Cyclooxygenase-2 Level in Bilharzial and Non-Bilharzial Related Bladder Cancer among Iraqi Patients

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Abstract

Bladder cancer has become common cancer globally. It is the most common urological cancer; it comprises a significant part of urologists' work. 80 % of bladder cancers are superficial at diagnosis; they have not invaded into the muscle. The residual 20% are muscle-invasive, which carry a much worse prognosis. Emerging evidence mark that some parasites such as the blood fluke Schistosoma haematobium, Clonorchis Sinensis and small liver flukes Opisthorchis viverrini are causative agents of malignancies such as bladder cancer caused by schistosomes and cholangiocarcinoma by liver flukes. Infection with Schistosoma haematobium leads to urogenital schistosomiasis, which has been correlated with the occurrence of bladder cancer. The mechanisms responsible for this association have not yet been clearly identified. This study clarifies the association between cyclooxygenase-2 (COX2) and bladder lesions associated with Schistosoma. The result shows there is a high expression of COX2 in the Bilharzia related bladder cancer (BBC) while there is low expression of COX2 in non-bilharzia related bladder cancer (NBBC). In conclusion, conceder the positive expression of COX2 among Iraqi patients with Schistosomal-related bladder lesions is high. There may be a strong association between high rates of bladder cancer and urinary schistosomiasis in Iraq, as the vast majority of COX2 lesions were positive.

Keywords: *Schistosoma, Bladder cancer, COX2, TCC, SCC.*

Introduction

The bladder cancer refers to any of several types of malignant growths of the urinary bladder. Abnormal cells multiply without control in the bladder in a disease. Urinary bladder tumors contribute greatly to the global incidence of human cancer with about 550,000 new cases worldwide each year. Of those, around 425,000 occur in men, and about two-thirds occur in high-income countries [(1)].

There are two main types of bladder cancer specified: Transitional cell carcinoma (TCC). It one of the most recurrent tumors correlating with exposure to exogenous carcinogens such as smoking and most prevalent in Western and industrialized countries. The second type is squamous cell carcinomas (SCC), which are more recurrent seen in some country of Middle Eastern and African countries, where urinary schistosomiasis is an endemic disease [(2)]. Around 75% of squamous cell carcinoma in the Middle East and Africa was caused by Schistosoma haematobium infection [(3)].

One of the main risks factor is Schistosoma haematobium infection that causes urogenital schistosomiasis, which is linked to bladder cancer. According to the (WHO), schistosomiasis infect 200 million individual and is endemic more than 76 tropical developing countries. Schistosomes are parasitic blood flukes, which have an intermediate invertebrate host: freshwater snails and a mammalian host [(4)].

Cyclooxygenase-2 (COX-2) is another inflammatory mediator involved in tumour growth [(5)]. It is an inducible

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enzyme (also called prostaglandin synthase) that converts arachidonic acid to prostaglandins [6] . For most normal human tissues, cyclooxygenase-2 (COX-2) is not detectable but can be found in inflammation [7]. COX-2 overexpression may play a role in carcinogenesis, the underlying mechanisms of COX-2 tumorigenicity linked to several factors, including apoptosis inhibition, angiogenesis promotion, and activation of carcinogens such as aromatic amines, immune surveillance modulation, and increased invasiveness of tumour cells. It was associated with the development and progression of different forms of bladder cancer, including bilharzia bladder cancer [8].

COX2 is useful as a signal marker for bilharzia-associated bladder cancer, which may clear the way for preventive and therapeutic approaches using Cyclooxygenase 2 inhibitors to control bladder cancer in addition to chronic inflammation. [8] .

Materials and Methods

The current study was done during the period from October 2019 to June 2020. The study includes 50 patient of Iraqi people with bladder carcinoma. The cases collected from Al-Sadr Teaching Hospital in Al-Najaf province, Ghazi al-Hariri hospital for surgery specialist/ Baghdad, Al kadhimiya teaching hospital/Baghdad and Al-Diwaniyah Teaching Hospital.

Fifty cases divided into two groups of patients, patients with bladder cancer with history of schistosomiasis, patients with bladder cancer without a history of schistosomiasis. The cases include 39 male and 11 female. The age of patient range from 21 to 75 with mean 51.6 years. The samples included two types, paraffin-embedded block from hospital (45 samples) archive and fresh tumor biopsies (5 samples). The fresh biopsy transferred to the laboratory in formalin 10% to prepare tissues blocks embedded in paraffin to prepare for immunohistochemistry.

Preparation of fresh biopsy and paraffin-embedded block sample for immunohistochemistry it is proceed by placed the fresh sample in 10% neutral buffered formaldehyde immediately after removal. Then dehydration in various concentrations of ascending ethanol (70, 80, 90 and 100%). Clearing with xylene and then infiltration with paraffin wax at 60°C. Embedding in molten paraffin templates and they were left at laboratory temperature. The two type of sample were Sectioned by the rotary microtome (5µm thick sections) and then place the section on the water path and then placed on positive charge slide. The sections were de-paraffinized with xylene and then hydrated through 100%, 90%, 70% and 50% ethanol.

After dewaxing and hydrate the paraffin section add retrieval solution (Sodium citrate buffer) and incubate at 95°C for 15-20min then cooling the slide for 15min. After the slide cooled washed with phosphate buffer solution (Elabscience china) for two minx3 times. Add 3% H2O2 to the slide over tissue part for 10 min to eliminate endogenous peroxidase activity. Wash with phosphate buffer solution for two minx3 times. Then drain the PBS with absorbent paper, and then add Normal Goat Serum to the section Incubate for 30 min at 37°C. Dry the liquid around the section with absorbent paper, and draw a circle around the tissue with pap pen. Add primary antibody with proper dilution ratio, incubate at 37°C for 1~2h. Wash with phosphate buffer solution 2 min ×3 times. Then add polymer helper and incubate at 37°C for 20 min. Wash with phosphate buffer solution 2 min ×3 times. Add Polyperoxidase-anti-Mouse/Rabbit IgG and incubate at 37°C for 20~30 min. Then wash with phosphate buffer solution 2 min ×3 times. Add DAB working solution and take control of the DAB colouration period, the colour of tan or brownish-yellow is the positive signal. Wash the section with deionized water terminates the chromogenic reaction. Drain the slide with absorbent paper. The tissues' sections were stained with hematoxylin. This process also includes immersion the sections in xylene and different concentrations of ethanol. The prepared sections were mounted with DPX and covered with a glass coverslip. After that, the slides were cleaned and labelled to become ready for microscopic examination .

Statistical Analyses

Version 26 of the SPSS statistical program was used for all statistical analyses. The chi-square Pearson test was used and statistically relevant P. values of 0.01 or less were considered .

Result

In this study, pathologic evidence of urinary

schistosomiasis was shown in 21 (42%) patients. However, the true frequency of such infection among Iraqi patients may be higher than the estimated figure depending on certain factors such as the biopsy site, the number of biopsies taken, and the intensity of infection. Besides, there may be other patients in the Iraqi society with urinary schistosomiasis who did not have the opportunity for urinary bladder biopsy due to neglected lower urinary tract symptoms or inadequate referral to urology departments in major hospitals.

The age range of patients was between 21 and 75 years, with a median age of 51.6 years. The high frequency was recorded in age group (45-52) years and (69-76) years, which constituted 44% of the total number of patients. The present investigation showed that 78% of the patients were males and only 22% were females).

The histopathology examination of the sample microscopically shows 34- sample diagnostic as transitional cell carcinoma with their percentage 68% of the entire cases. In addition, 16-sample diagnostic as squamous cell carcinoma with the percentage 32%. The result shows transitional cell carcinoma is the predominant type of bladder cancer, whereas in schistosomiasis - endemic regions, squamous cell carcinoma is the most common type . The result shows there are 15 samples contain the schistosomiasis ova and 35 samples not contain. The 15 samples divided to the two types of cancer; transitional cell carcinoma 2(13.33%) and squamous cell carcinoma 13(86.66) .the sample nil from ova divided to the two types of cancer transitional cell carcinoma 32(91.42) and squamous cell carcinoma 3(8.57%).

The gene expression of the cyclooxygenase-2 in the whole cases displayed in (table 1). The expression estimate by staining intensity among the positive cells ranged from weak to dark brown reaction product. The staining intensity divided into the four types; weak staining, low staining, medium staining and high staining. The result shows high expression of the cyclooxygenase-2 in the squamous cell carcinoma cancer type (10 cases) while the transitional cell carcinoma (4 cases). In addition, low and weak expression of the COX2 appears in a high number of cases in the type transitional cell carcinoma. The COX2 expressed in medium rate in transitional cell carcinoma (4 cases) and squamous cell carcinoma (5 cases).

Table 1 : The gene expression of the cyclooxygenase-2 in the whole cases

No.	COX Expression	TCC	SCC
1	Weak	12	0
2	Low	14	1
3	Medium	4	5
4	High	4	10
Total = 50		34	16

TCC : Transitional Cell Carcinoma ; SCC : Squamous Cell Carcinoma

The gene expression of COX2 based on the pathological characteristic of bladder sections shown in table (2). In the table, descript the COX2 expression in two groups. The two groups divided according to the patients if have bilharzia history or not; the group split to bilharzia bladder cancer and non-bilharzia bladder cancer.

The result shows high expression of Cox2 in 14 samples; all of them is bilharzia related bladder cancer cases. In contrast, the medium expression appears in nine samples divided to 6 bilharzia related bladder cancer cases, and three samples are non-bilharzia bladder cancer cases. The low expression appears in 15 samples divided to 1 sample Bilharzia related bladder cancer cases, and 14 samples are non-bilharzia related bladder cancer. The weak expression appears in 12 samples; all of them are non-bilharzia related bladder cancer.

Table 2 : The gene expression of COX2 based on the pathological characteristic of bladder sections

Expression	Entire Cases	BBC	NBBC	P.Value
Weak	12	0	12	<0.005
Low	15	1	14	
Medium	9	6	3	
High	14	14	0	
Total	50	21	29	

BBC : Bilharzia bladder cancer ; NBBC : Non-bilharzia bladder cancer

Discussion

Usually, COX-2 does not exist in tissues but is caused by certain stimuli, including inflammatory cytokines, growth factors and oncogenes [9]. However, COX-2 is not expressed in the normal human urinary bladder epithelium [10] .

Cyclooxygenase-2 expression showed in transitional cell carcinoma cases was not significantly than in the early stages. This is consistent with the insufficiency of COX-2 as an indicator of tumour aggression [7]. Other studies were unable to connect COX-2 associated expression to tumour status, including level, histological gradation or remote metastasis [11]. Wülfing et al. (2004) Found that TNM and histologic grading were not associated, but there was a significant interaction between transitional carcinoma of cells and squamous carcinoma of the cells [12] .

Most bladder squamous cell carcinoma expressed COX-2 markedly. Conversely, transitional cell carcinoma was less COX-2 expressed. These variations in expressions can be due to etiological variations. The development of SCC is closely linked to chronic inflammation of the urinary tract, so COX-2 plays a leading role in inflammatory carcinogenesis. T. Shirahama and Sakakura (2001), Moussa et al. (2009) agree with these findings; they shown the COX-2 is high in urinary bladder squamous cell carcinomas [7,9] .

Genetic and clinical research found that COX-2 is a critical aspect of carcinogenesis. COX-2 expression plays a significant role in cancer growth and tumorigenesis [13] . Increased COX-2 protein can contribute to tumorigenesis by affecting many biological characteristics like cell adherence, angiogenesis, including apoptosis and invasiveness [14].

The findings strongly show that Cyclooxygenase-2 is involved in the early carcinogenesis of squamous bladder cell. Unlike squamous cell carcinoma, transitional urinary bladder cell carcinoma expressed COX-2, as previously reported, less heterogeneously. This result nearly similar to [6]. The gene expression there are statistically significant differences between the infection size NBBC& BBC measured by COX2. The result showed a high expression level of COX2 in the BBC with the P-value <0.005 reverse NBBC that show low expression of COX2. The high level of COX2 expression in BBC probably related to the Schistosoma worm placed the ovum in the bladder wall, and the ovum caused damage to the bladder lumen by travelling through the urothelium. These travel casings are damaged by chronic inflammation through their interaction with host immune cells and subsequent formation of granuloma [15] . These chronic inflammation lead to macrophages activated at inflammatory sites that lead to increase the pro-inflammatory cytokines IL-1, TNF-a and TGF-b, are COX-2 expression inducers. This expression can contribute to the mechanisms by which chronic inflammation initiates BBC cell transformation [16].

Conclusions

Take care of people with schistosomiasis in their lives who are at risk of developing bladder cancer and

do periodic check-ups for them and Monitoring cox 2 levels for people who have had a previous infection with Bilharzia, and use anti-cox 2 if levels elevated. COX-2 are essential markers, which differentiate between Schistosoma-related bladder cancers and non-Schistosoma.

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