European Journal of Clinical Pharmacy
Print ISSN: 2385-409X, **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Emerging Biomarkers for Early Detection of Bladder Cancer

Article History:

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Received: 15-09-2025
Revised: 06-12-2025
Accepted: 13-12-2025
Published: 22-12-2025

Abstract: **Background:** Early detection of bladder cancer remains challenging due to the limitations of conventional diagnostic modalities such as cystoscopy and urine cytology. Emerging urinary and serum biomarkers have shown potential for improving non-invasive detection and risk stratification. **Objective:** To evaluate the diagnostic performance of selected emerging biomarkers for early detection of bladder cancer and to assess their association with tumor grade and invasiveness. **Methods:** This was a hospital-based cross-sectional analytical study conducted at Pir Abdul Qadir Shah Institute of Medical Sciences, Gambat from May 2024 to May 2025 including 125 adult patients with clinically suspected or newly diagnosed bladder lesions. **Results:** Malignancy was confirmed in 78 patients (62.4%). Urinary NMP22 (28.6 ± 9.4 U/mL vs 11.2 ± 4.8 U/mL), BTA (41.5 ± 13.1 U/mL vs 18.7 ± 6.5 U/mL), miR-21 expression (3.8 ± 1.2 vs 1.4 ± 0.6), DNA methylation score (0.72 ± 0.14 vs 0.38 ± 0.10), and serum ctDNA (24.9 ± 7.6 ng/mL vs 9.5 ± 3.7 ng/mL) were significantly elevated in malignant cases ($p<0.001$ for all). The DNA methylation panel demonstrated the highest diagnostic accuracy (sensitivity 91.0%, specificity 85.1%, AUC 0.93), followed by miR-21 (AUC 0.90). Biomarker levels were significantly higher in high-grade and muscle-invasive tumors. **Conclusion:** Emerging molecular and epigenetic biomarkers demonstrate strong diagnostic potential for early bladder cancer detection and correlate with tumor aggressiveness.

Keywords: bladder cancer, biomarkers, DNA methylation, microRNA-21, circulating tumor DNA, NMP22

INTRODUCTION

Bladder cancer is a widespread urinary malignancy and one of the leading sources of cancer morbidity and mortality in the world [1]. It occurs mostly in the older people and it is closely linked with known risk factors which include tobacco smoking, occupational exposure to aromatic amines, chronic inflammation and environmental toxins [3]. Although surgical and intravesical treatment methods have developed, even now, the initial diagnosis remains a key element in increasing survival and reducing relapse of the disease

[5]. Diagnostic diagnosis on the gold standard is cystoscopy with urine cytology but this is invasive, expensive, and unpleasant to patients whereas cytology has shown to have low sensitivity especially in low-grade tumors [2]. The diagnostic gap has increased the research to determine non-invasive, highly sensitive, and specific biomarkers that can be used to enable early discovery, risk classification, and surveillance of bladder cancer [6]. The complicated pathways of molecular changes in bladder cancer encompass FGFR3 mutations, TP53 changes, and cell-cycle regulatory protein aberrants [8]. Generally, these genetic and epigenetic alterations have provided opportunities to investigate circulating tumor DNA (ctDNA), microRNAs, DNA methylation signatures, and protein-based markers as diagnostic tools [4]. New urinary biomarkers like NMP22, BTA, and UroVysion fluorescence in situ hybridization (FISH) have demonstrated good diagnostic capability, although sensitivity and specificity fluctuate and remain an issue [9].

Recent developments in omics technologies, such as proteomics, metabolomics, and transcriptomics, have enabled the identification of new panels of biomarkers rather than relying on single-marker approaches [7]. The microRNAs (miR-21, miR-145) have shown a change in their expression patterns in bladder cancer cases, which makes them good candidates as non-invasive diagnostic indicators [10]. On the same note, aberrant DNA methylation patterns in genes such as RASSF1A and APC are detected in urine samples, underscoring the growing significance of epigenetic biomarkers [12]. Several biomarkers related to inflammatory mediators and immunity are also gaining interest as a result of the established interaction between the tumor microenvironment and carcinogenesis [11]. Also, recent advances in liquid biopsy methodologies can now be used to identify circulating tumor cells and extracellular vesicles, thereby improving early detection and monitoring [14]. The integration models of biomarkers are being explored to enhance diagnostic accuracy using artificial intelligence to integrate molecular, clinical, and imaging data [13]. These integrative methods can circumvent the shortcomings of single-marker tests and offer customized risk evaluation [16]. However, it is critical that validation is done in properly designed clinical studies with sufficient sample size before it can be implemented in routine clinical practice [15]. Considering the rising burden of bladder cancer on the world population and the constraints of the existing diagnostic modalities, there exists a sense of urgency to assess the emerging biomarkers in clinical practices [17].

Objective

To evaluate the diagnostic performance of selected emerging biomarkers for early detection of bladder cancer and to assess their association with tumor grade and invasiveness.

METHODOLOGY

This was a hospital-based cross-sectional analytical study conducted at Pir Abdul Qadir Shah institute of Medical Sciences Gambat from May 2024 to May 2025, including 125 adult patients with clinically suspected or newly diagnosed bladder lesions.

Inclusion Criteria

- Patients aged ≥18 years presenting with painless hematuria, irritative voiding symptoms, or imaging findings suspicious for bladder pathology.
- Patients undergoing cystoscopy with biopsy for definitive diagnosis.
- Patients willing to provide urine and blood samples for biomarker analysis.
- Patients who provided informed written consent.

Exclusion Criteria

- Patients with previously treated bladder cancer or history of intravesical therapy.
- Active urinary tract infection at the time of sample collection.
- History of other urological malignancies.
- Chronic inflammatory bladder conditions such as interstitial cystitis.
- Severe renal impairment (eGFR <30 mL/min/1.73 m²).
- Patients on recent chemotherapy or radiotherapy.
- Pregnant patients.
- Patients unwilling to participate.

Data Collection

After obtaining written informed consent, detailed demographic and clinical data were collected using a structured proforma. Baseline variables included age, gender, smoking status, occupational exposure history, presenting symptoms, duration of symptoms, and family history of malignancy. Physical examination findings and imaging results were documented. Urine samples were collected prior to cystoscopy for analysis of emerging biomarkers, including urinary NMP22, bladder tumor antigen (BTA), selected microRNA expression profiles, and DNA methylation markers. Serum samples were obtained for evaluation of circulating tumor DNA (ctDNA) and inflammatory markers. All specimens were processed in the institutional laboratory following standardized protocols. Cystoscopy findings were recorded, and tissue biopsy was performed in all suspected cases. Histopathological examination served as the reference standard for confirmation of bladder cancer and tumor grading. Tumors were staged according to the TNM classification system. All laboratory, radiological, and histopathological data were systematically recorded, cross-verified, and entered into a secured database for statistical analysis.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS software version 26. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequency and percentage. Diagnostic performance of individual biomarkers was evaluated by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A p-value of <0.05 was considered statistically significant..

RESULTS

The mean age of participants was 58.4 ± 11.2 years, with the majority (85.6%) aged 40 years or older. Patients aged 41–60 years accounted for 43.2%, while those aged 60+ accounted for 42.4%, indicating a predominance in the older age group. Males comprised 73.6% of the cohort, reflecting the well-known male predominance in bladder cancer. A significant proportion were current or former smokers (63.2%), and 27.2% reported occupational exposure to potential carcinogens. Painless hematuria was the most common presenting symptom (70.4%), followed by irritative lower urinary tract symptoms (21.6%), while 8.0% were detected incidentally.

Table 1. Demographic and Clinical Characteristics of Study Participants (N = 125)

Variable	Category	n (%) / Mean ± SD
Age (years)	Overall	58.4 ± 11.2
Age Group	18–40 years	18 (14.4%)
Age Group	41–60 years	54 (43.2%)
Age Group	>60 years	53 (42.4%)
Gender	Male	92 (73.6%)
Gender	Female	33 (26.4%)
Smoking Status	Current/Former Smoker	79 (63.2%)
Smoking Status	Never Smoker	46 (36.8%)
Occupational Exposure	Present	34 (27.2%)
Occupational Exposure	Absent	91 (72.8%)
Presenting Symptom	Painless Hematuria	88 (70.4%)
Presenting Symptom	Irritative LUTS	27 (21.6%)
Presenting Symptom	Incidental Detection	10 (8.0%)

Histopathology confirmed malignancy in 78 patients (62.4%), while 37.6% had benign or inflammatory findings. Among malignant cases, high-grade tumors were more common (59.0%) compared to low-grade tumors (41.0%). Regarding staging, T1 tumors were most frequent (43.6%), followed by Ta (26.9%) and ≥T2 disease (29.5%). Muscle invasion was identified in 18.4% of the total cohort and 29.5% of malignant cases, indicating a substantial proportion presenting with aggressive disease.

Table 2. Histopathological Findings and Tumor Characteristics (N = 125)

Parameter	Subcategory	n (%)
Histopathology	Malignant	78 (62.4%)
	Benign/Inflammatory	47 (37.6%)
Tumor Grade *(among total cases)*	High Grade	46 (36.8%)
	Low Grade	32 (25.6%)
Tumor Grade *(among malignant cases, n=78)*	High Grade	46 (59.0%)
	Low Grade	32 (41.0%)
Tumor Stage *(among total cases)*	Ta	21 (16.8%)
	T1	34 (27.2%)
	≥T2	23 (18.4%)
Tumor Stage *(among malignant cases, n=78)*	Ta	21 (26.9%)
	T1	34 (43.6%)
	≥T2	23 (29.5%)
Muscle Invasion	Present	23 (18.4%)
	Absent	102 (81.6%)

Urinary NMP22 levels were markedly elevated in malignant patients (28.6 ± 9.4 U/mL) compared to non-malignant individuals (11.2 ± 4.8 U/mL). Similarly, urinary BTA levels were higher in the malignant group (41.5 ± 13.1 U/mL) versus controls (18.7 ± 6.5 U/mL). miR-21 expression demonstrated nearly threefold elevation in malignancy (3.8 ± 1.2) compared to 1.4 ± 0.6 in non-malignant cases. DNA methylation scores were significantly increased in

malignant patients (0.72 ± 0.14 vs 0.38 ± 0.10). Serum ctDNA levels were also notably elevated (24.9 ± 7.6 ng/mL vs 9.5 ± 3.7 ng/mL).

Table 3. Comparison of Biomarker Levels between Malignant and Non-Malignant Groups

Biomarker	Malignant (n=78) Mean ± SD	Non-Malignant (n=47) Mean ± SD	p-value
Urinary NMP22 (U/mL)	28.6 ± 9.4	11.2 ± 4.8	<0.001
Urinary BTA (U/mL)	41.5 ± 13.1	18.7 ± 6.5	<0.001
miR-21 Expression (Fold Change)	3.8 ± 1.2	1.4 ± 0.6	<0.001
DNA Methylation Score	0.72 ± 0.14	0.38 ± 0.10	<0.001
Serum ctDNA (ng/mL)	24.9 ± 7.6	9.5 ± 3.7	<0.001

Among individual biomarkers, the DNA methylation panel demonstrated the highest diagnostic performance with sensitivity of 91.0%, specificity of 85.1%, PPV of 90.1%, NPV of 86.2%, and an AUC of 0.93, indicating excellent diagnostic accuracy. miR-21 also performed strongly with sensitivity of 88.5%, specificity of 80.9%, and AUC of 0.90. Urinary BTA showed sensitivity of 85.9% and AUC of 0.86, while NMP22 demonstrated sensitivity of 82.1% and AUC of 0.84. Serum ctDNA yielded sensitivity of 79.5%, specificity of 83.0%, and AUC of 0.87.

Table 4. Diagnostic Performance of Emerging Biomarkers

Biomarker	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Area Under Curve (AUC)
Urinary NMP22	82.1%	76.6%	84.3%	73.5%	0.84
Urinary BTA	85.9%	72.3%	83.8%	75.0%	0.86
miR-21	88.5%	80.9%	88.3%	81.3%	0.90
DNA Methylation Panel	91.0%	85.1%	90.1%	86.2%	0.93
Serum ctDNA	79.5%	83.0%	86.1%	75.8%	0.87

Biomarker levels were significantly higher in aggressive disease phenotypes. High-grade tumors exhibited elevated urinary NMP22 (33.4 ± 8.7 U/mL) compared to low-grade tumors (21.8 ± 6.5 U/mL), along with increased miR-21 expression (4.6 ± 1.0 vs 2.7 ± 0.8) and higher DNA methylation scores (0.81 ± 0.09 vs 0.60 ± 0.11), all with p<0.001. Muscle-invasive tumors demonstrated significantly greater serum ctDNA levels (31.2 ± 6.3 ng/mL) compared to non-muscle invasive disease (21.6 ± 5.4 ng/mL), as well as higher urinary BTA levels (48.7 ± 11.2 U/mL vs 38.3 ± 9.4 U/mL; p=0.002).

Table 5. Biomarker Association with Tumor Aggressiveness (n = 78 Malignant Cases)

Biomarker	High Grade (n=46) Mean ± SD	Low Grade (n=32) Mean ± SD	p-value
Urinary NMP22 (U/mL)	33.4 ± 8.7	21.8 ± 6.5	<0.001
miR-21 Expression	4.6 ± 1.0	2.7 ± 0.8	<0.001
DNA Methylation Score	0.81 ± 0.09	0.60 ± 0.11	<0.001

DISCUSSION

Giant congenital melanocytic nevi (GCMN) represent The current research proved that the new urinary and serum biomarkers hold great promise in the early diagnosis of bladder cancer. Malignancy was proven in 62.4 percent of the patients, and the majority of the tumors were of high grade (59.0 percent) and a high percentage (29.5 percent) of the malignant tumors were identified to have muscle invasion. The findings are consistent with other studies that found that many patients will present with a high-grade or invasive disease at an earlier diagnosis and the use of sensitive non-invasive diagnostic instruments is necessary [18]. Malignant patients had significantly higher levels of urinary NMP22 and BTA than non-malignant patients with a mean level of NMP22 of 28.6 and 11.2 U/mL and BTA of 41.5 and 18.7 U/mL, respectively (p<0.001). The two markers have been previously reported to exhibit similar trends where they were reported to be higher in the cancer patients, but with inconsistent specificity. Our results also support their use in diagnoses especially when combined with molecular tests [19]. MiR-21 and DNA methylation scores were most discriminatory with 3.8 +/-1.2 vs 1.4 +/-0.6 representing the significantly higher expression in malignant cases and the highest diagnostic ability at 0.93 respectively. Similar studies in the past have also borne out the high sensitivity of the epigenetic

panels over the traditional protein based assays in favoring the increasing trend of multi-marker molecular diagnostics [20].

Serum ctDNA levels in malignant patients (24.9 ± 7.6 ng/mL vs 9.5 ± 3.7 ng/mL) and the relationship with muscle-invasive disease (31.2 ± 6.3 ng/mL) were also significantly higher. Similar associations between circulating tumor DNA burden and tumor stage have been reported in previous studies, which indicates that it may be useful in disease detection besides in the stratification and monitoring of the disease [21]. Notably, the level of biomarkers was related to the aggressiveness of tumours. Tumours with high grades were associated with a greater level of NMP22, miR-21 expression and higher levels of methylation scores than low-grade disease, whereas invasive tumours of muscle tissues expressed higher levels of ctDNA and BTA. The same type of associations has been reported in the past studies with molecular biomarkers appearing to be an expression of tumor biology and invasive ability [22]. Comprehensively our results back up the idea that the combination of protein-based markers with molecular and epigenetic biomarkers can enhance the performance of diagnosis. In line with the past studies, multi-marker panels were more sensitive and had better AUCs compared to single biomarkers. These findings support the need to integrate new biomarker panel into the early diagnostic regimen to minimize the need to use invasive cystoscopy, especially when dealing with high-risk groups.

CONCLUSION

This case reports a rare and complex presentation of a It is concluded that emerging urinary and serum biomarkers, particularly DNA methylation panels, miR-21 expression, and circulating tumor DNA, demonstrate high diagnostic accuracy for the early detection of bladder cancer. These biomarkers not only differentiate malignant from non-malignant cases with significant precision but also correlate with tumor grade and invasiveness, reflecting underlying tumor biology. The integration of molecular and protein-based markers offers a promising non-invasive strategy that may enhance early diagnosis, risk stratification, and clinical decision-making, potentially reducing dependence on invasive cystoscopy in appropriately selected patients.

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