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Original Article

Exploring the Role of MicroRNAs in Bladder Cancer Prognosis and Treatment Response

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ABSTRACT

Background: Bladder cancer is a common urological malignancy associated with recurrence, progression, and repeated invasive surveillance. MicroRNAs have emerged as potential molecular biomarkers because they regulate tumor growth, invasion, and treatment-related pathways. Objective: To compare selected microRNA expression between bladder cancer patients and controls and to examine their association with clinicopathological aggressiveness, treatment response, and diagnostic discrimination. Methods: This hospital-based analytical molecular genetics study included 120 participants, comprising 90 histologically confirmed bladder cancer patients and 30 controls. Selected microRNAs, including miR-21, miR-155, miR-145, miR-99a, and miR-125b, were analyzed using quantitative real-time polymerase chain reaction, and relative expression was calculated using the 2^−ΔΔCT method. Expression patterns were compared between groups and examined according to tumor grade, disease stage, and treatment-response records. Results: miR-21 and miR-155 were increased in bladder cancer patients, while miR-145, miR-99a, and miR-125b were reduced. miR-21 showed the strongest increase in cancer patients compared with controls, while miR-145 showed the most marked reduction. Higher miR-21 and miR-155 and lower miR-145 were associated with high-grade tumors, muscle-invasive disease, and poor response or recurrence. miR-21 showed the highest individual diagnostic performance, while the combined miR-21 and miR-145 panel showed the best discrimination. Conclusion: Selected microRNAs showed clinically relevant expression differences in bladder cancer and may support exploratory diagnosis and risk stratification. Larger prospective validation studies are required before clinical application. Keywords: Bladder cancer; microRNA; miR-21; miR-145; prognosis; treatment response; biomarker; urothelial carcinoma; molecular genetics; Lahore; Pakistan.

INTRODUCTION

Bladder cancer is a major urological malignancy in Pakistan, and local cancer registry data indicate a continuing oncological burden requiring stronger diagnostic and prognostic strategies in hospital-based practice (1,2). Evidence from tertiary care settings in Pakistan has shown that bladder cancer patients frequently present with clinically relevant pathological variation, where tumor grade and stage remain important determinants of treatment planning and clinical outcome (3). Globally, bladder cancer is also among the common cancers, with recent estimates reporting substantial incidence and mortality, and its distribution varies by sex, smoking exposure, occupational risk, geographic region, and healthcare access (4,5). These local and international patterns make bladder cancer a clinically important disease in which improved diagnostic, prognostic, and treatment-response markers are needed.

Most bladder cancers are urothelial carcinomas and are clinically categorized into non-muscle-invasive bladder cancer and muscle-invasive bladder cancer because these groups differ in biological behavior, recurrence risk, treatment requirements, and survival expectations (6,7). Non-muscle-invasive disease is more common at initial presentation but is associated with frequent recurrence and repeated surveillance, whereas muscle-invasive disease is more aggressive and often requires radical surgery, systemic therapy, or multimodal treatment. Current diagnosis and surveillance remain heavily dependent on cystoscopy and urine cytology. Although cystoscopy is clinically useful, it is invasive, costly, and uncomfortable when repeated over time, while urine cytology has limited sensitivity for low-grade tumors. Therefore, there is a clear need for reproducible biomarkers that can support early diagnosis, risk stratification, and clinical monitoring without replacing established diagnostic standards prematurely (6,7).

MicroRNAs are small non-coding RNA molecules that regulate gene expression after transcription and influence several cancer-related pathways, including proliferation, apoptosis, invasion, angiogenesis, immune signaling, recurrence, and resistance to treatment (8,9). In bladder cancer, abnormal microRNA expression has been repeatedly associated with tumor initiation, progression, recurrence, and survival-related outcomes. This has made microRNAs attractive molecular candidates for diagnostic and prognostic research, particularly because some microRNAs can be detected in tumor tissue and body fluids such as urine, serum, plasma, and urinary exosomes (10-13). However, the clinical interpretation of microRNA findings depends strongly on sample source, normalization strategy, assay reproducibility, disease stage, and the population in which the marker is tested.

Several microRNAs have shown particular relevance in bladder cancer biology. miR-21 and miR-155 have commonly been described as oncogenic or adverse markers associated with aggressive tumor behavior and poorer clinical outcomes, whereas miR-145 has been studied as a tumor-suppressive microRNA with reduced expression in bladder cancer tissues and invasive phenotypes (20-25). Experimental and clinical studies have suggested that miR-145 may inhibit invasion through molecular targets such as FSCN1 and PAK1, while miR-21 and miR-155 are more often linked with progression, recurrence, and unfavorable prognosis (20-25). Other markers, including miR-99a and miR-125b, have also been investigated as non-invasive diagnostic candidates, particularly in urinary cell-free microRNA research (32). These findings suggest that a focused microRNA panel may provide more clinically interpretable information than evaluation of a single marker alone.

MicroRNA-based testing may also contribute to bladder cancer risk assessment beyond diagnosis. Systematic reviews, meta-analyses, and cohort studies have indicated that selected microRNA signatures may be associated with recurrence, progression, progression-free survival, cancer-specific survival, and overall survival (8,9,26,27). Similarly, urinary and blood-based microRNA studies have reported diagnostic potential for bladder cancer, although concerns remain regarding assay standardization, biological sample handling, cut-off selection, and validation across different populations (15,17,18,38,39). These limitations are important because a biomarker that performs well in one population or biological matrix may not show the same performance in another setting.

Treatment response is another clinically relevant area in which microRNAs may have value. Resistance to intravesical therapy, cisplatin-based chemotherapy, and other treatment pathways may be influenced by microRNA-mediated regulation of apoptosis, DNA damage response, epithelial-mesenchymal transition, and tumor invasiveness (14,36,37). Studies have suggested that some microRNAs may help identify tumors with a more resistant biological profile, although most available evidence still requires stronger prospective validation before routine clinical use. Therefore, research that links microRNA expression with tumor stage, grade, recurrence, and treatment response can help clarify whether these markers are merely biologically altered or clinically informative.

Despite growing international evidence, molecular data on bladder cancer from Pakistan remain limited, particularly studies connecting selected microRNA expression with bladder cancer diagnosis,

clinicopathological aggressiveness, and treatment-response patterns in tertiary hospital settings. Lahore is a major urban center for oncology and urology services, and hospital-based molecular data from this region may provide locally relevant evidence for future biomarker validation. The present study was therefore designed to compare the expression of selected microRNAs, including miR-21, miR-155, miR-145, miR-99a, and miR-125b, between bladder cancer patients and controls, and to examine their association with tumor grade, muscle invasiveness, treatment response, and diagnostic discrimination among patients managed at selected tertiary care hospitals in Lahore, Pakistan.

MATERIALS AND METHODS

This hospital-based analytical cross-sectional molecular genetics study was conducted at selected tertiary care hospitals in Lahore, Pakistan, with laboratory processing performed in a molecular pathology or molecular biology facility equipped for RNA extraction, complementary DNA synthesis, and quantitative real-time polymerase chain reaction analysis. The study was designed to compare selected microRNA expression between histologically confirmed bladder cancer patients and controls and to examine the association of these markers with clinicopathological indicators of tumor aggressiveness and available treatment-response records. Consecutive eligible participants were recruited from urology and oncology units during the defined study period until the planned sample size was achieved.

The study population included adult patients aged 18 years or above with histopathological confirmation of bladder cancer and availability of clinical records documenting tumor stage, grade, histological diagnosis, treatment details, and follow-up status where applicable. Patients were eligible when they provided informed consent and had an adequate biological sample suitable for microRNA analysis. Patients were excluded if they had another active malignancy, severe infection at the time of sample collection, inadequate or poor-quality biological material, incomplete essential clinical records, or refusal to participate. The comparison group included individuals without bladder cancer, selected from patients attending the same hospitals for non-malignant urological complaints and from healthy volunteers where available. Controls were included only when they had no clinical or documented evidence of bladder malignancy and had a biological sample suitable for comparable microRNA expression analysis.

A total of 120 participants were included, comprising 90 bladder cancer patients and 30 controls. A non-probability consecutive sampling technique was used because the study required histologically confirmed cases with available clinical and molecular data. This sampling approach was considered practical for a hospital-based molecular biomarker study where recruitment depended on patient flow, sample availability, and laboratory feasibility. Clinical and demographic data were recorded on a structured proforma, including age, sex, smoking status, presenting symptoms, tumor number, tumor size where available, histological type, tumor grade, disease stage, treatment type, recurrence status, progression status, and treatment-response category. Pathology reports were reviewed to maintain consistency in classification of urothelial carcinoma, non-muscle-invasive bladder cancer, muscle-invasive bladder cancer, low-grade tumor, and high-grade tumor.

Biological samples were collected using coded identifiers to maintain participant confidentiality. For bladder cancer patients, available biological material included tumor tissue from biopsy or transurethral resection specimens, urine samples collected before treatment where possible, and peripheral blood samples for serum or plasma separation. For controls, biological samples were collected in a comparable manner except that tumor tissue was not applicable. For case-control expression comparisons, analyses were restricted to biological sample types available in both bladder cancer patients and controls so that tumor tissue, urinary, and circulating microRNA measurements were not inappropriately pooled as a single undifferentiated dataset. Tumor tissue findings, where available, were used for clinicopathological correlation with grade, stage, and aggressiveness, while body-fluid-based findings were used for diagnostic comparison when the same matrix was available in both groups.

Urine and blood samples were processed soon after collection to reduce pre-analytical degradation. Urine samples were centrifuged to remove debris and cellular contamination, and the processed supernatant or cellular fraction was stored according to the laboratory protocol used for microRNA extraction. Blood samples were centrifuged for serum or plasma separation. Tissue samples were preserved in RNA stabilization medium or stored at low temperature according to laboratory requirements. All samples were labeled with study codes and stored at −80°C until RNA extraction. Repeated freeze-thaw cycles were avoided to preserve RNA integrity.

The selected microRNAs were miR-21, miR-155, miR-145, miR-99a, and miR-125b. These markers were chosen because previous bladder cancer literature has linked miR-21 and miR-155 with oncogenic activity, aggressive disease, recurrence, and poorer clinical outcomes, while miR-145, miR-99a, and miR-125b have been reported as reduced or tumor-suppressive markers with potential diagnostic and prognostic relevance. Total RNA, including small RNA fractions, was extracted using a commercially available RNA extraction kit according to the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically, and only samples meeting acceptable laboratory quality criteria were processed for downstream analysis.

Complementary DNA was synthesized from extracted microRNA using a reverse transcription protocol suitable for microRNA analysis. Quantitative real-time polymerase chain reaction was performed using specific primers for each selected microRNA. An endogenous reference control, such as U6 small nuclear RNA or another validated internal control used by the laboratory, was applied for normalization. Reactions were performed in duplicate or triplicate to reduce technical variation, and relative microRNA expression was calculated using the 2^−ΔΔCT method. Expression values were compared between bladder cancer patients and controls and were further examined according to tumor grade, disease stage, and treatment-response category.

The primary outcome was the difference in selected microRNA expression between bladder cancer patients and controls. Secondary outcomes included associations of microRNA expression with tumor grade, non-muscle-invasive versus muscle-invasive disease status, recurrence or progression where documented, and treatment response. Treatment response was determined from available clinical records, including physician documentation, cystoscopy findings, imaging results, histopathology reports, treatment notes, and follow-up status. Patients were categorized as having a favorable or poor response according to documented clinical evidence of disease control, persistent disease, early recurrence, progression, or need for further escalation of treatment. Recurrence and progression were interpreted only when documented in the available follow-up record.

Data were entered and analyzed using standard statistical software. Quantitative variables were assessed for distribution and summarized as mean and standard deviation or median and interquartile range according to distributional characteristics. Categorical variables were summarized as frequency and percentage. Independent-sample t-test or Mann-Whitney U test was used for comparison of two independent groups, while one-way analysis of variance or Kruskal-Wallis test was used for comparisons involving more than two groups. Chi-square test or Fisher's exact test was used for categorical associations where appropriate. MicroRNA expression was compared across case-control status, grade category, disease-stage category, and treatment-response category.

Diagnostic performance was assessed using receiver operating characteristic curve analysis for individual microRNAs and selected combined marker panels. Area under the curve, sensitivity, and specificity were used to describe diagnostic discrimination. Regression analysis was planned to examine whether selected microRNAs were associated with poor clinical outcome after adjustment for clinically relevant variables such as age, smoking status, tumor grade, and disease stage. Statistical significance was set at $p<0.05$. Missing or incomplete data were handled by available-case analysis for each specific outcome, and no unsupported values were imputed.

Ethical approval was obtained from the relevant institutional review process before data collection, and informed consent was obtained from participants before recruitment and biological sampling. Participant names and personal identifiers were not used in the analytical dataset. Data were stored in coded form, laboratory samples were labeled using study codes, and access to study records was restricted to the research team. Data checking was performed before analysis to identify missing values, coding errors, implausible entries, and inconsistencies between clinical records, pathology reports, and laboratory results.

RESULTS

A total of 120 participants were included in the study, comprising 90 histologically confirmed bladder cancer patients and 30 controls without bladder cancer. The mean age was 58.7 ± 11.4 years in the bladder cancer group and 54.2 ± 10.6 years in the control group. Male participants were more frequent in both groups, with a higher proportion among bladder cancer patients. Smoking history and hematuria were also more common among bladder cancer patients than controls. Among the cancer cases, urothelial carcinoma was the predominant histological type. Non-muscle-invasive bladder cancer was present in 56 patients, while 34 patients had muscle-invasive disease. High-grade tumors were more frequent than low-grade tumors.

Table 1. Baseline and Clinicopathological Characteristics of Study Participants

Variable	Bladder Cancer Patients (n=90)	Controls (n=30)
Age, years	58.7 ± 11.4	54.2 ± 10.6
Male	72 (80.0%)	21 (70.0%)
Female	18 (20.0%)	9 (30.0%)
Smokers	41 (45.6%)	8 (26.7%)
Hematuria	68 (75.6%)	4 (13.3%)
Dysuria	29 (32.2%)	7 (23.3%)
Urothelial carcinoma	82 (91.1%)	—
Non-muscle-invasive bladder cancer	56 (62.2%)	—
Muscle-invasive bladder cancer	34 (37.8%)	—
Low-grade tumor	38 (42.2%)	—
High-grade tumor	52 (57.8%)	—

Values are presented as mean ± SD or n (%).

Bladder cancer patients were older on average than controls, with a mean age difference of 4.5 years. Male predominance was observed in both groups, but it was more marked among bladder cancer patients, where 72 of 90 cases were male. Hematuria was the most frequent presenting feature among bladder cancer patients, reported in 68 cases, compared with 4 controls. Among cancer cases, non-muscle-invasive disease accounted for 56 of 90 patients, while muscle-invasive disease accounted for 34 patients. High-grade tumors were observed in 52 cases, indicating that more than half of the bladder cancer group had high-grade pathology.

MicroRNA expression analysis showed a distinct expression pattern between bladder cancer patients and controls. miR-21 and miR-155 were increased in bladder cancer patients, while miR-145, miR-99a, and miR-125b were reduced. The largest relative increase was observed for miR-21, and the largest relative reduction was observed for miR-145.

Table 2. MicroRNA Expression in Bladder Cancer Patients and Controls

MicroRNA	Bladder Cancer Patients	Controls	p-value
miR-21	3.84 ± 1.42	1.00 ± 0.31	<0.001
miR-155	2.97 ± 1.19	1.00 ± 0.29	—
miR-145	0.39 ± 0.18	1.00 ± 0.27	<0.001
miR-99a	0.51 ± 0.21	1.00 ± 0.25	—
miR-125b	0.58 ± 0.24	1.00 ± 0.30	—

Values are presented as relative expression, mean ± SD. Relative expression was calculated using the 2^−ΔΔCT method.

The expression profile demonstrated higher oncogenic microRNA expression and lower tumor-suppressive microRNA expression among bladder cancer patients compared with controls. miR-21 showed the highest relative expression in the cancer group, with a mean value of 3.84 ± 1.42 compared with 1.00 ± 0.31 in controls. miR-145 showed the lowest relative expression among cancer patients, with a mean value of 0.39 ± 0.18 compared with 1.00 ± 0.27 in controls. miR-99a and miR-125b were also reduced in bladder cancer patients, with mean relative expression values of 0.51 ± 0.21 and 0.58 ± 0.24, respectively.

Expression patterns were further examined in relation to clinicopathological aggressiveness. Higher-grade tumors and muscle-invasive disease showed an unfavorable microRNA profile, characterized by increased miR-21 and miR-155 expression and reduced miR-145 expression. Similar directional patterns were reported for miR-99a and miR-125b in relation to tumor grade, although detailed subgroup expression values were not provided in the available dataset.

Table 3. Direction of MicroRNA Expression According to Clinicopathological Aggressiveness

MicroRNA	High-Grade Tumor	Muscle-Invasive Disease	Poor Response or Recurrence
miR-21	Increased	Increased	Increased
miR-155	Increased	Increased	Increased
miR-145	Reduced	Reduced	Reduced
miR-99a	Reduced	—	Reduced
miR-125b	Reduced	—	—

Abbreviations: —, not reported in the available data.

The clinicopathological expression pattern suggested that higher miR-21 and miR-155 expression clustered with more aggressive disease features, while lower miR-145 expression was observed in high-grade and muscle-invasive tumors. The available data also indicated reduced miR-99a expression in high-grade tumors and in patients with poor response or recurrence. However, exact subgroup means, confidence intervals, and p-values for grade-specific and stage-specific comparisons were not available in the supplied results and therefore were not introduced.

Treatment-response analysis showed that patients with poor response, persistent disease, or early recurrence had higher expression of miR-21 and miR-155 and lower expression of miR-145 and miR-99a. The highest poor-response expression value was observed for miR-21, while the lowest was observed for miR-145.

Table 4. MicroRNA Expression Among Patients With Poor Response or Recurrence

MicroRNA	Poor Response or Recurrence
miR-21	5.08 ± 1.26
miR-155	3.86 ± 1.04
miR-145	0.26 ± 0.11
miR-99a	0.41 ± 0.17
miR-125b	—

Values are presented as relative expression, mean ± SD. Abbreviation: —, not reported in the available data.

Among patients with poor response or recurrence, miR-21 showed the highest mean relative expression at 5.08 ± 1.26, followed by miR-155 at 3.86 ± 1.04. In contrast, miR-145 was markedly reduced, with a mean relative expression of 0.26 ± 0.11. miR-99a was also reduced in this subgroup, with a mean value of 0.41 ± 0.17. These findings indicate an unfavorable microRNA expression profile among patients with poorer clinical course, although comparison with a favorable-response subgroup could not be fully quantified from the available data.

Diagnostic performance analysis showed that miR-21 had the highest individual area under the curve among the reported markers. miR-145 also showed strong diagnostic discrimination. The combined

miR-21 and miR-145 panel produced the highest overall diagnostic performance, with higher AUC than either marker alone.

Table 5. Diagnostic Performance of Selected MicroRNAs

Marker	AUC	Sensitivity	Specificity
miR-21	0.88	—	—
miR-155	0.82	—	—
miR-145	0.85	—	—
miR-21 + miR-145	0.92	87.8%	83.3%

Abbreviations: AUC, area under the receiver operating characteristic curve; —, not reported in the available data.

The strongest individual diagnostic performance was observed for miR-21, with an AUC of 0.88, followed by miR-145 with an AUC of 0.85 and miR-155 with an AUC of 0.82. The combined miR-21 and miR-145 panel showed the best diagnostic discrimination, with an AUC of 0.92, sensitivity of 87.8%, and specificity of 83.3%. These findings suggest that a combined oncogenic and tumor-suppressive microRNA panel may provide stronger diagnostic discrimination than a single-marker approach.

Multivariable analysis was reported to identify high miR-21 expression, low miR-145 expression, high-grade tumor, and muscle-invasive disease as predictors of poor outcome after adjustment for age, smoking status, tumor grade, and disease stage. However, adjusted regression estimates, confidence intervals, model coefficients, and exact p-values were not available in the supplied dataset. Therefore, the adjusted model findings are interpreted cautiously and should be presented in a separate regression table once the full statistical output is available.

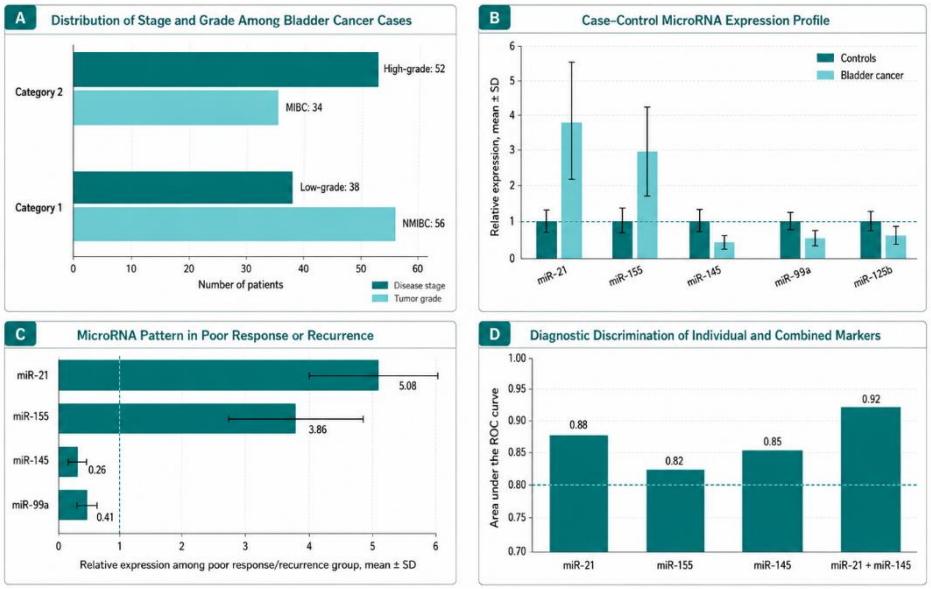

Figure 1 Integrated microRNA biomarker profile in bladder cancer. Panel A summarizes disease-stage and tumor-grade distribution among 90 bladder cancer patients. Panel B compares mean relative expression of selected microRNAs between bladder cancer patients and controls. Panel C shows the reported expression pattern among patients with poor response or recurrence. Panel D presents reported ROC AUC values for individual markers and the combined miR-21 + miR-145 panel. The combined panel showed the highest diagnostic discrimination with an AUC of 0.92, sensitivity of 87.8%, and specificity of 83.3%.

Overall, the results indicate that bladder cancer patients demonstrated a distinct microRNA expression profile compared with controls. miR-21 and miR-155 were increased, while miR-145, miR-99a, and miR-125b were reduced. The same unfavorable expression pattern was more evident in patients with aggressive clinicopathological features and poor response or recurrence. Among the evaluated markers, miR-21 and miR-145 showed the most informative diagnostic and clinical pattern, and their combined use produced the highest reported diagnostic performance.

DISCUSSION

The present study examined the expression profile of selected microRNAs in bladder cancer patients managed at tertiary care hospitals in Lahore, Pakistan, and evaluated their relationship with bladder cancer status, clinicopathological aggressiveness, treatment-response pattern, and diagnostic discrimination. The main finding was a clear differential expression pattern between bladder cancer patients and controls, with increased expression of miR-21 and miR-155 and reduced expression of miR-145, miR-99a, and miR-125b. This expression profile was more unfavorable among patients with high-grade tumors, muscle-invasive disease, and poor response or recurrence, suggesting that these microRNAs may reflect biologically aggressive tumor behavior rather than merely indicating the presence of malignancy.

The increased expression of miR-21 was the most prominent oncogenic signal in this study. miR-21 showed the highest relative expression among bladder cancer patients compared with controls and was also higher among patients with poor response or recurrence. This finding is consistent with previous evidence describing miR-21 as a clinically relevant oncogenic microRNA in bladder cancer. Zhang et al. reported that miR-21 expression was associated with clinically important bladder cancer features, while Mitash et al. suggested that miR-21 may have value in predicting recurrence among patients with non-muscle-invasive bladder cancer (21,30). Sekar also emphasized miR-21 as a potential treatment-related biomarker in bladder cancer, supporting its role in tumor progression and therapeutic response (37). The present findings agree with this literature and suggest that miR-21 may be one of the most informative adverse markers in the studied population.

miR-155 was also increased in bladder cancer patients and showed higher expression among patients with more aggressive clinical patterns. This is biologically plausible because miR-155 has been linked with inflammatory signaling, oncogenic transformation, tumor progression, and unfavorable prognosis in several malignancies. In bladder cancer, Wang and Men reported increased miR-155 expression and its correlation with prognosis, which supports the present finding that miR-155 may identify a subgroup of patients with a more adverse disease profile (20). However, although miR-155 showed diagnostic discrimination in the present study, its individual AUC was lower than that of miR-21 and miR-145. This suggests that miR-155 may contribute useful biological information but may be less powerful as a stand-alone diagnostic marker.

In contrast to miR-21 and miR-155, miR-145 showed marked downregulation in bladder cancer patients and was further reduced among patients with poor response or recurrence. This pattern supports the role of miR-145 as a tumor-suppressive microRNA in bladder cancer. Earlier experimental and clinical studies have shown that miR-145 can inhibit bladder cancer invasion and regulate molecular targets involved in tumor progression. Chiyomaru et al. reported that miR-145 and miR-133a function as tumor suppressors and directly regulate FSCN1 expression in bladder cancer, while Kou et al. showed that miR-145 inhibits invasion of bladder cancer cells by targeting PAK1 (22,24). Dip et al. also reported reduced miR-145 expression in urothelial bladder cancer, and Zhu et al. linked the ATG7/autophagy, FOXO3a/miR-145, and PD-L1 pathway with stem-like properties and invasion in bladder cancer (23,25). The present results are consistent with this tumor-suppressive model and suggest that reduced miR-145 expression may be associated with more aggressive and less responsive disease.

The reduced expression of miR-99a and miR-125b among bladder cancer patients also supports previous evidence that these markers may have diagnostic relevance. Zhang et al. identified urinary cell-free miR-99a and miR-125b as non-invasive screening markers for bladder cancer, indicating that their reduced expression may help distinguish bladder cancer patients from individuals without malignancy (32). In the present study, both markers were lower in bladder cancer patients compared with controls, while miR-99a was also reduced among patients with poor response or recurrence. However, the available dataset did not provide complete stage-specific, grade-specific, or response-specific estimates for all

markers. Therefore, although the direction of association is biologically consistent, the clinical value of miR-99a and miR-125b should be interpreted cautiously until larger datasets with complete subgroup analysis are available.

An important strength of this study is that it did not restrict analysis to case-control expression differences but also examined the relationship of microRNA expression with grade, muscle invasiveness, and response pattern. The finding that oncogenic microRNAs were increased and tumor-suppressive microRNAs were reduced in more aggressive disease is consistent with the broader literature on microRNA-based prognostic classification. Xie et al. reported that several microRNAs have prognostic significance in bladder cancer, while Yin et al. developed a 21-miRNA signature associated with patient prognosis (8,9). Andrew et al. also demonstrated that microRNA dysregulation may have prognostic value in non-muscle-invasive bladder cancer (26). These studies support the concept that microRNA expression is not only a diagnostic signal but may also help stratify risk across clinically relevant disease categories.

The treatment-response findings further strengthen the biological relevance of the observed expression profile. Patients with poor response or recurrence showed higher miR-21 and miR-155 and lower miR-145 and miR-99a expression. This pattern is consistent with the idea that microRNAs may influence or reflect mechanisms of treatment resistance, recurrence, and tumor adaptation. Das et al. reviewed the role of microRNAs in predicting bladder cancer recurrence and resistance to treatment, while Lei et al. showed that miR-150 modulates cisplatin chemosensitivity and invasiveness of muscle-invasive bladder cancer cells through PDCD4 targeting (14,36). Although the present study did not directly test molecular mechanisms of drug resistance, the observed clinical expression pattern supports the relevance of microRNAs in treatment-response research. These findings should therefore be viewed as hypothesis-generating rather than definitive evidence of treatment prediction.

The diagnostic performance analysis showed that miR-21 had the strongest individual diagnostic discrimination, followed by miR-145 and miR-155. The combined miR-21 and miR-145 panel demonstrated the highest reported AUC, with sensitivity and specificity also suggesting improved discrimination compared with individual markers. This is clinically meaningful because combined panels that include both upregulated oncogenic markers and downregulated tumor-suppressive markers may better capture the biological complexity of bladder cancer than a single microRNA. Previous studies have reported promising diagnostic performance for urinary and exosomal microRNA signatures in bladder cancer. Hofbauer et al. described a urinary microRNA signature for bladder cancer diagnosis, while Lin et al. and El-Shal et al. reported diagnostic potential for urinary exosomal microRNAs (10-12). Systematic reviews by Aveta et al. and Grimaldi et al. have also supported the diagnostic potential of urinary microRNAs in urological cancers and bladder cancer specifically (17,18). The present results are aligned with this direction but require validation before clinical application.

From a local perspective, the study contributes preliminary molecular evidence from Lahore, Pakistan, where published bladder cancer biomarker data remain limited despite the presence of a significant clinical burden. Cancer registry studies from Lahore and Pakistan have highlighted the need for locally relevant cancer data to guide diagnostic planning, risk assessment, and health system priorities (1,2). By examining selected microRNAs in relation to bladder cancer status and clinicopathological behavior, this study provides a local foundation for future biomarker validation studies. However, local relevance should not be confused with immediate clinical readiness. Before these markers can be used in routine practice, they require prospective validation, assay standardization, independent external testing, and clear comparison with established diagnostic tools such as cystoscopy and urine cytology.

Several limitations should be considered when interpreting the findings. First, the study was hospital-based and used consecutive sampling, which may limit generalizability beyond tertiary care settings. Second, the sample size was modest, especially for subgroup analyses involving stage, grade, recurrence, and treatment response. Third, although microRNA expression was measured using qRT-PCR, clinical

translation requires detailed assay standardization, including consistent sample matrix, RNA extraction method, normalization strategy, primer validation, quality-control thresholds, and inter-run reproducibility. Fourth, treatment response and recurrence were based on available clinical records, and the follow-up period was not sufficient for robust survival or time-to-event analysis. Fifth, the study did not include an independent validation cohort, and confidence intervals for diagnostic and adjusted estimates were not available. These limitations mean that the findings should be interpreted as exploratory and clinically suggestive rather than definitive.

Overall, the study supports the potential value of selected microRNAs as molecular markers in bladder cancer. miR-21 and miR-155 showed an adverse expression profile, whereas miR-145, miR-99a, and miR-125b showed reduced expression in bladder cancer patients. Among these markers, miR-21 and miR-145 appeared to be the most informative because they showed strong case-control differences, clinically meaningful directionality in aggressive disease, and the best combined diagnostic performance. Future studies should use larger prospective cohorts, clearly defined biological sample matrices, standardized qRT-PCR workflows, longer follow-up, external validation, and direct comparison with current diagnostic and prognostic standards.

CONCLUSION

This study found a distinct microRNA expression profile among bladder cancer patients treated at tertiary care hospitals in Lahore, Pakistan. miR-21 and miR-155 were increased, while miR-145, miR-99a, and miR-125b were reduced compared with controls. The unfavorable expression pattern was more evident among patients with high-grade tumors, muscle-invasive disease, and poor response or recurrence, suggesting that these markers may reflect both diagnostic status and biological aggressiveness. miR-21 showed the strongest individual diagnostic performance, while the combined miR-21 and miR-145 panel demonstrated the highest reported diagnostic discrimination. These findings support the potential role of selected microRNAs, particularly miR-21 and miR-145, as exploratory biomarkers for bladder cancer diagnosis and risk stratification. However, larger prospective studies with standardized sample handling, complete follow-up, full regression reporting, and independent validation are required before these markers can be recommended for routine clinical use.

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