR: 40–90) years, and 63 (IQR: 40–82) years, respectively. No significant difference in age was observed between the groups ($p = 0.627$).

Patients in the groups were staged according to histopathology results. Serum and urine endocan levels were compared among Ta, T1, and control groups. Serum endocan levels were statistically significantly higher, according to pathological staging, in the Ta and T1 groups than in the control group ($p = 0.012$ and $p < 0.001$, respectively). No statistically significant difference in serum endocan levels was found between patients with Ta and T1 stages ($p = 0.857$). The urine endocan level in group 2, consisting of T1 patients, was statistically significantly higher than that of the control group ($p < 0.001$). Urine endocan levels in group 1, including patients with Ta stage, were higher than those of the control group but with no statistically significant difference ($p = 0.271$). Table 1 shows demographic data, serum and urine endocan levels, and group comparisons.

The association of the patients' tumor diameter and number with serum and urine endocan levels was investigated. The correlation analysis revealed no positive correlation between tumor diameter and urine endocan levels. Table 2 presents the correlation study between serum and urine endocan levels and tumor size and number.

Comparisons in group 2, consisting of patients with T1 categorized as low-grade and high-grade, revealed no statistically significant difference in either serum or urine

Table 1: Comparison of Ta, T1, and control group patients in terms of demographic data and laboratory parameters.

Variables	Groups			p value
	Ta (1)	T1 (2)	Control (3)	
Number of patients (n)	14	52	41	
Age, years, median (IQR)	70.5 (38–83)	65.5 (40–90)	63 (40–82)	0.627*
Mean serum endocan ± SD, (mg/dl)	1434 ± 446	1504 ± 443	1039 ± 422	<0.001* 1 vs 2 0.857 1 vs 3 0.012 2 vs 3 <0.001
Mean urine endocan ± SD, (mg/dl)	675 ± 247	914 ± 362	502 ± 388	<0.001* 1 vs 2 0.077 1 vs 3 0.271 2 vs 3<0.001
Urine culture positivity, n (%)	2 (14.3)	3 (5.8)	0 (0)	0.064[&]

*One-way ANOVA, [&]Fisher's exact test; IQR: interquartile range, SD: standard deviation, mg: milligram, dL: deciliter, Ta: the tumour is only in the innermost layer of the bladder lining. T1: the cancer has started to grow into the connective tissue beneath the bladder lining.

Table 2: Correlation between serum and urinary endocan level, tumor size, and tumor number.

Spearman's rho*		Tumor diameter	Number of tumors
Serum endocan	CC	0.168	0.075
	Sig. (2-tailed)	0.178	0.550
Urine endocan	CC	0.084	0.014
	Sig. (2-tailed)	0.504	0.914

CC: correlation coefficient.

Table 3: Comparison of serum and urinary endocan levels of low grade and high grade patients in T1 group patients.

Variables	T1		p value
	LG	HG	
Number of patients	33	33	
Serum endocan	1447 ± 441	1530 ± 444	0.452*
Urine endocan	828 ± 325	899 ± 380	0.418*

*Independent sample t test, LG, low-grade, HG, low-grade.

endocan levels ($p = 0.452$ and $p = 0.418$, respectively). Table 3 shows data comparing low- and high-grade patients in group 2 based on serum and urine endocan levels.

Moreover, we evaluated the ability of urine endocan level as a marker for T1 disease in individuals with NMIBC using the ROC curve analysis (Figure 1, Table 4). We revealed an area under the curve of 0.735 and a cut-off value of 903.

No statistical difference in serum and urine endocan levels was detected between the risk-stratified groups, as summarized in Table 5.

Univariate and multivariate analyses were performed after classifying patients into low-, medium-, and high-risk groups (high- and very-high-risk groups). EAU evaluation according to risk groups revealed that urine endocan was statistically significantly higher in the univariate analysis performed in the high-risk groups (high- and very-high-risk group; $p = 0.034$). Table 6 shows data regarding univariate and multivariate analyses of serum and urine endocan levels according to the risk groups.

4. Discussion

Our results indicate a significant elevation of serum endocan levels in patients with Ta and T1 stages compared with the control group and reveal the potential of endocan in the non-invasive diagnosis of bladder tumors. Thus, our pioneering study provided a new avenue for early detection and risk assessment.

Bladder cancer is one of the 10 most prevalent malignancies globally, with approximately 550,000 new cases diagnosed annually [15]. Urothelial cancer, specifically, has been diagnosed in >90% of patients [16].

Typically, most of the diagnosed patients are in their 70s, but the average age of onset is >50 years. [17]. The treatment strategy for NMIBC is tailored following, the patient's prognostic profile. Various prognostic models are utilized to predict the risk of disease recurrence and/or progression, using some pathological features such as T-stage and disease degree [5].

Table 4: Receiver operating characteristic (ROC) analysis of urinary endocan level predictive value in predicting T1 disease.

	Cut-off value	Sensitivity-specificity (%)	AUC	95% CI	p value
Urine endocan (mg/dl)	903	(50.0–78.6)	0.735	0.584–0.886	0.007

ROC, receiver operating characteristic; AUC, area under the ROC curve; CI, confidence interval.

Table 5: Comparisons of serum and urine endocan levels between risk groups.

	RG 1	RG 2	RG 3	RG 4	p value
Number of patients	13	11	37	5	
Mean serum endocan ± SD, (mg/dl)	1310 ± 414	1492 ± 440	1557 ± 439	1441 ± 532	0.388*
Mean urine endocan ± SD, (mg/dl)	738 ± 280	829 ± 316	927 ± 389	791 ± 279	0.378*

SD, standard deviation; RG 1, low risk; RG 2, intermediate risk; RG 3, high risk; RG 4, very high risk. *One way ANOVA.

Table 6: Univariate and multivariate analysis for serum endocan, urine endocan levels in different risk groups of nonmuscle invasive bladder cancer.

	Univariate			Multivariate		
	OR	95% CI	p value	OR	95% CI	p value
Serum endocan	1.000	0.999–1.002	0.599	1.000	0.998–1.002	0.704
Urine endocan	1.003	1.000–1.006	0.034	1.003	1.000–1.006	0.056
EAU risk groups	48.455	5.701–411.863	<0.001	51.504	5.621–471.912	<0.001

OR, odds ratio; CI, confidence interval, SD: standard deviation; EAU, European association of urology.

Detecting the disease early, performing close monitoring, and implementing and modifying the treatment modality, as needed, is crucial because of the high recurrence rate and invasive nature of bladder tumors. However, noninvasive diagnostic methods, such as cytology and tumor markers, have not yet achieved optimal efficacy in early detection and prognosis or gauging tumor invasion in bladder cancer. Researchers have focused on noninvasive biochemical markers rather than invasive procedures with high morbidity and mortality because of the need for close patient monitoring. The promising use of markers, such as nuclear matrix protein-22, in demonstrating tumor and recurrence increased the interest in this type of research. Angiogenesis, defined as new blood vessel formation, is a crucial element in tumor growth, dissemination, and metastasis. Additionally, researchers have investigated the concentrations of endothelial markers involved in angiogenesis, tumor tissues, bloodstream, and urine concentrations [18–20].

The morphology of bladder tumors alone is insufficient to predict prognosis. Hence, biochemical studies are promising for elucidating the morphological characteristics, thereby gaining a deeper insight into tumor behavior. These studies have not conducted histopathological assessments of bladder tumors obsolete, but they have certainly improved our comprehension of the morphological transformations. Endocan is one of these molecules. Endocan plays a crucial role in tumor angiogenesis, progression, and recurrence, which are poor prognosis indicators. A correlation is noted between increased endocan mRNA levels and metastasis and poor prognosis in different cancers, including kidney, breast, liver, and lung cancer [12].

A study conducted on patients with hepatocellular cancer revealed high and low endocan levels, microvessel density, and overall survival time of patients to be statistically significantly different. In particular, elevated endocan levels were correlated with microvessel density and reduced survival duration [21].

Research indicates significantly elevated serum and urine endocan levels in patients with bladder tumors compared with those without [14].

Serum endocan levels, according to pathological staging, were significantly higher in patients with Ta and T1 stages compared with those of the control group ($p = 0.012$ and $p < 0.001$, respectively). However, no statistically significant difference in serum endocan levels was found between patients with Ta and T1 stages ($p = 0.857$). The patients with the T1 stage (group 2) demonstrated a significantly higher urine endocan level than both those with the Ta stage (group 1) and the controls ($p < 0.001$). In our study, serum and urine endocan levels in patients with NMIBC were higher than those of the control group, which is consistent with the literature [14].

Pathological staging revealed a statistically significant difference in urine endocan levels between pTa and pT1 tumors. Additionally, the univariate analysis performed in the high-risk groups (high- and very-high-risk groups) revealed a significantly higher urine endocan level. Urine endocan levels were higher than serum levels in pTa and pT1 tumors. This indicates a greater direct passage of the endocan through the bladder wall than the molecule's passage into the general circulation.

Our study has several limitations. First, the sample size is relatively small. Second, recurrent disease could not be evaluated. Third, bladder cancers other than urothelial

carcinoma could not be evaluated. However, to the best of our knowledge, this is the first clinical study to show the association of the urine endocan level with predicting NMIBC T1 disease and with high- and very-high-risk groups according to the EAU risk classification.

5. Conclusion

Our study verifies the potential diagnostic value of endocan levels in bladder cancer by investigating the association of the serum and urine endocan levels with the stage and grade of bladder tumors in patients with NMIBC to help in the risk stratification of these patients. However, prospective controlled studies in a larger series on this subject are warranted for a more conclusive statement.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Additional Points

Permission to Reproduce Material from Other Sources. Material from other sources was not used in the article.

Ethical Approval

Local ethics committee approval was obtained.

Consent

Informed consent was obtained from all patients included in the study.

Conflicts of Interest

All authors declare that are no conflicts of interest with this publication.

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