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Research Article

Fibroblast Growth Factor Receptor 3 Mutations in Bladder Cancer: A Marker for Early-Stage Diagnosis

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ABSTRACT

Background: Bladder cancer (BC) ranks as the tenth most common cancer globally, with a high recurrence rate. It is primarily caused by abnormalities in the epithelial layer lining the bladder and is classified from non-muscle invasive bladder cancer (NMIBC) to muscle-invasive bladder cancer (MIBC). Fibroblast Growth Factor Receptor 3 **(**FGFR3) S249C mutation is frequently observed in low-grade NMIBC but are rare in high-grade NMIBC and MIBC, leading to continuous receptor activation and promoting tumor growth.
Objective*:* This study aims to investigate the prevalence of FGFR3 S249C mutation in Iraqi BC patients, assess their association with tumor stage and grade, evaluate the potential of FGFR3 mutation as an early-stage diagnostic marker, and discuss implications for public health policy.
Subjects and Methods: The study was conducted at Al-Safeer Hospital, Karbala, Iraq. It included 60 individuals diagnosed with urothelial BC (50 males, 83.3%; 10 females, 16.4%) with a median age of 63 years. Tumor tissue samples from patients undergoing Transurethral Resection of Bladder Tumor (TURBT) were used. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples, and FGFR3 mutations were examined through direct DNA sequencing, focusing on the S249C mutation in exon 7. The prevalence of the S249C mutation was analyzed in relation to tumor stage and grade.
Results: The study revealed a significant prevalence (55%) of the S249C mutation. This mutation was more frequent among males and individuals over the age of 50 years. There was a higher incidence of BC in males (83.3%) compared to females (16.7%), especially in those aged 50 years and older. Histomorphological evaluations showed a considerable number of tumors classified as low-grade. Furthermore, pathological staging results identified pT1 as the most common stage, followed by pTa and pT2, underscoring the predominance of early-stage tumors within the study population.
Conclusions: This study highlighted the significant prevalence of FGFR3 S249C mutation in Iraqi BC patients. FGFR3 mutations were strongly associated with early-stage and low-grade tumors, indicating their potential as a molecular marker for early diagnosis.

Introduction

Bladder cancer (BC) counts tenth in global cancer incidence with a high recurrence rate (1). In 2020, GLOBOCAN recorded 573,000 new cases of BC globally, resulting in 213,000 deaths (2). The epithelial layer that lines the inner wall of the bladder is the primary cause of BC (3). In Iraq, particularly in Basra Governorate, a total of

2,579 cancer cases were recorded, with urinary BC representing 11.94% of the diagnosed cancers among adults. This indicates a significant presence of BC in the region, with the overall cancer incidence rate standing at 72 per 100,000 people (4).

BC can be categorized through various approaches. Based on standardized histomorphology traits established by the World Health Organization, the categorization approach distinguishes between high-grade and low-grade diseases. Tumor staging is an additional technique that quantifies the extent of bladder wall infiltration (Table 1). Non-muscle invasive bladder cancer (NMIBC) refers to BC that does not spread to the muscles and only affects the urothelium (stage Ta) or the lamina propria (stage T1). Muscle-invasive BC (MIBC) is cancer that spreads to or into the muscle (stage T2) or later (stages T3 and T4). Non-invasive cancers are treated differently (5). Carcinoma in situ (CIS) is an identifiable type of abnormal cell growth characterized by a high-grade, flat, noninvasive lesion. It is known for its several independent research groups that have discovered shared abnormalities in different genes commonly linked to low-grade NMIBC are FGFR3, STAG2, and the PIK3CA complex. Similarly, mutations in genes like p53, ERBB2, ARID1A, and KDM6A have been detected in high-grade MIBC (6).

FGFR3 signaling that is not working right is linked to many types of cancer, including urothelial carcinoma (7). FGFR3 point mutations are common genetic changes in BC, especially in low-grade NMIBC. They are infrequent in high-grade NMIBC and MIBC. This change happens in the tyrosine kinase part of FGFR3, which causes the receptor to stay active and mess up signaling pathways further down the line (8). BC frequently has a high recurrence rate due to the FGFR3 mutation, particularly in exon 7 of the FGFR3 gene. Exon 7 contains essential segments of the tyrosine kinase domain of the FGFR3 protein (9). Changes in this exon cause FGFR3 signaling to stay active, which helps bladder cancer grow and spread, especially in NMIBC (9). The most common genetic alteration is located at Ser249Cys in codon (TCC→TGC) inside exon seven (10). By using targeted inhibitors, it is possible to improve the effectiveness of therapy and reduce adverse side effects in tumors driven by FGFR3. This approach shows great potential for precision medicine in treating this disease (11).

In general, FGFR3 has a complex role in BC, acting as a marker for prognosis and diagnosis as well as providing a possible target for precision medicine techniques (12).

Table 1: Pathologic Stage of BC (13)

Tumor stage	Degree of invasion
Ta	Papillary cancer without invasion
T1	The lamina propria is invaded
T2	The muscularis propria is invaded
T3	The perivesical tissue is invaded
T4	Indicates extravesical extension into surrounding organs

Subjects and Methods

Study Design and Population:

We conducted a cross-sectional analysis at Al-Safeer Hospital, Karbala, Iraq. Samples were collected from 1st March to 30th December 2023. Formalin-fixed paraffin-embedded (FFPE) tumor tissue samples were collected from 60 patients who underwent TURBT. All grades selected in this study were under supervision of the histopathologist. Among the 60 cases, 50 (83.3%) were males, and 10 (16.4%) were females. The median age of the individuals was 63 years, with 57 patients (95%) being over the age of 50 years. Tumor samples were analyzed for FGFR3 S249C mutations through direct DNA sequencing, focusing on exon 7. The staging of all urothelial carcinoma was conducted per the Union for International Cancer Control (UICC) recommendations, while grading was determined based on the criteria published by the World Health Organization (WHO).

Inclusion criteria included confirmed bladder tissue with urothelial carcinoma showing adequate tissue material histologically. The exclusion criteria included patients with non-urothelial BC, those who had undergone previous BC treatment, and patients with prior therapy with FGFR inhibitors.

Ethical approval for the study was obtained from the Medical Ethics Committee of Al-Nahrain University, ensuring compliance with ethical standards for research involving human subjects.

Extraction of deoxyribonucleic acid (DNA):

The wax was removed from FFPE tumor tissue using xylene and ethanol. The samples were obtained by extracting DNA from paraffin-embedded tissues using the FFPE tissue DNA Extraction Micro (FAVORGEN, China), following the manufacturer's instructions.

Mutation analysis:

The FGFR3 gene displayed a mutation at locations Ser249Cys in codon (TCC→TGC) within exon 7. The mutation examinations were conducted through direct DNA sequencing. Table 2 contains the primers employed in this investigation.

PCR amplification:

The thermal cycling conditions specified in Table 3 were employed to perform PCR amplification. The PCR mix was prepared by combining 12.5 µl of the master mix, 1.25 µl of each 10 µM primer, and 5 µl of the extracted DNA. The mixture was then finalized in a total volume of 25 µl using nuclease-free water.

Gel electrophoresis:

To confirm the amplification of the target fragment, PCR products were electrophoresed on a 1.5% agarose gel, which was prepared by dissolving agarose powder in 1X TBE buffer. The mixture was boiled and then cooled to approximately 60°C before adding ethidium bromide at a concentration of 0.5 µg/mL for visualization. A 100 bp DNA ladder was used as a molecular size marker to estimate the size of the PCR products (figure 1).

Table 2: Primers used for amplification of FGFR3 gene (14).

Codon	Exon	Primer sequence	PCR product (bp)	Annealing Temperature °C
249	7	F 5' AGTGGCGGTGGTGGTGAGGGAG 3' R 5' GCACCGCCGTCTGGTTGG 3'	115	66

PCR: Polymerase Chain Reaction, bp: Base pair

Table 3: The program of PCR for the amplification FGFR3 gene S249C mutation.

Type of Cycle	Temperature °C	Time	No. of Cycles
Initial denaturation	95	10 min.	1 cycle
Denaturation	95	30 sec.	
Annealing	66	30 sec.	35 cycles
Extension	72	30 sec.	
Final extension	72	10 min.	1 cycle

Total time: 1:12:30

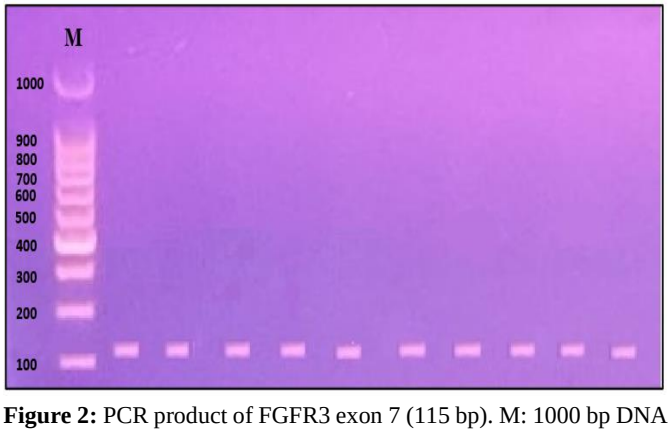

Figure 2: PCR product of FGFR3 exon 7 (115 bp). M: 1000 bp DNA ladder.

DNA sequencing:

Genetic analysis was conducted by directly sequencing the purified PCR products. After PCR gel electrophoresis, the bands were highly distinct and free of any unspecific bands or primer dimers. Consequently, the PCR products underwent Sanger sequencing using forward and reverse primers according to Sanger's technique by Macrogen company/Korea.

Statistical analysis:

SPSS software, version 26 (IBM, Armonk, NY, USA), was employed to conduct statistical analyses. The prevalence of the FGFR3 S249C mutation was examined in relation to tumor stage (pTa, pT1, pT2) and grade (low-grade, high-grade). Associations were determined using chi-square tests, with a p-value < 0.05 being considered statistically significant. Symbols, abbreviations, and statistical terms were defined in accordance with established conventions.

Results

The disease incidence was primarily observed in those above the age of 50 years. The gender distribution of patients exhibited a higher proportion of males compared to females. The histomorphology investigations of the tumors indicated a significant proportion of the tumors classified as low-grade. Furthermore, the majority of the tumors underwent pathological staging, with pT1 being the most common stage, followed by pTa and pT2 patients.

PCR and DNA sequencing were used to analyze the mutation in exon 7 (S249C). Table 4 displayed the association between demographic parameters and FGFR3 mutation. There was a strong correlation between the S249C mutation and low-grade BC as opposed to high-grade tumors. Furthermore, there was a notable correlation between FGFR3 mutation and tumor stage, with the FGFR3 mutation being more frequently observed in pT1 tumors, followed by pTa and pT2 tumors.

Table 4: FGFR3 mutation in relation to clinical and tumor characteristics

Characteristic n (%)			FGFR3 status		
			Wild n (%)	Mutant n (%)	P-Value
Gender	Male	50 (83.3)	24 (48)	26 (52)	0.5
	Female	10 (16.4)	3 (30)	7 (70)	
Age	<50	3	2 (67)	1 (33)	0.6
	>50	57	25 (44)	32 (56)	
Tumor stage (PT)	PTa	4 (7)	1 (25)	3 (75)	< 0.05
	PT1	44 (73)	15 (34)	29 (66)	
	PT2	12 (20)	11 (92)	1 (8)	
Tumor grade	Low	48 (80)	16 (33)	32 (67)	< 0.001
	High	12 (20)	11 (92)	1 (8)	

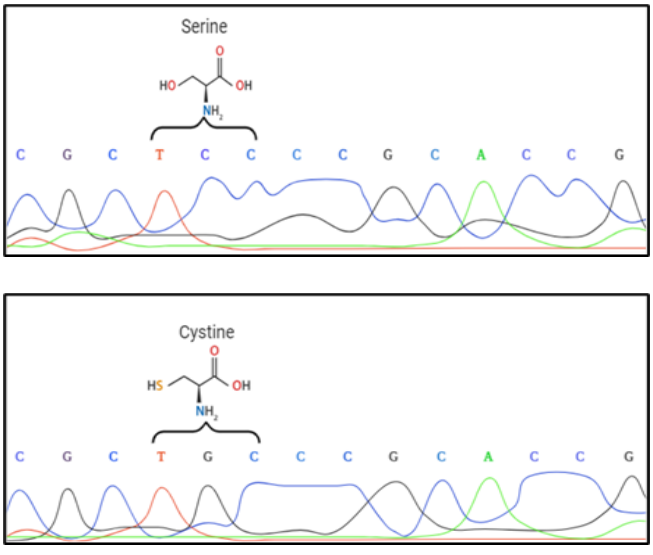

Figure 2: Comparison of DNA Sequencing Chromatograms: (A) Normal vs. (B) Mutant with S249C in exon 7. Panel A (Normal Sequence): Shows the wild-type codon TCC, which encodes Serine at position 249 in the FGFR3 gene. The chromatogram displays clear peaks for nucleotides T, C, and C. Panel B (Mutant Sequence - S249C Mutation): Displays the TGC codon, where a C to G mutation has occurred, resulting in the substitution of Cysteine for Serine at position 249. The change from TCC to TGC is evident in the sequencing chromatogram.

Discussion

In the current study, the FGFR3 mutation was detected in 55 % of BC patients which was higher than that reported in China population (15). Another study from Turkey demonstrated 50% prevalence of FGFR3 mutation (16) while a study in the Jorden, 59% FGFR3 mutation prevalence (17). Furthermore, a study in Iraq, which analyzed 30 samples, reported FGFR3 gene amplification in 13.3% of cases of urothelial carcinoma of the urinary bladder (18). This difference in the frequency can be due to variations in geographical region, sample size, and methodology used for mutation detection (19).

The current study found a greater prevalence of FGFR3 mutation in male patients compared to female patients, which is consistent with previous research findings (20, 21). Higher mutation rates in males may stem from gender-related biological differences and higher exposure to risk factors like smoking (22). The prevalence of FGFR3 mutation was higher in elderly people (> 50 years). Among patients in this age category, the mutation was observed in 56% of cases, while only 33% in patients under 50 years old. The mutation's higher presence in older patients may be linked to the accumulation of genetic mutations over time, reduced DNA repair, and longer carcinogen exposure (23). This result is in agreement with the published study by Yu *et al*. (24), Although there is disagreement with a study conducted in Turkey, which concluded that there is no significant association between the occurrence of FGFR3 mutations with the age or gender of patients (16).

In the current study, we assessed the association between the pathological tumor stage and grade and the presence of the FGFR3 mutation. The mutation was predominantly detected in early-stage cancers, specifically in 75% of pTa and 66% of pT1 tumors. Furthermore, the mutation was prevalent in low-grade tumors, manifesting in 67% of such instances. This is consistent with other investigations (25-27). FGFR3 mutations result in enhanced receptor activation, which stimulates cellular proliferation and survival, however, it generally does not induce significantly aggressive or invasive tumor characteristics. Patients with low-grade BC who include FGFR3 mutations typically experience more favorable outcomes compared to patients without these mutations (28, 29).

Investigating FGFR3 gene mutation in BC is crucial because it enhances both the accuracy of diagnosis and the selection of therapy options for BC. On January 19, 2024, Erdafitinib has been approved by the FDA for the treatment of urothelial carcinoma in adult patients who have FGFR3 genetic mutations that make them susceptible to the drug. This treatment has great potential as a therapeutic method for BC, providing advantages in terms of effectiveness and tolerance when compared to conventional chemotherapy or immunotherapy for this particular group of patients. New insights into the diagnosis and treatment of BC are anticipated to result from the recent increase in understanding of FGFR3, thereby extending the survival of patients (30).

Ultimately, the study showed that gender and age do not exhibit a significant correlation with FGFR3 status. However, tumor stage and grade display a significant link with FGFR3 mutations, suggesting their potential impact on the progression and severity of the disease.

Conclusion

This study detected a significant prevalence (55%) of FGFR3 mutations in Iraqi BC patients. There was a strong association between FGFR3 mutations and early-stage (pTa, pT1) and low-grade cancers. These findings highlight the importance of FGFR3 as a molecular marker in determining the tumor grade in difficult cases. Regarding therapeutic target, the FDA today approved erdafitinib, a treatment for adult patients with BC that has a significant impact on public health policies and costs compared to chemotherapy.

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Conflict of Interest

Authors declare no conflict of interest.

Data availability

Data are available upon reasonable request.

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