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ORIGINAL ARTICLE

Cyclooxygenase-2 expression in clear cell renal cell carcinoma

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Abstract

Introduction: Cyclooxygenase-2 (COX-2) promotes carcinogenesis by inducing proliferation and angiogenesis while decreasing apoptosis and immunosuppressive activity. It is overexpressed in many malignancies including renal cell carcinoma (RCC). The aim of this study was to investigate COX-2 expression in clear cell RCC and its association with tumour grades and demographic parameters. *Materials and Methods:* Thirty-six clear cell RCC cases were selected. There were 21 (58.3%) men and 15 (41.7%) women with median age of 56.6 years (range: 16-74 years). Chinese constituted 16 (44.4%) of the cases; Malays 14 (38.9%) cases and Indian 6 (16.7%) cases. There were 6 (16.7%) grade 1, 20 (55.6%) grade 2, 10 (27.8%) grade 3 and none was grade 4. The paraffin embedded tissues were cut at 4 μm thick and stained with COX-2 monoclonal antibody. *Results:* Eighteen (50%) of the RCC cases were immunopositive, of which all showed strong positivity. The immunopositive cases showed cytoplasmic membrane positivity. *Conclusion:* There was no significant association between COX-2 expression with grade, age, sex and ethnicity (p=0.457, p=0.054, p=0.389 and p=0.568 respectively). Strong positivity of COX-2 suggest that COX-2 may play a role in cell proliferation and in carcinogenesis.

Keywords: Cyclooxygenase-2, clear cell renal cell carcinoma, biomarker, prognosis

INTRODUCTION

Approximately 273,000 new renal cancer cases were diagnosed worldwide each year, representing nearly 2% of all cancers and 116,000 deaths in 2008.[1] The lifetime risk for developing renal cancer is about 1 in 63 (1.6%).[2] It affects 1.9 per 100,000 in the Malaysian population. Among all renal cancer, renal cell carcinoma (RCC) is the commonest. It has a poor prognosis and up to 30% to 40% of patients present with metastatic disease.[3]

Cyclooxygenase or prostaglandin (PG) endoperoxide synthase is an important enzyme for conversion of arachidonic acid to prostaglandins.[4] It exists in two isoenzymatic forms: COX-1 (a constitutive form) and COX-2 (an inducible form). COX-1 is expressed in most tissues and produces PGs for its physiological functions.[4-6] COX-2 is an immediate early gene involved in cellular proliferation and carcinogenesis.[7] It plays an important role in RCC carcinogenesis by inducing PG synthesis.[8-9] It converts procarcinogens to carcinogens, inhibits apoptosis, promotes angiogenesis, modulates inflammation and immune function and increases tumour cells invasiveness.[10-11] It is upregulated by growth factors and tumour promoters.[12-13]

Many RCC patients present with distant metastasis at the time of diagnosis. As the current treatment of RCC is invasive surgery (nephrectomy), the roles of surgery in these advanced cases are limited. Immune-based therapeutic approaches have been used in these patients but most showed inconsistent results.[14-15] Therefore, alternative non-invasive treatments need to be further explored. To date, there are only a few studies on COX-2 in RCC with conflicting results. A few studies have shown high COX-2 expression and have implied that COX-2 has prognostic significance in RCC.[10,16-17] Its inhibitors such as celecoxib have a therapeutic potential by inhibiting PG synthesis. It is thus of interest to assess COX-2 expression in RCC patients among our local populations. We

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performed an immunohistochemistry analysis to evaluate COX-2 expression and its association with tumour grades and thus prognosis.

MATERIALS AND METHODS

All histologically confirmed clear cell RCC cases were retrieved from the department archives. The study has been approved by Ministry of Health (NMRR-08-481-1636) and the Ethical Committee of Faculty of Medicine and Health Sciences, UPM. The cases were graded according to Fuhrman's grading system. The grades were based on the nuclear size, shape and nucleoli. They were divided into 4 grades (grade I-IV). Demographic data of the patients were obtained from the histopathological examination request forms.

Immunohistochemical staining for COX-2

COX-2 immunohistochemistry staining was performed using DAKO REAL EnVision kit (Dako, Ca, USA). Formalin-fixed and paraffin-embedded blocks were sectioned at 4 μm thickness, mounted onto poly-L-lysine glass slide and dried overnight at room temperature. These sections were dewaxed with absolute xylene, rehydrated with gradient alcohol and rinsed under running tap water. Microwave was used for antigen retrieval. It was set at 1000 W for 10 min at high temperature followed by 10 min at medium low temperature in citrate buffer (0.01 M, pH 6.0). The sections were cooled to room temperature for 20 min and the endogenous peroxidase activity was inactivated in 3% Hydrogen peroxide (H_2O_2) for 5 min. Then the sections were rinsed twice with phosphate buffered saline (PBS) for 5 min followed by 1 h incubation with monoclonal mouse anti-human COX-2 (clone CX-294, Dako, USA) at 1:100 dilution. Secondary antibody detection system (Dako, EnVision+ System-HRP labelled, Dako, USA) was added and incubated for 30 min at room temperature. Finally, a chromogen (diaminobenzidine; Dako, USA) was used to verify the immunoreaction, followed by counterstaining using haematoxylin stain[18]. Negative control was run simultaneously using the same samples and protocol; with the primary antibody replaced with antibody diluents. Colon adenocarcinoma cases that are known to express COX-2 were used as positive control.

Evaluation of results

The COX-2 stained slides were reviewed and scored. H-score (semi-quantitative method) was used to assess COX-2 expression in the tumour cells.[18] Cytoplasmic membrane staining intensity (0, negative; 1, weak; 2, moderate; 3, strong) was evaluated and the percentage of cells at each staining intensity level was assessed. A final expression score was calculated by multiplying a staining intensity score with a proportion score of the positively stained cells. This formula produces a H-score in the range of 0-300, where 0=0% of tumour cells stained and 300=100% of tumour cells stained strongly. A score of 100 or greater was considered strong/high positivity.[19] The COX-2 stained slides were reviewed and scored under light microscope by two independent histopathologists. If the staining was weak or equivocal, the slides were re-evaluated and were regarded as negative if an equivocal result was obtained again.

Statistical analysis

The results were analysed using SPSS version 21 (IBM Company). Spearman correlation test was used to analyse the association between clear cell COX-2 expression with grades and demographic parameters of RCC.

RESULTS

Thirty-six confirmed clear cell RCC cases were selected in the study. There were 21 males and 15 females with male: female ratio of 1.4:1. Sixteen (44.4%) of the cases were Chinese, 14 (38.9%) were Malays, and 6 (16.7%) were Indian. The patients' age were between 16 to 74 years with a mean age of 56.6 years. Of the 36 RCC cases, 6 (16.7%) cases were grade 1, 20 (55.5%) grade 2, 10 (27.8%) grade 3 and none from grade 4. The grades were assigned based on the highest-grade present in the tissues. Eighteen (50%) clear cell RCC cases demonstrated strong COX-2 expression. Eighteen (50%) RCC were immunonegative toward COX-2 (Fig. 1). Table 1 illustrates the characteristics of the RCC cases in the study. Table 2 shows association between COX-2 expression and patient characteristics. There was no significant association between grade of RCC and COX-2 expression (p=0.457). No significant association between COX-2 expression (H-score) with demographic parameters (age, sex, and ethnicity) was observed in this study (p=0.054, p=0.389 and p=0.568 respectively).

DISCUSSION

RCC represents more than 90% of all renal

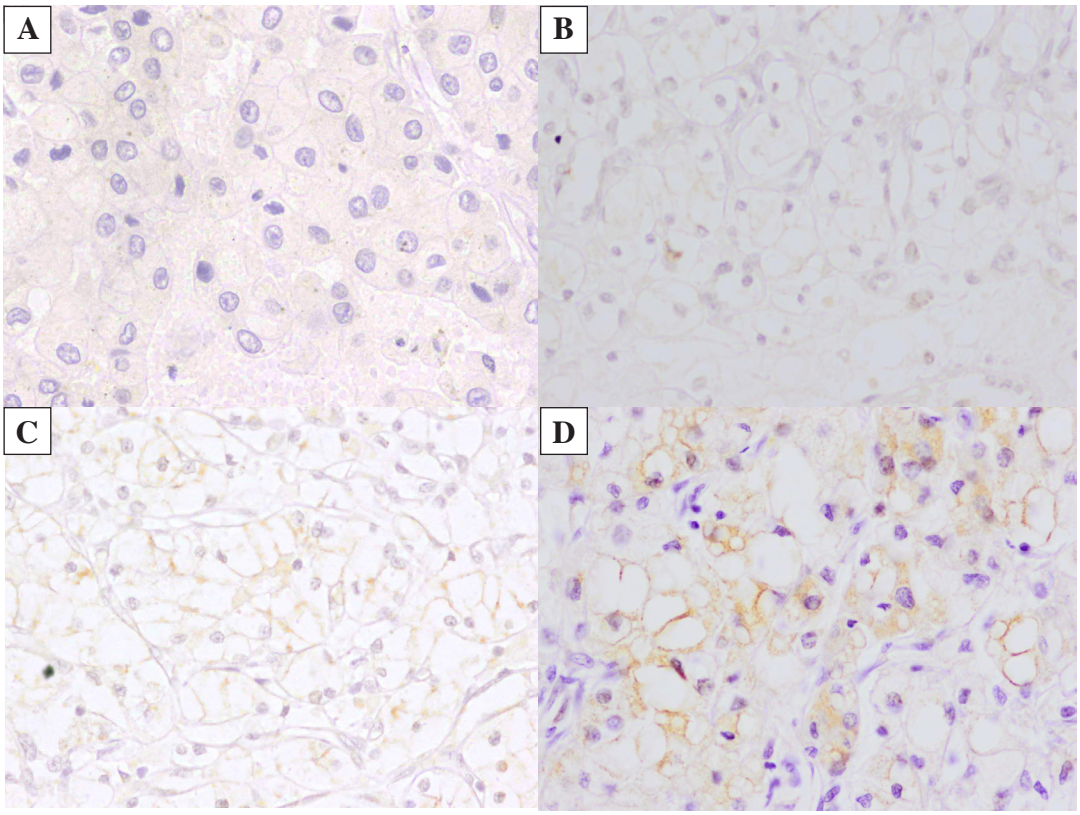

FIG. 1: COX-2 expression of clear cell renal cell carcinoma. (A) Negative, (B) 1+, (C) 2+, (D) 3+ (x200).

TABLE 1: Clinicopathological characteristics of patients with clear cell renal cell carcinoma (n = 36)

	No. of cases	%
Age		
< 50 years	8	22.2
≥ 50 years	28	77.8
Range	16-74 years	
Mean	56.6 years	
Gender		
Male	21	58.3
Female	15	41.7
Ethnic		
Chinese	16	44.4
Malay	14	38.9
India	6	16.7
Fuhrman grade		
Grade 1	6	16.7
Grade 2	20	55.5
Grade 3	10	27.8
Grade 4	0	0
COX-2 expression		
Low	18	50
High	18	50

malignancies.[20] In Malaysia, renal cancer ranked 14th most common cancer in men and 20th most common cancer in women.[21] In this study, there were more male patients than female patients with male:female ratio of 1.4:1. This finding is compatible with Malaysia National Cancer Registry report where RCC affects 2.5 of 100,000 males and 1.3 of 100,000 females.[22] Other study also showed similar trend, in which RCC was two to three times more common in men than women both in high and low risk countries.[23]

Fifty percent of clear cell RCC cases showed strong positivity towards COX-2. This finding is comparable with previous studies where 26% to 82% of RCC cases showed COX-2 expression.[8,9,16] Currently, it is widely acknowledged that COX-2 contributes to tumour development by promoting angiogenesis and then tumour invasiveness.[10-11,24-25]

COX-2 expression is regulated by many signals including mitogen-activated protein kinases (MAPK), protein kinase C (PKC), and p53. Subsequently, it regulates tumour cell proliferation, migration, and invasion.[26] Based on these observations, efforts have been made to determine the role of COX-2 in carcinogenesis

TABLE 2: Comparison between COX-2 expression and patient clinicopathological characteristics

	COX-2 expression		P-value
	Low n (%)	High n (%)	
Age			
< 50 years	4 (11.1)	4 (11.1)	0.054
≥ 50 years	14 (38.9)	14 (38.9)	
Gender			
Male	11 (30.6)	10 (27.8)	0.389
Female	7 (19.4)	8 (22.2)	
Ethnic			
Chinese	8 (22.2)	8 (22.2)	0.568
Malay	7 (19.4)	7 (19.4)	
India	3 (8.3)	3 (8.3)	
Fuhrman grade			
Grade 1	4 (11.1)	2 (5.6)	0.457
Grade 2	10 (27.8)	10 (27.8)	
Grade 3	4 (11.1)	6 (16.7)	
Grade 4	0	0	

in various malignancies,[26-30] with the expectation that COX-2 could be a novel target for cancer treatment and prevention.[24]

High COX-2 expression has been observed in canine[31] and human RCC.[8,32] There was a study which reported that increasing COX-2 expression was correlated with neoplastic changes from normal squamous epithelium, to dysplasia and to invasive oesophageal squamous cell carcinoma.[33] COX-2 was also expressed in both carcinoma *in situ* and transitional cell carcinoma of urinary bladder.[34] Hence, our studies suggested that COX-2 up-regulation has strong role in carcinogenesis.

Nuclear grade, after stage, is the most important prognostic factor of RCC.[15,17] A 4-tiered grading system has been used to assess the nuclear grade. COX-2 protein expression is associated with slow development of metastases, with favourable prognosis in metastatic RCC.[16] Nevertheless, our study did not show association between COX-2 expression and tumour grades. Cho *et al*. (2005) reported similar findings where COX-2 expression was insignificantly associated with tumour grade.[9] Hara *et al*. (2002) and Yoshimura *et al*. (2004) also found no association between COX-2 expression with tumour stage and grade.[28,35] Nonetheless, Miyata *et al*. (2003) reported that COX-2 expression was significantly associated with tumour grade and stage.[8]

In many cancers, COX-2 up-regulation is associated with disease progression and poor survival.[36-38] Conflicting results on the role of COX-2 in the disease progression and overall survival have been reported.[16,39] The prognostic outcome might be associated with the role of COX-2 in apoptosis résistance, cell proliferation, and angiogenesis *in vivo*.[40,41]

No significant relationship was seen between the COX-2 expression with the age and sex of the patients. Our findings were comparable with the studies conducted by Tuna *et al*. (2004) and Tabrizi *et al*. (2016).[42,43] No similar studies were conducted to compare ethnicity with COX-2 expression.

The limitations in our study are the relatively small sample size and the lack of comparison with a normal kidney. Other factors that lead to inconsistent findings among the studies include different scoring method, type of antibody, processing and staining techniques used. In conclusion, COX-2 might be associated with development and progression of RCC. Our results might contribute to the efforts to develop COX-2 inhibitor as an alternative therapy. However, further studies are needed to elucidate the association of COX-2 in RCC.

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