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Immunohistochemical expression of Cyclooxygenase-2 in colorectal carcinoma and its association with clinicopathological parameters

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Abstract

Background and objective: Inflammatory process and genetic factors play a key role in neoplasia of colorectal cancer. Cyclooxygenase-2 is involved in a variety of important cellular functions, including cell growth and differentiation, cancer cell motility and invasion, angiogenesis, and immune functions. This study aimed to evaluate the frequency of cyclooxygenase-2 in colorectal cancer and its correlations with different clinicopathological parameters.

Methods: A study carried out in the Department of Pathology in Rizgary Teaching Hospital and private laboratories in Erbil city from May 2015 to February 2016. Eighty formalin-fixed paraffin-embedded specimens of colorectal cancers were collected. The clinicopathological data was collected by the researcher from records of known cases of colorectal carcinoma in Erbil city and was done in a private laboratory in Dohuk city.

Results: High cyclooxygenase-2 expression was represented by 63.8% of colorectal carcinoma patients, and low expression was represented by 36.2% of them. There was a significant association between the mucinous subtype of colorectal carcinoma and low cyclooxygenase-2 expression (P = 0.01). A significant association was observed between moderately differentiated carcinoma and high cyclooxygenase-2 expression (P = 0.04). A significant association was also noted between increased tumor size and high cyclooxygenase-2 expression (P = 0.03).

Conclusion: Cyclooxygenase-2 expression is more likely to be prevalent among colorectal carcinoma patients and is correlated to tumor subtypes (low in mucinous subtypes), grade (high in moderately differentiated), and tumor size (high in the big sized tumor).

Keywords: Cylooxygenase-2; Colorectal carcinoma; Immunohistochemistry; Histopathology.

Introduction

Cancer of the large bowel continues to be one of the major public health problems, ranking as the second or third cause of cancer related death in many western countries with more than one million new cases diagnosed each year.[1] It is the second leading cause of cancer death in the United States.[1] According to the Iraqi cancer registry, colorectal cancer ranked seventh among the commonest ten cancers cases reported.[2] In the last few years, there have been many studies on the mechanisms involving the carcinogenesis of colorectal cancer. Inflammatory process and genetic factors play a key role in neoplasia of colorectal cancer.[3] The identification of an enzyme cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), catalyzing fatty oxidation as rate limiting step in progress from normal cell growth through hyperplasia or to neoplasia has opened up a whole new field of cancer search.[4] The up regulation of COX-2 prolongs the survival of abnormal cells and thereby

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favors the accumulation of sequential genetic changes, which increases the risk of tumor genesis.[5] However, the over-expression of COX-2 protein in colorectal cancers is likely to occur via several different mechanisms involving complex signaling pathways. Transformed epithelial cells and stromal cells, such as mononuclear cells, fibroblasts, endothelial cells and smooth muscle cells have been shown to express increased levels of COX-2.[6] COX-2 is involved in a variety of important cellular functions, including cell growth and differentiation, cancer cell motility and invasion, angiogenesis and immune functions.[3] The role of angiogenesis is very important in colorectal cancer and is a significant angiogenic factor in colorectal cancer tissue.[7] Cox-2 is over expressed in 80-90% of colorectal adenocarcinoma, mostly within the neoplastic epithelial cells. Selective inhibition of Cox-2 reduces colorectal cancer in different models of carcinogenesis.[8] This study aimed to evaluate the frequency of COX-2 immunoexpression in colorectal cancer and its correlations with different clinicopathological parameters.

Methods

A cross sectional study was conducted after the permission was granted by Kurdistan Board of Medical Specialties. A sample of 80 formalin-fixed paraffin-embedded specimens of colorectal cancers was collected by the researcher from records of known cases of colorectal carcinoma in the Department of Histopathology in Rizgary Teaching Hospital and private laboratories in Erbil city from May 2015 to February 2016. Sections were made and stained with Hematoxylin and Eosin H&E for reevaluation concerning the type and the grade of the tumor, lymphovascular invasion, lymph node status, and tumor stage. The information was obtained from the patient's records. Grading and TNM staging were done according to established criteria.[9]

Immunohistochemical method

Labeled polymer and enhanced polymer systems (DakoEnVision™ Flex) method, according to Dako recommendation, was used to stain the tissue by COX-2 antibody. The staining steps and incubation times are pre-programmed into the software of Dako autostainer link 48, where substrate Buffer, Envision TM FLEX Peroxidase-Blocking Reagent (as endogenous enzyme block), FLEX Anti-COX-2 Ready-to-Use (as primary antibody), Envision TM FLEX+ Mouse linker (as secondary reagent), EnVision/ HRP (as labeled polymer), DAB+ Chromogen (as substrate Chromogen), Envision TM FLEX hematoxylin (as counterstain) and distilled water were applied on the slides. Then slides removed from the autostainer link 48 and put in graded ethanol (70%,100%,100%, 2x2x2 minutes), respectively, then in xylene (2 minutes). The slides were mounted in Canada Balsam and examined under a light microscope. Positive and negative control slides were involved in each run of staining. Positive controls for COX-2 include sections of COX-2 positive colonic adenocarcinoma, while negative control slides were prepared from the same tissue block, but incubated with Tris Buffered Saline (TBS) instead of the primary antibody.

COX-2 immunoscore measurement

The slides were evaluated under the light microscope. In cancer cells, COX-2 expression was observed mainly in the cytoplasm and the nuclear envelope. The scoring system used in this study was based on the study of Almhanna et al.,[10] which divided the cases into two groups named as low COX-2 expression and high COX-2 expression. Scoring was established using a 0–6 scale based upon the sum of a percentage of stained cells score and intensity score. The intensity of staining was scored as 0 (negative), 1 (weak), and 2 (strong). Percentage of staining was scored as 0 (0%),

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1 (1-10%), 2 (11-50%), and 3 (> 50%),
Score 0-3: Low cox-2 expression
Score 4-6: High cox-2 expression
In cases of multifocal variable immune-reactivity, the average of the least intense and most intense staining was recorded. Sections in which the staining could not be well characterized were considered equivocal and were repeated.

Statistical Analysis

The collected data were analyzed using the statistical package for the social sciences (version 20). Multiple contingency tables were used, and appropriate statistical tests were performed. Chi-square was used for categorical variables, while Fisher's exact test was used when the expected count of more than 20% of the cells of the table was less than 5. In all statistical analyses, the level of significance (*P* value) was set at ≤0.05.

Results

Eighty colorectal carcinoma samples were included in the present study. Immune staining was performed for all of the cases, and all were included in the statistical analysis. The mean age was 53.7±15.2 years, 40% of them were aging ≥60 years. Female to male ratio was 1.2:1. The other clinicopathological data of the patients are summarized in Table 1.

Table 1: Clinicopathological data of the study sample.

Variables	Categories	No.	%
Clinical presentation	Rectal bleeding	28	35.0
	Obstruction	49	61.2
	Change in bowel habits	3	3.8
Tumor site	Colon(unspecified)	33	41.3
	Right side colon	6	7.5
	Left side colon	35	43.8
	Rectosigmoid area	6	7.5
Tumor size	<5 cm	21	26.2
	≥5 cm	59	73.8
Tumor subtype	Non specific	73	91.3
	Signet ring	1	1.3
	Mucinous	6	7.5
Tumor grade	Poorly differentiated	12	15.0
	Moderately differentiated	61	76.2
	Well differentiated	7	8.8
Lymphovascular invasion	Positive	31	38.8
	Negative	49	61.2
Tumor stage	I	10	12.5
	II	20	25.1
	III	47	58.7
	IV	3	3.7
Total	-	80	100

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The COX-2 expression was observed mainly in the cytoplasm and the nuclear envelope of the tumor cells. High COX-2 expression was present among 63.8% of patients, as shown in Figure 1, while 36.2% of them showed low COX-2 expression, as shown in Figure 2, with a mean COX-2 score of 4.5±1.8. The correlations between COX-2 expression and clinicopathological parameters of patients are illustrated in Table 2. A statistically significant association was found between the mucinous subtype of colorectal carcinoma and low COX-2 expression (P = 0.01). In addition, a significant association was observed between moderately differentiated carcinoma and high COX-2 expression (P = 0.04). A significant association was also noted between increased tumor size and high COX-2 expression (P = 0.03). COX-2 overexpression showed no statistically significant association with clinical presentation, tumor site, lymphovascular invasion and tumor stage.

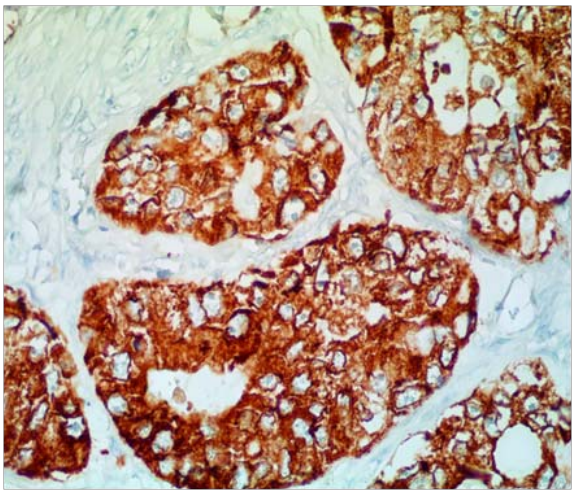

Figure 1: High COX-2 expression (IHC x400).

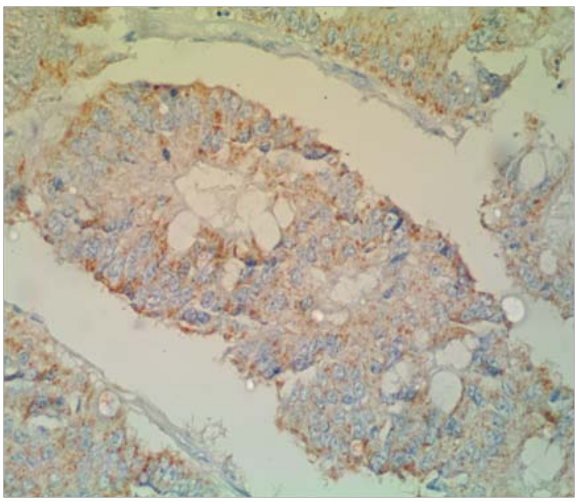

Figure 2: Low COX-2 expression (IHC x400).

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Table 2: The correlation between COX-2 overexpression and clinicopathological parameters.

Variables	Categories		COX-2		*P* value
			Low expression No. (%)	High expression No. (%)	
Clinical presentation	Rectal bleeding	28	11(39.3%)	17(60.7%)	0.4
	Obstruction	49	18(36.7%)	31(63.3%)	
	Change in bowel habits	3	0(0%)	3(100%)	
Tumor site	Colon(unspecified)	33	11(33.3%)	22(66.7%)	0.6
	Right side colon	6	1(16.7%)	5(83.3%)	
	Left side colon	35	14(40.0%)	21(60.0%)	
	Rectosigmoid area	6	3(50.0%)	3(50.0%)	
Tumor size	<5 cm	21	12(57.1%)	9(42.9%)	0.03
	≥5 cm	59	17(28.8%)	42(71.2%)	
Tumor Subtype	Non specific	73	23(31.5%)	50(68.5%)	0.01
	Signet ring	1	1(100%)	0(0%)	
	Mucinous	6	5(83.3%)	1(16.7%)	
Tumor grade	Poorly differentiated	12	8(66.7%)	4(33.3%)	0.04
	Moderately differentiated	61	18(29.5%)	43(70.5%)	
	Well differentiated	7	3(42.9%)	4(57.1%)	
Lymphovascular invasion	Positive	31	12(38.7%)	19(61.3%)	0.7
	Negative	49	17(34.7%)	32(65.3%)	
Tumor stage	I	10	4(40.0%)	6(60.0%)	0.5
	II	20	6(30.0%)	14(70.0%)	
	III	47	17(36.2%)	30(63.8%)	
	IV	3	2(66.7%)	1(33.3%)	

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Most of the tumor sizes were equal to or more than 5 cm (59 cases), of which 42 (71.2%) showed high expression of COX-2 with a statistically significant association (*P* = 0.03). Forty three out of 61 of cases were moderately differentiated adenocarcinoma with 70.5% showed high expression and 73 cases (91.3%) were conventional adenocarcinoma, NOS including 68.5% with high expression and their association with COX-2 expression were significant (*P* = 0.04 and 0.01 respectively). The commonest presentation of patient was intestinal obstruction (49 cases), of which 31(63.3%) showed high expression, followed by rectal bleeding (28 cases) with 17(60.7%) showed high expression. The least presenting symptom was a change in bowel habit (3 cases with 100% high expression), and all showed no significant association with COX-2 expression. Most of the tumors were located in left side colon (35 cases), of which 60.0% showed high COX-2 expression with no significant association. Most of the patients were negative for lymphovascular invasion (49 cases) rather than positive (31 cases, of which 65.3% showed high expression of COX-2 with no significant association. Regarding tumor stage, stage III was the highest stage (47 cases), of which 30 (63.8%) showed high expression, followed by stage II and I, and the least was stage IV, and the association with COX-2 expression was not significant.

Discussion

Colorectal adenocarcinoma is one of the most frequent malignant diseases worldwide.[11,12] Cyclooxygenase-2 expression is a frequent but not universal event in colorectal cancers, and it has been reported to be significantly increased in up to 85% of human sporadic colorectal adenocarcinomas.[11] In the present study, high COX-2 expression was present among 63.8% of CRC patients. This finding is close to the results of Kazemet al.[12] study in Egypt, which reported that 67.6% of CRC patients had a high COX-2 expression. This rate of high COX-2 expression is higher than that reported by Hussein et al.[13] study in Iraq in which the rate of COX-2 high expression was 42.5%. This difference might be attributed to differences in study methodology and differences in the technical aspects of COX -2 recording and possibly due to genetic and environmental factors.[12,13] The current study showed that there is no relationship between COX-2 expression and patients' age, gender and exact tumor site. This finding is consistent with results of Wu and Sun study[14] in China which stated that CRC patients' age, gender and tumor site were not significantly correlated with COX-2 expression. It has been reported that there were higher levels of COX-2 expression in patients with rectal cancer compared to patients whose tumors were located in other parts of the large bowel, possibly due to local variability in gene regulatory factors responsible for COX-2 expression.[13] Some other studies found no correlation between the location of colorectal tumor and COX-2 expression.[15] Our study found a significant association between increased tumor size and high expression of COX-2 (*P* = 0.03). This is consistent with the results of Wu and Sun[14] study, which found 81.5% of CRC with large tumor size showed COX-2 over-expression. In this study, there was a significant association between the mucinous subtype of CRC and low COX-2 expression (*P* = 0.01). This is consistent with the results of Kazem et al.[12] study in Egypt. With regard to the tumor grade, a significant association was observed between moderately differentiated carcinoma and high COX-2 expression (*P* = 0.04). This is consistent with the results of Masunaga et al.[16] in Japan and Yang et al.[17] in China, which found a significant association between moderately differentiated CRC and high expression of COX-2. In agreement with others,[18] no significant correlation was found in this work between COX-2 expression and whether the tumor is lymph

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node metastasis or staging. However, others reported that COX-2 expression is correlated with lymph node involvement and staging.[19] These discrepancies may be related to the different scoring systems and antibodies employed in immunohistochemistry. Our results suggest that increased expression of COX-2 plays a role in colorectal carcinogenesis, although the exact molecular mechanism of COX-2 over-expression is still unknown. Several studies have reported that COX-2 overexpressing tumor cells may regulate angiogenesis, inhibit apoptosis or enhance invasiveness by producing inflammatory prostaglandins.[21,22] Some studies have shown that selective COX-2 inhibitors prevent adenoma recurrence among patients with a prior history of adenoma. These studies support that there is an important role of COX-2 overexpression in colorectal carcinogenesis.[23,24] However, the role of COX-2 overexpression in determining the biologic behavior of CRC is still controversial. Previous reports are conflicting regarding the prognostic significance of COX-2 overexpression in CRC, some support and others refute that COX-2 overexpression has an independent adverse effect.[25,26] Likewise, no significant association was observed between COX-2 expression and the clinical presentation. This is consistent with a previous meta-analysis study,[28] which found no significant relationship between CRC clinical presentation and COX-2 expression.

Conclusion

COX-2 high expression is more likely to be prevalent among colorectal carcinoma patients and correlated to tumor subtype (low in mucinous carcinomas), grade (high in moderately differentiated carcinomas), and tumor size (high in big size carcinomas).

Competing interests

The authors declare no competing interests.

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