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UROTHELIAL CARCINOMA OF URINARY BLADDER;

IMMUNOHISTOCHEMICAL DEMONSTRATION OF COX-2 IN CORRELATION WITH VARIOUS GRADES

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ABSTRACT... In spite of the fact that inflammation has been regarded as a localized or generalized defensive component of the body to different types harmful stimuli, there has been becoming confirmation of its strong part in initiation or progression of different ailments particularly related with cancer. **Objectives:** Aim of this study was to recognize the pattern of expression and level of intensity of COX-2 in different grades of papillary urothelial carcinoma of urinary bladder along with significance of COX 2 in tumerogenesis of urothelial carcinoma of urinary bladder. **Setting:** Department of Pathology, BMSI, JPMC. **Period:** 1.1.2009 to 31.12.2012. **Methods:** The marker of COX-2 was investigated by using Immuno- histochemistry. **Results:** COX 2 was not detected in normal urothelium, but its intensity was expressed as 68% in low grade, 72 % in high grade and 80 % in invasive urothelial carcinoma. **Conclusion:** Results of the present study indicate that COX-2 as a component of inflammation play an important role in progression of urinary bladder tumor and encourage use of COX 2 inhibitors as potential antitumor agent.

Key words: Immunohistochemical staining, Cycloxygenase - 2

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INTRODUCTION

Malignancy of urinary bladder is the 9th most common cancer worldwide, accounting for two-third of all genitor-urinary tract cancers.[1] There were approximately 386,000 newly diagnosed cases of bladder cancer worldwide causing 150,000 deaths in 2008.[2] Each year approximately, 260,000 new cases are reporting in men and 76,000 in women.[3] The incidence of urothelial carcinoma is variable. Higher incidence rates of bladder cancer are seen in the developed countries while lower incidence is observed in Far Eastern countries.[4,5] In United States, urothelial carcinoma of the bladder is the 4th most common cancer in men and 8th in women with an incidence of 51,000 in the United States alone annually[6] According to SEER, 73,510 men and women were diagnosed out of which 14,880 men and women were died because of urinary bladder cancer in 2012 in United States[7] According to American Cancer Society (2007), globally male/female ratio is 10:3[8] Annual incidence & deaths reports are not available at the national level in Pakistan. In a study conducted by Shaukat Khanum Cancer Hospital (2005), cancer of the urinary bladder was ranked 9th in the list of malignancies registered from 1994 to 2004.

Bhurgri et al in 2005, indicated that in Pakistan from the year 1998 till 2002, carcinoma of the urinary bladder ranked 4th with an age-standardized rate of 6.8/100,000[9] and in another study, as the 8th most common cancer amongst Afghan refugees[10] Epidemiological, experimental and genetic studies suggest that urothelial cancer results from complex interaction between genetic polymorphism in tumor suppressor genes, inflammation and certain contributors like cigarette smoking, exposure to acryl amine and artificial sweeteners[11,12,13,141,5] Chronic inflammation has been indicated as a well recognized cause of urothelial cancer since several decades.[16,17] Arachidonic acid metabolism by cyclooxygenase – 2, initiate the formation of prostaglandins which in turn are

involved in different steps of bladder carcinogenesis.[18,19]

Pro-inflammatory nature of Cox – 2 express just because of specific stimuli e.g. mitogens, cytokines and growth factors.[20] Therefore targeting COX 2 may provide an opportunity to fight against urothelial cancers in future.[21] We has designed this study to assess the immunohistochemical demonstration of COX-2 in correlation with various grades of bladder cancer. No information with respect to COX 2 over expression in urinary bladder cancer could be retrieved in our population. It was therefore decided to carry out this study as a likely aid in providing an insight regarding the etiology and genetic factors involved in our population.

MATERIALS AND METHODS

Tissue sample

Present study was based on the analysis of urinary bladder carcinoma biopsies, received at the Department of Pathology, BMSI, JPMC from 1.1.2009 to 31.12.2012. Approximately sixty selected formalin fixed tissue samples with various grades along with normal bladder tissues cases were analyzed for immunohistochemical staining for COX-2. Histo-pathological reports were available on all tissue samples.

Immunohistochemical Methods

Paraffin embedded tissues (3-4 μm sections) were cut and mounted on (+) vely charged poly-lysine coated glass slides (Cyto test, China). Tissue sections were processed with trilogy (Cell Marque, USA). Hydrogen peroxide (H2O2) applied for 05 minutes. Washed with 01 change of IHC wash buffer. Antigen retrieval was achieved by steamers technique. Washed with 5 changes of IHC washed buffer.

At room temperature, antibody was applied and incubated for ten minutes. Rinced with 3 changes of IHC wash buffer. Amplifier (Cell Marque, USA) applied and incubated for 10 minutes. Rinsed with 3 changes of IHC wash buffer. HRP polymer (Cell applied and incubated for 10 minutes. Rinsed with 3 changes of IHC wash buffer. Tissue covered with chromogen (Cell Marque), incubate for 30 seconds to 20 minutes at room temperature as necessary to allow for proper color development. Slides rinsed in Distilled water, counter stain and dehydrate and mounted. To treat control slides either no antibody or with isotype matched IgG serum. All slides were reviewed by supervisor (Dr Shahnaz). The immunostaining of COX-2 was graded according to the scale 1-3, where (1+) weak staining, (2+) moderate staining, (3+) strong staining. Epithelial cells with COX-2 expression in various grades of urothelial cancer was expressed as the (%) percentage of epithelial cells with (+ve) staining by counting at least 100 cells. It was graded on a scale of 1-4 where (1+) 5-10 % cancer cells, (2+) 11-40 % cancer cells (3+) 41-70 % cancer cells, (4+) 71-100 % cancer cells.

COX - 2 intensity for immunoreactivity was recorded as 1+ mild, 2+ moderate and 3+ strong. Results were statistically analyzed. No primary antibody used to treat control slides. Cases of adenocarcinoma of colon are taken as positive control cases.

RESULTS

Sixty tissue samples of urothelial carcinoma of various grades with distribution of twenty five cases each of low grade and high grade, ten cases of invasive grade of urothelial carcinoma of urinary bladder along with five cases of normal urothelium were examined by Immunohistochemistry. Pattern of Cox - 2 expressions in various grades of cancer was presented in following table.

In non invasive low grade, majority of cases i.e., 17 (68%) out of 25 showed moderate (2+) cytoplasmic staining for COX 2. All these 17 cases showed reactivity (extent) not more than (2+) or 40 % of cells. Remaining 8 (32%) cases out of 25 showed mild (1+) cytoplasmic staining and extent only in (1+) or 10% of cells. None of the malignant cells in low grade showed strong (3+) intensity or extent (3+ or 4+). None of these cases showed negative staining reaction.

In non invasive high grade, majority of cases i.e.,

Morphological grades	Total No. of cases	Proportion				P-value
		1+	2+	3+	4+	
Non invasive low grade	25	8 (32%)	17 (68%)	0 (0%)	0 (0%)	
Non invasive High grade	25	0 (0%)	5 (20%)	18 (72%)	2 (8%)	0.001
Invasive	10	0 (0%)	2 (20%)	6 (60%)	2 (20%)	

Table-I. Cytoplasmic Proportion Of Cox 2 In Various Grades Of Papillary Urothelial Carcinoma

Significant association of different grade with proportion in COX 2 p<0.05

Proportion: 1+ (05-10 %) cells. 2+ (11-40 %) cells. 3+ (41-70 %) cells. 4+ (71-100 %) cells.

Morphological grades	Total No. of cases	Proportion			P-value
		1+	2+	3+	
Non invasive low grade	25	8 (32%)	17 (68%)	0 (0%)	
Non invasive High grade	25	0 (0%)	7 (28%)	18 72%)	0.001
Invasive	10	0 (0%)	2 (20%)	8 (80%)	

Table-II. Cytoplasmic Intensity Of Cox 2 In Various Grades Of Papillary Urothelial Carcinoma

Significant association of different grade with proportion in COX 2 p<0.05

Intensity: 1+ Mild 2+ Moderate 3+ Strong

18 (72%) out of 25 showed strong (3+) cytoplasmic staining as well as extent in between 41-71 % (3+) of malignant cells. 7 (28%) out of 25 cases showed moderate (2+) cytoplasmic staining while 5 (20%) cases showed extent in between 11-40 % of neoplastic cells (2+). One case showed extent in between 71-100 % i.e., (4+). None of the malignant cells express mild (1+) intensity or extent less than 10 %. In invasive urothelial carcinoma, majority of cases i.e., 8 (80%) cases out of 10 showed strong cytoplasmic staining (3+) while 02 (20 %) cases showed moderate (2+) staining. None of the case showed mild cytoplasmic staining. Most of the cases i.e., 6 (60%) showed strong extent i.e., 3+. 02 (20 %) cases showed strong proportion of staining i.e., 4+ showing beyond 70 % staining. None of the case showed mild cytoplasmic staining or extent less than 10 %. Normal transitional epithelium failed to express any cytoplasmic intensity or extent.

DISCUSSION

In the present study, basic relationship between COX 2 immuno-reactivity and tumor evaluating has been observed. Intrusive urothelial malignancies demonstrated 80 % immune-reactivity, high grade non invasive demonstrated 72 % followed by low grade 68 %. Comparable results were accounted for by[22], demonstrating 86 % of invasive urothelial carcinomas and 78 % of non invasive urothelial carcinomas.[23] indicated the consequences of COX 2 immuno-staining in thirty five patients with urothelial cancer of the urinary bladder, and it has been observed that COX 2 expression in 20% of pT1 and 45 % of carcinomas with muscular layer attack by immunoblotting, proposing that invasive carcinomas have expanded representation of COX 2.[24] likewise reported 38 % reactivity of high review urothelial growth with COX 2 with none of the low grade quality demonstrating any inspiration proposing that COX 2 increments with the stage and grade of urothelial carcinomas. The findings demonstrate a positive relationship between the repeat of COX 2 expression and the assessment and period of illness.

Positive immunostaining in carcinoma in situ has reported by[25], yet all the cases included in our arrangement were overtly malignant and non indicated in situ changes. This is could be because of the way that JPMC got cases for the most part from low financial groups of patients who are generally uneducated henceforth ignorant of any sign and indications identified with right on time detection. Additionally, none of the biopsies specimens demonstrated any contiguous typical transitional epithelium or intra epithelial malignancy as most biopsies were from tumor regions indicating overt malignant changes. Subsequently it was impractical to confer on CIN cases in our

study. At the same time keeping in view the way that just about all cases demonstrated chronic inflammatory changes in lamina propria and increasing concentration of COX 2 in all evaluations of urothelial carcinoma we reached the conclusion that inflammation brings about the change of basic cystitis into a deadly ailment like cancer. The worldwide burden of urinary bladder disease has stimulated wide concern universally and the diagnosis and treatment for bladder cancer are in a condition of advancement and unfolding. Now, it has been proposed that chemotherapy represents to vital modality for patients with bladder malignancy. The results are not agreeable because of low response rate and severe side effects. Impedance with the inflammatory microenvironment has been affirmed to backing antitumor activities. It is typical that altering the provocative microenvironment by using against COX 2 prescriptions, possibly update chemotherapy of urothelial carcinomas. Since many years, we have abundant learning regarding the distinguish mechanisms by which carcinoma and inflammation intersect and this is the right time to interpret great part of the fundamental information picked up thus far and use it to add new researches of various types of cancer treatments. So, COX 2 is an amazing target particle by which the discovery and treatment of urothelial carcinoma may be conceivable at ahead of schedule stage. Keeping in view the pattern of COX 2 immunostaining, we presumed that there was a strong relationship between progressively increasing expressions of Cycloxygenase - 2 with the advancement to different grades of carcinomas. Since COX 2 is a rate constraining catalyst in inflammatory process, anti COX 2 medications may potentiate or improve the impact of chemotherapy, so that the patient can be shielded from prolonged administration of chemotherapic prescriptions. Besides, wide scale mass instruction and guiding projects and in addition concentrating on risk variable and role of inflammation in bladder malignancy may give critical experiences into how to avoid urothelial disease.

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"You never fail until you stop trying."

Albert Einstein

AUTHORSHIP AND CONTRIBUTION DECLARATION

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1	Muhammad Salman Zafar	Main author	
2	Muhammad Asadullah	Co-author / Conducted the literature search / extracted and entered data / analyzed results.	
3	Usman Ahmad	Co-author / Wrote the protocol / Assisted in laboratory work.	