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Evaluation of The Diagnostic Power of New Urinary Molecular Biomarkers in The Early Diagnosis of Urinary Bladder Cancer

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Abstract

Background: Urinary molecular biomarkers attracting interests as potential tools for early detection and prognosis of urinary bladder cancer (UBC). This study was performed to assess the diagnostic ability of Nuclear Matrix Protein 22 (NMP22), Bladder Tumor Antigen (BTA), microRNA-21 (miR-21) and survivin (BIRC5) in urinary bladder cancer patients.

Methods: A case–control study conducted at Al-Forat A-Awsat Oncology Center, Najaf, Iraq during July 2025 to February 2026. A total 110 subjects were included: 62 patients with histopathological confirm of urinary bladder malignancy, and 48 healthy controls. Standardized collection of fresh urine samples NMP22 and BTA were measured in urine using enzyme-linked immunosorbent assay (ELISA) kits. Methods qRT-PCR was used to measure the expression levels of miR-21 and survivin (BIRC5).

Results: The urinary levels of NMP22 (24.85 ± 8.37 U/mL vs. 9.42 ± 3.18 U/mL), BTA (42.71 ± 12.65 U/mL vs. 18.53 ± 6.27 U/mL), and miR-21 expression (3 Standardized DNA tests for the diagnosis of bladder carcinoma on urine) were significantly higher in patients compared with healthy controls (p <0 001). As a result, survivin had the highest diagnostic with an area under the operating curve (AUC) of 0.945 (sensitivity and specificity: 93.5% and 89.6%), and it was followed by miR-21 AUC = 0.927(91.9% sensitivity,87.5% specificity). AUC value was good for NMP22 (0.841) and BTA 0.812 also, which showed that these two diagnostic tests are doing important role in diagnosis of bladder cancer.

Conclusions: NMP22, BTA, miR-21 and survivin were differentially dysregulated in UCB patients and associated with disease progression. Both survivin and miR-21 displayed better diagnostic and prognostic ability in all biomarkers.

Keywords: NMP22, BTA, miR-21, Survivin, Urinary bladder cancer

Introduction

Urinary bladder cancer (UBC) is one of the most common malignancies that afflict the urinary tract and remains a global public health challenge. Recent epidemiological estimates suggest that bladder cancer is one of the 10 most common cancers diagnosed worldwide with a high incidence especially among men and significant mortality (Guo et al., 2024). Around 75-80% of newly diagnosed patients have non-muscle-invasive bladder cancer (NMIBC), a group remarkably associated with high recurrence rates and, in about 40%, disease progression to more aggressive forms of this disease. With the development of multiple therapeutic strategies but advances in early diagnosis still are critical for improving survival and decreasing disease related morbidity (Fan et al., 2024).

At present, cystoscopy with urine cytology needs to be regarded as the gold determinants for the diagnosis and surveillance of bladder cancer. Cystoscopy, while very accurate for diagnosis, is invasive, costly, and painful to patients; moreover, due to the high rate of recurrence of the tumor in affected bladder tissues, cystoscopy requires repeated examination (Guldhammer et al., 2023). Although highly specific, urine cytology has a low sensitivity (most notably for the detection of lesser grade lesions). These challenges have stimulated large research efforts into development of robust, noninvasive biomarker discovery strategies in the hope that they may enable earlier detection and improve management (Heard & Mitra, 2024).

Abstract Urine is an attractive biological specimen for biomarker discovery as it can be obtained noninvasively and contains proteins, nucleic acids, and tumor-Shed molecules expelled directly from bladder tumors. Recently, advances in molecular diagnostics have suggested the role of urinary biomarkers that could improve diagnostic sensitivity and specificity while minimizing invasive procedures. Therefore, various serum- or urine-based markers for proteins or RNA have been studied as potential and promising tools for diagnosis in bladder cancer (Harsanyi et al., 2024).

One of the most investigated protein biomarkers is Nuclear Matrix Protein 22 (NMP22), which is a nuclear mitotic apparatus protein that originates from apoptotic and necrotic urothelial tumor cells (Tang et al., 2020). Increased urinary concentrations of NMP22 have been related to bladder cancer as well, the assay has been approved by the United States Food and Drug Administration (FDA) as an adjunctive diagnostic test. NMP22 highlights many recent studies emphasizing on NMP22 as a legacy biomarker and improving its analytical performance via novel biosensor technologies. However, a major limitation remains the false-positive results related to hematuria and benign urological conditions (Cheng et al., 2024).

Bladder Tumor Antigen (BTA), is another established urinary marker that detects the presence of complement factor related proteins secreted by neoplastic urothelial cells. Since they are more sensitive than conventional cytology, especially for low-grade tumors, the use of BTA assays has been tested in different clinical situations (Yang et al., 2025). However, urinary tract infections, stones and inflammatory disorders may restrict their specificity and thus independent clinical utility. Therefore, BTA is to be used in conjunction with other molecular markers as they may enhance the diagnostic ability (Heard & Mitra, 2024).

The significant stability of microRNAs in all biological fluids along with their clear role as efficient regulators of the process of carcinogenesis, have made them very promising biomarkers. MicroRNA-21 (miR-21) is one of most studied oncogenic microRNAs among them. MiR-21 targets multiple signaling pathways and acts as an oncogene that promotes tumor initiation, proliferation, invasion and resistance to apoptosis. Concurrently, up-regulated expression of miR-21 has also been implicated in multiple urological malignancies including bladder cancer, supporting its development as a diagnostic and prognostic biomarker (Gan et al., 2024). Additionally, accumulating evidence suggests that miRNA promoter methylation-based assays may be more sensitive compared to other commonly used biomarkers for detecting early-stage disease (Torres-Bustamante et al., 2024).

Another attractive molecular marker in bladder cancer is survivin, which is encoded by the Baculoviral Inhibitor of Apoptosis Repeat Containing 5 (BIRC5) gene. Survivin is part of the inhibitor of apoptosis protein family, and it is a key regulator of cell division and inhibition of programmed cell death (Xiao & Li, 2015). The anti-apoptotic protein survivin is over-expressed in malignant tumors and has been linked to tumor progression, recurrence, and poor prognosis. Survivin has significant diagnostic impact and could be a valuable noninvasive biomarker to detect bladder cancer have recently been reported in a systematic review and meta-analysis. Due to the biological importance and cancer specificity, survivin is an attractive target for molecular diagnosis (Zhou et al., 2024).

Due to the heterogeneous nature of bladder cancer and the restrictions associated with available single marker approaches, there is growing interest in assessing multiple urinary molecular

biomarkers as opposed to using a sole marker. Using protein and nucleic acid biomarkers together, like NMP22 and BTA in combination with miR-21 and survivin, would improve diagnosis levels of early-stage cancer (Ahangar et al., 2024). Thus, the present study was designed to examine urinary molecular biomarkers for their diagnostic performance in the early detection of urinary bladder cancer and also assess their antipicated utility as promising noninvasive clinical tools that have potential use for enhancing the accuracy of disease detection.

Patients and Methods

Study Design and Setting

This case-control study was conducted at Al-Forat A-Awsat Oncology Center, Najaf Province, Iraq in the period from July 2025 to February 2026. The aim of the study was to assess the diagnostic expression of selected urinary molecular biomarkers: Nuclear Matrix Protein 22, Bladder Tumor Antigen, microRNA-21 and survivin (BIRC5) in the early detection of urinary bladder cancer.

Ethical Considerations

The study protocol was approved by the Institutional Ethical Committee of Al-Diwaniya Oncology Center and legal health authorities. All procedures were conducted in accordance with the ethical principles contained within the Declaration of Helsinki. All participants provided written informed consent for participation in the study.

Study Population

Of 150 subjects, there were 62 patients with the diagnosis of beautiful cancer bladder; and 88 apparently healthy individuals who served as controls. Age of participants ranged from 25 to 75. Diagnosis of urinary bladder cancer was made by specialist urologists and oncologists clinically and through cystoscopic, histopathological analysis of bladder biopsies, urine cytology, and radiologic studies when necessary. Tumor staging was assigned according to the American Joint Committee on Cancer (AJCC, 8th edition) tumor-node-metastasis (TNM) classification system, and tumor grading according to the World Health Organization (WHO) classification of urothelial tumors. All patients were newly diagnosed and had not undergone any chemotherapy, radiotherapy, immunotherapy targeted or other therapy when the sample was collected.

Inclusion and Exclusion Criteria

The study population included patients with histopathologically confirmed urinary bladder cancer. The control group consisted of apparently healthy individuals, matched for age and sex, with no history of malignant diseases or chronic inflammatory disorders. Subjects were excluded from the study if they had other malignancies, autoimmune disorders, chronic inflammatory diseases, active urinary tract infections, renal or hepatic insufficiency, diabetes mellitus with complications, a history of organ transplantation, recent surgical intervention, or previous chemotherapy or radiotherapy. In addition, individuals with any medical condition that could potentially affect the measurement or interpretation of urinary biomarkers were also excluded from the study.

Clinical Assessment

Demographic and clinicopathological data were collected using a structured questionnaire designed by us through medical records. Data was collected on age, sex, residency area, smoking status, family history of bladder cancer, body mass index (BMI), tumor size, histopathological subtype and grade (low-grade or high-grade), muscle-infiltrative pathology (non-muscle-invasive or muscle-invasive bladder carcinoma) lymph node involvement; presence of distant metastasis and TNM stage. Specialist physicians and at al-Diwaniya Oncology Center performed clinical examinations and disease stages.

Sample Collection and Processing

Aseptic collection for fresh midstream urine specimens (20–50 mL) were performed with all the participants in sterile urine containers prior to any therapeutic intervention. Samples were transferred to the laboratory as soon as possible and centrifuged at 3000 rpm for 10 min to remove cell debris. Aliquot of the supernatant was made into sterile Eppendorf tubes and stored at −80°C for biochemical and molecular evaluations. Urinary sediments were maintained after centrifugation, frozen at −80°C until RNA extraction for molecular studies.

Urinary NMP22 and BTA measurements

NMP22 test and determine urinary concentrations of Bladder Tumor Antigen (BTA) were both measured using commercially available diagnostic enzyme-linked immunosorbent assay (ELISA) kits according to the respective manufactures' instructions. All assays were conducted in accordance with standardized laboratory processes, and all samples were analyzed in duplicate for reproducibility and analytical precision. Quality-control procedures (internal) were conducted from start to finish in the analysis.

Quantification of miR-21 Expression

Total RNA, comprising of small RNAs, was isolated from urinary sediment samples by using a commercially available RNA extraction kit according to the manufacturer's protocols. Complementary DNA (cDNA) was then synthesized using a reverse transcription kit. miR-21 expression levels were further measured by performing quantitative real-time polymerase chain reaction (qRT-PCR). U6 small nuclear RNA was used as the endogenous control for normalization. Comparative expression levels were quantified relative to 2^-ΔΔCt method.

Measurement of Expression Levels of the Survivin (BIRC5) Gene

Urinary sediment samples were reverse-transcribed into complementary DNA (cDNA) with total RNA extracted. All above mentioned gene-specific primers were used to measure q-PCR levels of survivin (BIRC5)-gene expression. Housekeeping gene for normalization was Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The expression levels in relative amounts were calculated according to the 2^-ΔΔCt method.

Statistical Analysis

All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) software, version 26.0 (IBM Corp., Armonk, NY, USA). Presentation of study variables. Continuous variables are expressed as mean ± standard deviation (SD), and categorical variables as frequencies and percentages. The Shapiro-Wilk test was used to assess the normality of data. The biomarker levels among bladder cancer patients and healthy controls were analyzed using the independent-samples t-test, for normally distributed variables. Biomarker levels were associated with clinicopathological characteristics (tumor grade, muscle invasion, lymph node involvement and TNM stage) using Pearson's correlation. To evaluate the diagnostic performance of NMP22, BTA, miR-21 and survivin alone or in combination, receiver operating characteristic (ROC) curve analyses were performed. Sensitivity, specificity, positive and negative predictive values, area under the curve (AUC) and cut-off values. Multivariate logistic regression analysis was used to identify independent predictors of urinary bladder cancer, as well as the contribution of each biomarker in diagnosis after adjusting for other confounding factors. $P < 0.05$ was regarded as statistically significant for the study.

Results

Demographic characteristics of patients with urinary bladder cancer and healthy controls are shown in Table 1. Most participants in both arms were aged 55 years and older, which reflected the higher incidence of bladder cancer at that age. Most subjects were male, again confirming the well-known male predominance of bladder cancer. For residence, a greater proportion of participants resided in urban areas in either group. Statistical analysis showed no significant differences between patients and controls in terms of age ($P = 0.35$), sex ($P = 0.57$) or residence ($P = 0.12$), suggesting that both groups were well matched for baseline demographic characteristics

Table 1. General information of patients with urinary bladder cancer and comparison with healthy control group

Indicators		`Patients (No. = 62)		Control (No. = 88)		Chi Square	P value (Sig.)
		Freq.	%	Freq.	%		
Age/Years	25-34	4	6.5	7	8	1.98	0.35 (NS)
	35-44	9	14.5	14	15.9		
	45-54	15	24.2	22	25		
	55-64	20	32.3	26	29.5		
	≥ 65	14	22.5	19	21.6		

Gender	**Male**	**49**	**79**	**67**	**76.1**	**0.31**	**0.57 (NS)**
	Female	**13**	**21**	**21**	**23.9**		
Residence	**Rural**	**25**	**40.3**	**32**	**36.4**	**2.43**	**0.12 (NS)**
	Urban	**37**	**59.7**	**56**	**63.6**		

NS: Non-significant at P>0.05

Figure 1 exhibited the distribution of patients with urinary bladder cancer according to stages of urinary bladder cancer, it shows that the prevalence of T(tumor) stage of urinary bladder cancer (n=34, 54.84%) was the most common among the studied patients, followed by N(Nodule) stage (n=19; 30.64%); M(Metastasis) stage of urinary bladder cancer (n=9; 14.52%).

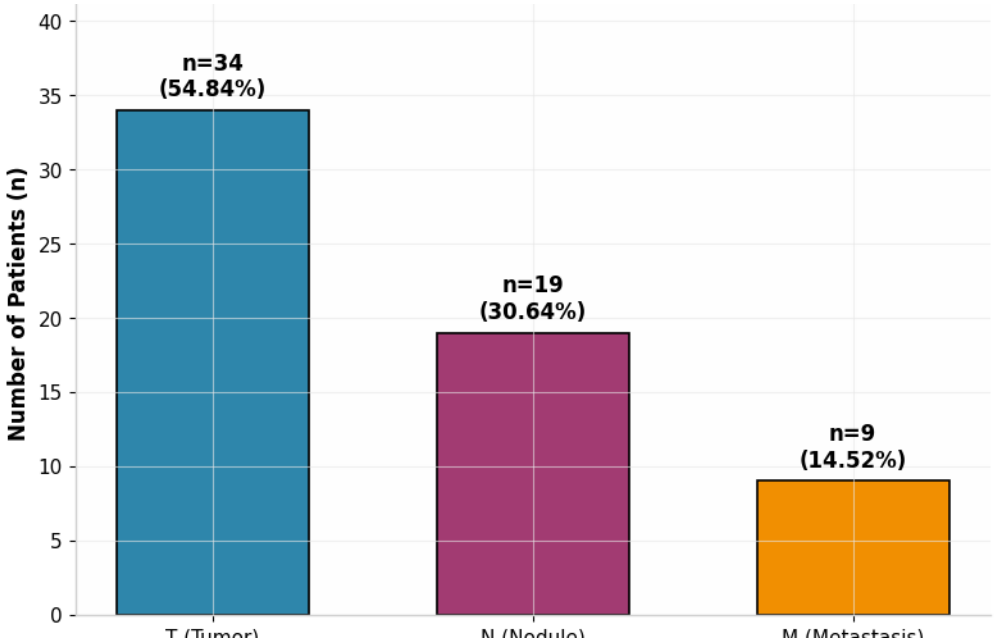

Figure 1. Distribution of patients with urinary bladder cancer according to stages of urinary bladder cancer

Table 2 show that all urinary biomarkers measured levels were significantly ($P < 0.001$) increased in patients with urinary bladder cancer when compared to healthy controls. In the corresponding urinary study, urinary concentrations of NMP22 were approximately 2.6-fold higher in patients compared to controls, whereas the levels of BTA showed a substantial increase, indicating that malignant urothelial cells shed more tumor-associated proteins into urine. Similarly, expression levels of miR-21 and survivin were highly upregulated in the patient group, suggesting activation of oncogenic pathways and inhibition of apoptosis through activation of targets involved in bladder carcinogenesis. The survivorsin was found to be the most varied molecular marker among patients and controls; complemented with miR-21, but very closely associated with it, thus indicating that these molecular markers have a greater discriminatory capacity.

Table 2. Measurement of markers levels between patients with urinary bladder cancer and control subjects

Groups	Patients Mean ± SD	Control Mean ± SD	T Test (P Value)
NMP22 (U/mL)	24.85 ± 8.37	9.42 ± 3.18	t = 13.94 p < 0.001 (HS)
BTA (U/mL)	42.71 ± 12.65	18.53 ± 6.27	t = 14.08 p < 0.001 (HS)
miR-21 (Fold change)	3.89 ± 1.34	1.07 ± 0.42	t = 15.72 p < 0.001 (HS)
Survivin (Fold change)	4.42 ± 1.51	1.18 ± 0.48	t = 16.28 p < 0.001 (HS)

HS: High significant at P<0.001

Results of multiple linear regression analysis in order to find the independent predictors of urinary bladder cancer severity and staging, with the results shown in Table 3. The regression coefficients of all four biomarkers were also positive, indicating higher biomarker levels are associated with more advanced disease stage. Of the two independent variables identified by multivariate analysis, survivin possessed the strongest predictive value (β = 0.421, P < 0.001), followed by miR-21 (β = 0.356, P < 0.001), which indicates that these two molecular markers may be useful as indicators of aggressiveness in tumors closely related to tumor progression. On the other hand, it was NMP22 (β = 0.214, P = 0.013) and BTA (β = 0.183, P = 0.020), which were associated more weakly but still statistically significantly with disease stage.

Table 3. Multiple linear regression analysis for predictors of urinary bladder cancer severity/staging

Predictor	β (Standardized Coefficient)	B	SE	t-value	P-value
NMP22	0.214	0.173	0.067	2.58	0.013*
BTA	0.183	0.146	0.061	2.39	0.020*
miR-21	0.356	0.284	0.073	3.89	<0.001**
Survivin	0.421	0.337	0.079	4.27	<0.001**

The summarized diagnostic performance of the analyzed biomarkers in distinguishing subjects with urinary bladder cancer from controls is shown in Table 4. All biomarkers discriminated (P < 0.001) with AUC values equal to or greater than 0.80 (i.e., good to excellent accuracy). Survivin (AUC = 0.945) had the best overall sensitivity (93.5%) and specificity (89.6%) within the evaluated markers, followed by miR-21 (AUC = 0.927) with corresponding sensitivity and specificity values of 91.9% and 87.5%, respectively. Thus, both molecular markers are excellent classifiers for bladder cancer. In contrast, NMP22 and BTA, the two traditional markers of urinary protein were somewhat less discriminatory. NMP22 had an area under the curve (AUC) of 0.841 with a sensitivity/specificity of 82.3%/79.2% while BTA had the lowest diagnostic accuracy (0.812) among the markers investigated.

Table 4. Diagnostic power parameters of studied markers for the diagnosis of urinary bladder cancer

Biomarker	(AUC)	Sig. p-value	Cut-off Point	Sensitivity (%)	Specificity (%)
NMP22	0.841	<0.001 (HS)	14.5	82.3	79.2
BTA	0.812	<0.001 (HS)	26.8	79	77.1
miR-21	0.927	<0.001 (HS)	1.85	91.9	87.5
Survivin	0.945	<0.001 (HS)	2.15	93.5	89.6

AUC: Area Under the curve

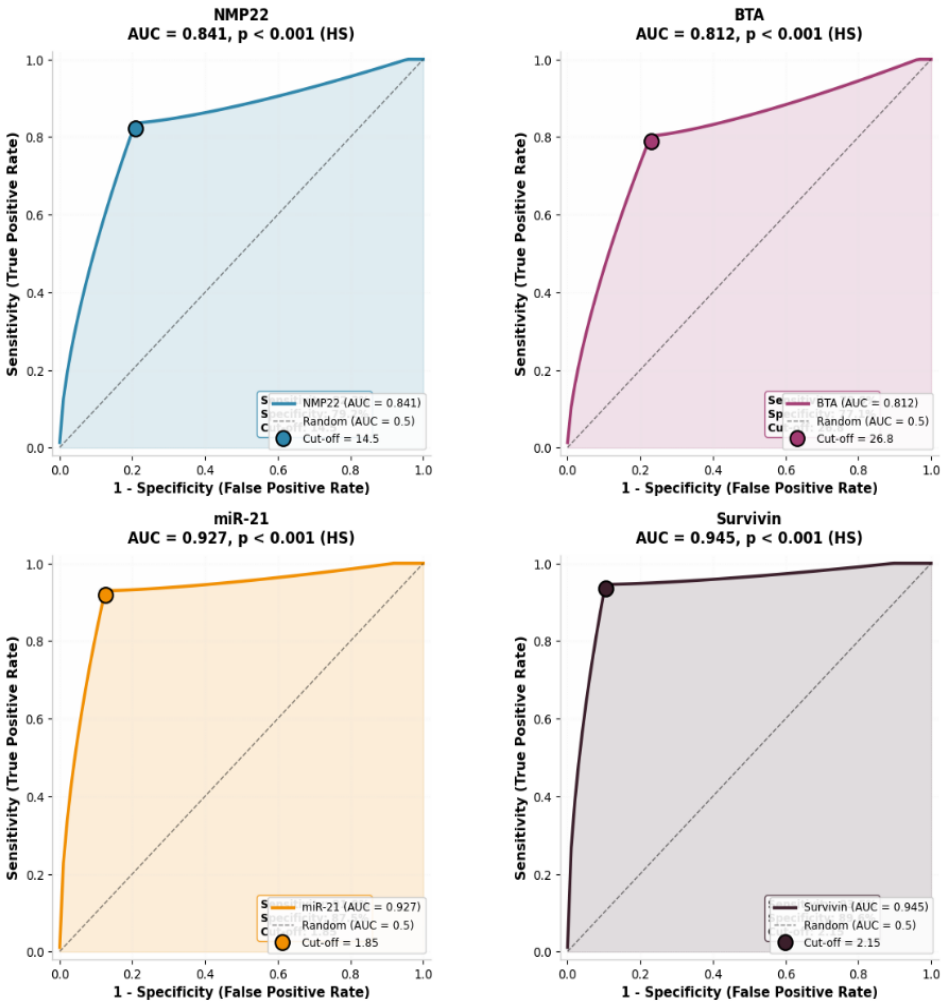

Figure 2. ROC Curve of the studied markers for the diagnosis of urinary bladder cancer

Discussion

The current study investigated the diagnostic value of four urinary markers, Nuclear Matrix Protein 22 (NMP22), Bladder Tumor Antigen (BTA), microRNA-21 (miR-21) and survivin (BIRC5), in patients with urinary bladder cancer. Results showed all biomarker levels were significantly higher in patients than healthy controls, and indicated the presence of biomarkers had diagnostic value with survivin and miR-21 being the most efficacious. Moreover, regression analysis showed that survivin and miR-21 were the most significant independent predictors of disease severity and staging. The results of this study substantiate the increasing evidence that molecular biomarkers can supplement or replace standard urinary markers for early detection and risk stratification of bladder cancer.

Patients and controls were matched successfully, as there were no significant differences in demographic characteristics (age, sex, residency). While most patients were male, which is in agreement with the generally known epidemiology of bladder cancer, there were no significant demographic differences so this limits potential confounding effects. Lenis et al (2020) made similar observations, and stated that bladder cancer is more prevalent in elderly men due to hormonal factors and environmental exposures. Thus, the strong comparability of the study groups increases the validity of biomarker analyses.

Current results revealed remarkably higher urinary concentrations of NMP22 in patients with bladder cancer than control individuals. Urinary NMP22 is a nuclear mitotic apparatus protein that is released from both apoptotic and necrotic urothelial tumor cells, its urinary concentration reflects increased cellular turnover within malignant tissues (Xia et al., 2020). The results are consistent with those reported by Cheng et al. (2024), referring NMP22 as one of the better-established urinary markers, which 0068 are guaranteed to be raised in case bladder carcinoma is present. On the same line, Heard and Mitra (2024) have shown that NMP22 has a better sensitivity than urine cytology especially regarding low grade tumors. However, its specificity may be reduced due to benign diseases including hematuria and urinary tract inflammation; this might account for the lower diagnostic performance of NMP22 compared with molecular biomarkers observed in the current study. On the other hand, Shariat et al. (2010) found that CDX2 was demonstrated in 5/19 (26%) urothelial carcinomas, 7/64 (11%) lung adenocarcinomas, 5/30 (17%) large cell/sarcomatoid lung carcinomas, and 4/19 (21%) esophagus squamous cell carcinomas.

However, urine BTA values were also significantly raised in patients with bladder cancer. BTA assesses the presence of secreted complement factor H-related proteins by malignant urothelial cells, thereby mirroring tumor-associated biological activity. Current results are in line with those of Heard and Mitra (2024) who demonstrated that BT assays are reasonably sensitive, but less specific as elevated levels may also arise from urinary tract infections and inflammatory disorders. Thus, while BTA continues to be a potentially important add-on marker for bladder cancer testing, its diagnostic performance seems less strong than tissue-specific molecular biomarkers.

Out of these, miR-21 showed one of the most optimal diagnostic performance. miR-21 is a well-studied oncomiR that drives tumorigenesis by regulating cell proliferation, invasion, angiogenesis and apoptosis. At this point, miR-21 expression was significantly upregulated in patients and excelled at sensitivity and specificity. The results presented corroborate with the findings reported by Torres-Bustamante et al. (2024), who showed the considerable potential of dysregulated miRNAs for bladder cancer stratification and diagnosis. Similarly, Gan et al. (2024) indicated that the overexpression of miR-21 promotes tumor growth and is associated with adverse clinical presentations in urologic malignancies. This study presents microRNA signature for diagnosis of stage1 NSCLC and reinforces the increasing interest in microRNA-based liquid biopsy methods.

The diagnostic accuracy of survivin evaluated by ROC analysis, represented the largest AUC (0.92) with higher tissue sensitivity and specificity than other biomarkers investigated in this context. Survivin (BIRC5), a member of the inhibitor of apoptosis protein family, is essential for amelioration from programmed cell death and helping with the abnormal proliferation of cells (Kelly et al., 2011). This notably selective over-expression in cancerous tissues accounts for its amazing discrimination power. Zhou et al. confirmed these findings (2024) whose systematic review and meta-analysis suggested that survivin is a very favorable biomarker for bladder cancer diagnosis. Likewise, Fan et al. (2024) recently recognized survivin as one of the most clinically relevant molecular biomarkers involved in bladder carcinogenesis and disease progression.

In the multiple linear regression analysis, all of them were positively correlated with disease severity and staging but among those biomarkers survivin and miR-21 had the most significant independent predictive values. Collectively, these data support a central role for aqueous alterations in apoptosis inhibition and microRNA dysregulation in bladder cancer progression. Guo et

al. (2024) also came to similar conclusions, they observed that molecular alterations correlate intrinsically beginning from non-muscle invasive fall to aggressive disease phenotypes. This suggests that molecular biomarkers may further enhance prognostic prediction beyond what is provided by conventional clinicopathological parameters.

Receiver operating characteristic analysis also confirmed that survivin and miR-21 exhibit superior diagnostic accuracy than the traditional urinary biomarkers (NMP22 and BTA), with area under the curve values > those of each respective conventional biomarker. These data suggest that the RNA based biomarkers and apoptosis-related genes might better discriminate malignant from non-malignant conditions. Harsanyi et al. (2024) found consensus agreement that novel molecular biomarkers demonstrate improved specificity and sensitivity compared to traditional protein-based markers. In addition, the compound of different classes of biomarkers may enhance diagnostic performance and decrease positive false.

However, the small number of patients and its single-center design are potential limitations. Thus, these findings require confirmation in multicenter studies with larger cohorts and standardization of cut-off values prior to broad clinical application. Acknowledgments: Despite these limitations, the present study provides additional support for using urinary miR21 and survivin as promising non-invasive biomarkers of urinary bladder cancer diagnosis and progression.

Conclusion

The current study showed that levels of NMP22, BTA, miR-21 and survivin in urine were significantly higher in bladder cancer patients than in healthy controls. All investigated biomarkers had a good diagnostic performance but miR-21 and survivin exhibited higher sensitivity, specificity, and area under curve (AUC) values. The second finding identified survivin and miR-21 as particularly strong independent predictors of disease severity and staging. These observations indicate that molecular biomarkers may facilitate improved diagnostic and prognostic clinical utility compared to conventional urinary protein markers. Conclusions: Thus, miR-21and survivin found in urine represent useful non-invasive biomarkers for the identification and risk stratification of patients with urinary bladder cancer. More extensive multicenter studies are required to confirm our findings as well as transition into routine clinical practice.

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