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Evaluation of serum fibronectin levels and fibronectin gene polymorphism in patients receiving intravesical BCG therapy for non-muscle invasive bladder cancer and its prognostic value

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Abstract

Background Bladder cancer continues to be a significant health issue, leading to ongoing research into novel biomarkers and treatment strategies. This study aims to evaluate the potential of serum fibronectin levels and fibronectin gene polymorphisms as biomarkers for predicting the recurrence and treatment response in patients with NMIBC undergoing intravesical BCG therapy.

Methods Between June 2022 and December 2022, data of 73 patients who applied to the Mersin University Urology Clinic due to NMIBC and were followed and treated in our clinic, receiving intravesical BCG treatment, when necessary, as well as 56 individuals without any malignancy, were prospectively examined. Serum fibronectin levels were measured using the enzyme-linked immunosorbent assay method. PCR testing was applied for the fibronectin gene RS10202709 and RS 35,343,655 gene polymorphisms by using Real-Time PCR.

Results The mean serum fibronectin level in the patient group was 76.794 ± 66.998ng/ml. Simultaneously, it was 50.486 ± 25.156ng/ml in the control group, and these differences in serum fibronectin levels were statistically significant($p = 0.003$). Out of the 73 patients included in the study, recurrence of bladder cancer was observed in 53 of them. They were divided into two groups based on the recurrence times: early recurrence and late recurrence. The mean fibronectin level in the early recurrence group was 102 ± 86.1 ng/ml, while it was 44.7 ± 11.8 ng/ml in the late recurrence group. Emphasize the significance of the higher fibronectin levels in the early recurrence group by stating, patients with early recurrence exhibited significantly higher serum fibronectin levels compared to those with late recurrence ($p < 0.001$), suggesting a potential role for fibronectin as a prognostic biomarker.

Conclusions The statistically higher concentrations of serum fibronectin levels in patients with bladder cancer observed in our study are a noteworthy finding. These findings suggest that serum fibronectin levels could serve

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as a valuable prognostic biomarker for early recurrence in NMIBC patients, although their predictive value for BCG treatment response remains limited.

Keywords BCG, Bladder cancer, Fibronectin, Gene Polymorphism

Introduction

Bladder cancers, ranked 10th among the most commonly diagnosed cancers worldwide, range from noninvasive tumors, which are generally nonaggressive and have low disease-specific mortality, to aggressive and invasive tumors with high mortality rates that often necessitate long-term invasive surveillance of patients [1, 2]. The high prevalence of tobacco smoking, infections and occupational exposures may be major causes for bladder cancer [3, 4]. Urothelial carcinoma ranks among the most common malignancies worldwide, with approximately 430.000 new diagnoses annually. While the majority of patients are diagnosed with nonmuscle invasive bladder cancer (NMIBC) at the time of diagnosis, approximately 25% present with muscle-invasive bladder cancer (MIBC), advanced, or metastatic disease [5, 6]. Therefore, accurate diagnosis and close monitoring play a crucial role in the management of NMIBC.

Currently, the gold standard method for diagnosing bladder cancers is cystoscopic examinations. Cystoscopy offers high sensitivity, particularly for low-grade NMIBC and carcinoma in situ (CIS) tumors, but it has limited specificity. Additionally, it is an expensive and invasive diagnostic method [7–9]. Urine cytology, often used in conjunction with cystoscopy, is highly specific for diagnosing CIS tumors, but its sensitivity ranges from 15 to 75% [10–13]. In cases where any suspicious focus is detected during cystoscopy, a biopsy is taken for histopathological evaluation to establish a definitive diagnosis [14]. Based on clinical and histopathological parameters, patients are initially classified into risk groups, which then guide the selection of further treatment options.

One of the commonly used additional treatment options for NMIBC is intravesical therapy. In intravesical immunotherapy, the primary step involves the direct binding of Bacillus Calmette Guerin (BCG) applied intravesically to Fibronectin (FN) in the intraluminal part of the bladder, leading to an immunological response and an angiogenic process.

FNs are high-molecular-weight glycoproteins that mediate a wide range of cellular interactions with the extracellular matrix (ECM), playing crucial roles in cell adhesion, migration, growth, and differentiation [15, 16]. FN has been highly expressed in various types of cancer, revealing its potential role in tumor formation and progression. A meta-analysis demonstrated that urinary FN has a sensitivity of 81% and a specificity of 80% in the diagnosis of bladder cancer. In various types of cancer, the inhibitor of protein phosphatase 2 A (CIP2A), a human oncoprotein, has been shown to have significant effects on cancer cell proliferation. In bladder cancer, the relationship between CIP2A overexpression and abnormal cell proliferation has been confirmed by previous studies, and it has been found that the cause of CIP2A overexpression in bladder cancer is associated with FN.

Studies have shown that FN mediates the intraluminal binding of intravesically applied BCG and that BCG binding via FN is a necessary step in BCG-mediated antitumor activity [17]. If FN is essential for initiating antitumor activity, modulation of the BCG-FN interaction could provide a tool to enhance intravesical BCG therapy [18]. Given the characteristics of the FN gene, the most frequently observed polymorphisms in this gene, namely, the RS 10,202,709 and RS 35,343,655 polymorphisms, may also impact the response to intravesical BCG therapy, supporting this hypothesis.

Due to the high recurrence and progression rates of NMIBCs, regular cystoscopies have been necessary over the years, as they adversely affect the quality of life and healthcare expenditures of patients [3, 4]. Therefore, developing a reliable and noninvasive method for selecting suspicious patients and predicting the risk of recurrence and treatment response is crucial in the management of bladder cancer. Many urinary biomarkers have been developed to reduce the frequency of unnecessary cystoscopies. However, none have been included in current guidelines due to inadequate sensitivity or negative predictive value (NPV) [19–24].

In this study, we aimed to investigate the differences in serum FN levels and FN gene polymorphisms between patients with NMIBC receiving intravesical BCG treatment and the controls. Additionally, we aimed to anticipate the impact of these values on the diagnosis, prognosis, and treatment response of the disease. This study is the first to investigate the prognostic value of serum fibronectin and its genetic variations in the context of BCG treatment for NMIBC.

Materials and methods

Study population

The data of 118 patients who presented to the Mersin University Urology Clinic between June 2022 and December 2022 who were followed and treated in our clinic for NMIBC and who received intravesical BCG treatment when necessary, as well as 56 individuals without any malignancies, were prospectively examined. Individuals with invasive and/or metastatic bladder cancer, those under 18 years of age, those with NMIBC

who did not receive intravesical BCG treatment, those with malignancies other than bladder cancer, and those who did not sign informed consent forms were excluded from the study. Patients with other malignancies were excluded from the study to ensure that the results were not influenced, as the fibronectin molecule or fibronectin gene polymorphisms may play a role in the formation of various malignancies. Among a total of 118 NMIBC patients, 73 underwent intravesical BCG treatment once a week for a minimum of 6 weeks, starting two weeks after the initial diagnosis. Patients who completed at least the induction phase of intravesical BCG therapy, along with 56 healthy individuals without any malignancy, were included in the study. All individuals' demographic data, such as age, weight, height, body mass index (BMI), family history, and smoking status, were examined. Informed consent forms were signed by all individuals included in the study. Ethical approval for the research was obtained from the Mersin University Faculty of Medicine Local Ethics Committee with the decision dated 23.03.2022, numbered 2022/200. Informed consent forms were signed by all individuals included in the study.

Sample collection and storage

The analysis of serum FN levels and the detection of the FN gene polymorphisms RS 10,202,709 and RS 35,343,655 were conducted at the Mersin University Medical Biochemistry Laboratory. Blood samples were collected from patients and healthy individuals and put into 2 ml EDTA tubes for polymorphism analysis. For enzyme-linked immunosorbent assay (ELISA), blood samples were collected into 5 ml plain biochemistry tubes. The blood samples collected into simple biochemistry tubes were centrifuged at 4000 rpm for 10 min, and the sera were separated. Storing serum samples at -80 °C slows down protein degradation, thereby preserving the long-term stability of biomolecules like fibronectin, which enhances the reliability of research results. Therefore, the serum samples were stored at -80 °C until the analysis day. The blood samples collected in EDTA tubes were stored at +4 °C.

Measurement of fibronectin levels

Serum FN levels were determined using the ELISA method, following the protocol recommended by the manufacturer. This was carried out using an ELISA washer and ELISA reader (Thermo Scientific) devices. For each analysis, the concentrations were calculated for each sample using the optical density values corresponding to known standards by utilizing the curves and equations drawn.

DNA isolation and polymorphism analysis

DNA isolation was carried out from the blood samples stored at +4 °C using a DNA isolation kit (Roche Diagnostics, Mannheim, Germany). The FN gene polymorphisms RS 10,202,709 and RS 35,343,655 were detected in the isolated DNA samples using a real-time PCR (polymerase chain reaction) device (Roche LightCycler 480; Roche Diagnostics, Mannheim, Germany). The reaction mixtures were prepared according to the protocol specified by the manufacturer, and the PCR conditions listed in Supplementary Table 1 were applied.

Statistical

In this study, continuous measurements were assessed for normality using the Shapiro-Wilk test. This test was selected due to its robustness and widespread use in determining whether a continuous variable adheres to a normal distribution, a critical assumption for many parametric statistical analyses. The hypothesis tested here was that the continuous variables follow a normal distribution, which is essential for the validity of subsequent parametric tests.

For comparing constant measurements between patient and control groups, Student' s t-test was employed. This test is appropriate for evaluating the means of two independent groups when the data is continuous and normally distributed. The hypothesis tested was that there is no significant difference in the means of the continuous variables between the patient and control groups.

Descriptive statistics, including means and standard deviations, were reported to summarize the central tendency and variability of continuous variables.

To analyze differences in categorical variables between groups, Pearson' s chi-squared test, Fisher' s exact chi-squared test, and the likelihood ratio chi-squared test were utilized. Pearson' s chi-squared test was applied for larger sample sizes, while Fisher' s exact test was used for small sample sizes or sparse data. The likelihood ratio chi-squared test served as an alternative that may offer more reliability in specific situations. The hypothesis tested was that there is no association between categorical variables and group membership.

Counts and percentages were provided as descriptive statistics for categorical variables to offer a clear understanding of the distribution of these variables across groups.

Binary logistic regression analysis was conducted to identify potential risk factors influencing bladder cancer incidence. This method is suitable for modeling the relationship between multiple independent variables and a binary outcome, such as the presence or absence of bladder cancer. The hypothesis tested was that certain independent variables are significant predictors of bladder cancer incidence.

Table 1 Distribution of continuous measurements between groups n: number of samples, BMI: body mass index, p: values of significance with difference of each group

	Control (*n* = 56)	Patient (*n* = 73)	*p*
Age	56.0 ± 18.0	73.0 ± 10.3	0.095
BMI	28.18 ± 4.54	28.54 ± 4.77	0.666
Fibronectin level (ng/ml)	50.486 ± 25.156	76.794 ± 66.998	**0.003**

n: Number of samples, BMI: Body mass index, p: Values of significance with difference of each group

Hardy-Weinberg equilibrium analysis was performed to verify whether gene polymorphisms were in equilibrium within the population, an essential consideration for genetic association studies to ensure the sample' s representativeness of the general population. The hypothesis tested was that the observed genotype frequencies do not deviate from those expected under Hardy-Weinberg equilibrium.

Counts and percentages were also presented as descriptive statistics to provide a comprehensive overview of the data.

A paired samples t-test was used to evaluate differences between early and late recurrence groups. This test is appropriate for comparing two related samples within the same group of patients, allowing for the assessment of changes over time or between conditions. The hypothesis tested was that there is no significant difference in the means of the continuous variables between the early and late recurrence groups.

Furthermore, a one-way ANOVA test was employed to examine genotype differences in gene polymorphisms concerning continuous measurements. One-way ANOVA is suitable for comparing means across three or more groups, enabling the detection of overall differences between genotypes. The hypothesis tested was that there are no significant differences in the means of continuous variables across different genotypes.

Levene' s test was conducted to assess the homogeneity of variances, ensuring the validity of tests such as ANOVA and t-tests. The hypothesis tested was that the variances are equal across groups.

A significance level of $p < 0.05$ was considered statistically significant throughout the analyses, indicating that the probability of observing the results by chance is less than 5%.

Results

A total of 73 (56.6%) patients with NMIBC who were followed and treated in our clinic for NMIBC received intravesical BCG treatment, and 56 (43.4%) individuals without any malignancies were included in the study. When examining the differences between groups for continuous measurements, only the mean levels of FN were found to be significantly different. The mean FN concentration in the patient group was 76.794±66.998 ng/ml, while the mean FN concentration in the control group was 50.486±25.156 ng/ml ($p = 0.003$) (Table 1). This significant difference in FN levels suggests that elevated FN may be associated with NMIBC, potentially serving as a biomarker for identifying patients at risk.

Within the NMIBC patient group, descriptive statistics were also examined for predisposing risk factors (e.g., occupational exposure, chronic urinary system infection, history of radiotherapy, history of chemotherapy, family history) and tumor characteristics (e.g., stage, pathological tumor grade, presence of CIS, papillary/solid differentiation, tumor size, number of tumor formations) (Supplementary Table 2). Understanding these factors can aid in tailoring personalized treatment plans and surveillance strategies for patients.

The Hardy-Weinberg equilibrium was investigated for the RS 35,343,655 and RS 10,202,709 FN gene polymorphisms in both the patient and control groups, and it was determined that the population was in equilibrium. Descriptive statistics (counts and percentages) for genotypes and alleles, along with p values, are provided in supplementary Table 3. This equilibrium suggests that the sample is representative of the general population, supporting the validity of genetic association findings.

The results obtained when genotypes were included in the logistic regression model are provided in supplementary Table 4. No significant differences were found in mean FN levels among genotypes for RS 35,343,655 and RS 10,202,709 polymorphisms. Similarly, there were no significant differences in BCG response among genotypes for both polymorphisms. These findings indicate that these specific polymorphisms may not influence FN levels or BCG treatment response, suggesting that other genetic or environmental factors may play a more significant role. The complete findings are summarized in Tables 2, 3 and Supplementary Table 2.

As shown in Tables 4 and 53 of the 73 patients included in the present study experienced bladder cancer

Table 2 Distributions of continuous measurements for gene polymorphisms n: number of samples

	RS35343655				RS10202709			
	CC (*n* = 56)	CT (*n* = 56)	TT (*n* = 17)	P	CC (*n* = 95)	CT (*n* = 33)	TT (*n* = 1)	*p*
Fibronectin level	69.6 ± 55.3	61.1 ± 57.1	65.6 ± 43.6	0.714	69.7 ± 60.7	53.6 ± 28.8	39.9±…	0.309

p: values of significance with the difference of each group

Table 3 Relationships between the RS35343655 gene polymorphism and BCG Treatment responsiveness

		CC		CT		TT		p
		n	%	n	%	n	%	
BCG refractory tumor	No	22	73.3	25	75.8	7	70.0	0.931
	Yes	8	26.7	8	24.2	3	30.0	
BCG unresponsive tumor	No	22	73.3	25	75.8	7	70.0	0.931
	Yes	8	26.7	8	24.2	3	30.0	
BCG intolerance tumor	No	26	86.7	25	75.8	8	80.0	0.546
	Yes	4	13.3	8	24.2	2	20.0	

n: number of samples, p: values of significance with the difference of each group

Table 4 Statistical analysis of fibronectin levels and recurrence durations in patients with a history of recurrence

	Number of patients ($n=53$)	FN level Mean ± Standard Deviation (ng/ml)	*p*
Early Relapse (First six months)	21	102 ± 86.1	**< 0.001**
Late Relapse (relapses after six months)	32	44.7 ± 11.8	

n: Number of samples, p: Values of significance with the difference of each group

recurrence. The patients were divided into two groups based on the recurrence time: early recurrence (recurrence within the first six months) and late recurrence (recurrence after six months), and their FN levels were compared. There were 21 patients with early recurrence and 32 patients with late recurrence. The average FN concentration in the early recurrence group was 102 ± 86.1 ng/ml, while in the late recurrence group, it was 44.7 ± 11.8 ng/ml. FN levels were significantly greater in the early recurrence group than in the late recurrence group ($p<0.001$). This outcome is likely attributable to the aggressive behavior of the tumor or the variability in the immune response of the human body. This suggests that higher FN levels could be indicative of a higher risk of early recurrence, highlighting the potential of FN as a prognostic marker to guide surveillance strategies and early interventions.

Discussion

Currently, the greatest challenge in the management of bladder cancer is the frequent and prolonged surveillance with cystoscopy, which is an invasive and costly diagnostic method. In patients with a history of NMIBC, a highly sensitive biomarker is necessary to distinguish patients who can prevent tumor recurrence and avoid unnecessary cystoscopies. Despite extensive investigation into urinary biomarkers to reduce cystoscopies in NMIBC surveillance, none have been applicable in clinical practice due to their low sensitivity or specificity [19–24].

Our study revealed significantly higher serum FN levels in patients with NMIBC (76.794 ± 66.998 ng/ml) compared to the control group (50.486 ± 25.156 ng/ml, $p=0.003$). This finding suggests that serum FN could potentially be integrated into NMIBC clinical protocols as a biomarker. For instance, patients with lower FN levels might require less frequent cystoscopies, reducing the invasiveness and cost of surveillance. A risk-stratified approach based on FN levels could be developed, where patients with FN levels below a certain threshold undergo cystoscopy at longer intervals, while those with higher levels maintain the current follow-up schedule. This approach could significantly improve patient quality of life and reduce healthcare costs without compromising oncological outcomes.

Importantly, our study found that FN levels were significantly higher in the early recurrence group (102 ± 86.1 ng/ml) compared to the late recurrence group (44.7 ± 11.8 ng/ml, $p<0.001$). This finding suggests that serum FN levels could serve as a prognostic marker for early recurrence. Clinicians could use this information to tailor follow-up protocols, intensifying surveillance for patients with higher FN levels and potentially identifying recurrences earlier.

The allelic frequencies and genotypes (CC, CT, TT) of the FN gene polymorphisms RS 35,343,655 and RS 10,202,709 were examined in both the patient and control groups. However, neither group exhibited statistically significant differences between the genotypes and FN levels. Additionally, no statistically significant differences were found in the response to intravesical BCG therapy among different types of unresponsive tumors (BCG-refractory tumors, BCG-unresponsive tumors, BCG-intolerant tumors, and BCG-relapsing tumors) (all $p>0.05$).

In a literature review, studies have investigated urinary FN levels, tissue FN levels, and serum FN levels in different populations with and without bladder cancer, and the findings revealed increased FN levels in individuals with bladder cancer [25–32]. Our study's results are in line with the literature, supporting the hypothesis that serum FN could serve as a potential biomarker for bladder cancer diagnosis. Additionally, our study is the first

to examine the effects of the FN gene polymorphisms RS 3,534,365 and RS 10,202,709 on treatment response in patients with NMIBC treated with intravesical BCG therapy. While statistically significant differences were found in serum FN levels between the groups in our study, no statistically significant differences were observed in FN gene polymorphisms concerning bladder cancer diagnosis and treatment response. Additionally, in this study, we anticipate that by predicting the recurrence time of known recurrent NMIBC using serum FN values, changes can be made in early and effective treatment approaches.

While our study provides promising results, it' s important to acknowledge its limitations. The small sample size (73 NMIBC patients and 56 controls) and single-center design limit the generalizability of our findings to broader, more diverse populations. These limitations may have affected our ability to detect significant associations between FN gene polymorphisms and bladder cancer occurrence or BCG therapy response. To address these issues, future research should focus on larger, multi-center studies that include more diverse patient populations. Such studies would provide more robust evidence and potentially uncover associations that our study may have missed due to limited statistical power.

To validate FN as a biomarker for NMIBC, we propose a large-scale, multi-center prospective cohort study. This study should include a diverse patient population and standardized protocols for FN measurement and follow-up. Additionally, it should incorporate long-term follow-up to assess the predictive value of FN for recurrence and progression over time. Such a study would provide the necessary evidence to potentially incorporate FN testing into clinical guidelines for NMIBC management.

Furthermore, our findings open up new avenues for research into the extracellular matrix' s role in bladder cancer. Future studies should explore other components of the extracellular matrix, such as laminin, collagen, or proteoglycans, to develop a more comprehensive understanding of the tumor microenvironment in bladder cancer. This could lead to the identification of additional biomarkers or potential therapeutic targets.

In conclusion, our study demonstrates the potential of serum FN as a biomarker in NMIBC, particularly for predicting early recurrence. The significantly higher serum FN levels in bladder cancer patients, especially in the early recurrence group, are noteworthy findings. Measuring serum FN levels could provide significant advantages in the diagnosis and prognosis of bladder cancer, considering its applicability and cost-effectiveness. However, larger, multi-center studies are needed to confirm these findings and establish standardized protocols for FN testing in clinical practice. As we continue to unravel the complex biology of bladder cancer, integrating biomarkers like FN into clinical decision-making holds promise for improving patient care and outcomes in NMIBC.

Conclusions

Our findings indicate that serum fibronectin levels could serve as a valuable prognostic biomarker for NMIBC, particularly in predicting early recurrence. Incorporating FN level monitoring into routine clinical practice could enhance patient stratification and guide more personalized treatment strategies.

Abbreviations

NMIBC	Nonmuscle invasive bladder cancer
MIBC	Muscle-invasive bladder cancer
CIS	Carcinoma in situ
BCG	Bacillus Calmette Guerin
FN	Fibronectin
ECM	Extracellular matrix
NPV	Negative predictive value
BMI	Body mass index
ELISA	Enzyme-linked immunosorbent assay

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12894-024-01592-8.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

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Author contributions

Ali Nebioğlu contributed as the writer and was involved in materials, data collection, and processing. Rojda Tanrıverdi participated in materials, data collection, and processing, and conducted the literature review. Mert Başaranoğlu handled analysis and/or interpretation, materials, data collection, and processing. Barış Saylam was responsible for the literature review, materials, data collection, and processing. Ercüment Ulusoy provided critical review and conducted the literature review. Murat Bozlu supervised the project and contributed to its conception. Erdem Akbay worked on analysis and/or interpretation and the literature review. Lülüfer Tamer was in charge of the design and involved in analysis and/or interpretation. Semra Erdoğan contributed to analysis and/or interpretation.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Ethical approval for the research was obtained from the Mersin University Faculty of Medicine Local Ethics Committee with the decision dated 23.03.2022, numbered 2022/200. Informed consent forms were signed by all individuals included in the study. It has been confirmed that all data were processed in accordance with relevant data protection regulations and patient confidentiality was maintained.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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