Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2021; Vol. 19(1)

Fibroblast Growth Factor Receptor 3 (FGFR3) Gene Amplification in Patients with Urothelial Carcinoma of the Urinary Bladder

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Abstract

Background The fibroblast growth factor receptors (FGFRs) family includes five tyrosine kinase receptors, which play an important role in many cellular mechanisms including proliferation, migration and survival. Deregulations in the genes that coding for these receptors, mainly the FGFR3 gene, had been reported in patients with urothelial carcinoma of the urinary bladder.

Objective To estimate the frequency of FGFR3 gene amplification in patients with urothelial carcinoma of the urinary bladder and its relation to some clinico-pathological parameters of the tumor.

Methods The present retrospective study included 30 paraffin blocks of urothelial carcinoma tissue (trans-urethral resection of bladder tumor (TURBT)), and 10 samples of normal bladder tissue (control group). Sections were taken from each paraffin block for studying FGFR3 gene amplification by fluorescent in situ hybridization (FISH) technique.

Results FGFR3 gene was shown to be amplified in 4 (13.3%) out of 30 urothelial carcinoma cases. There was significant relation between FGFR3 gene amplification and tumor's pathological stage (p=0.037). FGFR3 gene wasn't statistically correlated with the patient's age, gender, tumor's grade or the lympho-vascular permeation.

Conclusion FGFR3 gene amplification was detected in 13.3% of urothelial carcinoma of the urinary bladder cases, which may reflect its role in carcinogenesis process. This amplification was statically correlated to tumor's pathological stage suggesting its relation to tumor progression.

Keywords FGFR3, FISH, TURBT, urothelial carcinoma, gene amplification

Citation Al-Marashi MA, Qasim BJ. Fibroblast Growth Factor Receptor 3 (FGFR3) gene amplification in patients with urothelial carcinoma of the urinary bladder. Iraqi JMS. 2021; 19(1): 116-125. doi: 10.22578/IJMS.19.1.15

List of abbreviations: FFPE = Formalin-fixed paraffin-embedded, FGFR3 = Fibroblast growth factor receptors 3, FISH = Fluorescent in situ hybridization, H&E = Hematoxylin and eosin, TURBT = Trans-urethral resection of bladder tumor

Introduction

Bladder cancer is the tenth most common cancer worldwide and the fourth most common cancer in Iraq. Male predominance is highly observed (Male: Female about 4:1) [(1)].

Urothelial (transitional cell) carcinoma account for 90% of all bladder cancers. Other rare types include squamous cell carcinoma, adenocarcinoma and small cell carcinoma account for 9% of bladder tumors. It is worth to mention that although squamous carcinoma is a rare type, it accounts for 81% of bladder cancer in area where schistosomiasis is endemic [(2)].

At molecular level, recent studies show that urothelial carcinoma follow two different pathways of gene deregulation. Fibroblast growth factor receptors 3 (FGFR3), phosphoinositide 3-kinases/ protein kinase B (PI3K/AKT) pathway and Rat Sarcoma (RAS)

pathway are usually associated with low grade, non-invasive tumors, while high grade, muscle invasive tumors usually follow different pathway include deregulation in tumor suppressor gene P53, p16 and RB [3].

Fibroblast growth factor (FGF) pathway plays an important role in various cellular functions, including cellular differentiation, proliferation, apoptosis and migration. Also, the fibroblast growth factor/fibroblast growth factor receptor (FGF/FGFR) signaling pathway have role in embryogenesis, angiogenesis, tissue homeostasis and wound healing process. Deregulation of FGF/FGFR signaling pathway shown to be play important role in carcinogenesis. The mechanisms by which this pathway can be deregulated include mutation, translocation or gene amplification [4].

Deregulations in FGF/FGFR signaling pathway has been reported to promote the development of different type of cancer through oncogenesis, neoangiogenesis and anti-cancer drug resistant mechanisms [5].

Alteration in FGFRs genes, especially FGFR3 gene, had been reported in patients with urothelial carcinoma of the bladder. These alterations could be in form of activating mutation, gene amplification or chromosomal rearrangement. These finding make the FGF/FGFR signaling pathway a potential therapeutic target for many types of cancers [6].

Many clinical trials had been conducted to evaluate the efficacy of FGFR target therapy in urothelial tumors that harbor specific alteration in FGFR3 gene [7,8].

Recently, the Food and Drug Administration (FDA) grants an accelerated approval for the pan-FGFR inhibitor Erdafitinib, a selective tyrosine kinase inhibitor for patients with urothelial carcinoma whom have alteration in FGFRs genes [7].

So, the aim of the study to estimate the frequency of FGFR3 gene amplification among patients with urothelial carcinoma in order to identify those who will get benefit from recently released, FDA approved target therapy.

Methods

Sample collection

The present retrospective study included 30 formalin-fixed paraffin embedded (FFPE) tissue blocks of urinary bladder biopsies from patients whom had been diagnosed with urothelial carcinoma of the urinary bladder during 2017 and 2018, were collected from Al-Basra Teaching Hospital Laboratory, Al-Mawanea General Hospital Laboratory and Doctor Sawsan Al-Haroon Private Laboratory.

Also, 10 samples of normal bladder tissue (control group) collected from autopsy cases from Forensic Medicine Unit in Basra and were processed as FFPE blocks in the Department of Pathology and Forensic Medicine, College of Medicine/ Al-Nahrain University.

Clinical and pathological information were collected from patient admission case sheets and pathology reports.

Sectioning

From each FFPE tissue block, two sections of 5 µm thickness were obtained. One section was processed and stained with hematoxylin and eosin (H&E) stain for revision of the diagnosis, and the other section were applied onto positively charged slides and submitted to a two days procedure of fluorescent in situ hybridization (FISH) study for FGFR3 gene.

Cytogenetic study procedure

Sections which applied onto positively charged slides were processed through two days procedure for FISH study of FGFR3 gene using ZytoVision FGFR3/4p11 dual-color probe (Germany).

In day one, slides were incubated at 70°C for 10 minutes then incubated in xylene solution twice for 10 minutes each time. Slides then rehydrated by series of descending concentrations of ethyl alcohol (100%, 100%, 90% and 70%) for 5 minutes each time. The slides then washed twice with distilled water for 2 minutes each time, and incubated in pre-warmed Heat Pretreatment Solution Citric at 98°C for 15 minutes duration. Furthermore, the slides washed two times in distilled water for two minutes each time, the allowed to dry.

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Pepsin drops then applied over the specimen and the slides then incubated in humidity chamber at 37°C for 25 minutes. The slides then washed with Wash Buffer saline-sodium citrate (SSC) for 5 minutes and then washed with distilled water for one minute. Slides then dehydrated by series of ascending concentrations of ethyl alcohol (70%, 90% and 100%) for one minute each time and then allowed to dry. Ten µl of ZytoLight SPEC FGFR3/4p11 dual color probe was applied onto each specimen and cover-slip was applied and sealed with rubber cement. Denaturation was allowed for 10 minutes at 75°C then slides were transferred to humidity chamber to hybridize overnight at 37°C.

Next day, slides were removed from humidity chamber, rubber cement was removed carefully and the slides were submerged in pre-prepared 1X Wash Buffer A for 3 minutes at 37°C to remove the cover slips, and then washed twice with the 1X Wash Buffer A twice for 5 minutes duration each time, followed by incubation in a series of ascending concentration of ethyl alcohol (70%, 90% and 100%) for one minute each time. Slides then allowed drying in dark area and 25 µl of diamidino-2-phenylindole (DAPI) solution was applied followed by cover slip. Finally, slides incubated for 15 minutes in dark area then transferred to the refrigerator at 6°C and stored till the time of examination.

Evaluation of FGFR3 gene had been carried out using Zeiss Axio Imager Z2 fluorescent microscope with 64X oil immersion subjective lens. Three filters had been used Texas Red, DAPI and fluorescein isothiocyanate (FITC) filter for specimen evaluation.

Image capturing had been done using CoolCube 1 Digital High-resolution Camera and photo analyzed by fluorescence imaging system by MetaSystem Company.

The FGFR3/4p11 Dual color probe composed of green-labeled poly-nucleotides (ZyGreen) that target FGFR3 gene region and orange-labeled poly-nucleotides (ZyOrange) that target a specific sequence in chromosome 4 in the chromosomal region 4p11.

The number of FGFR3 and 4p11 signals was counted in 50 nuclei and identified to be amplified if has one of the following criteria: [6,9]

1. FGFR3/4p11 ratio is ≥ 2.
2. The average number of FGFR3 signals per tumor cell is ≥ 6.
3. Ten percent of the tumor cells or more contain ≥ 15 FGFR3 signals or large clusters.
4. Fifty percent of the tumor cell or more contain ≥ 5 FGFR3 signals.

The statistical analysis of this study was performed with the statistical package for social science (SPSS Version 25) and Microsoft Excel 2013. Numeric variables were described as mean, median and standard deviation. Independent-samples ttest was used to estimate the statistical difference between them. Categorical data were formulated as numbers and percentages. Chi-square test was used to measure the association between categorical variables. P-value was considered to be significant if it was less than 0.05.

Results

Out of total 30 urothelial carcinoma cases, 24 (80%) patients were males and only 6 (20%) patients were females with a male to female ratio were 4:1.

The overall mean age was 65.73±8.38 years (range: 51-77 years).

Eighteen patients (60.0%) out of 30 cases with urothelial carcinoma had high grade tumors (Figure 2); while low grade tumors had been recorded in the remaining 12 cases (40.0%) (Figure 1) with no case had met the criteria for papillary urothelial neoplasm of low malignant potential (PUNLMP) category.

Fourteen cases (46.67%) out of 30 shown to have muscle invasion features (T2) (Figure 3), while the remaining 16 cases (53.33%) where have lamina propria invasion only (T1). No cases with a feature of Tis or Ta subcategories had been recorded.

Lymphovascular permeation was observed in 18 (60%) out of 30 urothelial carcinoma cases (Figure 4), while the remaining 12 cases (40%) were didn't have lymphovascular permeation.

Four (13.3%) cases out of 30 urothelial carcinoma cases show feature of FGFR3 gene amplification (Figure 5), while the remaining 26 (87.7%) cases didn't meet any feature of FGFR3 gene amplification (Figure 6).

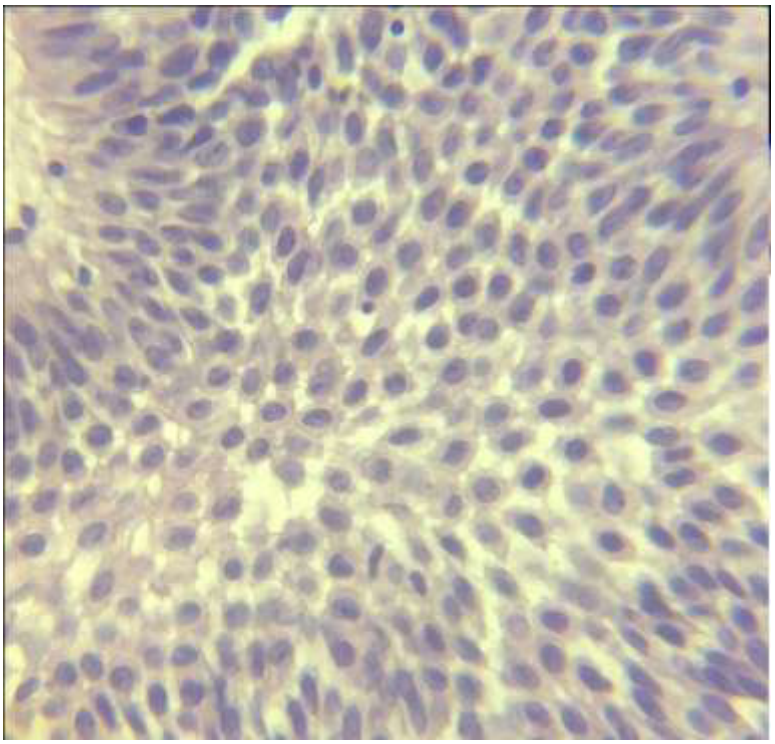

Figure 1. Low grade, papillary urothelial carcinoma of the urinary bladder shows minimum cytological atypia, minimum nuclear pleomorphism with infrequent mitosis. (40X)

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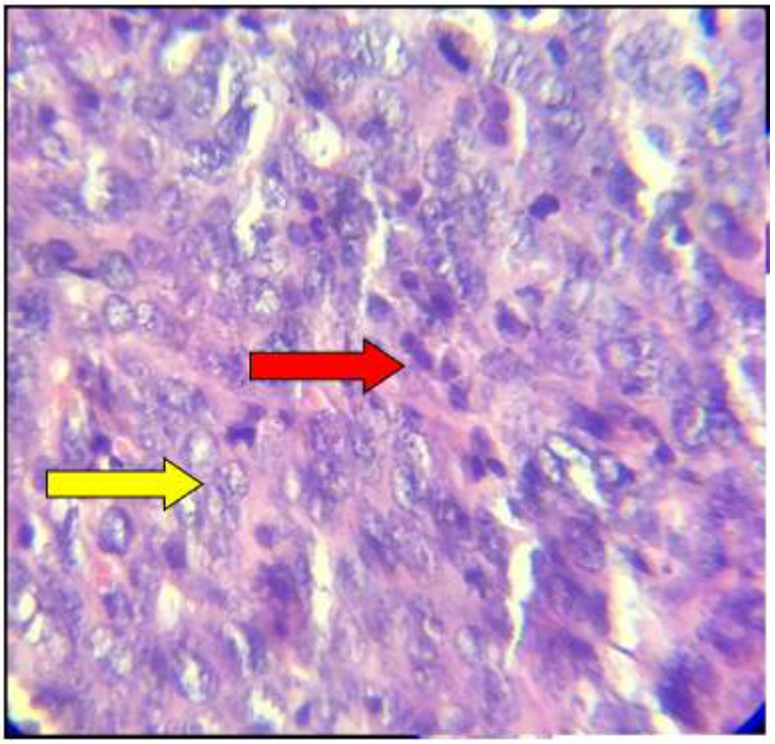

Figure 2. High grade urothelial carcinoma of the urinary bladder shows marked cytological atypia, loss of polarity, nuclear pleomorphism, prominent nucleoli (yellow arrow) and increased mitotic figure (red arrow). (40X)

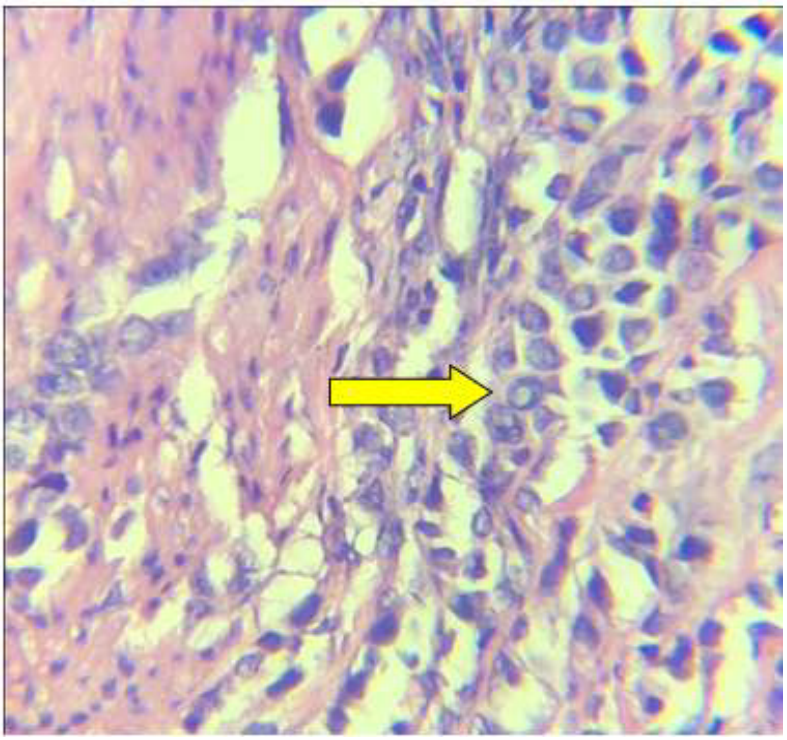

Figure 3. High grade urothelial carcinoma, pathological stage T2. Cluster of tumor cells with marked pleomorphism, hyperchromasia and prominent nucleoli invading muscular layer (Arrow). (40X)

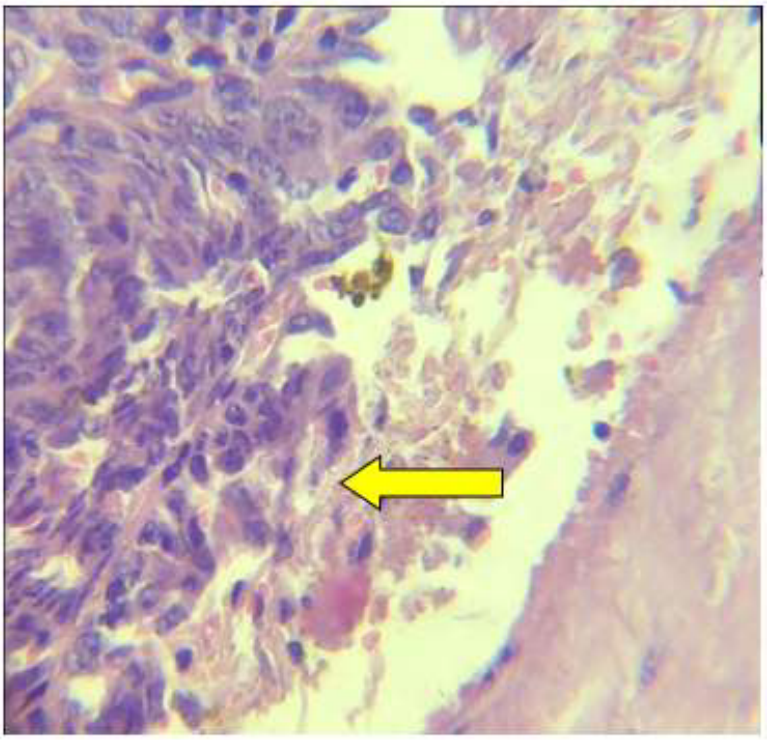

Figure 4. High grade urothelial carcinoma with lymphovascular invasion, shows nest of tumor cells with large pleomorphic nuclei and prominent nucleoli invading vessel wall (Arrow). (40X)

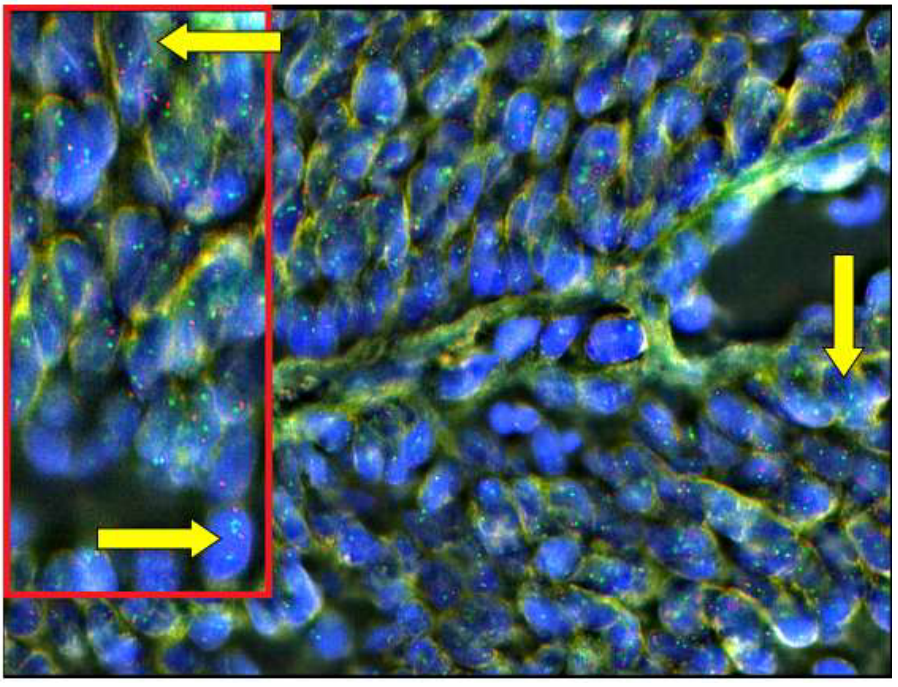

Figure 5. High grade urothelial carcinoma of the urinary bladder with FGFR3 gene amplification. FGFR3 dual color probe hybridize to malignant cells showing FGFR3 gene amplification as indicated by two chromosome 4 (Red) signals and >2 FGFR3 (Green) signals in the nucleoli (Arrows). (64X)

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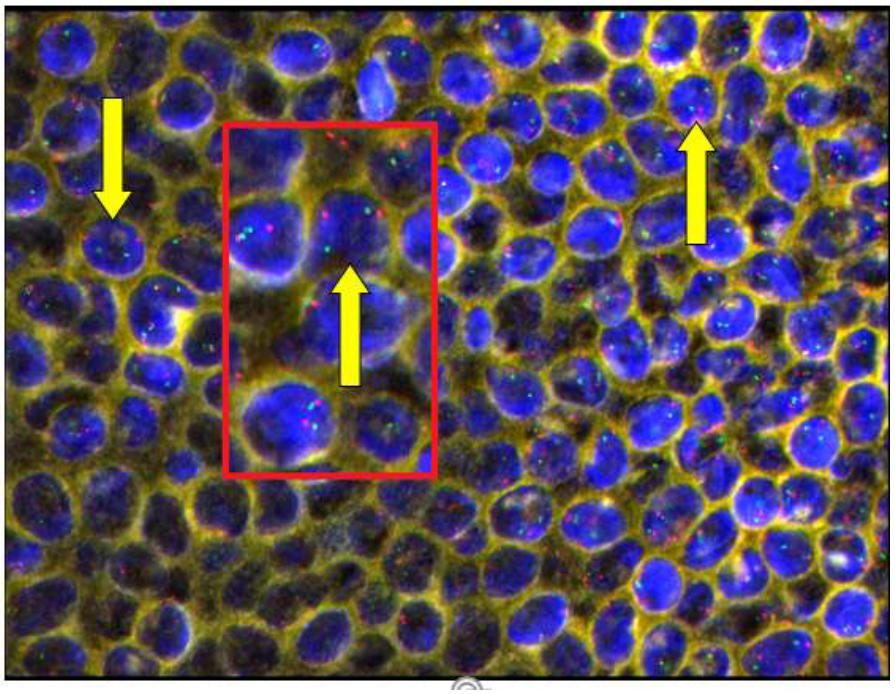

Figure 6. Low grade urothelial carcinoma of the urinary bladder with no amplification in FGFR3 gene. FGFR3 dual color probe hybridize to malignant cells showing no amplification in FGFR3 gene as indicated by two chromosome 4 (Red) signals and two FGFR3 (Green) signals in the nucleoli (Arrows). (64X)

Mean age of FGFR3 gene amplified group was 65.0±5.77 in years, while the mean age of FGFR3 gene non-amplified group was 65.85±5.79 in years. There was no statistically significant difference in mean age between FGFR3 amplified and FGFR3 gene non-amplified groups (P value=0.855).

Two (33.3%) out of the total 6 female patients and 2 (8.3%) out of total 24 male patients had an amplified FGFR3 gene (P value=0.169). FGFR3 gene was shown to be amplified in 2 (16.67%) out of 12 cases of low-grade urothelial carcinoma and in 2 (11.11%) out of total 18 high grade cases (P value=1.00). FGFR3 gene were shown to be amplified in 4 (22.2%) out of 18 cases with lymphovascular permeation, while all 12 non-lymphovascular invasive cases were associated with no amplification in FGFR3 gene. (P value =0.13), (Table 1).

Four (28.57%) case out of 14 muscle invasive (T2) cases, were shown to have amplified FGFR3 gene, while all the 16 lamina propria invasive (T1) cases shown to have no amplification in FGFR3 gene. There is a significant correlation between pathological stage and FGFR3 gene amplification (P value=0.037), (Table 1).

Table 1. Relation between FGFR3 gene amplification to patient's clinico-pathological parameters

Parameter		No. of patients	FGFR3 gene amplified	P value
Gender	Male	24	2	0.169
	Female	6	2	
Grade	High	18	2	1.00
	Low	12	2	
Stage	pT1	16	0	0.037
	pT2	14	4	
LVI*	Absent	12	0	0.13
	Present	18	4	

* LVI: Lympho-vascular invasion

Discussion

FGFR3 is a tyrosine kinase receptor that is encoded by FGFR3 gene, which located in 4p 16.3 region [10]. Its signaling pathway is involved in many cellular physiological processes, including cellular proliferation, differentiation, migration and survival. Alterations in FGFR3 gene will lead to aberrations in this signaling pathway and play an important role in carcinogenesis process, tumor progression and acquired resistance to anti-cancer therapies in many types of human cancers. These alterations could be in form of activating mutation in FGFR3 gene, chromosomal rearrangement or gene amplification [5].

Alterations in FGFR3 gene had been recorded frequently among patients with urothelial carcinoma of the urinary bladder. This may reflect that FGFR3 may have role in the carcinogenesis process of these tumors and suggest that the FGFR3 may be a potential therapeutic target for urothelial tumors [11].

Among these alterations, the activating mutation in FGFR3 gene have been found to be the most common alteration that may affect the FGFR3 gene among patients with urothelial carcinoma and associated mainly with low grade, non-invasive tumors [6]. Mostly, these mutations involve the extracellular and transmembrane domain and lead to constitutive activation and ligand-independent dimerization of this receptor [12].

Additionally, chromosomal rearrangement involving FGFR3 gene, which forms a constitutively activated fusion gene had been reported in about 6% of invasive urothelial tumors [12]. The transforming acid coiled coil 3 (TACC3) gene, which located within 48kb of FGFR3 on 4p16.3 region, had been found to be the most common fusion partner [13].

The present study was conducted to estimate the frequency of FGFR3 gene amplification among patients with urothelial carcinoma of the urinary bladder and to assess its relation to other clinical and pathological parameters.

In this study, FGFR3 gene was shown to be amplified in 13.3% of patients with urothelial carcinoma of the bladder. This result was higher than the result of a German study by Fischbach et al. which reported that FGFR3 gene was found to be amplified in 3.4% of bladder cancer cases [6].

Other study conducted in USA by Helsten et al. [11] reported that FGFR3 gene was amplified in 2% of cases of urothelial carcinoma.

In Egypt, a study by Hammam et al. [14] shows that the amplification of FGFR3 gene was documented in 88.2 % of cases with urothelial carcinoma of the urinary bladder, mostly cases of bilharzias associated carcinoma. This may explain the high percentage of reported FGFR3

gene amplification in this study due to endemicity of bilharzias in this region.

Present study revealed that there is significant correlation between tumor pathological stage and FGFR3 gene amplification that is parallel to the Egyptian study done by Hammam et al. 14 but, disagree with the German study by Fischbach et al. [6]. This correlation could be explained by that FGFR3 gene amplification is a late event in the development of urothelial carcinoma.

The present study also shows no significant correlation between FGFR3 gene amplification and tumor grade which is parallel to the German study by Fischbach et al. [6], which found that FGFR3 gene amplification were almost equally distributed in low- and high-grade tumors.

Furthermore, no significant correlation between FGFR3 gene amplification and lymphovascular invasion was found in the present study.

In conclusions, FGFR3 gene amplification was detected in 13.3% of urothelial carcinoma of the urinary bladder cases which may reflect its role in carcinogenesis process. This amplification was statically correlated to tumor's pathological stage suggesting its relation to tumor progression.

Acknowledgement

The authors would like to thank laboratory staff of Department of Pathology and Forensic Medicine in College of Medicine, Al-Nahrain University.

Author contribution

Al-Marashi: Collection of samples, perform the laboratory procedures, and analyze the result statistically. Dr. Qasim is the supervisor of this study. She contributed by slide revision, FGFR3 gene amplification scoring revision and assesses the results of this study. Both authors contributed in writing the manuscripts.

Conflict of interest

The authors declare no conflict of interest.

Funding

Self-funding.

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Received Feb. 19th 2020
Accepted Aug. 9th 2020

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