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Immunohistochemical Expression of Fibroblast Growth Factor Receptor 3 and Cyclooxygenase-2 in Urinary Bladder Carcinomas with Correlation of Schistosomiasis in Egyptian Patients

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Abstract

BACKGROUND: In Egypt, schistosomal infestation is a leading cause of bladder cancer. A mutation in the fibroblast growth factor receptor 3 (FGFR-3) gene is the most common and most specific genetic abnormality in bladder cancer. Similarly, cyclooxygenase-2 (COX-2) is an inducible, pro-inflammatory enzyme with previous studies showing higher expression in schistosomal-associated bladder cancer.

AIM OF THE STUDY: The aim of the study was to evaluate the immunohistochemical expression of FGFR3 and COX2 in bladder carcinoma and correlates their expression to the associated schistosomal infestation to implicate possible therapeutic treatments.

MATERIALS AND METHODS: This retrospective study included a total of 90 cases of archived, formalin fixed, paraffin-embedded tissue blocks that included variable subtypes and grades of urothelial carcinomas. Immunohistochemistry for expression of FGFR-3 and COX2 was performed using a standard avidin-biotin-peroxidase system.

RESULTS: About 73.3% of the total cases (66 cases) showed variable positive reactivity for FGFR3, of which 33.3% (22 cases) were associated with bilharzia infection. A statistically significant correlation was detected between FGFR-3 and tumor size, grade, histologic subtype, LN status, lymphovascular invasion, and stage. About 83.3% of the total cases (75 cases) showed variable positive immunoreactivity for COX-2, of which 37.3% (28 cases) were bilharzial-associated. A positive correlation was established between COX-2 and grade, concomitant *in situ* changes and cases associated with bilharzia infection.

CONCLUSION: FGFR-3 can be used as a prognostic marker for low-grade urothelial tumors. Results also portray that COX-2 has an inflammatory inciting role in bladder carcinoma development, especially in patients with a history of schistosomiasis (bilharziasis). Both COX-2 and FGFR-3 should be explored further for its use alone or in combination with conventional treatment, to reduce the recurrence rate and progression of superficial (low grade) tumors.

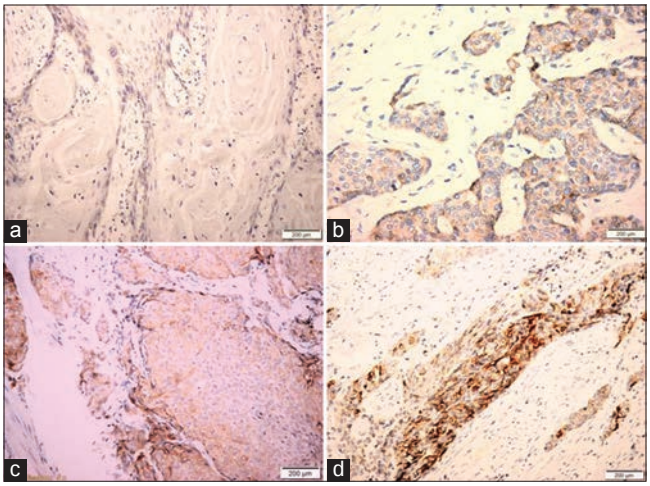
Introduction

Bladder cancer is the sixth most common cancer throughout the world [1]. It is the most common malignancy among Egyptian males. Schistosomal infection is considered a major risk factor for developing squamous cell carcinoma (SCC) [32].

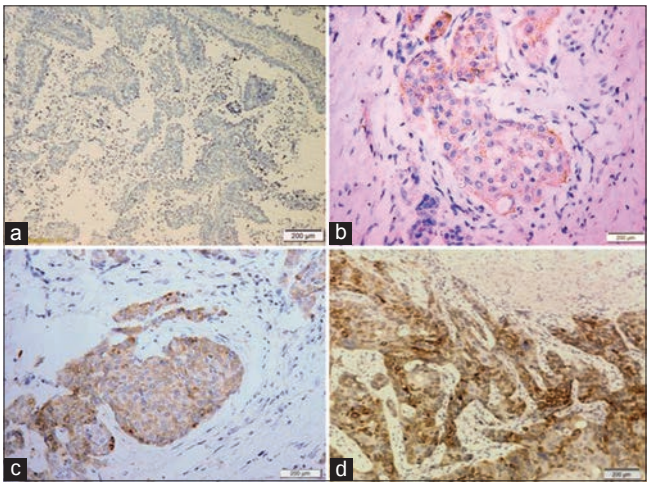
At present, there are no validated prognostic molecular biomarkers such as T-cell receptor clonality and tissue or blood-based immune-gene profiling to guide the clinical management of urinary bladder cancer. Crucial therapeutic decisions are based on risk tables that include tumor size and number, previous history, in addition to histopathological criteria which are often limited by observer variability and have relatively low reproducibility [2]. Factors that include choice of antibody, fixation technique, and selection of tumor areas to measure staining methods, signal detection methods, criteria for positive staining, data interpretation guidelines, and stratification criteria are all major challenges for the clinical application of tissue-based biomarkers in urothelial bladder management [27].

Overall, urothelial cancer (UC) management would greatly benefit from cost-effective methods for screening and surveillance to predict the risks of recurrence and progression so that more targeted therapeutic regimes and intensive monitoring could be focused on patients at higher risk [2], [16], [17].

Fibroblast growth factor receptor 3 (FGFR3) has been revealed to possess an oncogenic role in numerous types of cancer, especially bladder [3]. Patients with FGFR3 alterations tend to have a low likelihood of response to chemotherapy or immune checkpoint [27]. FGFR3 mutations are found in a large proportion of low grade, low stage bladder cancers, the development of a robust test detecting these mutations would be the most obvious step. It would be a useful molecular marker in bladder cancer diagnosis and prognosis and could – in theory – provide an excellent urine marker for follow-up of patients with low grade and low stage bladder cancer [4], [5]. It could also guide the management lines in treatment-resistant



AQ6 *Figure 1: Variable staining degrees of expressing fibroblast growth factor receptor 3. (a) Negative staining of low-grade squamous cell carcinoma (×200), (b) weak cytoplasmic and membranous staining of squamous cell carcinoma (×200), (c) moderate cytoplasmic staining of papillary transitional cell carcinoma (TCC) (×200), (d) strong membranous and cytoplasmic staining of invasive TCC (×400)*



AQ6 *Figure 2: Variable staining degrees of expressing cyclooxygenase-2. (a) Negative staining of papillary transitional cell carcinoma (TCC) (×200), (b) weak cytoplasmic staining of low-grade squamous cell carcinoma (×200), (c) moderate membranous and cytoplasmic staining of papillary TCC (×200), (d) strong membranous and cytoplasmic staining of squamous cell carcinoma (×200)*

patients to shift into FGFR inhibitors, e.g., erdafitinib and BGJ398 [28].

Cyclooxygenase-2 (COX-2) is an induced inflammatory mediator involved in the development of tumors responsible for the conversion of arachidonic acid to prostaglandins. Overexpression of COX-2 may play a role in carcinogenesis through cellular proliferation, angiogenesis, apoptosis, invasion/motility, and immune responses [18]. It is not detectable in most normal tissues; however, it is induced at sites of inflammation by cytokines, growth factors, and tumor promoters that result in upregulation of PD-L1, which increase the myeloid-derived suppressor cells in peripheral blood and eliminating CD8 T cells from urinary bladder cancer [27].

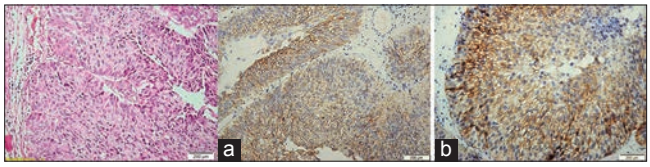


Figure 3: Non-invasive papillary transitional cell carcinoma, low-grade, (×200) original magnification, hematoxylin and eosin stain. (a) Strong membranous fibroblast growth factor receptor 3 immunostaining, (b) strong membranous cyclooxygenase-2 immunostaining

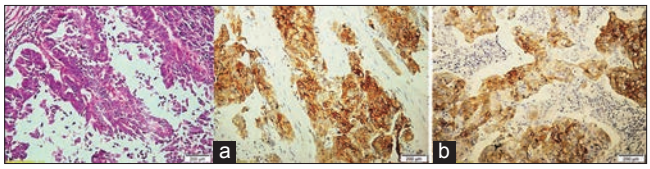


Figure 4: Invasive papillary transitional cell carcinoma, high grade, (×200) original magnification, hematoxylin and eosin stain. (a) Strong membranous and cytoplasmic fibroblast growth factor receptor 3 immunostaining, (b) Strong membranous and cytoplasmic cyclooxygenase-2 immunostaining

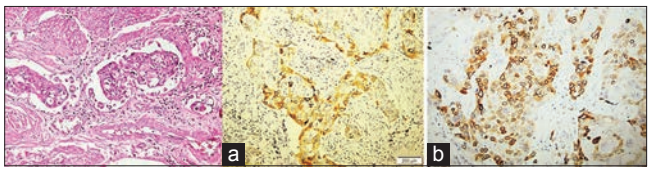


Figure 5: Squamous cell carcinoma, high grade, with evident lymphovascular emboli (×200) original magnification, hematoxylin and eosin stain. (a) Strong membranous and cytoplasmic fibroblast growth factor receptor 3 immunostaining, (b) strong membranous and cytoplasmic cyclooxygenase-2 immunostaining

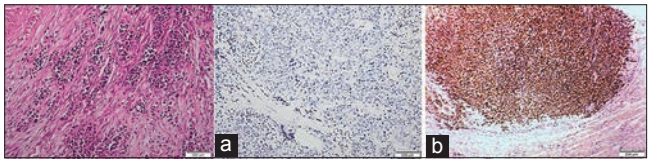
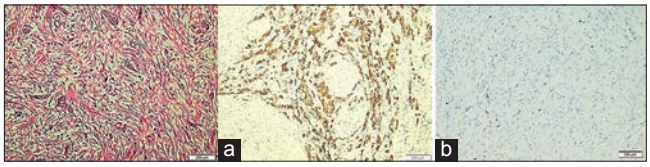


Figure 6: Small cell carcinoma, high grade, (×200 original magnification), hematoxylin and eosin stain. (a) Negative fibroblast growth factor receptor 3 immunostaining, (b) strong membranous and cytoplasmic cyclooxygenase-2 immunostaining

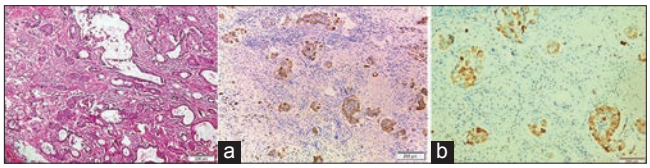
Schistosomiasis has been linked to induce urinary bladder cancer by increasing the proliferation of urothelium and decreasing apoptosis. The concurrent inflammation and presence of possibly mutagenic parasite molecules could explain the observation of increased chromosomal damage. In addition, there is an increase cell proliferation in endothelial cells, resulting in angiogenesis with egg granuloma formation. The resulting keratinous squamous metaplasia has been associated with the increased risk of developing squamous cell carcinoma, with approximately one-half of the cases arising subsequent to the metaplasia [29].

This study aimed at the assessment of immunohistochemical expression of FGFR3 and COX-2 in urinary bladder carcinoma in Egyptian patients, to investigate their potential role in urothelial carcinogenesis. Further assessment is targeted to



AQ6 **Figure 7**: *Sarcomatoid carcinoma with diffuse spindling, high grade, (×200 original magnification), hematoxylin and eosin stain. (a) Strong membranous and cytoplasmic fibroblast growth factor receptor 3 immunostaining, (b) Negative cyclooxygenase-2 immunostaining*

the association of their expression to the presence of bilharzia infection of these cases with the possible role of both FGFR3 and COX-2 as possible usage of therapies targeting such receptors.



AQ6 **Figure 8:** *Transitional cell carcinoma with focal adenoid differentiation, high grade, (×200) original magnification, hematoxylin and eosin stain. (a) Strong membranous and cytoplasmic fibroblast growth factor receptor 3 immunostaining, (b) Strong membranous and cytoplasmic cyclooxygenase-2 immunostaining*

Materials and Methods

This retrospective study was carried out on 90 cases of randomly chosen formalin-fixed paraffin-embedded tissue blocks of patients diagnosed with bladder carcinoma with or without coexistent schistosomal infestation from the archives of our pathology department from the period of September 2017 to July 2018. Patients were operated by radical cystectomy or transurethral resection biopsy. Clinicopathological data of all cases were recorded from the patient records and tabulated. Ethical clearance was obtained by the institutional review board prior to the study.

Each paraffin block was by cut by rotator microtome at 5 μ thickness then mounted on glass slides to be stained by hematoxylin and eosin (H&E) for histopathological re-evaluation by two pathologists and on charged glass slides for immunostaining process. The slides deparaffinized and hydrated in xylene. Then, they were treated for antigen retrieval (using a microwave oven for 30 min) an automated Omnis DAKO immunostainer at a high pH of 8.

Afterthis,thesectionswereretreatedwithantibodies using avidin-biotin-peroxidase 3% (Thermo Scientific) for 30 min. Diaminobenzidine tetrahydrochloride was used as a substrate and chromogen. Hematoxylin (Biogenex) was used as a counterstain. The primary antibody was a mouse monoclonal antibody of anti-FGFR3 which was manufactured by Santa Cruz Biotechnology, Inc. (Dallas, TX, clone B-9, SC-13121) and used at a dilution of 1:50.

Tumor tissue sections were examined and scored under Leica DM500 microscope at low power than high power magnification by two independent pathologists who were not informed of the histological diagnosis.

Assessment of FGFR3 expression in the tumor cells was designated as brownish cytoplasmic and/or membranous staining in more than 10% of tumor cells (threshold point of positivity). A minimum of 500 cells was counted. A case of normal skin was also sectioned and underwent the same procedure for each run performed to serve as a positive control case. FGFR3 positivity in the background stroma and in the muscle fibers served as a positive internal control.

The same steps were performed for COX-2 staining but antigen retrieval was done at a lower pH (pH 6) and a case of the normal colon was also sectioned and underwent the same procedure for each run to serve as a positive control. The primary antibody was a mouse monoclonal antibody against COX2 which was manufactured by Santa Cruz Biotechnology, Inc. (Dallas, TX, clone H-3, (sc-376861) and used at a dilution of 1:100. Assessment of COX-2 expression in the tumor cells was designated as brownish cytoplasmic and/or membranous staining in more than 10% of tumor cells (threshold point of positivity). A minimum of 500 cells was counted. COX-2 positivity in the background stroma, endothelial cells, and muscle fibers served as a positive internal control.

The data were tabulated and Chi-square test was used to examine the relation between qualitative variables. $p < 0.05$ was considered significant.

All slides were screened on Leica DM500 microscope and all photos were imaged by HD digital microscope camera, named Leica ICC50 HD, connected to the same microscope.

Results

This study included 90 cases of urinary bladder cancer, in which the ages of the patients ranged from 41 to 79 years with a mean age of 64 years. As regards to the gender, 73 of the 90 cases were found to be males (representing 81.1% of the study) and the remaining were females (17 cases; 18.9%). FGFR3 expression was established in 66 cases (73.3% of the total cases). As for COX2, it was expressed in 75 cases (83.3% of total cases). Only one-third of cases (35.5%) showed schistosomal affection.

The majority of the cases were found to be of high grade (54 cases; 60%) and 36 cases were of low grade (representing 40% of the study). A large percentage of the low-grade tumors showed positive expression for FGFR3 ($p = 0.028$; 69.4% of the group). A similar pattern was also noted among the low-grade

tumors showing a positive COX2 expression (p = 0.020; 83.3% of the group). Further findings also showed a positive statistical correlation of positive nodal metastasis (p = 0.0006) and positive lymphovascular invasion (LVI) (p = 0.0028) in relation to FGFR3; however, these results were only seen in regards to LVI to COX2 expression.

As regards to tumor size in this study, FGFR3 demonstrated a positive correlation (p = 0.028) unlike COX2 which did not. The bladder carcinoma cases with adjacent carcinoma *in situ* (CIS) were seen in 34.4% of this study group and showed a strong relation with COX2 (p = 0.034) but showed an insignificant finding in correlation to FGFR3 (p = 0.068).

The relationship between FGFR3 and COX2 expression and the clinicopathological variables are shown in Tables 1 and 2, respectively. The main histological subtypes included 31 cases of the conventional urothelial cell carcinoma constituting (34.4%), 22 cases of squamous cell carcinoma (25.6%), and urothelial cell carcinoma with squamous cell differentiation (13.3%). The remaining cases included micropapillary (4 cases), small cell (4 cases), sarcomatoid (4 cases), and urothelial cell carcinoma

Table 1: Clinicopathological characteristics of the studied cases of bladder carcinoma and its correlation with FGFR3 expression

Parameters	n (%)		Positive FGFR3 expression		Negative FGFR3 expression		p value
	(n=90, 100%)	%	(n=66, 73.3%)	%	(n=24, 26.7%)	%	
Age							
≤60 years	43	47.77	30	45.45	13	54.2	0.195
>60 years	47	52.22	36	54.54	11	45.8	
Gender							
Male	73	81.11	56	84.88	17	70.83	0.191
Female	17	18.88	10	15.12	7	29.17	
Tumor size							
<3 cm	30	33.33	23	34.88	7	29.17	0.028
3–5 cm	31	34.44	25	37.89	6	25	
>5 cm	29	32.22	18	27.23	11	45.83	
Grade							
Low grade	36	40	25	37.89	11	45.83	0.028
High grade	54	60	41	62.11	13	54.17	
Presence of nodal metastasis							
Positive	25	27.77	20	30.33	5	20.83	0.0006
Negative	65	72.22	46	69.67	19	79.17	
Muscle invasion							
Present	79	87.78	57	86.34	22	91.67	0.064
Absent	11	12.22	9	13.66	2	8.33	
Lymphovascular invasion							
Present	54	60	25	37.89	11	45.83	0.0028
Absent	36	40	41	62.11	13	54.17	
Perineural invasion							
Present	46	51.11	24	36.34	12	50	0.088
Absent	44	48.89	42	63.66	12	50	
Carcinoma *in situ*							
Present	31	34.44	43	65.12	16	66.67	0.068
Absent	59	65.56	23	34.88	8	33.33	
Coexisting bilharzial infestation							
Present	32	35.56	22	33.33	10	41.67	0.015
Absent	58	64.44	44	66.67	14	58.33	

FGFR3: Fibroblast growth factor receptor 3.

Table 2: Clinicopathological characteristics of the studied cases of bladder carcinoma and its correlation with COX-2 expression

Parameters	n (%)		Positive COX2 expression		Negative COX2 expression		p value
	(n=90, 100%)	%	(n=75, 83.3%)	%	(n=15, 16.7%)	%	
Age							
≤60 years	43	47.77	34	45.33	9	60	0.041
>60 years	47	52.22	41	54.66	6	40	
Gender							
Male	73	81.11	63	84	10	66.67	0.187
Female	17	18.88	12	16	5	33.33	
Tumor size							
<3 cm	30	33.33	26	34.67	4	26.66	0.069
3–5 cm	31	34.44	24	32	7	46.66	
>5 cm	29	32.22	25	33.33	4	26.66	
Grade							
Low grade	36	40	30	40	6	40	0.020
High grade	54	60	45	60	9	60	
Presence of nodal metastasis							
Positive	25	27.78	19	25.33	6	40	0.074
Negative	65	72.22	52	69.33	13	86.66	
Muscle invasion							
Present	79	87.77	55	73.33	14	93.33	0.106
Absent	11	12.22	10	13.33	1	6.66	
Lymphovascular invasion							
Present	36	40	29	38.66	7	46.66	0.018
Absent	54	60	46	61.33	8	53.33	
Perineural invasion							
Present	44	48.88	36	48	8	53.33	0.12
Absent	46	51.11	39	52	7	46.67	
Carcinoma *in situ*							
Present	31	34.44	25	33.33	6	40	0.034
Absent	59	65.55	50	66.66	9	60	
Coexisting bilharzial infestation							
Present	32	35.55	28	37.33	4	26.67	0.00
Absent	58	64.44	47	62.66	11	73.33	7

COX-2: Cyclooxygenase-2.

with focal adenoid differentiation (2 cases). There is no significant correlation between expression of FGFR-3 and COX2 in bladder cancer and histological subtypes in this study group as seen in Table 3.

This study also focused on the schistosomal (bilharzial) association in Egyptian patients. One-third of the cases (35.5%) showed concomitant affection of this helminth. Table 4 illustrates the further analysis performed on the common significant parameters seen in both FGFR3 and COX2 positive cases associated with schistosomiasis. COX2-associated bilharzial cases demonstrated a positive correlation to CIS (p = 0.009). Both FGFR3 and COX2 both showed a positive correlation (p = 0.025) and (p = 0.028), respectively, in regards to low-grade tumors with bilharzial affection in this study. On the other hand, schistosomal affected cases did not display significant findings in relation to the high-grade tumors that showed FGFR3 and COX2 expression. Likewise, no significant findings were established in regards to size or LVI.

Discussion

Contrary to the leading etiology of smoking and occupational exposures in Western countries, chronic bladder infection with *Schistosoma haematobium* has been the most important risk factor for bladder cancer in Egypt [8].

The purpose of this study is to determine any correlation between primary bladder cancers and FGFR-3 and COX-2 protein expression. Furthermore, if there is a possible connection between bilharzial-association in these tumors and these two markers as the assessment of combined biomarkers instead of a single one will potentially provide a more accurate reflection of the underlying biological complexity of the antitumor immune response. This present study included 90 cases of urothelial bladder carcinomas in Egyptian patients. The ages of the patients ranged from 41 to 79 with a mean age of 64. These results were close to the results recorded by Arsalan *et al*. [7] which displayed a mean age of 62.1 years with 89.1% of the cases diagnosed at more than 40 years of age. Regarding the gender of the cases in this study, the majority were males (81%), similar to Gust *et al*. [9], in which 77% were males.

As for immunostaining for FGFR-3 expression, the current study showed that only 24 cases showed negative staining and the remaining 66 cases (73.3% of total cases) showed positive cytoplasmic and/or membranous. Hammam *et al*. [11] also found that 72% of malignant cases in their study were also positive for FGFR3 immunostaining, which is compatible with another study [10] that stated that expression of FGFR3 was found in approximately 70% of both low and high-grade tumors, as well as equally distributed between invasive and non-invasive urothelial carcinoma. Evaluation of the results of immunostaining

Table 3: Histological subtypes and staging of the studied cases and correlation with FGFR3 and COX2 expression

Parameters	n (%)		Positive FGFR3 expression		Negative FGFR3 expression		p value	Positive COX2 expression		Negative COX2 expression		p value
	(n=90, 100%)	%	(n=66, 73.3%)	%	(n=24, 26.7)	%		(n=75, 83.3%)	%	(n=15, 16.7%)	%	
Histological subtype												
Urothelial carcinoma	31	34.44	22	33.33	9	37.5	0.121	24	32	7	46.66	0.144
Urothelial w/squamous cell differentiation	12	13.33	10	15.15	2	8.33		11	14.66	1	6.66	
Squamous cell carcinoma	23	25.55	17	25.75	6	25		17	22.66	6	40	
Micropapillary	4	4.444	4	6.06	0	0		4	5.33	0	0	
Small cell	4	4.444	2	3.03	2	8.33		2	2.66	2	13.33	
Sarcomatoid	4	4.444	0	0	4	16.66		0	0	4	26.66	
Non-invasive TCC*	10	11.11	10	15.15	0	0		10	13.33	0	0	
TCC w/ focal adenoid differentiation	2	2.22	1	1.515	1	4.16		1	1.33	1	6.66	
Stage												
Ta/T1	19	21.11	16	24.24	3	12.5	0.031	17	22.66	2	13.33	0.1
T2a	7	7.77	6	9.09	1	4.16		4	5.33	3	20	09
T2b	7	7.77	5	7.57	2	8.33		5	6.67	2	13.33	
T3a	14	15.55	8	12.12	6	25		10	1.33	4	26.67	
T3b	30	33.33	21	31.81	9	37.5		20	26.67	10	66.67	
T4a	12	13.33	10	15.15	2	8.33		4	5.33	3	20	
T4b	1	1.11	0	0	1	4.166		1	1.33	0	0	

*TCC: Transitional cell carcinoma, FGFR3: Fibroblast growth factor receptor 3, COX2: Cyclooxygenase-2.

Table 4: Schistosomal-associated cases with positive FGFR3 and COX2 expression and their correlation to similar tumor parameters

Schistosomal-associated cases	n (%)		Positive FGR3 expression		p value	Positive COX2 expression		p value
	(n=32, 35.5%)	%	(n=22, 33.3%)	%	0.015	(n=28, 37.3%)	%	0.007
Tumor size								
<3 cm	10	31.25	8	36.3		10	35.7	0.154
3–5 cm	14	43.75	7	31.8	0.075	12	42.8	
>5 cm	8	25	7	31.8		6	21.4	
Grade								
Low grade	19	59.3	14	63.3	0.025	17	60.7	0.028
High grade	13	40.6	8	36.3	0.109	11	39.3	0.097
Lymphovascular invasion								
Present	17	53.1	10	45.4	0.173	15	53.5	0.088
Absent	15	46.8	12	54.5		13	46.4	
Carcinoma *in situ*								
Present	14	43.7	9	40.9	0.094	13	46.4	0.009
Absent	18	56.2	13	59.1		15	53.6	

FGFR3: Fibroblast growth factor receptor 3, COX2: Cyclooxygenase-2.

for COX-2 showed that only 15 cases were negative with the remaining 75 cases (83.3% of total cases) showed positive cytoplasmic and/or membranous reactivity. Similar findings were reported in another study, in which 71.6% of the cases showed COX2 immunoreactivity [30].

When analyzing the tumor grade in this study, close results were similarly reported by Maeng *et al*. [13], in which the majority (81.3%) of the lower-grade tumors (PUNLMP and low-grade UC) demonstrated cytoplasmic positivity for FGFR-3, whereas only 47.8% of high-grade urothelial carcinomas did p = 0.022. In a study by Tomlinson *et al*. [14], FGFR3 was higher in non-invasive (pTa) compared to invasive (pT2) tumors and was associated with low grade compared with high grade. Both COX-2 and FGFR-3 showed positive correlations to tumor grade (p = 0.020, p = 0.028), respectively. Further, multiple-response analytical statistics were performed, in which low-grade tumors that were positive to COX-2 and FGFR-3 showed a positive correlation (p = 0.028). This strongly suggests that both COX-2 and FGFR-3 show over-expression more commonly in lower grade urothelial tumors.

The tumor size in this study showed a positive correlation for FGR-3 staining (p = 0.028.) This may be attributed to many studies that postulate the link between FGFR-3 expression in lower grade tumors, with a smaller size and lower stage [15]. On the other hand, this study displayed no linear correlation between COX-2 expression and tumor size. Thus, these interesting results similarly supported by Hammam *et al*. [11], who argued that COX-2 may have more of a tumorigenic role rather than a prognostic impact.

From a histologic standpoint, invasive urothelial carcinoma was the predominant subtype in this study and accounted for 31% of all cases. There was no significant correlation seen regarding FGFR3 (p = 0.121), similar to Tomlinson *et al*. [12], who also showed no significant association between FGFR3 mutation status and histologic subtype (p = 0.106). Youssef *et al*. [18] showed a positive correlation of histologic type and COX-2 (p ≤ 0.005). This discrepancy may be due to a large amount of schistosomal cases (n = 205) in comparison to non-schistosomal cases (n = 110) in their study which is associated more commonly with squamous cell histologic variant.

In this study, concomitant *in situ* changes were evident in only 31 cases (34.3%). Showing a smaller percentage, Youssef *et al*. [18] demonstrated that only 29 cases (9.2%) showed associated carcinoma *in situ*. This may be due to a larger percentage of non-papillary late-stage tumors in their study. Similarly, in our study, an insignificant correlation was seen between FGFR-3 and concomitant *in situ* changes; however, a positive statistic finding was seen when correlating COX-2 (p = 0.034). Jacobs *et al*. [20] additionally reported that 93% of CIS showed COX-2 expression. Moreover, another study [19] reported that 86% of invasive transitional cell carcinomas, 78% of non-invasive transitional cell carcinomas, and 75% of CIS were COX-2 positive. In addition, in 53% of cases, morphologically normal epithelium adjacent to the cancer lesion was COX-2 positive. They reported that this indicates that morphologically normal epithelial cells may change into tumor cells due to a paracrine effect caused by increased cytokines and/or growth factors [19], [20].

Regarding metastatic tumor deposits to regional lymph nodes, a study performed in Egypt on 315 cases of bladder cancer, showed a positive nodal metastasis of 30% and positive LVI emboli of 30.5%. However, in our study, a higher percentage (60%) of lymphatic invasion was seen [18]. Previous studies have reported that the rate of LVI ranges from 35% to 55% in muscle-invasive bladder carcinoma. Regarding COX-2, no statistical relationship was developed in this study between it and lymph node status nor LVI (p = 0.074, p = 0.018), respectively. On similar terms, a large study performed on 773 bladder cancer patients also showed no statistical relationship to COX-2 regarding lymph node status or invasion (p = 0.296, p = 0.869), respectively. Yamada *et al*. [23] observed positive findings between primary COX-2-positive patients and metastatic COX-2-positive patients (p = 0.03). This finding is significant since it suggests that metastatic lymph nodes are likely to be COX-2 positive when the primary tumor is COX-2 positive.

A significant correlation was also detected between FGFR3 expression and nodal status, in which FGFR3 expression was seen in 80% of the cases with positive regional lymph node metastasis (p ≤ 0.0006). In relation to LVI, another strong correlation was also established in this study (p = 0.0028). On the other hand, different findings were found in the study led by Baldia *et al*. [21], in which no significant finding was seen (p = 0.087). This may be due to the fact that the cohort study group was only squamous cell carcinoma patients and did not include other histologic variants.

A positive correlation was analyzed between tumor stage and FGFR3 (p = 0.031). Similar to our findings, Poyet *et al*. [15] showed a significant association between tumor stage and grade and FGFR3 immunoreactivity (both p < 0.001). Prognostic research involving the grade of bladder carcinomas explained that the overall survival was significantly better in patients with low-grade tumors compared to high-grade tumors (p = 0.011) and that patients with stage pTa had a significantly better overall survival than patients with invasive tumors [31].

The diagnosis of schistosomal bladder cancer was determined based on histological proof of bilharziasis (calcified eggs of *S. haematobium*) in the studied cases. Of the ninety cases, 32 cases (35.6%) showed bilharzial ova. Statistical analysis showed a positive correlation for both COX-2 and FGFR-3 (p = 0.007, p = 0.015), respectively. Another study

El-Sheikh *et al*. [26] showed COX-2 overexpression was associated with pathological T stage (p = 0.01), grade p < 0.001), LVI (p = 0.041), and bilharziasis p = 0.045). Similar results supported that COX-2 expression was significantly higher in schistosomal-associated TCC than in non-schistosomal-associated TCC (p < 0.01).

In relation to FGFR3, Hammam *et al*. [11] showed that 54.5% of their study cases showed FGFR3 immunostaining and demonstrated a positive correlation of bilharzial bladder cancers and FGFR-3 (p ≤ 0.001). Further Chi-square analytical studies were performed to show if a relation between COX2 and FGFR3 exists between the schistosomal-associated cases, in which positive correlation was established between the bilharzial-associated low-grade COX2 and FGFR3 cases (p = 0.025, p = 0.028), respectively.

Our second interpretation of the results goes in hand in hand with the fact that normal urothelial cells predominantly express high levels of cyclooxygenase (COX)-1, while bladder cancer cells show COX-2 overexpression [26]. COX2 expression, according to these results, is higher, especially when associated with inflammatory conditions such as schistosomiasis which is predominant in Egypt. According to Kluth *et al*. [25], combining celecoxib with BCG for schistosomal-associated cases was found to increase tumor infiltration of CD4+ T cells with a significant reduction of tumor burden. Thus, similar to the results of Yamada *et al*. [23], patients may benefit from treatment with selective COX-2 inhibitors in addition to the usual BCG treatment administered to the schistosomal cases in Egypt to limit progression of the tumor.

Conclusion

FGFR3 has been revealed to possess an oncogenic role in bladder cancer and mutations are found in up to 80% of primary low-grade tumors [24]. Our results have shown that FGFR3 expression is positive with low grade and low stage tumors and is statistically supported. Detection of FGFR3 mutations in urine should be employed for general population screening aimed at the early detection of primary tumors [22].

The results of this study established a positive correlation of FGFR3 in relation to tumor size, stage, grade, nodal status, and LVI. As for COX2, we have proven the significant findings in relation to grade, LVI and CIS. Schistosomiasis showed a clear cut correlation to both FGFR3 and COX2 in this study with a strong association to low-grade tumors and CIS areas.

Our recommendation is the consideration of FGFR-3 and selective COX-2 inhibitors for their use alone or in combination with conventional treatment such as intravesical BCG in cases associated with schistosomal infection.

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