Oncology and Radiotherapy ©
Vol.17 Iss.11: 001-009 • RESEARCH ARTICLE

Prognostic value of FGFR3 expression in Urinary bladder cancer

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Abstract

The aim of this study is to determine the expression of the FGFR3protein in human bladder cancer, and significant correlations between these parameters and clinico pathologic variables like (grade and stage. the bladder cancer is one of the most common cancers worldwide. the incidence and mortality of this disease increased during last decades alarmingly in Iraq. The current study is designed to detect the role of FGFR3expression in bladder carcinoma as a possible marker for detecting the biological behavior of malignancy and its correlation with grade and non-muscle invasive bladder cancer NMIBC. for both diagnostic and prognostic purposes. The study focuses on a technique of immunohistochemistry for detection FGFR3expression in bladder cancer. The samples are collected randomly in southern Iraq, in AL-Nasiriya city, from AL Hussein teaching hospital. Number of samples is 100 ,70 bladder cancer tissue and 30 controls benign tissue. Results of this study reveal that FGFR3expression is positive in 49 of 70 sample. The study demonstrated FGFR3 expression is increased in low grade bladder cancer represent (96.30%), while in high grade (53.49%), FGFR3expression high in Ta stage (100%) , and in T1(100%) T2(51.28) T3 (50%) expression FGFR3excessive in non-muscle invasive (92.85%), whilst the low expression for FGFR3in the muscle invasive type (54.76%).

So FGFR3can be used as a marker for assessment of bladder cancer aggressiveness. This study represents an important step because there are few of studies about this topic in Iraq; we are needing more studies to prove the function of this FGFR3in biological behavior of bladder cancer.

Key words: Bladder cancer, FGFR3, Immunohistochemical.

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Word count:3595 **Tables:** 03 **Figures:** 04 **References:** 29

Received:16 October, 2023, Manuscript No. OAR-23-112437
Editor assigned: 08 November, 2023, Pre-QC No. OAR-23-112437 (PQ)
Reviewed: 19 November, 2023, QC No. OAR-23-112437 (Q)
Revised:23 November, 2023, Manuscript No. OAR-23-112437 (R)
Published:30 November, 2023, Invoice No. J-112437

INTRODUCTION

Bladder cancer was had the most common extreme conditions among malignant tumor of the urinary tract that require amalgamation of some techniques and immunological tests to influence the kind of tumor, and despite the progress of pioneering techniques in the ground of treatment, the exact diagnosis of the case had not been prepared without trusting on conquest a biopsy from bladder tissue [1]. Based on their deepness of invasion within bladder wall, they have been divided into either (non-muscle-invasive bladder carcinoma (NMIBC) or muscle-invasive bladder carcinoma (MIBC)). NMIBC Represent for approximately over70% of wholly newly detected BCs [2]., containing non-invasive papillary urothelial carcinomas (stage pTa), carcinoma in situ (CIS) and invasive carcinomas restricted to the lamina propria (stage pT1) [2].

Kinds of bladder tumor contain Transitional cell carcinoma (TCC) has been the most communal kind about (97%), followed by squamous cell carcinoma (SCC) about (2%) and the lowest predictability was had adenocarcinoma as (1%) [3]. Bladder tumor has been the fourth most communal tumor in men, with approximately 60,000 new-fangled diagnosis each year ranking as the eighth principal reason of cancer associated deaths in the United States, with about 12,000 deaths yearly, Specifically, in 2017, there have been 79, 030 cases of bladder tumor and 16,870 associated deaths in the United States [4]. In Iraq, bladder malignancy has been the fifth of ten tumors, the third tumor in men and the eighth in women. 866 men had been diagnosed with bladder tumor, Comparison to 297 women, agreeing to the Iraqi Cancer Registry (2011)[5]. As for the year 2021, it would have ranked fifth among other most communal tumors, with an infection rate of 1,769, as the number of male infections has been 1,360, while females have been 409, and this designates that male infections have been greater than females [6]. Whereas the current suggested management for advanced bladder tumor has been systemic cisplatin-based chemotherapy, immunotherapy has been developing as a viable salvage management where first-line chemotherapy failed to control the illness [6].

The chief problems after first therapy have been greater chances of recurrence, development, and metastasis[7].. Conventional predictive influences, including cancer staging, grading, size, and multifocality, could not prophesy clinical outcome in most patients with bladder

tumor. Thus, some efforts to achieve indicators which could predict reappearance, progression, therapeutic reaction, and survival have been being prepared[7,8].

Fibroblast Growth Factor Receptor 3 (FGFR3) has been a receptor tyrosine kinase with frequent genetic variations in BCA [9]. Inhibition of Fibroblast Growth Factor Receptor (FGFR) poses another newfangled target for Urothelial Carcinoma (UC) management. Genomic variations in FGFR3 have been among the most recurrent reported somatic mutations in Urothelial Carcinoma (UC) of the Upper Urinary Tract (UUT) and bladder [10]. In addition, gene enlargements of FGFR1 and FGFR3 have been common in Urothelial Carcinoma (UC) with reported rates of 6% and 13%, correspondingly [11].

While primarily related to UC of low malignant potential, FGFR variations have been present in UC as well, albeit the prevalence has been poorly examined [12]. Genetic indication shows that urothelial carcinoma could progress from two molecular passageways: FGFR3, which play a important role in non-invasive bladder urothelial carcinoma; and TP53, FGFR itself has been a high-affinity tyrosine kinase transmembrane receptor that has been significant for embryogenesis, cell growth, difference, proliferation, and angiogenesis. FGFR had been 4 active isoforms, which have been FGFR1, FGFR2, FGFR3, and FGFR4. FGFR3 signaling passageway particularly could stimulate the Androgen Receptor (AR) activity [13].

As stated by Knowles and Hurst, fibroblast growth factor receptor-3 (FGFR3) has been a tyrosine kinase that reasons a proliferation in bladder cell growth and has been found to conversion in 75% of Non-Muscle Invasive Bladder Cancer (NMIBC) cases. Lerner et al also found that expression of FGFR3 was eight times developed in NMIBC cases [13].

One of the most important factors in the incidence of bladder cancer is smoking, as there is a direct relationship between the increase, the number of cigarettes, years of smoking, and the incidence of bladder cancer, as the absorption of nicotine, which is a toxic substance, leads to a lack of blood absorption of oxygen that the body needs and is replaced by carbon dioxide that is rapidly absorbed by the blood, which leads to hemoglobin saturation [14].

The aim of this study was to evaluate the expression of FGFR3, in bladder cancer cases by using IHC, and to investigate a possible association between the level of FGFR3, expression and tumor grade, stage. We also sought to bring out the clinic pathological features of bladder cancer among Iraq's patients.

MATERIALS AND METHODS

Collection of Samples

This project included100 bladder tissue samples divided into two groups: 70 samples (patients' group) of bladder cancer tissues and 30 samples (control group) of benign bladder lesion. The age range was between 20-80 years old. All samples in study were collected from Al-Hussein Hospital and a Private Laboratory in Thi-Qar province during the period between January 2022and February 2023 All samples kept at 10% formaldehyde and room temperature for histopathological and immunohistochemical analysis.

Immunohistochemistry assays (IHC)

In this study the immunohistochemical technique was been performed as designated earlier [15]. First step has been the deparrafination, accomplished by soaking samples in xylene and then rehydrated through a chain of graded alcohol. After that, Antigen retrieval by placing the slides in Retrieval Solution in high temperature Then left in room temperature for 20 min to cooled, subsequently adding of peroxidase to eliminate internal peroxidase action of tissue for 10 min and slides had been again washed 3 times for 10 min in phosphate buffered saline. Then let sections overnight for incubated with primary antibodies FGFR3, (FGFR3, - Rabbit Monoclonal antibody) (FGFR3, 1:100). In the next day, the slides imperiled to washing 3 times with (PBS) for 10 min. Then dropping of anti- Rabbit labeled polymer-HRP primary anti body and incubated then at room temperature with gentle vibrator for 30 min. Then, the slides washed three times with PBS buffer for 5 min each. After that drops DAP Chromogen additional to the slides and incubated for 10 min at room temperature agreeing protocol Dako. Next step, slides washed with D.W (distilled water) for 10 min. Then, drops of the counter stain (Haematoxylin) has been utilized onto the slides and let for incubation at room temperature for 2 min, to stain the nucleus of cells, then, slides washed or cleaned by the running tap water for 2 min and distilled water for 1 min . After that, the slides dehydrated by soaked in ascending concentrations of alcohol (70, 95, and 100%) for 1 min each one. After dehydration slides soaked in xylene 2 times for 2 min each. Then drops of DPX practical onto slides and protected with cover slides (22 X 22 mm) and let them dry.

Statistical analysis

The data has been analyzed by the statistical package for available from SPSS., percentages have been utilized to display the data. The Pearson Chi-square test has been utilized to determine the significance of the difference

between significant qualities (qualitative data). Whenever the P-value has been less than 0.05, statistical significance has been considered.

RESULTS

The study shows that 49 case out of 70 bladder cancer show over expression of FGFR3 (70%) and 2 out of 30 controls are positive for FGFR3. high expression of in Ta stage (100%), and in T1(100%) T2(51.28) T3 (50%) as in table 1. The study demonstrated FGFR3 expression is increased in low grade bladder cancer represent (96.30%), while in high grade (53.49%), (p. value < 0.001) as in table 2 expression FGFR3 excessive in non-muscle invasive (92.85%), whilst the low expression for FGFR3in the muscle invasive type (54.76%) as in table 3.

A Total Score (TS) is the sum of PS plus IS (ranging from 0, 2–8). A positive result for FGFR3 is defined as TS ≥ 3, which was validated in numerous large clinical studies

Tab. 1. Represent FGFR3 expression, T stages of tumor

Stage	Positive FGFR3 No.	%	Negative FGFR3 No.	%	Total No.	%
Ta	19	100	0	0	19	27.14
T1	9	100	0	0	9	12.86
T2	20	51.28	19	48.71	39	55.71
T3	1	50	1	50	2	2.86
T4	0	0	1	100	1	1.43
Total	49	70	21	30	70	100

CalX2= 291.35 TabX2= 9.49 DF= 4 p. value < 0.001high-sig

Tab 2. Represent FGFR3expression and grades tumor

Grade Tumor	Positive FGFR 3 No.	%	Negative FGFR3 No.	%	Total No.	%
Low grade	26	96.3	1	3.7	27	38.57
High grade	23	53.49	20	46.51	43	61.43
Total	49	70	21	30	70	100

CalX2= 48.664 TabX2= 3.84 DF= 1 p. value < 0.001high-sig
OD high/low grad 0.047 (0.016 – 0.138)

Tab 3. Correlation FGFR3expression and muscle invasive of bladder cancer

Type of Tumor	Positive FGFR3 No.	%	Negative FGFR 3 No.	%	Total No.	%
Muscle invasive	23	54.76	19	45.23	42	60
Non-muscle invasive	26	92.85	2	7.14	28	40
Total	49	70	21	30	70	100

CalX2= 12.478 TabX2= 3.84 DF= 1 p. value < 0.001high-sig
OD invasive/non-invasive 0.324 (0.171 – 0.613)

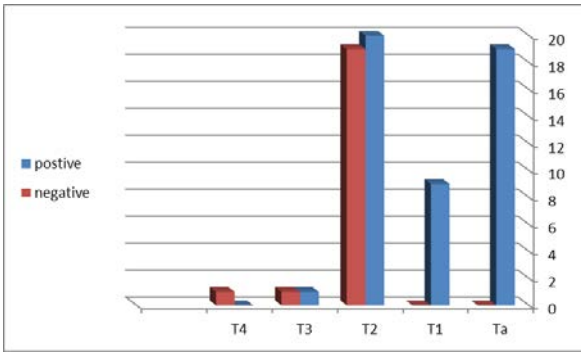

Fig 1. Represent FGFR3expression, T stages of tumor

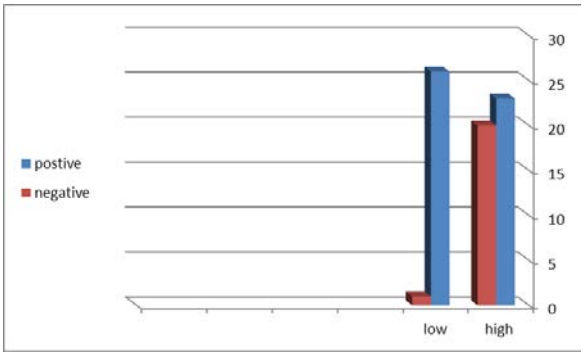

Fig 2. Represent FGFR3expression and tumor grades

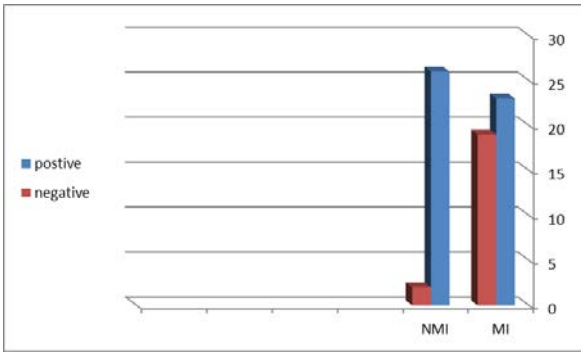

Fig 3. Correlation FGFR3expression and muscle invasive of bladder cancer

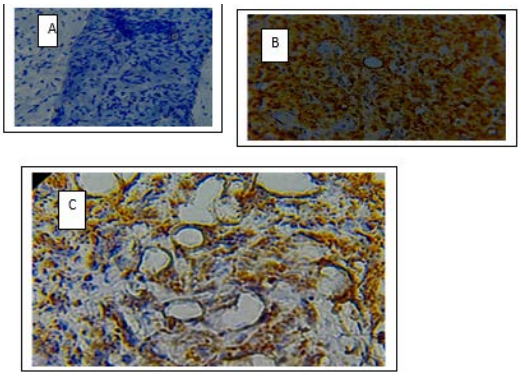

Fig 4. (A) show no expression of PD-L1 (10X) (B) high- grade urothelial carcinoma, show high expression of FGFR3 (20X), (C) low grade papillary urothelial carcinoma, no expression FGFR3 (40X)

DISCUSSION

The study shows that 49case out of 70 bladder cancer show over expression of FGFR3 (70.0%) and 2 out of 30 control are positive for FGFR3. high expression of FGFR3in stage Ta Ta stage (100%) , and in T1(100%) T2(51.28) T3 (50%) as in table 1. Agreement Syah et al [16].

FGFR3 has been the chief gene known in bladder tumor that metamorphosed selectively in this illness and could be seen with clinical parameters [17]. Lindgren et al found that FGFR3 expression associated with the FGFR3 metamorphosis, in which overexpression of FGFR3 was chiefly due to a metamorphosis in the FGFR3 gene, thus growing the FGFR3 receptor. A dysregulation of the growth receptor signing passageway due to FGFR gene perversions, containing amplifications, fusions, and metamorphoses, enhances tumor propagation, invasion, angiogenesis, and immune evasion through reduced cytokine signing [17]. These perversions, might affect the cancer microenvironment with consequently reduced cancer infiltrating lymphocytes (TILs). This could be part of the elucidation for the observed poorer reaction rates to immune checkpoint inhibitor (ICI) for cancers with FGFR gene metamorphoses in Lopez-Beltran et al. [18].

We had been demonstrated that overexpression of FGFR3 protein has been communal in UC and that increased expression has been more frequent in tumours of low stage. Two previous studies have examined FGFR3 protein expression in UC by immunohistochemistry [19]. Gomez- Roman et al reported like results to our study with increased FGFR3 protein expression in 69.5% pTa, 72 % pT1 and 49 % of pT2 tumours [20]. Mhawech-Faucegli et al showed increased protein expression in 76.6% pTa and 46.9% pT1 tumours [21].

It had been shown that in bladder cancer cell lines expressing high levels of wildtype FGFR3, targeted inhibition by antibodies results in reduced propagation [22]. The results of this study shows that (96.30%) cases of (70%) positive FGFR3expression of bladder cancer have been of low grade urothelial carcinoma and low expression FGFR3in high grade of bladder cancer (53.49%) (p. value < 0.001)as in table 3 0expression FGFR3excessive in non-muscle invasive 81.08%), whilst the low expression for FGFR3in the muscle invasive type (57.58%) as in Table 3 this result agreement Syah et al.

FGFR3 has been a tyrosine kinase, which increases the growth of bladder cells. It has been found to be metamorphosed growth of bladder cells. It has been found to be metamorphosed in 92.85% of cases of non-muscle invasive bladder cancer. Lerner et al reported an increase in FGFR3 expression that had been eight times higher in NMIBC. In this study, we found positive FGFR3 immunohistochemistry in 70.0% of subjects. In the NMIBC group, 92.85% of subjects had positive FGFR3 immunohistochemistry. The expression of FGFR3 was significantly related with the occurrence of non- muscle invasion FGFR3 immunohistochemistry had an 0.324 times higher chance in the MIBC group. Bladder tumor with a positive FGFR3 had a lower recurrence rate comparison with bladder tumor with a negative FGFR3 (p. value < 0.001). Some studies have also found high significant differences in FGFR3 expression in NMIBC and MIBC [23].

Results investigator in this research have been in line with the research conducted by van Rhijn et al., which stated that low FGFR3 expression has been a strong indicator in non-invasive bladder carcinoma recurrences [24]. The assumed recurrences affected by the remaining cancer stem cells that had been still intact on the primary tumor site after primary tumor resection [24]. On the low FGFR3 immunoexpressed tumor, cancer stem cells suspected of undergoing a slower propagation rate. They also act as weak cell-cell interface and poor stromal strength, altogether causing tumor cells to reimplant in the bladder epithelium easily. They migrated to inside the epithelium as well as important to trouble-free stromal invasion. Patients with low FGFG3 immunoexpression have been likely to experience recurrences in numerous sites and multiple growths [24].

Lindgren et al found a significant association between FGFR3 metamorphosis and expression. When the mutation existed, the overexpression of FGFR3 occurred, which might be because: 1) cells that had the FGFR3 metamorphosis Lindgren et al [17]. found that FGFR3 expression associated with the FGFR3 metamorphosis, in which overexpression of FGFR3 was principally due to a metamorphosis in the FGFR3 gene, thus increase the FGFR3 receptor. We, therefore, studied markers at a time that predictable to provide a more precise prediction for the occurrence of MIBC [25]. showed that FGFR3has been induced by hypoxia in bladder cancer cell lines. tumor hypoxia may represent an additional mechanism for increased levels of FGFR3 in bladder tumor. FGFR3 overexpression may provide a positive influence by avoiding development to MIBC illness [26,27].

The FGFR3 metamorphosis was found in 92.85% of cases with NMIBC(Lerner et al.,2016). van Rhijn et al

found the FGFR3 mutation in65% of subjects with bladder tumor (stage pTaG1-2)[28].In that study, the level of FGFR3 was determined in a semiquantitative fashion, and they did not identify a significant relationship between FGFR3 expression levels and tumor characteristics. It has been important to note has been that no established cut-off points have been developed for FGFR3 IHC staining.

CONCLUSION

FGFR3 expression is significantly associated with two important prognostic factors; stage and grade. While FGFR3 expression was significantly correlated with the tumour grade and degree of invasion suggesting the suitability of FGFR3 as prognostic markerof urothelial carcinoma. Non-invasive urothelial carcinoma patients with multiple tumor characteristic which has high FGFR3 immunoexpression more than patients' muscle invasive

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