The expression of Matrix metalloproteinase-2 and cyclooxygenase-2 by immunohistochemistry in patients with colorectal carcinoma

Areej A. Hussein* **BSc; MSc, PhD**
Basim M. Ibrahim **BSc; MSc**
Basim M. Kashman **BSc; MSc**

Summary:

Background: Human colorectal carcinogenesis is a complex, multistep and multigenetic process. Matrix metalloproteinase-2 and cyclooxygenase-2 are key enzyme in degradation of extracellular material, are over expressed in several epithelia like colorectal adenocarcinoma.

Objectives: This study was designed to detect the expression of matrix metalloproteinase-2 and cyclooxygenase-2 in patients with colorectal carcinoma and their correlation with age, gender, tumor grade and presence or absence of muscle invasion.

Materials and methods: Immunohistochemical staining of MMP-2 and COX-2 was determined in 40 tissue samples from colorectal patients, from teaching laboratories in Baghdad medical city. In addition twelve apparently normal colorectal autopsies were used as a control group.

Results: The expression of matrix metalloproteinase-2 was detected in 30 of 40 colorectal cancer cases (75%) while cyclooxygenase-2 was detected in 31 0f 40 colorectal cancer cases (77.5%). All normal colorectal specimens showed negative results. The incidence of both of them was higher for grade II than those in earlier grade.

Conclusion: Cancer cells to have a more aggressive behavior may show an up regulation of tumor cell derived matrix metalloproteinase-2 and cyclooxygenase-2.

Fac Med Baghdad 2011; Vol. 53, No. 3
Received Jan. 2011
Accepted May 2011

Keywords: Colorectal neoplasm, Matrix metalloproteinase-2, Cyclooxygenase-2, Metastasis

Introduction:

Colorectal cancer is the third leading cause of cancer-related death in world [1]. According to Iraqi cancer registry. Human Colorectal Cancer ranked seventh among the commonest ten cancers cases reported, accounting for 392 and 254 in males and females respectively [2]. Unfortunately, the peak incidence for colorectal cancer is 60-70 years of age and highest among Chinese. Colorectal cancer, including the rectum, is the host to more primary neoplasms than any other organ in the body. About 90% of the colon cancers are characterized as adenocarcinoma whereas the other 10% are mucinous adenocarcinomas [3]. Matrix metalloproteinase (MMP)-2 (72-kDa gelatinase A, type IV collagenase) is zinc-dependent endopeptidases belonging to the large family of MMPs [4]. Matrix metalloproteinase-2 and MMP-9 are potent proteolytic enzymes and play a role in tumor invasion, metastasis, and neovascularization based their capacity to remodel tissue via extracellular matrix and basement membrane degradation. It has long been suggested that cancer cells likely produce their own proteolytic enzymes or recruit them from host cells in an effort to degrade extracellular matrix for migration and invasion of the surrounding tissue [5].

* *Dept. of Microbiology, College of Medicine, Diyala Unversity.*
** *Dept. of Oral Pathology, College of Dentist, University of Baghdad.*
*** *Dept. of Microbiology, College of Medicine, University of Baghdad.*

Cyclooxygenase-2 (COX-2), a key enzyme in arachidonic acid metabolism, is over expressed in several epithelial malignancies including colorectal cancer [6]. As a pro-inflammatory enzyme, COX-2 expression can be induced by pro-inflammatory and mitogenic stimuli. Emerging evidence demonstrates that chronic inflammation is related to colorectal neoplasia and that up-regulation is believed to be an early event in colorectal carcinogenesis [5]. The present study was undertaken to investigate the correlation of MMP-2 and COX-2 expression with different clinico-pathological variables like: age, gender, grade and presence or absence of muscle invasion in colorectal carcinoma.

Materials and methods:

Patients and specimens.

A total of 40 colorectal carcinoma samples were obtained from the archives of the Department of Pathology, teaching laboratories in Baghdad medical city, from February 2008 to October 2010. Samples were derived from patients who were solely surgically treated. There were 21 men (52.5%) and 19 women (47.5%) with a range 25- 89 years (58.0 ± 10.5), 22 patients with colon and 18 patients with rectal tumors. Twenty seven patients (67.5%) had histologically confirmed lymph node metastases, whereas the remaining 13 patients (32.5%) were found to have no clinical or histopathologic evidence of lymph node involvement. According to the World Health

Organization classification, tumors of 10 (25%) patients were well differentiated adenocarcinoma, that of 26 patients (65%) were moderately differentiated adenocarcinoma and that of 4 (10%) patients were poorly differentiated adenocarcinoma.

Immunohistochemistry. Tissues were fixed in 10% buffered formalin, processed, and stained with Hematoxylin and Eosin (H and E). Hematoxylin and Eosin-stained slides of all samples were reviewed to confirm the diagnosis. One paraffin block with the bulk of tumor tissue was used for immunohistochemical studies. Sections, 4 microns-thick, of formalin-fixed paraffin embedded tissues were cut and mounted on positive charched slides. The sections were deparaffinized in Xylene and rehydrated in a descending Ethanol series. Heat induced epitope retrieval techniques were used for antigen retrieval as follows: citrate buffer (pH 6.0) and a water bath at 95°C-98°C for 30 minutes. Sections were incubated for 10 minutes in 3% hydrogen peroxide to quench endogenous tissue peroxidase. Then drop of protein block were added at 37°C for 5 minutes. The sections were immunostained with a monoclonal antibody (Clone MMP-2,CA-4001/CA719E3C,ab 3158) at a 1:40 dilution directed against MMP-2 and (clone COX-2, ab 10940-500, Ab.com.UK) at a dilution of 1:50 directed against COX-2 as recommended by the manufacturer instruction (Cambridge Science Park. England, cat. number ab 64259, kit contents). Tissue sections were incubated with the primary antibody over night at 4°C. After washing with phosphate-buffered saline, a Super picture secondary antibody were used 10 minutes at room temperature. Followed by the addition of the streptavidin-HRP for 10 minutes at room temperature. After washing with phosphate-buffer saline, the samples were treated with substrate chromogen solution for 10 minutes at room temperature, then counterstained with hematoxylin for 30 sec. Slides were washed well in running tap water for 5 minutes, then dehydrated by serial 70%, 80%, 95%, 100% ethanol and xylene then mounted with permanent-mounting medium (DPX) and the examining and scoring were done under light microscope by a pathologist at power 400 according to the scoring system of [7] [Score 1, less than 10 %, Score 2, more than 10 % and less than 50% and Score 3, more than 50 %].

Statistical analysis. Was performed using the Chi-square with Fischer exact test.

P value < 0.05 was regarded as significant.

Results:

The current results revealed a significant increased in the cellular expression of MMP-2 and COX-2 among the 40 investigated colorectal adenocarcinoma as showed in Table (1) and Figure (1) and (2), which demonstrated that 30 cases (75%) of colorectal adenocarcinoma cases were positive for MMP-2, while 31 cases (77.5%) of colorectal adenocarcinoma cases were positive for COX-2. On the other hand there was no positive result among control groups.

Table (1): The Expression of MMP-2 and COX-2 in Patients with Colorectal Adenocarcinoma.

Result of Immunohistochemistry			MMP-2 Expression	COX-2 Expression	Comparison of Significance	
					p- value	Sig.
Patients	Positive	N %	30 (75%)	31 (77.5%)	0.0005	Highly Sig. P<0.01
	Negative	N %	10 (25%)	9 (22.5%)		
	Total	N %	40 (100%)	40 (100%)		
Control	Positive	N %	0	0		
	Negative	N %	12(100%)	12 (100%)		
	Total	N %	12(100%)	12 (100%)		

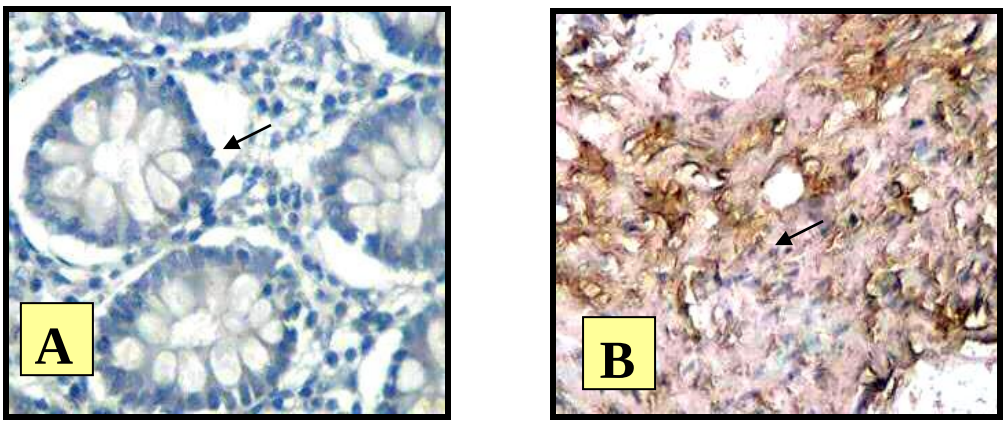

Figure (1):Immunohistochemistry for MMP-2 in colorectal adenocarcinoma tumor section, stained by DAB chromogen and counter stained with heamatoxylin is shown as reddish brown in positive cases (magnification power, 400X). A-Negative expression, B- MMP-2 positive expression,

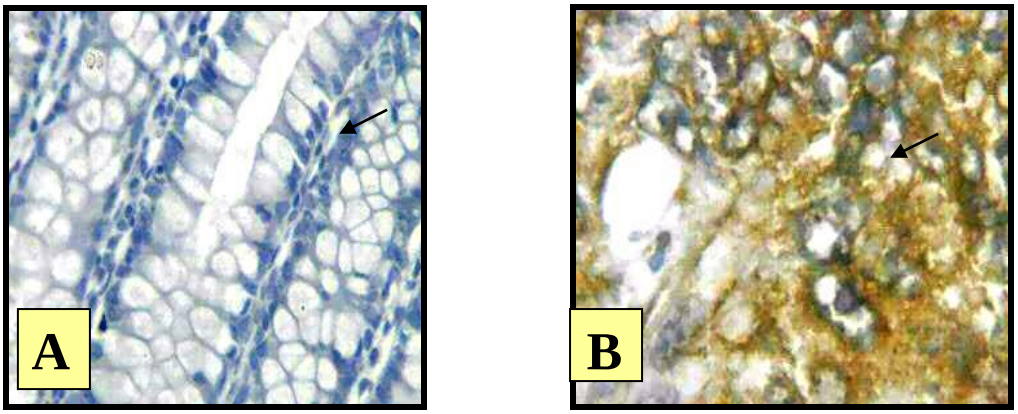

Figure (2):Immunohistochemistry for COX-2 in colorectal adenocarcinoma tumor section, stained by DAB chromogen and counter stained with heamatoxylin is shown as radish brown in positive cases (magnification power, 400X). A-Negative expression, B- COX-2 positive expression,

Table (2), (3), (4) and (5) demonstrated the correlation between expression of MMP-2 and COX-2, with different variables. The results showed that there were no significant differences between immunohistochemistry expression of both MMP-2 and COX-2 with age, grade, and muscle invasion while significant correlation with gender based on Chi-square test of analysis was detected.

Table (2): Immunohistochemistry expression of positive and negative MMP-2 as related to clinicpathological profile of patients with colorectal adenocarcinoma.

Variables		MMP-2 positive	MMP-2 negative	Comparison of Significance	
				p-value	Sig.
Age	<= 50	9 (45%)	11 (55%)	0.14	Non Sig. (P>0.05)
	> 50	21 (65.6%)	11 (34.4%)		
Gender	Male	0	5 (100%)	0.03	Sig. (P<0.05)
	Female	13 (50 %)	13 (50 %)		
Tumor grade	I	8(80%)	1(20%)	0.46	Non Sig. (P>0.05)
	II	20(76.9%)	6(23.1%)		
	III	2(50%)	2(50%)		
Muscle invasion	Invasive	20(74.1%)	7(25.9%)	0.84	Non Sig. (P>0.05)
	Non invasive	10(76.9%)	3(23.1%)		

Table (3): Immunohistochemistry expression of positive and negative COX-2 as related to clinicpathological profile of patients with colorectal adenocarcinoma.

Variables		MMP-2 positive	MMP-2 negative	Comparison of Significance	
				p-value	Sig.
Age	<= 50	10 (50%)	10 (50%)	0.26	Non Sig. (P>0.05)
	> 50	21(65.6%)	11 (34.4%)		
Gender	Male	0	5 (100%)	0.01	Sig. (P<0.05)
	Female	15 (57.7%)	11 (42.3%)		
Tumor grade	I	8 (80%)	2(20%)	0.38	Non Sig. (P>0.05)
	II	21(80.8%)	5(19,2%)		
	III	2(50%)	2(50%)		
Muscle invasion	Invasive	21 (77.8%)	6(22.2%)	0.95	Non Sig. (P>0.05)
	Non invasive	10(76.9%)	3(21.3%)		

Table (4): Correlation of MMP-2 scores as related to different parameters.

Parameters		MMP-2 scores			Comparison of Significance	
		Low	Intermediate	High	P-value	Sig.
Age	<= 50	2(22.2%)	3(27.3%)	4(40%)	0.67	Non Sig. (P>0.05)
	> 50	7(77.8%)	8(72.7%)	6(60%)		
Gender	Male	5(55.6%)	7(63.6%)	5(50%)	0.81	Non Sig. (P>0.05)
	Female	4(44.4%)	4 (36.4%)	5(50%)		
Tumor grade	I	4(44.4%)	2(18.2%)	2(20%)	0.18	Non Sig. (P>0.05)
	II	5 (56.5%)	9(81.8%)	6(60%)		
	III	0	0	2(20%)		
Muscle invasion	Invasive	7(77.8%)	6 (54.4%)	7(70%)	0.52	Non Sig. (P>0.05)
	Non invasive	2(22.2%)	5 (45.5%)	3(30%)		

Table (5): Correlation of COX-2 scores as related to different parameters.

Parameters		COX-2 scores			Comparison of Significance	
		Low	Intermediate	High	P-value	Sig.
Age	25-39	2(28.6%)	2(28.6%)	6(35.3%)	0.97	Non Sig. (P>0.05)
	55-70	5(71.4%)	5 (71.4%)	11(64.7%)		
Gender	Male	3(42.9%)	4(57.1%)	9(52.9%)	0.08	Non Sig. (P>0.05)
	Female	4(57.1%)	3 (42.9%)	8(41.1%)		
Tumor grade	I	2(28.6%)	0	6(35.3%)	0.34	Non Sig. (P>0.05)
	II	5 (71.4%)	6(85.7%)	10(58.8%)		
	III	0	1(14.3%)	1(5.9%)		
Muscle invasion	Invasive	6(85.7%)	5 (71.4%)	10(58.8%)	0.68	Non Sig. (P>0.05)
	Non invasive	1(14.3%)	2 (28.6%)	7(41.2%)		

Discussion:

Matrix metalloproteinase's (MMPs) are involved in cellular proliferation, migration, invasion and metastasis [8]. Matrix metalloproteinases-2 and MMP-9 are thought to play a central role in these processes, in view of their ability to degrade many Extracellular Matrix (ECM) components and other substrates [9]. In general, the role of MMPs in carcinogenesis seems to be very complex, sometimes even controversial, according to some preclinical findings [10].The results of present study had demonstrated that MMP-2 was over expressed in colorectal adenocarcinoma. These results might possibly reflect the association between cellular expression of MMP-2 and colorectal adenocarcinoma tumor genesis. This was in agreement with the findings of Mook, Bjo¨rklund and Jung-Cheng. Since they found over expression of this enzyme in colorectal adenocarcinoma. Also this result was in line with other reports indicating that MMP production in colorectal adenocarcinoma was present in both tumor stroma cells[13][14][15]. This raises the question why MMPs expression was rare in benign tumor, we know that benign tumor have no metastasis and no invasion, so that there is no need for additional degradation of ECM, and finally no need for exaggerated MMPs expression. In fact, analysis of both primary and metastatic tumors demonstrated increased MMPs at the metastatic site had pointed out their role in tumor migration and spread [16]. In this study, the results showed no correlation between MMP-2 expressions and age, grade and muscle invasion while significant correlation with gender observed. In the present study the results were in agreement with [17] Who did not find any association between the expression of MMP-2 immunoreactions protein in colorectal adenocarcinoma cancer tissue and age, grade muscle invasion. While disagreement with most studied which mention the higher incidence rate of colorectal adenocarcinoma occurring in males than females. The differences in the time trends in colorectal cancer in males and females could be explained by cohort effects in exposure to some gender-specific risk factor; one possibility that has been suggested is exposure to estrogens[18]. There is, however, little evidence of an influence of endogenous hormones on the risk of colorectal cancer. In contrast, there is evidence that exogenous estrogens such as hormone replacement therapy (HRT), tamoxifen, or oral contraceptives might be associated with colorectal tumors [19]. Cyclooxygenase-2, the inducible isoform, is regularly expressed at low levels in colonic mucosa. Cyclooxygenase-2 may contributes to carcinogenesis and the malignant phenotype of tumor cells by inhibiting apoptosis, increasing angiogenesis, increasing invasiveness, modulating inflammation or immuno-suppression and conversion of procarcinogens to carcinogens[20]. COX-2 mRNA expression detected at higher levels than in normal colorectal mucosa[21]. The current result revealed a significant increase in the cellular expression of COX-2 among colorectal

adenocarcinoma patients. These results were in agreement with previous studies by Eberhart, Kargman and Williams who reported that COX-2 is highly expressed in colorectal adenocarcinoma and with the result of Maihofner *et al.*, (2003) who detected of COX-2 in 80-90% of colorectal adenocarcinomas. This findings conclude that MMP-2 and COX-2 are expressed at higher level during colorectal tumorogenesis. Therefore, MMP-2 and COX- 2 and may contribute to the early stage of development of colorectal adenocarcinoma.

References:

1-*Cao H, Zhong X, Hap L, Xiao-ding L, and Shao-lin L: The-765C allele of the cyclooxygenase-2 gene as a potential risk factor of colorectal cancer: A meta-analysis: Tohoku J. Exp. Med., 2010, 222, 15-21.*

2-*Iraq Cancer Board, results of Iraqi Cancer Registry (1999-2004), Ministry of health, Iraq Cancer Registry Centre, Baghdad - Iraq, 2008.*

3-*Mohd. NS, Boo SS, Wan KW, Azlina Z, Norashikin S and Thuaibah H: Detection and Localization of Anti and Pro-apoptotic Mrna Genes in Human Colorectal Cancer Using in situ RT-PCR. American Journal of Biochemistry and Biotechnology, 2010; 6 (3): 164-171*

4- *Jung-Cheng K, Jin-Shuen C, Chien-Hsing L, Jao-Jen Chang and Yi-Shing S: Intratumoral Macrophage Counts Correlate with Tumor Progression in Colorectal Cancer. J. Surg. Oncol. 2010; 102: 242-248.*

5-*Noe¨l A, Jost M, Maquoi E: Matrix metalloproteinases at cancer tumor–host interface. Semin. Cell Dev. Biol. 2008; 19:52–60.*

6-*Chan AT, Ogino S and Fuchs CS: Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N. Engl. J. Med. 2007; 356:2131-2142.*

7-*De Vicente J C, Manuel F F, Lucas V, Jose A V, and Gonzalo H V: Expression and clinical significance of matrix metalloproteinases-2 and matrix metalloproteinases-9 in oral squamous cell carcinoma. Oncology. 2004;8:13.*

8-*Smola-Hess S., Jenny P, Cornelia M, Paola Z, Hans S and Herbert JP: Expression of membrane type 1 matrix metalloproteinase in papillomavirus-positive cells: role of the human papillomavirus (HPV) 16 and HPV8 E7 gene products. J. Gen. Viral. 2005; 86: 1291-1296.*

9-*Katori H, Akinori N and Mamoru T: Increased Expression of Matrix Metalloproteinase-2 and 9 and Human Papilloma Virus Infection Are Associated with Malignant transformation of sinonasal Inverted Papilloma. J. Surg. Oncol. 2006; 93: 80-85.*

10-*Deryugina, EI and Quigley JP: Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rec. 2006; 25:9-34*

11-*Mook OR, Frederiks WM, Van Noorden CJ: The role of gelatinases in colorectal cancer progression and metastasis. Biochim. Biophys Acta. 2004; 1705: 69-89.*

12-*Bjo¨rklund M, Koivunen E: Gelatinase-mediated migration and invasion of cancer cells. Biochim Biophys Acta. 2005; 1755: 3-69.*

13-*Papadopoulou S, Scorilas A, Arnogianaki N and et al.: Expression of gelatinase-A (MMP-2) in human colon cancer and normal colon mucosa. Tumor Biol. 2001; 22: 383-389.*

14-*Lubbe WJ, Zhou ZY, Fu W and et al.: Tumor epithelial cell matrix metalloproteinase 9 is a target for antimetastatic therapy in colorectal cancer. Clin. Cancer Res. 2006; 12:1876-1882.*

15-*Hersze´nyi L, Sipos F, Galamb O and et al.: Matrix metalloproteinase-9 expression in the normal mucosa-adenoma-dysplasiaadenocarcinoma sequence of the colon. Pathol Oncol Res 2008; 14:31–37.*

16-*Sutinen M, Kainulainen T, Hurskainen T and et al.: Expression of matrix metalloproteinases (MMP-1 and -2) and their inhibitors (TIMP-1, -2 and -3) in oral lichen planus, dysplasia, squamous cell carcinoma and lymph node metastasis. Br J Cancer 1998; 77: 2239-2245.*

17*-Al-Ibrahimi, S H M: Role of matrix metalloproteinase-2 and 9 in situ mRNA expression and immunohistochemical staining of PECAM-1 and VWF based intratumoral microvessel density during colorectal tumor progression. M.Sc. Thesis, College of Medicine, Al-Nahrain University, Iraq; 2005.*

18*-Dos Santos S I, Swerdlow AJ: Sex differences in time trends of colorectal cancer in England and Wales: the possible effect of female hormonal factors. Br. J. Cancer 1996; 73 (5): 692-697.*

19*- Beral V, Banks E, Reeves G, Appleby P: Use of HRT and the subsequent risk of cancer. J Epidemiol Biostat.1999; 4 (3): 191-210.*

20*-Dempke, W, Rie C, Grothey A and Schmoll HJ: Cyclooxygenase-2: A novel target for cancer chemotherapy. J. Cancer Res. Clin. Oncol. 2001; 127: 411-417.*

21-*Hasegawa K, Ichikawa W, Fujita T, Ohno R and Okusa T and et al.,: Expression of Cyclooxygenase-2 (COX-2) mRNA in human colorectal adenomas. Eur. J. Cancer 2001; 37: 1467-1474.*

22-*Eberhart, CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S and et al.: Up regulation of cyclooxygenase-2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology.1994; 107: 1183-1188.*

23-*Kargman SL, O'Neill GP, Vickers PJ, Evans J and Mancini F and et al.,: Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. Cancer Res 1995; 55: 2556-2559.*

24-*Williams CS; Luongo C, Radhika A, Zhang T and Lamps LW and et al.,: Elevated cyclooxygenase-2 levels in min mouse adenomas. Gastroenterology. 1996; 111: 1134-1140.*

25-*Maihofner C, Charalambous MP, Bhambra U, Lightfoot T, Geisslinger G and et al.,: Expression of cyclooxygenase-2 parallels expression of interleukin-1 beta, interleukin-6 and NF-kappa B in human colorectal cancer. Carcinogenesis, 2003; 24: 665-671.*