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Overexpression of fibroblast growth factor receptor 3 in urothelial carcinoma of urinary bladder among Iraqi patients and its correlation with different clinicopathological parameters

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Abstract
Background: Urothelial carcinoma, previously known as transitional cell carcinoma, constitutes about 90% of bladder cancer cases and is highly prevalent in Iraq. It is linked to risk factors like tobacco smoking, chemical exposure, and schistosomiasis, and varies from low-grade tumors that rarely spread to aggressive high-grade forms that are prone to invasion and metastasis. FGFR3 mutations play a significant role in its pathogenesis, making FGFRs a potential target for cancer therapy.
Aim of study: To evaluate the immunohistochemical expression of FGFR3 in urothelial carcinoma and correlate the expression with different cliniocopathological parameters.
Materials and Methods: Sixty-three cases of Urothelial carcinoma of Urinary Bladder were included in this study. Immunohistochemical study for tissue sections were done to detect the reactivity of FGFR3 and correlate the results with different clinicopathological parameters.
Results: FGFR3 expression in low grade tumors was positive in 76.1% of the cases whereas 47.1% of high grade cases were positive for FGFR3 ($P<0.05$). Immunohistochemical expression of FGFR3 in muscle invasive(T2) urothelial carcinoma was positive33.3%, non-invasive urothelial 81.08% (pta 89.5%, T1 72.2%) ($P<0.05$).
Conclusion: The expression of FGFR3 is observed in urothelial cancer. Our findings have demonstrated that low grade and low stage cancers have positive FGFR3 expression. supported statistically, FGFR3 protein expression has the potential to serve as an additional molecular marker, alongside tumor grading and staging, in the prediction of prognosis.

Keywords: Overexpression, fibroblast, growth factor receptor 3, urothelial carcinoma, urinary bladder, Iraqi patients.

Introduction

Urothelial carcinoma (UC) also known as transitional cell carcinoma includes carcinomas of the bladder, ureters, renal pelvis. UC is the fourth common cancer in men [1]. It is comprising approximately 90% of all primary cancer of bladder [2]. It is more common after 60 years of age. Tobacco smoking, chemical exposure and Schistosomiasis are the major risk factors [3]. Urothelial carcinomas morphology ranges from papillary to flat, noninvasive to invasive and low grade to high grade. Papillary urothelial carcinoma represents 70-80% of newly diagnosed bladder cancer patients and present with noninvasive or early invasive disease (stages Tis, Ta, or T1). The risk of recurrence for patients with superficial bladder tumors can be as high as 70% but low progression rate [4]. Pathological stage is the most important factor that determines the prognosis and the mode of therapy of the UC [5]. The carcinoma without basal lamina invasion is staged as pTa (noninvasive papillary urothelial carcinoma) and pTis (carcinoma in situ). While that with lamina propria and muscularis mucosal (MM) invasion by the tumor is staged as pT1, and muscularis propria (MP) invasion is staged as pT2. Stage pT3 is given for perivesical soft tissue extension [6]. The outcome of invasive tumors depends on the stage. Distant metastasis is associated with very poor prognosis and poor response to adjuvant therapy [7]. Non-muscle invasive bladder UC can be treated using transurethral bladder tumor resection and intravesical therapy [8]. Muscle-invasive bladder cancer is associated with a poor prognosis and is treated with neoadjuvant chemotherapy followed by cystectomy [9].

New treatment for UC such as immune checkpoint inhibitors is used for advanced and metastatic UC [10]. The aim of study is to evaluate the immunohistochemical expression of FGFR3 in urothelial carcinoma and correlate the expression with different cliniocopathological parameters.

Methods

This retrospective cross-sectional study was conducted at the Department of Pathology and Forensic Medicine, Faculty of Medicine, Babylon University, involving formalin-fixed, paraffin-embedded tissue blocks from 63 newly diagnosed bladder urothelial carcinoma cases. These cases were selected from archived materials at Al-Hilla Teaching Hospital and private laboratories between December 2022 and September 2023. The patients had undergone Transurethral Resection of Bladder Tumor (TURBT), and clinicopathological data such as age, gender, histological diagnosis, grades, and stages were collected from medical reports. For each case, two 5 µm sections were prepared from the tissue blocks. One section was stained with Hematoxylin and Eosin (H&E) for histological reassessment of tumor type, grade, and invasiveness. The other section underwent immunohistochemical staining for FGFR3 using a mouse monoclonal antibody (Clone BSB_150) derived from cell culture supernatant. This antibody, of the IgG2a isotype, shows cytoplasmic and membranous expression and was used in a prediluted 7ml format at a pH of 7.5. The presence of FGFR3 was determined by the brown staining of the membrane and/or cytoplasm in the stained sections. The study adopted a semi-quantitative scoring system for evaluating FGFR3 expression. The system included the Q score, ranging from 0 to 3 based on the intensity of staining, and a percentage staining score graded from 0 to 4. The final Q score was calculated by multiplying the intensity score by the percentage staining score, with scores of 0 and 1 considered negative and scores of 2 to 12 considered positive [11]. The statistical analysis of the collected data was performed using SPSS Version 27, employing the Pearson Chi-square test to identify significant differences in FGFR3 expression between the positive and negative tumors. A p-value of <0.05 was considered statistically significant. Inclusion criteria for the study encompassed microscopically proven cases of urothelial carcinomas of the urinary bladder across all grades and stages, affecting both males and females of any age. Exclusion criteria included urothelial carcinoma originating from the kidney and ureter, other types of bladder malignancies, inadequate biopsies, and cases with incomplete medical records.

Results

Distribution of patients with urothelial carcinoma according to FGFR3 immunoreactivity was as following; Positive FGFR3 immunohistochemical results seen in 43 patients (68.3%) while the remaining 20 patients (31.7%) revealed negative results. as shown in Table (1).

Table 1: Patients Distribution according to tumor characteristics.

Clinicopathological Variables		Case No. (%)
Age at Diagonsis	≤60	(23/63) 36.5
	>60	(40/63) 63.5
Gender	Female	(23/63) 36.5
	Male	(40/63) 63.5
Histological Grade	Low	(46/63) 73
	High	(17/63) 27
Stage	Pta	(19/46) 41.3
	T1	(18/46) 39.14
	T2	(9/46) 19.56
FGFR3 Immunoreactivity	Positive	(43/63) 68.3
	Negative	(20/63) 31.7

The male gender was the predominant in the current study, where the total of males was 40 (63.5%) out of the total of 63 studied cases. Shown in Fig (1). In this study of UC, the low grade UC (lGUC) was reported in 46/63 (73%) cases, while the high grade UC (HGUC) represent only 17/63 (27%) cases out of the total number of the study. As shown in Figure (2).

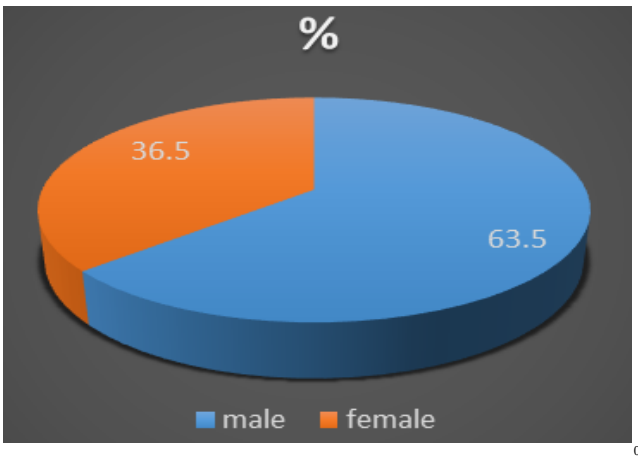

Fig 1: Distribution of sex in urothelial carcinoma

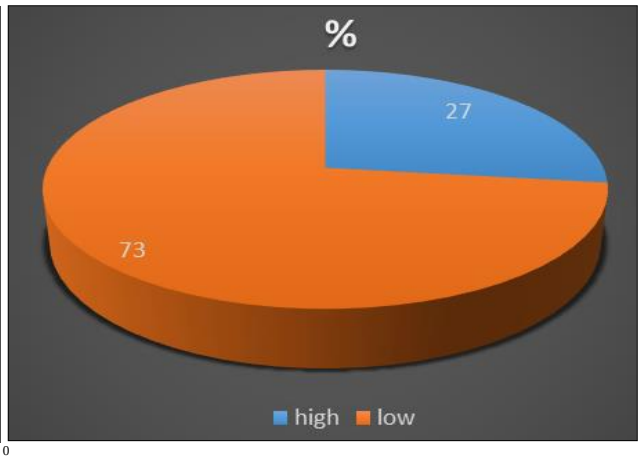

Fig 2: Grades distribution of urothelial carcinoma

About 65.2% of cases with ≤60-year-old were positive for FGFR3 compared to 70% in those patients above 60-year-old, however; no statistical significance was noted (p value 0.781), shown in Table (2).

Table 2: $FGFR_3$ association with Age(N=63).

Age	Positive	Negative	Total	P-Value < 0.05
≤ 60	15/23 (65.2)	8/23 (34.8)	23/63 (36.5)	0.781
>60	28/40 (70)	12/40 (30)	40/63 (63.5)	
Total	(43/63) 68.3	(20/63) 31.7	63/63 (100)	

*P-Value less than 0.05 consider statistically significant

From the data shown in Table 3 most of the cases with low grade reveal positive Immunohistochemical expression for FGFR3 (76.1%) while only 8 cases (47.1%) of high grade revealed positive expression. this association was statistically significant (p-value 0.037).

Table 3: The association between FGFR3 immunoreactivity and grading of bladder cancer

Grade	Positive no&%	Negative &%	Total &%	P-Value
Low	35/46 (76.1)	11/46 (23.9)	46/63 (73)	0.037*
High	8/17 (47.1)	9/17 (52.9)	17/63 (27)	
Total	(43/63) 68.3	(20/63) 31.7	63/63 (100)	

*P-Value less than 0.05 consider statistically significant.

Out of 46 patients, seventeen out of nineteen stage pTa, thirteen out of eighteen stage T_1 and three out of nine cases of T_2 revealed positive Immunohistochemical expression of FGFR3. This correlation was statistically significant. (p-value 0.99) as shown in Table 4, while FGFR3 cytoplasmic expression in Muscle invasive urothelial carcinoma was Illustrated in Figure 3.

Table 4: The correlation between FGFR3 immunoreactivity and stage of bladder cancer

Stage	Positive no&%	Negative no&%	Total no&%	P value
PTa	17/19 (89.5)	2/19 (10.5)	19/46 (41.3)	0.009*
PT1	13/18 (72.2)	5/18 (27.8)	18/46 (39.1)	
PT2	3/9 (33.3)	6/9 (66.7)	9/46 (19.6)	
Total	33/46 (71.7)	13/46 (28.3)	46/46 (100)	

*P-value less than 0.05 consider statistically significant

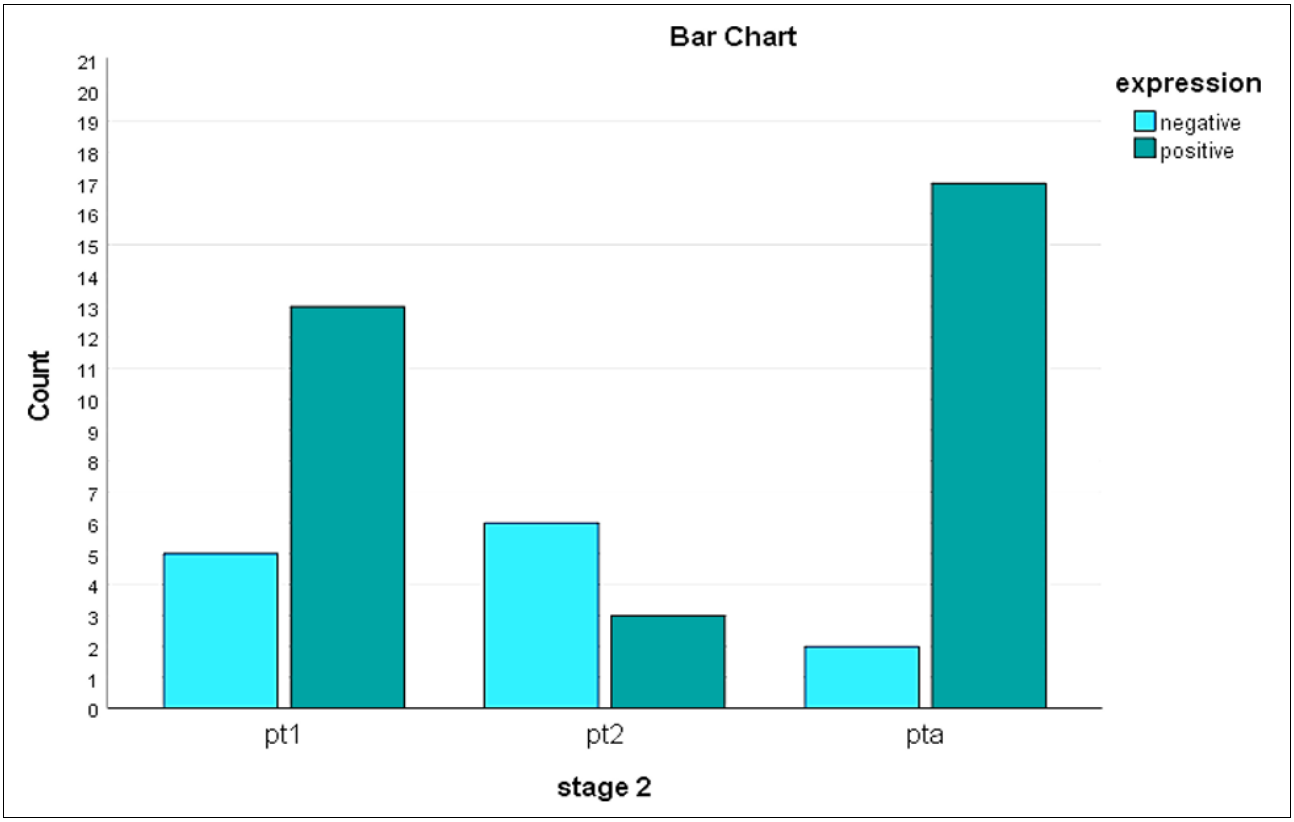

Fig 3: The correlation between FGFR3 immunoreactivity and stage of bladder cancer.

Most of non-muscle invasive were low grade (31 out of 37) with 83.8% positive Immunohistochemically for FGFR3. However, this was statistically not significant (p-value 0.32) shown in Table 5.

Table 5: The association between FGFR3 immunoreactivity and grade among non-invasive urothelial carcinoma.

Grade	Positive &%	Negative &%	Total &%	P-Value
Low	26/31 (83.8)	5/31 (16.2)	31	0.32
High	4/6 (66.6)	2/6 (33.4)	6	
Total	30 (81)	7(19)	37	

*P value less than 0.05 consider statistically significant

Figures (4-6) show H&E and different cytoplasmic FGFR3 expression in urothelial carcinoma.

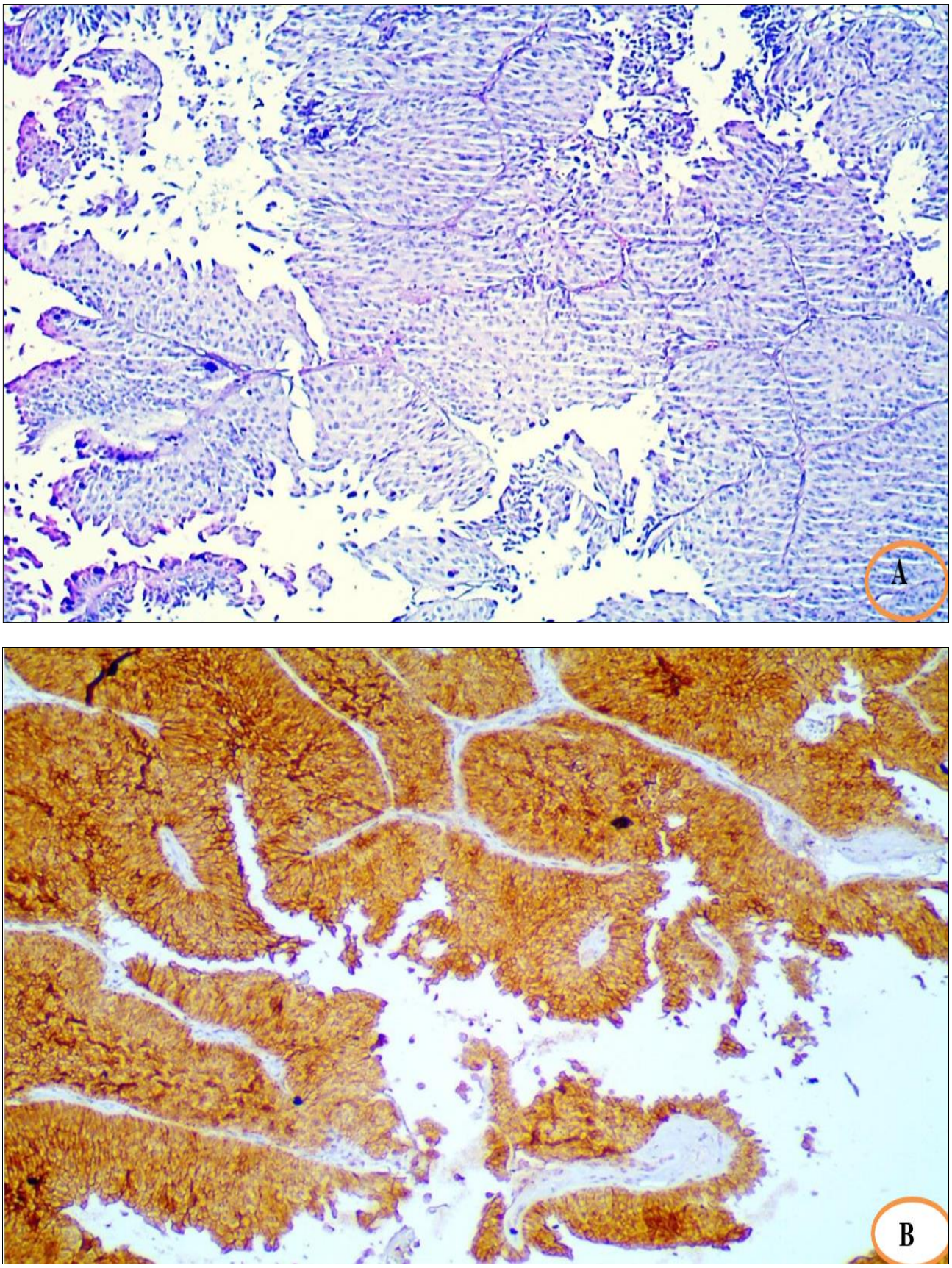

Fig 4: Low grade papillary urothelial carcinoma (A) H&E, (B) with intense cytoplasmic staining of FGFR3 expression (Score 9-12), (100X)

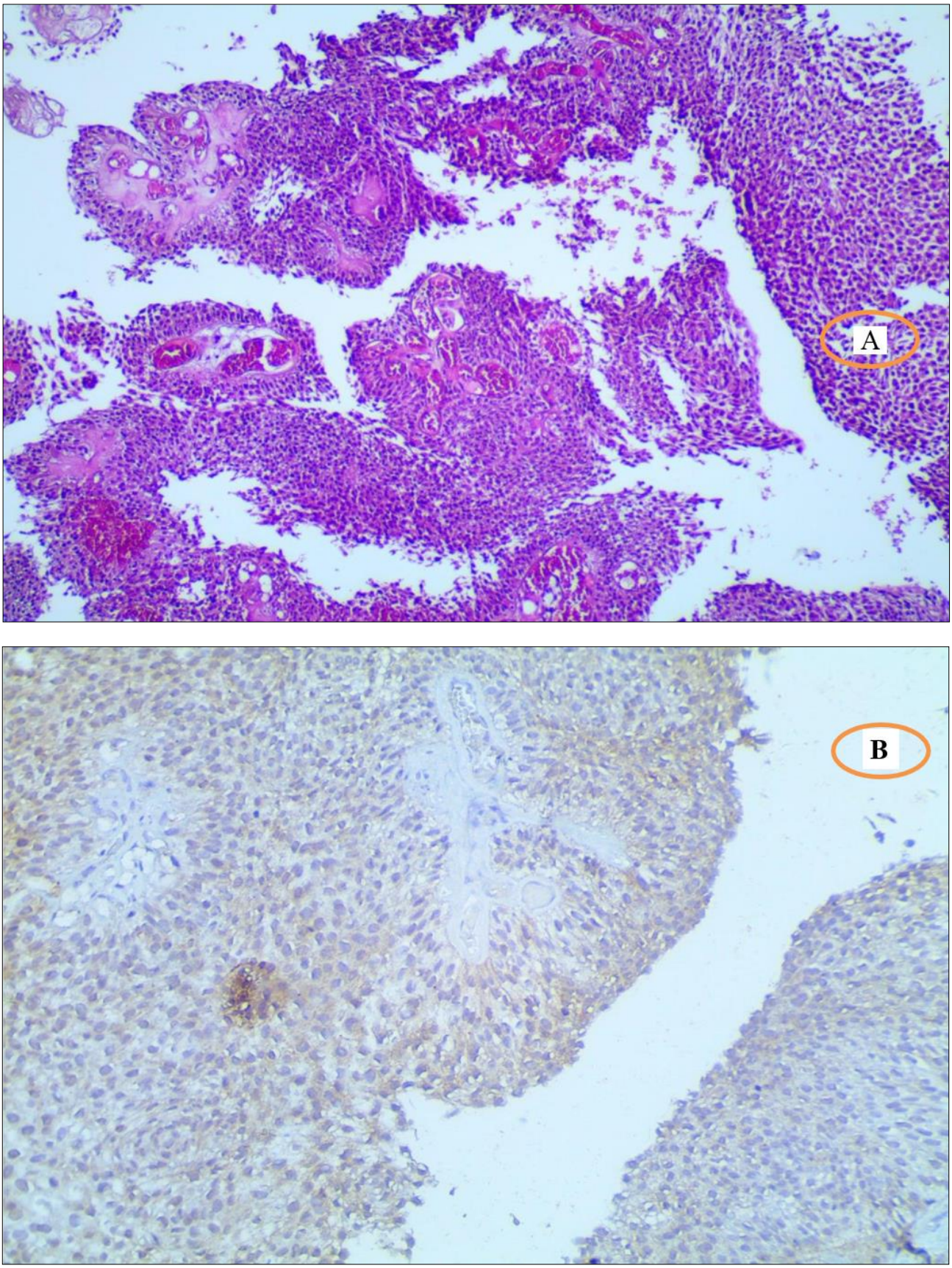

Fig 5: Low grade papillary urothelial carcinoma (A) H&E, (B) with weak FGFR3 cytoplasmic expression (score 2-5), (100x).

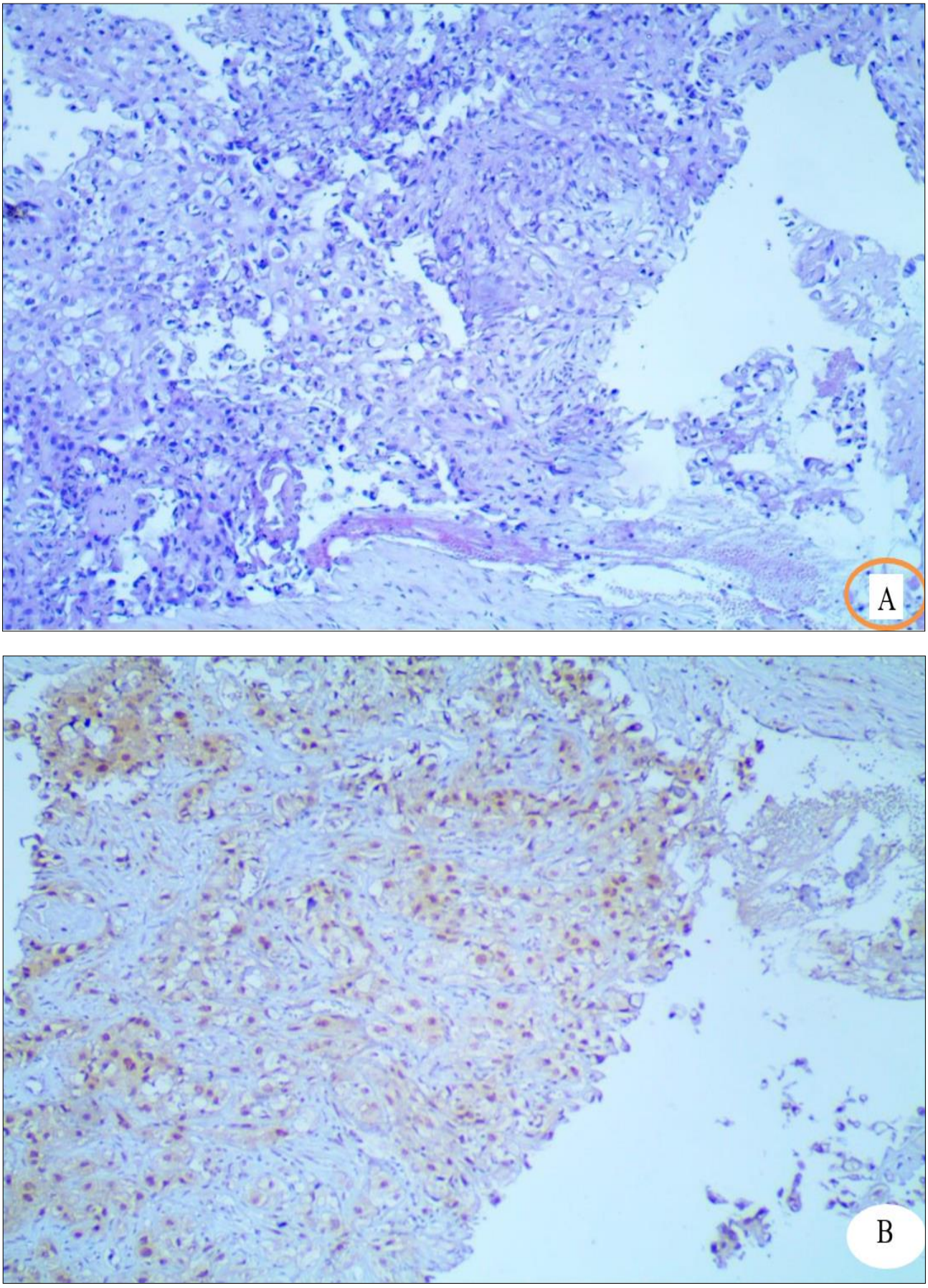

Fig 6: (A) muscle invasive urothelial carcinoma H&E, (B) with weak FGFR3 cytoplasmic expression (score 2-5), (100x).

Discussion

This study, conducted on 63 Iraqi patients with urothelial carcinoma of the bladder, assessed the FGFR3 protein expression using immunohistochemistry and its correlation with clinicopathological features like tumor grade and stage. Patients' samples were collected and processed for analysis, with tissue blocks stained for FGFR3 expression and evaluated using a semi-quantitative method known as the Q

score, which considers both the intensity of staining and the proportion of positively stained cells. The study's inclusion criteria covered all grades and stages of microscopically confirmed urothelial carcinomas of the bladder, while exclusion criteria omitted urothelial carcinomas from other locations, other bladder malignancies, inadequate biopsies, and incomplete medical records. The majority of the cases (68.3%) demonstrated positive FGFR3 expression, indicative of its potential role in bladder carcinogenesis and as a therapeutic target. Clinicopathological data indicated a mean patient age of 62.2, with ages ranging from 36 to 82. There was no significant correlation found between FGFR3 expression and patient age, aligning with findings from other studies such as Randa Khaled *et al*. [12] and Arsalan *et al*. [13]. Regarding tumor grade, 73% were low grade and 27% high grade. Positive FGFR3 expression was significantly more common in low grade than in high grade tumors, with 76.1% of low grade tumors showing positive staining compared to 47.1% of high grade tumors, which is consistent with results from previous studies like Young-Hee Maeng *et al*. [14] and Akanksha *et al*. [11]. Statistical analysis revealed a significant association between FGFR3 expression and tumor grade ($p = 0.037$) and stage (p values of 0.009 and 0.018 for different aspects of staging). Most notably, low grade and early-stage tumors (pta and T1) showed higher FGFR3 expression scores (9-12), whereas higher stage tumors (T2) typically scored lower (2-5). These findings underline the potential for FGFR3 as a marker for non-invasive, lower grade bladder tumors. The study also paralleled findings from other regional studies, such as Hammam *et al*. [15] and Tavakkoly-Bazzaz J *et al*. [16], but unlike some studies, it found no significant correlation between FGFR3 gene amplification and tumor grade. The research, however, does affirm the significant correlation between FGFR3 expression and both tumor pathological stage and grade, suggesting a conserved pattern across different populations. Overall, the results support the oncogenic role of FGFR3 in bladder cancer, predominantly in low-grade and low-stage tumors, and confirm its utility as a biomarker for selecting patients for targeted therapy. The study's implications extend to the potential development of FGFR3-targeted treatments that could benefit patients with specific tumor profiles, emphasizing the importance of molecular characterization in the management of bladder cancer.

Conclusion

The expression of FGFR3 is observed in urothelial cancer. Our findings have demonstrated that low grade and low stage cancers have positive FGFR3 expression. supported statistically, FGFR3 protein expression has the potential to serve as an additional marker, alongside tumour grading and staging, in the prediction of prognosis as good prognostic factor.

Conflict of Interest:
Not available

Financial Support:
Not available

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