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Short Communication

Diagnostic accuracy of urinary cytokeratin fragment-19 (CYFRA21-1) for bladder cancer

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Abstract

Bladder cancer (BC) is known for its high recurrence rate and requires constant patient monitoring. To confirm the diagnosis, a tissue sample from a cystoscopy is required, which the patient often avoids. Urine has the potential to be utilized as a diagnostic fluid because of its non-invasive nature and various biomarker contents. The aim of this study was to determine the diagnostic value of cytokeratin fragment-19 (CYFRA21-1) levels in urine for diagnosing BC. This single-center cross-sectional study included adults aged ≥18 years who presented with hematuria and had suspected BC based on imaging findings. Patients with a history of intravesical chemotherapy, radiotherapy and immunotherapy were excluded. Urine samples were collected prior to the cystoscopy. Detection of urinary CYFRA21-1 was carried out using the enzyme-linked immunosorbent assay (ELISA) method. Of 154 patients included in the study, the diagnosis of BC was confirmed in 92 patients. Patients with BC had significantly higher urinary CYFRA21-1 levels compared to the non-bladder cancer group. The sensitivity, specificity, positive and negative predictive value, and positive likelihood ratio of the CYFRA21-1 were 80.4%, 43.5%, 67.9%, 60% and 1.425, respectively. The area under the curve (AUC) for CYFRA21-1 was 0.608, computed from a receiver operating curve (ROC) with a cut-off value of 13.3 ng/mL. In conclusion, urinary CYFRA21-1 levels have moderate diagnostic accuracy in determining BC among suspected individuals. Due to its high sensitivity, this biomarker could potentially be used alongside other screening tools for BC detection.

Keywords: Bladder cancer, CYFRA21-1, urinary biomarker, diagnostic accuracy, tumor marker

Introduction

According to the 2022 cancer statistics, bladder cancer (BC) ranks as the ninth most common cancer worldwide, with a prevalence of approximately 3%. The ratio of male-to-female prevalence is approximately 5:1 [1]. Smoking is the most significant risk factor in almost 50% of cases [2,3]. Of all cancers, bladder cancer has the highest recurrence rate (30–70%) and necessitates close patient observation for several years [4,5]. Tumor specimens are typically obtained for histopathological evaluation through cystoscopy with a bladder biopsy. The primary drawback of the procedure is its invasive nature and potentially high costs, which often result in significant discomfort for the patient [6]. Therefore, high-sensitivity and specificity non-invasive techniques are required for the early detection of initial tumors and recurrences.

Finding effective biomarkers for BC may reduce the number of unnecessary cystoscopies. Urine is considered an ideal body fluid for detecting pathophysiological changes since it is the

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product of blood filtration by the kidneys and contains several soluble biomarker proteins that are in direct contact with the bladder [7,8]. Urine is readily available and can be collected without invasive procedures, making it an appropriate specimen for biomarker studies [9].

Proteins are macromolecules that are highly adaptable and directly contribute to biological processes. Analyzing the alterations in tumor-specific proteins is critical for understanding the molecular mechanism of carcinogenesis and development [10]. Cytokeratin, an intermediate filament of the epithelial cytoskeleton, is excreted in the urine following the demise of epithelial cells. Research has demonstrated a substantial association between the advancement of bladder cancer and cytokeratin 8, 18, 19, and 20 [11]. In muscle-invasive bladder cancer (MIBC), overexpression of cytokeratin-19 has been observed and can be excreted in both blood and urine [12]. The group of cytokeratin-19 fragments identified using those antibodies in a two-step sandwich enzyme-linked immunosorbent assay (ELISA) is known as CYFRA21-1 [13].

A previous study on bladder cancer revealed that urinary CYFRA21-1 had a sensitivity and specificity of 96.9% and 67.5%, respectively [14]. A separate investigation has shown that the presence of CYFRA21-1 in urine had a sensitivity and specificity of 67.3% and 88.4%, respectively [15]. The presence of numerous normal cells and metabolic substances in urine could potentially impact the levels of cytokeratin and other protein biomarkers, leading to inconsistent results. The overexpression of CYFRA21-1 in MIBC raises the possibility that it could aid in distinguishing between NMIBC and MIBC. As a result, further investigation is required to determine its clinical relevance in bladder cancer. Therefore, the aim of this study was to evaluate the efficiency of urinary CYFRA21-1 in diagnosing bladder cancer.

Methods

Study design and participants

The study was conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from October 2023 to March 2024, with a cross-sectional design. This study investigated all patients who visited the urology polyclinic or emergency room with gross hematuria. Patients aged ≥18 years with suspicion of bladder cancer from radiographic findings were included. The study excluded patients who had previously undergone chemotherapy, immunotherapy, radiation, or intravesical chemotherapy. Samples were chosen through consecutive sampling with a minimum required sample size of 112, determined using the Lemeshow formula [16].

The study participants were divided into two groups (bladder cancer and control groups). The bladder cancer group included patients diagnosed with bladder cancer, while the control group included patients with a history of hematuria but without any evidence of bladder tumors on cystoscopy or histological evaluation. All patients provided written informed consent, and the study received approval from the ethics committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, with registration number 0611/KEPK/III/2023.

Study variable and data collection

Demographic data, including age and gender, was collected by reviewing the electronic medical records (EMR) system within the hospital. Additionally, imaging data related to the patients were retrieved and analyzed based on the diagnostic and radiological reports stored within the EMR database. Urine samples and histopathological results were collected prospectively as part of the ongoing study. The urine samples were analyzed for CYFRA21-1 levels using an ELISA at the Clinical Pathology Laboratory of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The diagnosis of bladder cancer was established through histological examination, conducted by a pathologist, which served as the gold standard for evaluating the diagnostic accuracy of CYFRA21-1 levels. The specimen for histological examination was obtained through transurethral resection of bladder tumor (TURB).

Bladder cancer patients were classified into two subgroups according to the degree of tumor infiltration into the bladder wall: non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC). In NMIBC, tumors were limited to the mucosa and submucosa, which include carcinoma in situ, pTa, and pT1 tumors, as classified by the tumor, node, and metastasis (TNM) system. On the other hand, tumor cells invaded the muscle layer and

covered tumor stages pT2-pT4 in MIBC. The grading of BC was determined according to the 2004/2022 World Health Organization (WHO) criteria.

Urinary CYFRA21-1 levels test

Following imaging (ultrasound or contrast-enhanced computed tomography), urine samples were collected before the patient underwent bladder biopsy or transurethral resection of bladder tumor. The urine samples were preserved at -80°C until further assays were performed. This study used the Human CYFRA21-1 ELISA kit by Elabscience (Catalogue No. E-EL-H2077). Prior to usage, all reagents and specimens were adjusted to room temperature. The urine samples were centrifuged at 3,000 rpm for 10 minutes at 4°C to separate cellular debris before testing for CYFRA21-1 levels. The standard curve equation was determined by standard plotting. Serial dilutions (1:10 to 1:200) were performed on a urine sample that had a high marker. The type of microplate reader used was the HumaReader Single Plus.

Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate the normality of the numerical data. The Kruskal-Wallis and Mann-Whitney tests were conducted to evaluate the continuous data due to the abnormal distribution of the data. The diagnostic efficacy of urine CYFRA21-1 was evaluated through an analysis of receiver operating characteristic (ROC) curves. The optimal threshold was determined to be the one with the highest Youden index, and the sensitivity and specificity were calculated accordingly. The data was analyzed using IBM SPSS Statistics 25.0 (IBM, New York, USA). Statistical significance was defined as a *p*-value less than 0.05. Figures were generated using GraphPad Prism software (GraphPad Software, Boston, USA) and SPSS.

Results

Subjects study characteristics

The characteristics of the study subjects are presented in **Table 1**. The bladder cancer group consisted of 75 (81.5%) male and 17 (18.5%) female patients. Histopathological evaluation detected 88 cases of urothelial carcinoma (**Table 2**). Based on staging, 12 patients were diagnosed with NMIBC, and 80 patients were diagnosed with MIBC. The non-bladder cancer group comprised 35 (56.5%) male and 27 (43.5%) female patients.

Table 1. Characteristics of the study subjects

Variables	Bladder cancer (n=92)	Non-bladder cancer (n=62)	*p*-value
Demographic data			
Age, mean±SD (years)	59.44±13.46	52.51±16.00	0.005
Sex (male/female)	75/17	35/27	0.001
Tumor stage, n (%)			
NMIBC	12 (13.1)	NA	NA
MIBC	80 (86.9)	NA	NA
Tumor grade/histology classification, n (%)			
Papillary urothelial neoplasm of low malignant potential	5 (5.4)	NA	NA
Low-grade	9 (9.8)	NA	NA
High-grade	78 (84.8)	NA	NA
Urinary CYFRA21-1, mean±SD (ng/ml)	145.3 (0.5–3,677)	51.42 (0–1,313.5)	<0.001

MIBC: muscle invasive bladder cancer; NA: not applicable; NMIBC: non-muscle invasive bladder cancer

Table 2. Histopathological characteristics of the study subjects

Group	n (%)	CYFRA21-1 (ng/ml)
		Median (min-max)
Bladder cancer	92 (59.75)	145.30 (0.5–3,677)
Urothelial carcinoma	88 (57.14)	
Adenocarcinoma	2 (1.30)	
Myxoid liposarcoma	1 (0.65)	
Squamous cell carcinoma	1 (0.65)	
Non-bladder cancer	62 (40.25)	51.4 (0–1,313.5)

Group	n (%)	CYFRA21-1 (ng/ml) Median (min-max)
Renal cell carcinoma	18 (11.68)	0.645 (0–301.3)
Adenocarcinoma prostate	8 (5.20)	70.8 (1.5–356.6)
Testicular tumor	3 (1.95)	104 (5.5–140.9)
Urolithiasis	8 (5.20)	265.8 (10.2–466.5)
BPH/LUTS	7 (4.54)	121.95 (5.5–430)
Cystitis	6 (3.90)	9.25 (1.8–460.3)
Gynecology tumor	12 (7.79)	418.55 (0.6–1,313.5)

BPH: benign prostatic hyperplasia; LUTS: lower urinary tract symptoms

Correlation between urine CYFRA21-1 and clinical features

The Kruskal-Wallis test indicated no significant difference in urine CYFRA21-1 level across bladder cancer grades (p=0.411), as presented in **Figure 1**. However, the test indicated a significant difference in urine levels of CYFRA21-1 between NMIBC, MIBC, and non-bladder cancer groups (p=0.003). Post-hoc comparisons using Dunn's method with a Bonferonni correction for multiple tests indicated that the urine CYFRA21-1 level in the MIBC group was significantly higher than that in the non-bladder cancer group (p=0.011). However, there was no significant difference in comparisons of NMIBC versus MIBC group (p=0.053) and non-bladder cancer versus NMIBC group (p=1.000).

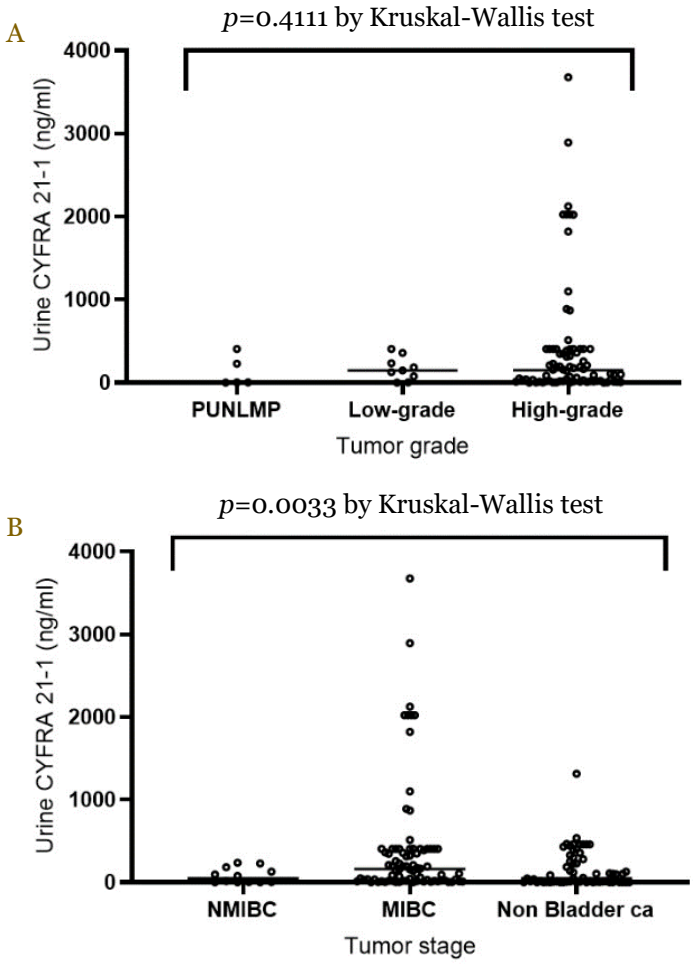

Figure 1. Association between urine CYFRA21-1 and clinical characteristics. Analysis of urine CYFRA21-1 levels in individuals with different grades of bladder cancer showed no significant difference in tumor grading across bladder cancer (Kruskal-Wallis test; p=0.4111) (A). Analysis of urine CYFRA21-1 levels in individuals with different stages of bladder cancer showed a significant difference in tumor stage across bladder cancer (Kruskal-Wallis test; p=0.0033) (B).

Diagnostic value of urine CYFRA21-1 for bladder cancer

The concentration of urine CYFRA21-1 was markedly elevated in the BC group compared to the non-bladder cancer group (p<0.001). The ROC curve illustrating the diagnostic efficiency of

urinary CYFRA21-1 for detecting bladder cancer in individuals with radiographic suspicion is presented in **Figure 2**. The area under the curve (AUC) was 0.608 (95%CI: 0.515–0.701). The AUC has been classified as moderate with a statistical significance (p<0.001). The optimal threshold determined based on the constructed ROC curve was 13.3 ng/mL. The sensitivity, specificity, positive and negative predictive value, positive likelihood ratio (LR+) and negative likelihood ratio (LR-) of the CYFRA21-1 were 80.4%, 43.5%, 67.9%, 60%, 1.425 and 0.45, respectively. The sensitivity and specificity of CYFRA21-1 in diagnosing NMIBC were 58.33% and 43.55%, respectively.

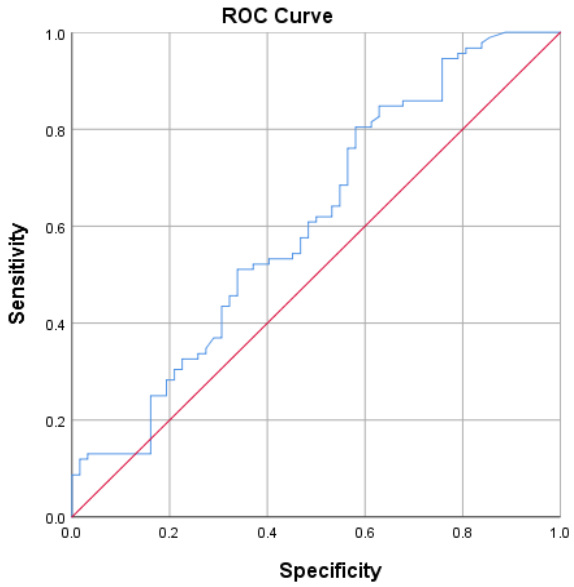

Figure 2. Receiver operating characteristic (ROC) curve of diagnostic efficiency of urine CYFRA21-1 in diagnosing bladder cancer among individuals with radiographic suspicion.

Discussion

The present study found that urinary CYFRA21-1 has a sensitivity of 80.4% and a specificity of 43.5%, respectively, with a maximum cut-off value of 13.3 ng/mL. A previous study demonstrated that the urinary CYFRA21-1 test had a sensitivity of 61.9% and a specificity of 75%, estimated at a cut-off value of 2.8 ng/mL [17]. Another study reported that the sensitivity and specificity of CYFRA21-1 in urine were 96.9% and 67.5%, respectively, with a threshold of 4 ng/mL [15]. High sensitivity is crucial for non-invasive tests to identify tumors accurately. The threshold for determining positivity affects the differences in sensitivity and specificity. Therefore, sensitivity and specificity cannot be used to evaluate the index test's diagnostic accuracy.

Compared to individuals without BC, the BC group's urine CYFRA21-1 concentration was noticeably higher. Cellular injury can cause the release of cytokeratin-19 into serum and urine samples, potentially leading to cellular differentiation and exophytic growth [18]. A prior study demonstrated a positive association between CYFRA21-1 expression and higher stages and grades, as reported in urine by other studies [19]. The increased protease activity of the apoptosis regulator caspase 3 causes an acceleration of cytokeratin breakdown in neoplastic epithelial cells. This fragment release causes an increase in CYFRA21-1 levels [20]. In the present study, significant differences were observed between the non-bladder cancer group and the MIBC group, while no significant differences were found between the NMIBC and non-bladder cancer groups. This suggests that CYFRA21-1 may be more effective in distinguishing MIBC from non-bladder cancer.

The AUC is widely accepted as a measure that represents the comprehensive accuracy of an index test, with values ranging from 0.5 to 1 [21]. Our study's results demonstrate that urine CYFRA21-1 has a modest level of diagnostic efficiency, as reflected by an AUC value of 0.608 [22]. Our results were somewhat lower than those of a previous study, which reported an AUC of 0.74 for urine CYFRA21-1 [19]. Other studies have shown AUC values of 0.797 and 0.72 [14,23]. The differences may be caused by variations in the spectrum and prevalence of diseases within the

cohort being studied [24,25]. One of the best performance indicators for a diagnostic test is the likelihood ratio. A positive likelihood ratio of more than 10 suggests the presence of diseases, while a negative likelihood ratio below 0.1 can effectively exclude the possibility of disease [26]. Our study revealed that the likelihood of the test achieving a positive result in individuals with BC was 1.425 times greater than in people without BC. Thus, the CYFRA21-1 concentration in urine did not meet the requirements for clinical practice and should be adjusted before it can be used in a clinical setting.

The strength of our study includes the use of cystoscopy for all participants, thus minimizing the potential for partial verification bias. Furthermore, this investigation is conducted using a double-blind method. The patients' medical histories were not disclosed to the laboratory personnel, and the clinicians who diagnosed them were similarly blind to the urine CYFRA21-1 test results. Furthermore, all study participants displayed symptoms of bladder cancer, thereby guaranteeing adequate representation of the study group. The limitation of this study is the lack of a standardized method for preparing urine samples to detect CYFRA21-1, which limited the ability to replicate the results with greater accuracy. The complex composition of urine causes challenges in the advancement of accurate and consistent protein quantification technologies. An assessment of the impact of confounding factors, such as hematuria, on the results of the ELISA test, is necessary. The limitations of the assays are a result of the challenges encountered in creating reliable ELISA tests for urine in clinical settings [9]. Another limitation of this study is the need for a more discerning selection of participants. This is because diagnostic tests based on cytokeratin can be easily influenced by benign conditions like urolithiasis or infection, which can elevate the level of cytokeratin in urine [27].

Conclusion

Urinary CYFRA21-1 levels have modest diagnostic accuracy and could be used as a screening tool for BC due to its high sensitivity. However, the test has low specificity for benign diseases, making it unsuitable for bladder cancer diagnosis. Urine CYFRA21-1 diagnostic effectiveness for BC patients requires further prospective, large-scale, multicenter clinical studies.

Ethics approval

The study was approved by the ethics committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, with registration number 0611/KEPK/III/2023. All of the subjects, or their legal representatives, provided informed consent.

Acknowledgments

The authors have nothing to declare.

Competing interests

All the authors declare that they have no known conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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