Research Article

Immunohistochemical Expression of HER-2 and COX-2 in Urothelial Carcinoma of the Urinary Bladder

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Abstract
Background: Urothelial carcinoma is the commonest type of urinary bladder tumours. HER-2 is known to contribute to physiological mechanisms of cell proliferation by tyrosine-kinase-activity. Overexpression of HER-2 is shown to influence malignant cell proliferation, and metastasis in a variety of tumours. COX-2 a critical enzyme in the conversion of arachidonic acid to prostaglandins influences human tumours, being involved in carcinogenesis, and differentiation. **Methods:** Immunohistochemical staining for HER-2, and COX-2 on 100 cases of urothelial carcinoma divided into; grade1 (n=14), grade 2 (n=42), and grade 3 (n=44). We evaluated HER-2, and COX-2 immunoexpression, the relationship between both markers, and with clinicopathological data. **Results:** Positive HER-2 expression was detected in 31% of cases studied. A significant association was observed between HER-2 expression and tumour grade, stage, and depth of muscle invasion (*p* value= 0.007, 0.005, and 0.002 respectively). COX-2 expression was found to be positive in 71% of cases studied. A significant association was observed between COX-2 expression and tumour grade, stage, and depth of muscle invasion (*p* value= 0.007, 0.028, and 0.002 respectively). No significant association was found between each marker and patient's age, gender, lymph node metastasis, or bilharziasis. A significant positive correlation between HER-2 and COX-2 expression (r=0.197, and *p*=0.050) was noted. **Conclusion:** These results suggest that HER-2 and COX-2 could be valuable target molecules in the evaluation of patients with urothelial carcinoma, and alternative or complementary to modalities in the treatment of urothelial bladder carcinoma.

Key Words: HER-2- COX-2- Urothelial Bladder Carcinoma- Immunohistochemistry

Introduction
Carcinoma of the urinary bladder is a world-wide health problem, and it ranks 9th in cancer incidence rates. In 2010, bladder cancer was responsible for about 170,000 deaths world-wide[1-3]. In developed countries, 90% of urinary bladder cancers are urothelial carcinoma, with 4:1 male to female ratio, and median age at diagnosis is 68 years[4].

In Egypt, urinary bladder cancer represents about 12.7% of total malignant tumours. It ranks as the 2nd between males after liver cancer, with 4:1 male to female ratio, and the median age at presentation is 60.5 years. Urothelial carcinoma of the bladder (UCB) represents about 73% of all bladder tumours[5-7].

Smoking, exposure to arsenic in drinking water, and occupational exposure to aromatic amines are well known risk factors for bladder cancer[8]. In Egypt, chronic bladder infection with urinary schistosomiasis has been the most important risk factor for bladder cancer[9]. The decline in the relative frequency of bladder cancer is associated with a decline in schistosomal egg positivity and is probably related to better control of schistosomiasis. This was accompanied by a change in the histological profile of tumours, with significant predominance of UCB and an increase in the age of patients, a pattern rather similar to that in western reports[6].

HER-2 is a proto-oncogene located at the long arm of chromosome 17q21, encoding a protein with a molecular weight of 185 kDa. It belongs to the epidermal growth factor receptor (EGFR) family along with 3 other receptors: epidermal growth factor receptor; HER1, HER3, and HER4. It encodes for a tyrosine kinase transmembrane growth factor receptor. HER-2

contributes to physiological mechanisms of cell proliferation and differentiation by intrinsic tyrosine-kinase activity[10].

HER-2 signaling promotes cell proliferation through MAPK pathway and inhibits cell death through the phosphatidylinositol 3'-kinase-AKT-mammalian target of rapamycin (mTOR) pathway[11]. In malignant tumours, HER-2 overexpression is the direct result of gene amplification. It is strongly associated with increased disease recurrence and a poor prognosis. HER-2 is overexpressed in around 20–30% of breast cancers[10, 12].

HER-2 overexpression was first described in bladder carcinoma by[13]. HER-2 overexpression in UCB ranges from 8.5% to 81%. This variability could be attributed to tumour heterogeneity, tissue sampling, and methods used to detect it. Indeed, heterogeneous HER-2 expression within tumours and differences in expression between primary tumours and metastases was also reported[14, 15].

It was suggested that 12.5% of patients with invasive bladder carcinoma may benefit from HER-2 targeting drug therapy. Moreover, it was suggested that patients suitable for this therapy should be identified by cost-effective IHC first, and then by performing gene amplification analysis using FISH in IHC 3+ patients[17, 18].

Studies have obtained in vitro evidence that prostaglandin E2 (PGE2) participates in carcinogenesis and angiogenesis[19,20]. The biosynthesis of PGE2 requires three sequential enzymatic reactions: the release of arachidonic acid from membrane glycerophospholipids by phospholipase A2 (PLA2), the conversion of arachidonic acid to the unstable intermediate PGH2 by cyclooxygenase 1 (COX-1) or cyclooxygenase 2 (COX-2), and the isomerization of PGH2 to PGE2 by PGE2 synthase (PGES). COX-2 and PGES collaboratively mediate the induction of matrix metalloproteinase-9 (MMP-9), which plays a crucial role in cancer invasion by basement membrane degradation[21].

COX-2 is not detectable in most normal tissues; however, it is induced at sites of inflammation by cytokines, growth factors and tumour promoters[22]. COX-2 activation mediates cellular processes also implicated in carcinogenesis such as angiogenesis, cell survival, proliferation and apoptosis[23].

Growing evidence indicates that chronic inflammation may increase the risk of UCB. At high concentrations, NSAIDs have anticarcinogenic properties operating through COX-2 dependent pathway. The results of a meta-analysis of 17 observational studies indicated that use of acetaminophen, aspirin or non-aspirin NSAIDs was not associated with bladder cancer risk. However, non-aspirin NSAIDs use might be associated with a reduction in risk of bladder cancer for nonsmokers[24].

It was reported by [25] that HER-2 regulated the expression of COX-2 in a study using a colorectal cancer cell line. In colorectal cancer, activation of HER-2 induces activation of COX-2 promoters. Up-regulation of COX-2 in Estrogen receptor (ER) negative and HER-2 positive breast tumours is a marker of poor outcome. A combination of COX-2 and HER-2 inhibitors may be particularly effective in patients with ER-negative/HER2-positive breast cancer[26]. Regarding the correlation between HER-2 and COX-2 expression in UCB, some reported no statistically significant relation between both[18], while others found positive significant correlation[27].

Material and Methods

Cases selection

The present study included one hundred cases of UCB, which were chosen from the archive of the Department of Pathology, Minia University Hospital in the period from 2010 to 2014. Tumour tissues were obtained from 70 patients who had undergone radical cystectomy and 30 patients had done transurethral resection (TUR).

Clinicopathological data

Clinicopathological data were obtained from pathology reports of the selected cases. These data included patients' age, gender, tumour grade, and tumour stage. Lymph node metastasis was evaluated in radical cystectomy cases. The data is summarized in table 1.

Tumour classification was performed according to WHO classification of urothelial tumours of

the urinary tract**.** Grading was done according to WHO grading system into grade 1, 2, and 3[(28)]**.** Staging was done according to AJCC TNM staging system of the urinary bladder cancer[(29)]**.**

Tissue specimens from all cases were fixed in 10% neutral buffered formalin, processed, and embedded in paraffin wax. Five µm serial sections were prepared and stained with haematoxylin and eosin stain to revise the histological findings of all cases regarding tumour typing, grading, and staging.

Immunohistochemistry for HER-2 and COX-2

Five µm sections were prepared for immune-histochemistry for HER-2 and COX-2 primary antibodies, utilising the avidin biotin-peroxidase complex with diaminobenzidine (DAB) chromagen detection system. Slides were stained with *c-erbB-2/HER-2 antibody* (Monoclonal mouse antibody clone e2-4001 + 3B5, 7ml Ready to use, Lab Vision Laboratories, incubated overnight), and *COX-2 antibody* (Polyclonal rabbit antibody, 7ml Ready to use, Lab Vision Laboratories, incubated overnight) using standard techniques. Regarding HER-2, the positive control was known HER-2 (+3) positive ductal carcinoma of the breast. Sections of colon adenocarcinoma were used as positive control for COX-2 expression.

Scoring of immunostaining

To assess positive staining for HER-2, the entire tissue section was screened for positive tumour cells, defined as cells with membranous staining. In case of COX-2, tissue section was screened for positive tumour cells, defined as cells with cytoplasmic staining. Screening of sections was done under light microscope magnification X200.

- HER-2 expression

HER-2 immunostaining was evaluated for each case, with each slide's percentage of cells staining as follows: 0 = Absence of the reaction or <10% membrane stain of the cells, +1 = Weak membrane stain /incomplete for >10% of the cells, +2 = Membrane stain completed, weak /moderate for >10% of the cells, and +3 = Intense membrane stain completed for >30% of the cells. Cytoplasmic staining was considered as a non-specific pattern [(30)].

- COX-2 expression

For COX-2, cytoplasmic staining was considered positive. The frequency of staining was scored by proportioning the total area with carcinoma with positively stained area: (%0)=0, (1- 25%)=1, (26-50%)=2, (51-75%)=3, (76-100%)=4. The intensity of staining was classified as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). During the statistical evaluation, we first evaluated staining intensities and percentages separately, and then reevaluated them after determining the final staining score. The final staining score (0-7) was obtained by combining staining intensity and frequency. Further, tumours having a final staining score $\geq$ 4 were considered to be positive and tumours with a score of $\leq$ 3 were considered to be negative [(31)]**.**

Statistical analysis

Associations between immunoreactivity and different clinicopathological data were done by Chi-Square test. Pearson's rho coefficient was used for continuous variables to test the correlation between the two markers. Statistical analysis was done using SPSS® Release 16 (SPSS, Inc.). Statistical significance was determined at p value of $\leq$0.05.

Results

On studying HER-2 expression, positive membranous expression (2+ and 3+) was found in 31/100 (31%) of the cases. HER-2 overexpression (3+) or high positive staining was observed in 20/100 (20%) of the cases, while 11/100 (11%) cases showed (2+) or low positive expression. Negative expression (including 0 and 1+ scores) was found in 69/100 (69%) cases, stratified as; 0 score in 43/100 (43%), and 1+ score in 26/100 (26%) cases (Figures 1-A, B, C).

In the present study, there was a significant association between HER-2 immunoexpression and tumour grade (*p* value = 0.007). HER-2 overexpression was found more frequently in grade 3 (31.82%), than in grade 2 (14.29%), while none of grade 1 showed HER-2 overexpression. Regarding tumour stage; none of stage Ta cases showed HER-2 over-expression, and there was an increase in HER-2 overexpression with increase in T stages. The association between HER-2 immunoexpression

and different tumour stages was statistically significant (*p* value = 0.005) (Table 2).

HER-2 overexpression was observed more in muscle-invasive carcinomas (24.65%) than non-muscle invasive ones (7.4%). Negative expression was frequent in non-muscle invasive (70.37%) than in muscle- invasive carcinomas (68.49%). There was a significant association between HER-2 immunoexpression and tumour invasiveness (*p* value = 0.002). Cases with positive lymph node metastasis (N1 & N2) showed more HER-2 overexpression in comparison to those cases without metastasis (N0 & Nx). Such result was not statistically significant (*p* value = 0.436) (Table 2).

HER-2 immunoexpression was not significantly correlated with patient's age, gender, or bilharziasis (*p* value= 0.926, 0.109, and 0.06 respectively). There was a trend towards positive HER-2 expression in older patients, in female gender, and in tumour without associated bilharzial infection (Table 2).
COX-2 expression was found to be positive in 71 (71%) of cases studied. COX-2 positivity was observed more in grade 3 cases (77.27%), than in both grade 2 (76.19%), and grade 1 (35.71%) cases. A positive significant association was detected between COX-2 expression and advanced grade (*p* value= 0.007). The frequency of COX-2 expression was increased by stage. This result was statistically significant (*p* value = 0.028) (Table 3) (Figures 2-A, B).

COX-2 positivity was observed in the muscle-invasive carcinomas (79.45%) more than the non-muscle invasive ones (48.15%). Negative expression was more frequently seen in non-muscle invasive (51.85%) than in muscle-invasive carcinomas (20.55%). There was a significant association between COX-2 immunoexpression and tumour invasiveness (*p* value = 0.002). Although no significant correlation was found between COX-2 expression and lymph node metastasis (*p* value = 0.413), positivity was more frequent in cases with positive lymph node metastasis (N1 & N2) (88.24%), than in absent metastasis (Nx and N0) cases (60%), and (76.75%) respectively (Table 3).

Although there was no significant association between COX-2 expression and patient's age, gender, or bilharziasis (*p* value = 0.862, 0.055, and 0.258 respectively). The present study demonstrated an increase inCOX-2 expression in male cases and in cases with associated bilharziasis as compared with its expression in female cases and in absence of associated bilharziasis (Table 3).

The current study, detected a significant positive correlation between HER-2 immuno-expression and COX-2 immunoexpression (*r*=0.197, and *p*=0.050). Twenty five of the 31 positive HER-2 cases were also positive for COX-2 expression. These cases were distributed as 18 cases with HER-2 score 3+ and 7 cases with HER-2 score 2+. Twenty three cases showed negative expression for both markers (Table 4).

Table (1): Clinicopathological data of the cases

Clinicopathological data		Number (%)
Age in years **Median (range)**	63.5 (32-86)	
Gender	Males Females	86 (86%) 14 (14%)
Grade	1 2 3	14 (14%) 42 (42%) 44 (44 %)
Stage	Ta T1 T2 T3 T4	11 (11%) 17 (17%) 23 (23%) 40 (40%) 9 (9%)
Muscle invasion	Muscle-invasive Non-muscle invasive	27 (27%) 73 (73%)
LN metastasis	Nx N0 N1 & N2	10 (14.28%) 43 (61.44%) 17 (24.28%)

Table (2): Relationship of HER-2 immunoexpression and clinicopathological variables (Total 100 cases)
Test of significance: Chi square test

Clinicopathological variables	Negative HER-2 (n=69)		Positive HER-2(n=31)		*p* value
	0 (n=43)	1+ (n=26)	2+ (n=11)	3+ (n=20)	
Age **<63.5 years (n=50)**	22(44%)	14(28%)	5(10%)	9(18%)	0.926
>63.5 years (n=50)	21(42%)	12(24%)	6(12%)	11 (22%)	
Gender **Male (n=86)**	39 (45.34%)	24(27.9%)	9(10.46%)	14(16.28%)	0.109
Female (n=14)	4 (29.6%)	2(14.8%)	2(14.8%)	6(42.8%)	
Bilharziasis **Positive (n=21)**	14(66.66%)	2(9.52%)	1(4.76%)	4(19.04%)	0.06
Negative (n=79	29(36.7%)	24(30.37%)	10(12.65%)	16(20.25%)	
Grade **G1 (n=14)**	5 (35.71%)	6 (42.85%)	3 (21.44%)	0 (0%)	0.007*
G2 (n=42)	17 (40.47%)	11 (26.19%)	8 (19.05%)	6 (14.29%)	
G3 (n=44)	21 (47.73%)	9 (20.45%)	0 (0%)	14 (31.82%)	
Stage **Ta (n=10)**	5 (50%)	2 (20%)	3 (30%)	0 (0%)	0.005*
T1 (n=17)	2 (11.6%)	10 (58.8%)	3 (17.64%)	2 (11.67%)	
T2 (n=24)	10 (41.7%)	5 (20.8%)	4 (16.6%)	5 (20.83%)	
T3 (n=40)	23 (57.5%)	7 (17.5%)	1 (2.5%)	9 (22.5%)	
T4 (n=9)	3 (33.33%)	2 (22.22%)	0 (0%)	4 (44.44%)	
Muscle invasion **Muscle-invasive (n=73)**	36 (49.3%)	14 (19.17%)	5 (6.84%)	18 (24.65%)	0.002*
Non-muscle invasive (n=27)	7 (25.92%)	12 (44.44%)	6 (22.22%)	2 (7.4%)	
Lymph node metastasis Nx (n=10)	3 (30%)	2 (20%)	1(10%)	4 (40%)	0.436
N0 (n=43)	22 (51.61%)	8 (18.6%)	4 (9.3%)	9 (20.9%)	
N1 & N2 (n=17)	9 (52.9%)	4 (23.5%)	0 (0%)	4 (23.5%)	

***p* value ≤ 0.05 is considered significant**

Table (3): Relationship of COX-2 immunoexpression and clinicopathological variables (Total 100 cases)

Clinicopathological variables	Negative COX-2 (n=29)	Positive COX-2 (n=71)	*p* value
Age			
<63.5 years (n=50)	14 (28%)	36 (72%)	0.826
>63.5 years (n=50)	15 (30%)	35 (70%)	
Gender			
Male (n=86)	24(27.91%)	62(72.09%)	0.550
Female (n=14)	5(35.71%)	9(64.29%)	
Bilharziasis			
Positive (n=21)	4(19.04%)	17(80.96%)	0.258
Negative (n=79)	25 (31.65%)	54(68.35%)	
Grade			
G1 (n=14)	9 (64.28%)	5 (35.71%)	**0.007***
G2 (n=42)	10 (23.8%)	32 (76.19%)	
G3 (n=44)	10 (22.7%)	34 (77.72%)	
Stage			
Ta (n=10)	6 (60%)	4 (40%)	
T1 (n=17)	8 (47.05%)	9 (52.95%)	0.028*
T2 (n=24)	4 (16.7%)	20 (83.3%)	
T3 (n=40)	10 (25%)	30 (75%)	
T4 (n=9)	1(11.1%)	8 (88.9%)	
Muscle invasion			
Muscle-invasive (n=73)	15 (20.55%)	58 (79.45%)	0.002*
Non-muscle invasive (n=27)	14 (51.85%)	13 (48.15%)	
Lymph node metastasis			
Nx (n=10)	4 (40%)	60 (60%)	
N0 (n=43)	10 (23.25%)	33 (76.75%)	
N1 & N2 (n=17)	2 (11.76%)	15 (88.24%)	0.413

Test of significance: Chi square test
***p* value ≤ 0.05 is considered significant**

Table (4): Correlation between HER-2 and COX-2 immunoexpression

	COX-2 positive	COX-2 negative	Total	p value
HER-2 positive	25	6	31	
HER-2 negative	46	23	69	0.050*
Total	71	29	100	

Pearson correlation test was used.
Correlation is significant at the 0. 05 level (2-tailed).

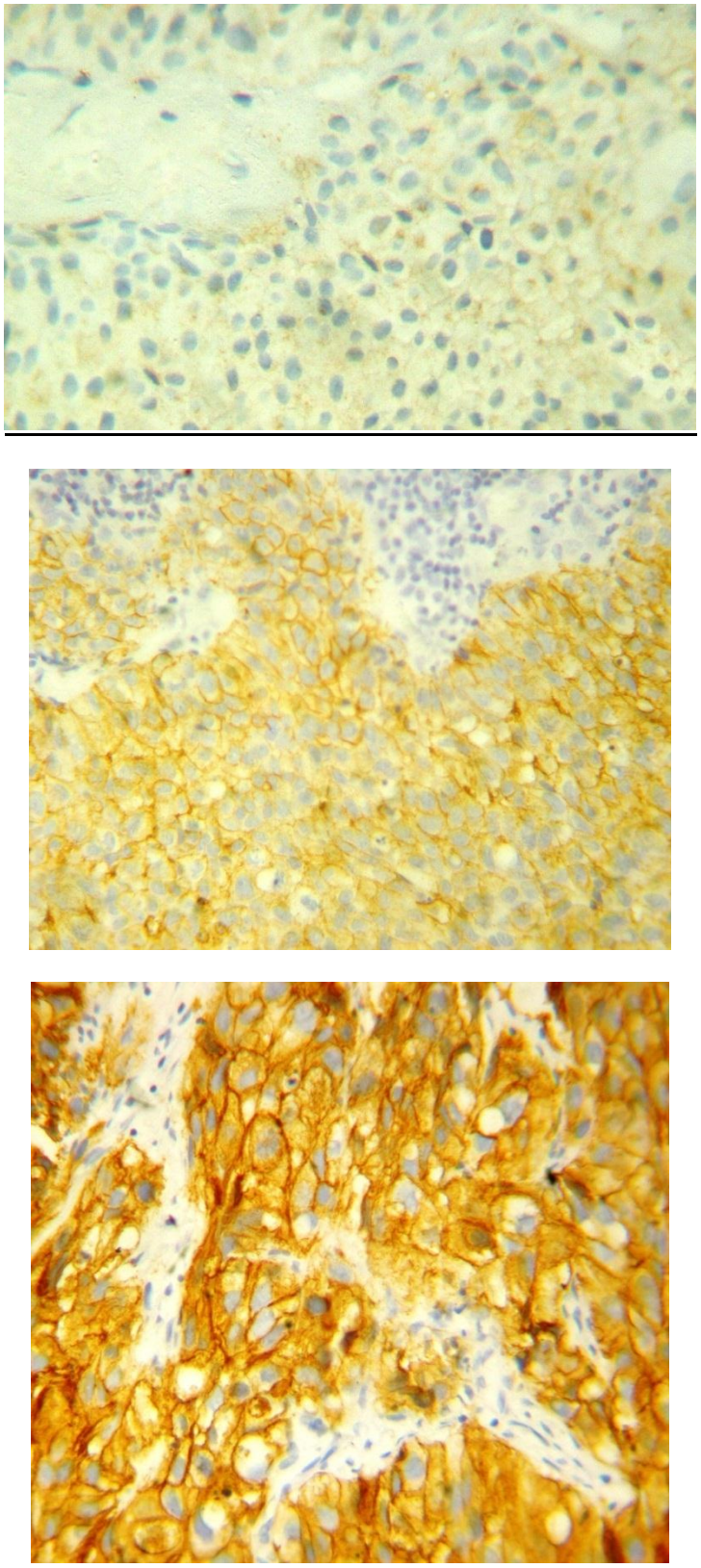

Fig. (1-A, B, C): HER-2 immunostaining (A) negative HER-2 expression score 1+ in grade 1 Urothelial carcinoma (B) positive membranous score 2+ HER-2 expression in grade 2 urothelial carcinoma (C) positive membranous score 3+ HER-2 expression in grade 3 urothelial carcinoma (X400)

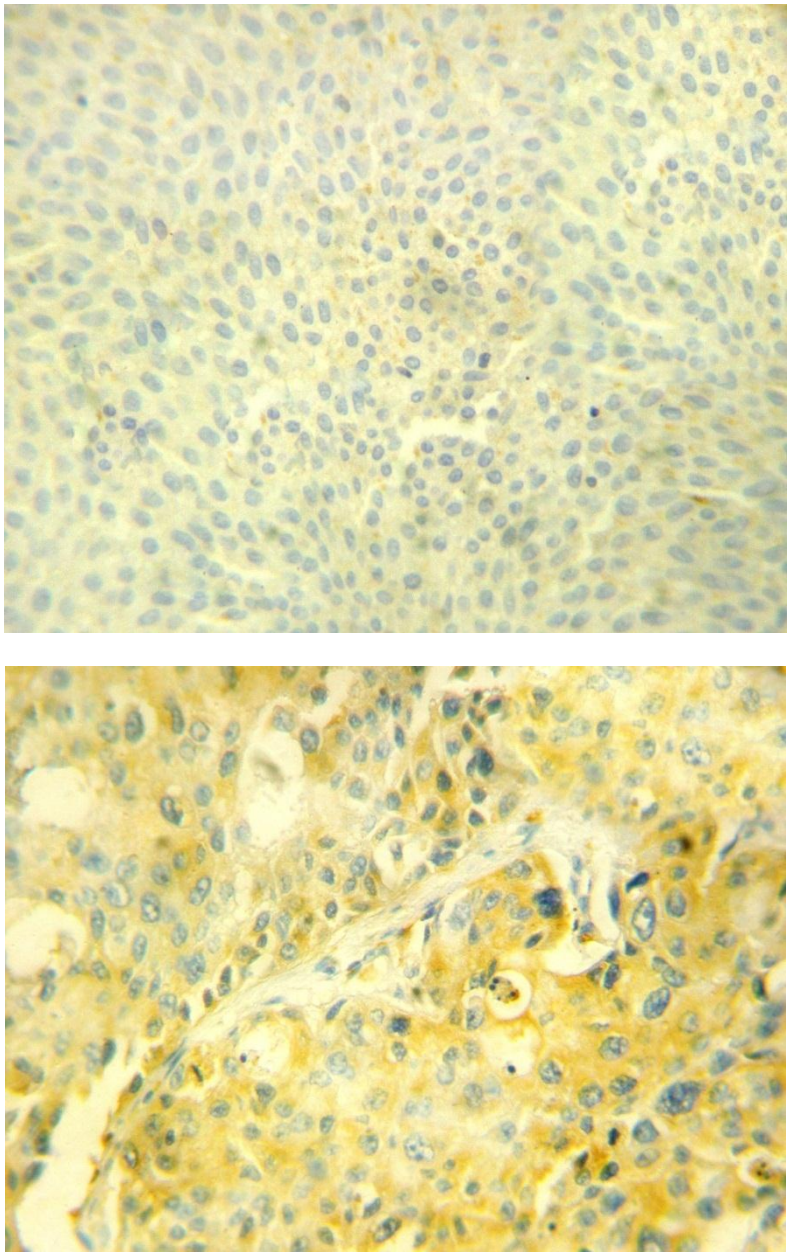

Fig. (2-A, B): COX-2 immunostaining (A) negative cytoplasmic COX-2 expression in grade 1 urothelial carcinoma (B) positive cytoplasmic COX-2 expression in grade 3 urothelial carcinoma (X400).

Discussion

Carcinoma of the urinary bladder is a world-wide health problem, with several molecular pathways involved in tumoregenesis[1, 32].

In the current study, HER-2 positive expression was found in 31% of cases studied. Score 2+ was observed in 11%, and score 3+ in 20% of the cases. Negative expression was detected in 69% of the cases (0 in 43%, 1+ in 26%). Those rates matched what was previously described by Naruse et al., who reported that 21.7% of the cases were score 3+, 10.9% were score 2+, and negative expression was in 67.3% of the subjects[18]. On the other hand, El Gehani et al., found score 3+ in 27.8%, score 2+ in 25%, and negative staining in 47.2% of the cases[33].

In the current study, no statistically significant association was found between HER-2 expression and patient's age, or gender. There was a trend towards HER-2 overexpression in females; as 42.8% of them showed score 3+, while only 16.28% of the male cases did. This

was in the line with El Gehani et al.,, who reported that 50% of the female cases were score 3+, also found no statistical significant association between HER-2 expression and patient's age[33]. Therefore, female patients could benefit more from HER-2 targeted therapy. No statistical significant association between HER-2 expression and gender was found by Bolenz et al., but on the contrary to our findings, HER-2 positivity was detected almost equally in males (28.2%) and females (26.2%)[34].

Our results showed significant association between HER-2 overexpression (score 3+) and high grade tumours. This was in the line with [4,30,35]. All these studies found significant positive correlation with high grade tumours.

On the other hand, no significant association between grade and HER-2 positivity was reported by previous studies[33,36]. This difference from the current study could be due to the small number of urothelial carcinoma cases as they used (40 and 36, respectively), and usage of polyclonal antibody. But all these studies found that the expression was higher in high grade cases, and this agreed with our results as 31.82% of grade 3 samples showed score 3+ but only 14.29% of grade 2 did, while none of grade 1 showed 3+ expression.

Our findings and those reported by these studies that HER-2 overexpression is more common in high grade than in low grade UCB, indicate that HER-2 expression is associated with poor prognosis.

In the present study, a significant association between HER-2 overexpression (score 3+) and advanced tumour stages (T2, T3, and T4) was detected. A finding which was in accordance with other studies [3, 4, 30, 35-37]. Collectively these studies found that the expression was significantly correlated with advanced stages. On the other hand, no significant association was found between stage and HER-2 positivity [17]. This difference from the current study could be due to the sample size (40), and the usage of polyclonal primary antibody.

Regarding the association between HER-2 immunoexpression and muscle invasiveness, a statistically significant relation was detected. HER-2 overexpression was found in 24.65% of muscle invasive, while only 7.4% of the non-muscle invasive cases did. This finding was in the line with[3,4,18,30,36-37]. All of these studies found that the expression was higher in muscle invasive than in non-muscle invasive cases.

In a previous study, non-muscle invasive and muscle-invasive tumour samples from the same patient were compared to assess HER-2 expression during the disease progression from non-invasive to invasive disease. Those results suggest that overexpression of HER-2 occurs prior to and persist with the onset of invasive disease[16].

It therefore appears that in tumours progressed to T2 stage have already acquired HER-2 abnormalities that occur before the onset of detrusor muscle invasion. So the application of anti-HER-2 therapy to tumours of Ta or T1 stages that are most likely to progress to T2 disease, based upon the presence of HER-2 protein overexpression might lower the rate of disease progression. The proposed decision algorithm is to carry out calibrated HER-2 IHC evaluation in all muscle-invasive urothelial bladder carcinomas as a screening test, confirmed by FISH results[12].

Those findings suggest that HER-2 over-expression is more common in high grade, advanced stage, and muscle-invasive UCB. So HER-2 overexpression may be considered as a poor prognostic marker, taking in consideration that high grade and advanced tumor stage with muscle invasion are the most important bad prognostic factors for urinary bladder carcinoma.

Considering the association between lymph node metastasis and HER-2 expression, no statistically significant association was detected. This agreed with[18, 36]. While another study detected significant association between lymph node metastasis and HER-2 expression[38].

Our results demonstrated no significant correlation between HER-2 expression and bilharzial-associated cases. However, there was a trend towards increased positivity in tumours without associated bilharzial infestation. Such result disagreed with that reported by Hammam

et al., who found significant correlation between HER-2 expression and associated bilharzial infestation[3]. This difference could be due to the use of different scoring system and the number (33) of UCB cases in the study.

COX-2 was considered as a risk factor for development and invasion of urinary bladder carcinoma[39]. COX-2 expression in UCB was reported to be 13.6%[40], 52.5%[41] and 98%[42].

In the current study, COX-2 showed positive expression in 71(71%) cases, and was negative in 29(29%) of cases studied. These rates matched what was previously described by Yildirim et al., and Diamantopoulou et al., who reported positivity in 70.1%, and in 76.9% of the cases respectively [31,43].

On the other hand, Wadhwa et al., found COX-2 positive staining was seen in 84.2% of the patients, this difference may be because they used 38 cases in their study [44]. Also positive staining was found in 57.4% of the 68 cases studied [22]. This difference from the current study could be due to the number of urothelial carcinoma cases examined in their study and different method of IHC staining evaluation.

Overexpression of the inducible form of COX-2 is known to increase the invasive ability of tumour cells in vitro and has been involved in the development of UCB [43]. This supports the suggestion that tumour recurrence and progression may be reduced by using combi-nations of COX-2 inhibitors in positive cases.

Our results detected a significant association between COX-2 immunoexpression and tumour grade. There was a trend towards increased COX-2 positivity and advancement of tumour grade. This was in the line with what was found by previous studies who reported a significant association between COX-2 and tumour grade[31,44,45].

From these observations by us and others, COX-2 was expressed in high grade carcinomas more than in low grade carcinomas, this reveals that this marker can be involved in tumour differentiation and induced in high grade UCB; however this need to be confirmed by further follow up studies.

On the other hand, no significant correlation between COX-2 expression and tumour grade was observed by previous studies [18, 46]. The difference from ours could be the number of cases studied (46 and 28, respectively).

In the present study, a significant association between COX-2 expression and tumour stage was detected. This finding was in accordance with[44,47]. While no significant association between stage and COX-2 positivity was detected in other reports[18,45]. The difference from ours could be due to the usage of different IHC scoring system and different primary monoclonal COX-2 antibody.

In the current study, a statistically significant relation was detected between COX-2 immuno-expression and tumour muscle invasion. COX-2 overexpression was found in 79.45% of muscle-invasive cases, while only 48.15% of the non-muscle invasive cases did. This finding was in the line with[23,48], who found that COX-2 expression was higher in muscle-invasive than in non-muscle invasive cases. On the contrary, other studies found no statistical significant association between COX-2 expression and invasiveness[43,45]. The difference from the current study might be the result of the different number examined in these studies (134 and 92 respectively), and the usage of different IHC evaluation system.

The current study and previous ones detected that COX-2 was expressed in muscle-invasive tumours that are at a more advanced stage than non-muscle invasive tumours.

There is much evidence that the COX-2 gene is involved in features of tumor aggressiveness, such as invasiveness. Human colon cancer cells transfected with a COX-2 expression vector have increased activity of metalloproteinase-2, which is necessary for the degradation of extracellular matrix, resulting in increased tumour cell migration. However, there is no evidence that COX-2 is associated with invasiveness and metastasis in human tumors[43]. Thus, it would be interesting to determine in additional studies whether COX-2 is a prognostic factor in UCB.

Those findings suggest that COX-2 positive expression is more common in high grade,

advanced stage, and muscle-invasive UCB. So COX-2 positivity may be associated and considered as an indication of worse prognosis, taking in consideration that high grade and advanced tumour stage with muscle invasion are the most important bad prognostic factors for urinary bladder carcinoma.

Considering the association between lymph node metastasis and COX-2 expression, no statistically significant association was detected in the present study. This agreed with[23,45]. However, a previous study reported a significant association between COX-2 expression and lymph node metastasis[18]. The difference from ours could be due to the number of cases (46) in that study.

The present study demonstrated an increase in COX-2 positive expression in cases with associated bilharziasis (80.96%), but this result was not statistically significant. This agreed with what was detected by previous studies who found that 85.2% and 90% of bilharzial associated cases respectively, were positive for COX-2[22,48]. These findings could support the role of inflammatory process accompanying bilharziasis in the initiation and progression of bilharzial-associated urothelial carcinoma.

Our results detected a significant positive correlation between HER-2 and COX-2 expression. Co-expression was found in 25 (25%) of the cases examined. These cases were distributed as 18 cases with HER-2 score 3+ and 7 cases with HER-2 score 2+, while 23 (23%) cases showed negative expression for both markers.

Co-expression was reported by Eltze et al., in 33% of the cases[27]. They concluded that combined treatment with HER-2 and COX-2 inhibitors may be beneficial for the HER-2 and COX-2 expressing tumours and this should be assessed in further futuristic studies.

On the contrary, Naruse et al., reported no statistically significant relationship between both markers, and only 15.2% (7/46) of the cases were positive for both HER-2 and COX-2 expression [18]. This difference could be the result of different number of cases examined (46), different primary monoclonal COX-2 antibody, and the different IHC scoring method for both HER-2 and COX-2 expression.

In gastric cancer, the frequency and the magnitude of COX-2 expression were markedly enhanced in HER-2 positive tumours. These findings support the hypothesis that the HER-2 status may be one of the determinants of COX-2 expression[49]. Also the overexpression of COX-2 and HER-2 are relatively common events in pancreatic cancer[50].

It was reported that combination of COX-2 selective inhibitors with HER-2 inhibitors had provided an additive antitumour effect on breast cancer and colorectal carcinoma in experimental animal models[51,52]. It was suggested that COX-2 may be a therapeutic target for the treatment of ER-negative/HER-2-positive breast cancer. A combination of COX-2 and HER-2 inhibitors may be particularly efficacious in these patients[26].

Conclusion

From observations in this study, it is suggested that HER-2 and COX-2 can play essential roles in the pathogenesis, aggressiveness, invasion, and progression of urothelial carcinoma. So studying the expression of those key molecules could serve as potential targets for prognosis and treatment of bladder urothelial carcinoma, and may assist in the identification of cases that are at greater risk and may have an unfavorable clinical outcome.

Prospective studies on the efficacy of HER-2-targeted therapies in urothelial carcinomas are warranted. Also studying the efficacy of COX-2 inhibiting NSAIDs are important to determine if COX-2 could be a valuable biological target molecule in the treatment of patients with UCB.

Further studies are recommended to demonstrate the relation between HER-2 and COX-2 on molecular basis. These should include in vitro molecular studies and transfer data to a large scale of cases with survival information to confirm their prognostic significance. This in turn will help in identification of patients with aggressive tumours in order to select cases that can benefit from therapy.

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