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Immunohistochemical Expression of COX2 in Urothelial Carcinoma of the urinary bladder

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Abstract

Background and objectives: Urothelial carcinoma of urinary bladder constitutes 90% of bladder cancer cases. Cyclo-oxygenase 2 as an immune-histochemical marker, is not expressed in normal tissues, while it is expressed in some cancers including urothelial carcinoma. The purpose of this study is detecting the frequency of expression of Cyclo-oxygenase 2 in urinary bladder's urothelial carcinoma in association with certain clinicopathological variables.

Methods: A retrospective study was done on seventy-nine cases (fixed with formalin and embedded in paraffin) of urothelial carcinoma which were chosen haphazardly from a private laboratory in Erbil city over two years (between October 2019 to October 2021). In the present study, Cyclo-oxygenase 2 expression was assessed on the urothelial carcinoma slides.

Results: Cyclo-oxygenase 2 was positive in 45.6% of the cases. It was positive in 80% of non-papillary tumors, while it was less expressed (40.6%) in papillary tumors, which means that significant correlation was seen between Cyclo-oxygenase 2 positivity and tumor pattern (papillary vs non-papillary) ($p = 0.037$). In this study, no important association was found between Cyclo-oxygenase 2 expression and other clinicopathologic features like age ($p=0.236$), gender ($p=517$), tumor grade ($p=0.106$), lymphovascular invasion ($p=0.696$) and T stage ($p=0.061$).

Conclusion: In this study Cyclo-oxygenase 2 was expressed in less than half of our urothelial tumor samples. A strong association was found between Cyclo-oxygenase 2 positivity and tumor pattern (papillary and non-papillary), but no important association was found between Cyclo-oxygenase 2 positivity and other variables such as age, gender, tumor grade, lymphovascular invasion and tumor stage.

Keywords: COX2, Immunohistochemistry, Urothelial carcinoma.

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Introduction

Bladder cancer ranks the tenth most common cancer worldwide. Consistent rising in its incidence is recorded globally, especially in developed nations.[1,2] Incidence of bladder cancer is the highest in South and West of Europe and North of America.[3] In Iraq the incidence rate of bladder cancer was raising from 3.44 in 2000 to 3.75 in 2016.[4] A survey in Sulaymaniah, Kurdistan revealed that the bladder cancer is among the top ten major cancers in both sexes within all age groups with an incidence rate of 2.2%.[5] Men are more commonly affected by bladder cancer with a 4:1 ratio, and among them it ranked as the sixth most commonly diagnosed cancers and ninth leading cause of cancer fatality.[1] The major risk for urothelial carcinoma (UC), which constitutes about 90% of bladder cancer cases, is tobacco smoking, followed by exposure to chemical carcinogens at particular occupations,[3] these risk factors may have contributed to male predominance in bladder cancer incidence.[1]Cyclooxygenase 2 (COX2), the prostaglandin E2 (PGE2) generating enzyme, is encoded by the prostaglandin endoperoxide synthase (PTGS2) gene and it is important for changing arachidonic acid to prostaglandin H2 which is converted later to prostaglandins PGD2, PGE2, PGF2α, prostacyclin (PGI2), or thromboxane A2 by tissue-specific isomerases. COX2 is significantly involved in inflammation-related carcinogenesis. It has an impact in suppressing immune system, apoptosis prevention, neovascularization, invasion, and metastasis.[6-8] It has been supposed that COX2 has a crucial function in neoplasm initiation and development, also in regulating stem cell proliferation and differentiation in inflammation-related urinary bladder carcinogenesis.[9] it appears that PGE2 participates in cancer stem cells (CSCs) regeneration of various malignancies such as bladder cancer.[10,11] Mechanistically, the role of COX2/PGE2 in CSC repopulation is by activating signaling pathway of JAK2 (Janus kinase 2)/STAT3 (signal transducer and activator of transcription 3).[12] Cyclo-oxygenase 2 cannot be found in normal tissues and normal conditions, but it is released by the effect of growth factors and inflammatory mediators,[13] it is overexpressed in multiple premalignant and malignant circumstances, like urinary bladder cancer,[14] and this marker is found in high grade and stage cases of bladder carcinomas.[15] It was found that there is important effect of selective COX2 inhibitors in decreasing the incidence of human bladder carcinoma.[16,17] The aim of this study is to evaluate COX2 expression in UC of urinary bladder by immunohistochemistry, as well as to investigate its association with some clinicopathologic variables, like age, sex, tumor pattern (papillary and non-papillary), tumor grade, lymphovascular invasion and tumor size (T) as part of the TNM staging.

Material and methods

A retrospective study was done on 79 blocks of urothelial carcinoma cases which were collected from the documents of a private laboratory in Hawler city through October 2019 - October 2021. Two sections were taken from every block, Hematoxylin & Eosin (H&E) was used for staining one of the sections for histological examination and the other section was used for immunohistochemical assessment regarding COX2 positivity. Urothelial carcinoma cases were graded as high grade and low grade according to the 2016 WHO classification system,[18] and pathologically staged according to the American Joint Committee on Cancer (AJCC) eighth edition.[19]This was ethically approved by Kurdistan Higher Council of Medical Specialties. Immunohistochemical staining was performed using Dako recommendations with COX2 marker (CX-294, dilution 1:50). Each block was used to prepare two sections measuring 4-µm, one of them was stained

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with H&E stain, and the other was used for the analysis of COX2 expression. The blocks were de-paraffinized, rehydrated by ethanol, and incubated for 10 min in 3% H2O2 to prevent endogenous hydrogen peroxidase activity. Antigen retrieval was done by autoclaving in citrate buffer (pH=6) for 10 min. the next step was applying a primary antibody of COX2 at 4°C overnight. After rinsing in phosphate buffer saline, sections were treated with a peroxidase-labeled polymer attached to mouse antihuman COX2 antibody as a secondary antibody (Dako) for 30 min at 37°C followed by counterstaining with hematoxylin. The slides were assessed by two pathologists, colonic cancer tissue was used as the positive control. For negative controls, the primary antibody was omitted in each run. According to the literature, COX2 reactivity is mainly expressed in cytoplasm[20]. A semi quantitative method was used for the evaluation of COX2 expression, which includes calculating the percentage of the cells with brown cytoplasmic staining (COX2 positive cells). The used threshold is a cutoff point of 10%. COX2 positive result is when 10% of the cells or more are stained, while COX2 negative result is when less than 10% of the cells are stained.[20,21] The Statistical Package for Social Sciences (SPSS, version 26) was used for the analysis of the data. Chi square test of correlation was used to compare between two or more groups. When the expected value (result) was found to be <5 of >20% of the cells of the table, Fisher's exact test was used. A p value of ≤ 0.05 was considered as statistically important.

Results

Seventy-nine cases were taken in this study. The mean age (SD) was 68.1 (10.9) years, ranging from 40-91 years. The median was 70 years. The majority of the samples (77.2%) were aged more than 60 years, and the majority (77.2%) were males, Table (1).

Table (1): Distribution of age and gender.

	No.	(%)
Age (years)		
≤ 60	18	(22.8)
> 60	61	(77.2)
Mean (SD)	68.1	(10.9)
Gender		
Male	61	(77.2)
Female	18	(22.8)
Total	79	(100.0)

The majority (87.3%) of the tumors were of the papillary type, and 74.7% of them were of high grade. Only 8.9% had lymphovascular invasion, and the largest proportion (68.4%) of the sample was of stage T1, Table (2).

Table (2): Tumor characteristics.

	No.	(%)
Tumor pattern		
Papillary	69	(87.3)
Non-papillary	10	(12.7)
Tumor grade		
Low grade	20	(25.3)
High grade	59	(74.7)
Lymphovascular invasion		
Positive	7	(8.9)
Negative	72	(91.1)
T stage		
Ta	8	(10.1)
T 1	54	(68.4)
T 2	15	(19.0)
T 3	2	(2.5)
Total	79	(100.0)

The COX2 expression was positive in 45.6% of cases. As shown in Table (3), there is strong connection between COX2 positivity and tumor pattern (papillary and non-papillary), it showed that COX2 was positively expressed in 80% of cases with non-papillary tumors, comparing with 40.6% of cases with papillary tumors (p = 0.037). No significant correlation was found between

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COX2 positivity and other variables as shown in Figure (1),including: age (p = 0.236), sex (p = 0.517), tumor grade (p = 0.106), lymphovascular invasion (p = 0.696), and T stage (p = 0.061). It is crucial to mention that COX2 was expressed more in tumors with advanced stages (73.3% and 50% in patients with T2 and T3 stages respectively) compared with patients with stage Ta and T1 (25% and 40.7% respectively), Table (3).

Table (3): Expression of COX 2 by the studied factors.

	COX 2 expression			
	Positive	Negative	Total	p
	No. (%)	No. (%)	No. (%)	
Age (years)				
≤ 60	6 (33.3)	12 (66.7)	18 (100)	
> 60	30 (49.2)	31 (50.8)	61 (100)	0.236*
Gender				
Male	29 (47.5)	32 (52.5)	61 (100)	
Female	7 (38.9)	11 (61.1)	18 (100)	0.517*
Tumor pattern				
Papillary	28 (40.6)	41 (59.4)	69 (100)	
Non-papillary	8 (80.0)	2 (20.0)	10 (100)	0.037**
Tumor grade				
Low grade	6 (30.0)	14 (70.0)	20 (100)	
High grade	30 (50.8)	29 (49.2)	59 (100)	0.106*
Lymphovascular invasion				
Positive	4 (57.1)	3 (42.9)	7 (100)	
Negative	32 (44.4)	40 (55.6)	72 (100)	0.696**
T stage				
Ta	2 (25.0)	6 (75.0)	8 (100)	
T1	22 (40.7)	32 (59.3)	54 (100)	
T2	11 (73.3)	4 (26.7)	15 (100)	
T3	1 (50.0)	1 (50.0)	2 (100)	0.061**

*By Chi square test.
**By Fisher's exact test.

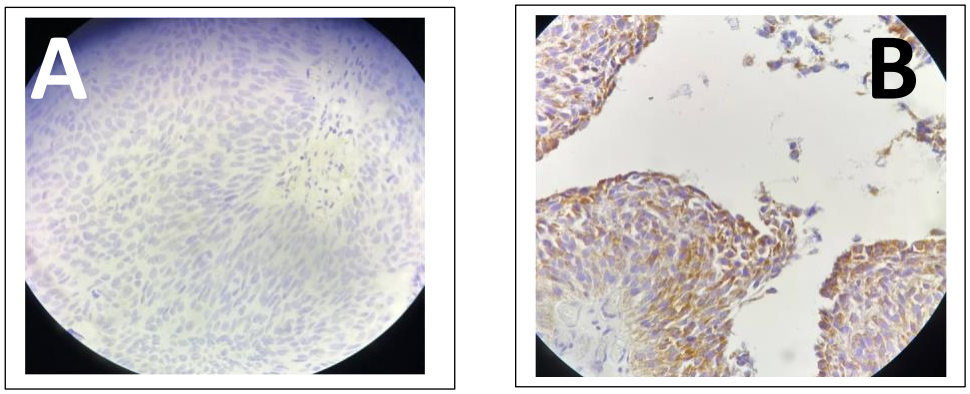

Figure (1): COX2 expression
(A) Negative COX2 expression. (B) Positive COX2 expression (power

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Discussion

Cyclo-oxygenase 2 is not released in normal circumstances, it is produced by the effect of inflammatory mediators, growth factors and oncogenic agents.[22] Recently many studies showed that COX2 is expressed in many human cancers, including urothelial carcinoma of the urinary bladder.[23-25] Authors showed that selective COX2 inhibitors can have anti-tumor effects,[23] this lead to designing trials aiming for treating cancers in humans like breast, colon and lung cancer (non-small cell type) by combining COX2 inhibitors with chemotherapy or radiation.[26]In various studies that were carried out, different expressions of COX2 for urothelial carcinoma were reported, ranging from 37% [20,27] to 84%.[28] In this study, positive results for COX2 expression where seen in 45.6% of slides, comparable results were found by other researches, as Tabriz et al which recorded 50% ,[29]Maghrabi et al in 37%. [20]While COX2 was positive in 83% by Wulfing et al.[26] 84% by Wadhwa et al.[28]93% by El-Anwar et al.[30] This difference in results may be due to different sample sizes, different antibody sources and variable properties of the antibodies. Our study contained 79 samples of urinary bladder urothelial carcinoma. The patients age ranged from 40-91 years, the median was 70 years, the mean age (SD) of the patient was 68.1 (10.9) years. Large group of the patients (77.2%) were aged more than 60 years, similar to what was obtained by Al-Maghrabi et al.[20] No important correlation between COX2 positivity and age of the patients, this result was in harmony with what obtained by Wulfing et al.[26]Our study included 61 men and 18 women, 77.2% of the cases were men, like what was observed by Al-Maghrabi et al.[20] This male predominance mainly is due to occupational exposure to chemical hazards and cigarette smoking in males rather than females. Strong association was not found between COX2 positive expression and sex of patients, in agreement to what was recorded by Agrawal et al.[27]In this study, COX2 expression in non-papillary urothelial carcinoma was higher than in papillary type, as COX2 was expressed in 80% of non-papillary urothelial carcinoma cases, whereas 40.6% of papillary urothelial carcinoma cases expressed COX2. According to our findings, there was an important statistical relation between COX2 positivity and tumor pattern, this finding was similar to what was found by Badary et al.[31]This study showed higher rates of COX2 expression among advanced T stages (73.3% and 50% among patients with T2 and T3 stages respectively), compared with 25% and 40.7% among patients with stages T1 and Ta respectively. This result was in alignment to that observed by Al-Maghrabi et al and Wadhwa et al.[20,28] Our research showed no important correlation between expression of COX2 and tumor grade, similar to what was found by El-Anwar et al.[30] unlike what was found by Wadhwa et al, which demonstrate strong relation between COX2 expression and tumor grade. In our research there was no significant association with lymphovascular invasion, the same results were found by Tabriz et al.[29]

Conclusion

This study concluded that COX2 was expressed in less than half of our urothelial carcinoma cases. A strong relation was found between COX2 reactivity and the pattern of the tumors (papillary and non-papillary), but no strong association was seen between COX2 positivity and other factors like age, sex, lympho-vascular invasion, tumor grade and tumor stage. According to this research, no significant correlation was found between COX2 positivity and all of the clinicopathological factors we used except type of the tumor, so further researches and studies are recommended to confirm the

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usefulness of using COX2 for diagnosing urothelial carcinoma cases.

Conflicts of interest:

The authors declare no conflicts of interest.

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