Egyptian Journal of Surgery Vol 26, No 4, Oct., 2007

ORIGINAL ARTICLE

THE PROGNOSTIC SIGNIFICANCE OF GELATINASES (MMP-2 & MMP-9) EXPRESSION IN COLORECTAL CANCER IN UPPER EGYPT

By
Mostafa Thabet,[1] Ola Omran[2]
[1] Department of Surgery, [2] Pathology, Faculty of Medicine, Assiut University, Egypt.

Correspondence to: Mostafa Thabet, Email: mostafaharera@yahoo.com

Aim: ***Prognosis of colorectal carcinoma depends on many factors, such as: age & sex of patient-location, multiplicity, local extent and size of tumor-bowel obstruction or perforation as well as tumor microscopic type &grade- vascular & perineural invasion, nodal and distant metastasis. The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes strongly implicated in tumor invasion and metastasis, hence in tumor prognosis. The purpose of our study was to assess the role of MMP-2 and MMP-9 expression in colorectal tumorigenesis, invasion and metastasis , hence their prognostic values.***
Method: ***Immunohistochemical analysis of MMP-2 and MMP-9 glycoproteins in colorectal cancer cells, an immunohistochemical score based on the intensity of immunoreactivity and proportion of immunoreactive cells that established for each MMP and correlation of this expression with the established prognostic factors.***
Results: ***MMP-2 was expressed in 81.8% (strong expression in 40%) of cases, and MMP-9 expressed in 72% (strong expression in 35%) of cases.***
Conclusions: ***MMP-2 and MMP-9 are widely expressed in colorectal carcinoma suggesting significant diagnostic and prognostic values in these tumors. Increased levels of MMP-2 and MMP-9 protein expression in colorectal carcinoma tissues as compared to normal tissues suggest their association with colorectal tumor invasion and metastasis.***

Keywords: *Tumor invasion, Immunohistochemistry, Matrix metalloproteinases.*

INTRODUCTION

Colorectal carcinoma is one of the most common malignant tumors and represents the third cause of cancer mortality in the world.(1) Despite major advances in the diagnosis and treatment of this disease, its mortality has remained unchanged during the last 2 decades. In Egypt, there is an increasing incidence of colorectal cancinoma(2) especially in patients less than 40 years.(3) The prognosis of colorectal cancer depends on many factors.(4) Tumor aggressiveness is attributed to its ability for local invasion and distant metastasis which may be helped by the presence of matrix metaloproteinases expression by the tumor cells. Tumor invasion and metastasis are considered to be the most clinically useful prognostic indicator and the major causes of death in colorectal carcinoma patients. However, tumors of the same stage can follow significantly different clinical courses, indicating the necessity for the identification of novel prognostic factors.(3,4) Recent researches in the field of mechanism of tumor invasion and metastasis have demonstrated that the degradation of extracellular matrix (ECM) and basement membrane (BM) is a prerequisite and the contribution of matrix metalloproteinases (MMPs) is very important during this process.(5-8) MMPs are a family of zinc-dependent endopeptidases that are collectively capable of degrading most components of the BM and ECM.(9) To date, 26 MMP genes have been identified in humans, and many are implicated in cancer.(10) These enzymes are divided according to their target protein into several families including collagenases, gelatinases,

stromelysins, and membrane-type MMPs.[10] MMPs form a complex biological system strictly controlling degradation of ECM not only through their direct role in degrading ECM but also by interaction with other biological systems implicated in tumor invasion, including cell adhesion molecules, cytoskeletal proteins, and growth factors.[11,12] The balance of secreted MMPs and their specific inhibitors (Tissue Inhibitors of Metalloproteinases, TIMPs) plays an important role in maintaining connective tissue homeostasis and the imbalance is supposed to be linked to the invasive character of tumor cells.[13-15] MMP-2 and MMP-9 have been implicated to play a role in colorectal cancer progression, invasion and metastasis in animal models.[16] MMP-2 and MMP-9 are related to tumor invasion and metastasis by their capacity for tissue remodeling via ECM as well as BM degradation and induction of angiogenesis.[17,18] These gelatinases are secreted as zymogens and cleaved to the active form, and their function is tightly regulated by several different mechanisms.[17,18]

Several studies have proved that there is high expression of MMPs in many kinds of tumors, such as carcinoma of esophagus, lung, stomach, etc. Some authors have found that plasma levels of MMP-2 and MMP-9 were significantly elevated at all stages of colorectal cancer patients and a significant reduction was seen following curative resection.[19] Image analysis of the intensity of the bands detected by western blotting (72 KDa for MMP-2 and 92 KDa for MMP-9) showed that the increase in MMP-2 and MMP-9 was 1.8-fold and 2.9-fold, respectively, in colon cancer tissue compared with normal colonic mucosa.[20] However, there is no extensive studies on the immunohistochemical expression of MMP-2 and MMP-9 in colorectal cancer. Therefore, the aim of the present study was to investigate the expression characteristics of MMP-2 and MMP-9 in colorectal carcinoma tissues and to explore the relationship between them and colorectal carcinoma progression, trying to provide a valuable marker for its clinical prognosis.

PATIENTS AND METHODS

This study included 55 patients presenting with colorectal cancer proved histopathologically, obstructed and perforated cases were excluded. They were admitted to surgical department, Assiut University hospital in the period between May 2004 and Dec. 2006. All patients were subjected to full medical history, through clinical examination, routine laboratory investigations including blood picture, blood urea, serum creatinine, blood sugar, liver function and prothrombin time and concentration. Radiological evaluation including X ray chest, abdominal sonar, C.T. scan of the abdomen and barium enema were done for all cases. Diagnostic colonoscopy was done for every patient taking biopsy for histopathological study. After this preoperative work up, chemical and mechanical colonic preparation was done followed by laparotomy.

In the operative room: Routine intraoperative evaluation was done regarding tumor site, size, mobility, nodal and hepatic spread, peritoneal seeding and malignant ascitis. After operations the resected specimens were examined grossly, opened longitudinally and fixed in 10% formalin then sent for histopathology.

In the histopathology lab.: Histopathological evaluation was done with particular attention to microscopic type, grade according to Broders system,[21] and extent of the tumor according to the Dukes system.[22] Lymph node involvement and features of the tumor border were also evaluated.

Immunohistochemical staining for the MMP-2 and MMP-9 was demonstrated using Labelled Streptavidin –Biotin immunoperoxidase technique. In brief, the sections were deparaffinized in xylene, rehydrated in EtOH, and washed twice with distilled water. For better antigen retrieval, the samples were boiled three times for 5 minutes in a citrate buffer in a microwave oven (pH 6.0). Endogenous peroxidases were blocked by 5% hydrogen peroxidase treatment for 5 minutes. The samples were washed with PBS (pH 7.2) and incubated in 1.5% normal horse serum for 35 minutes to prevent nonspecific antigen binding. The primary antibody for MMP-2 was a mouse monoclonal antibody toward a human proform of MMP-2 (Ab-1; clone CA-4001; Neomarkers, Fremont, CA), used at a working dilution of 1:25. The primary antibody used for detection of MMP-9 was a mouse monoclonal antibody, which recognizes both the pro- and active forms of human MMP-9 (MAB3309; clone 56-2A4; Chemicon, Temecula, CA), used at a dilution of 1:2,500. The samples were incubated with the primary antibody overnight at 4°C. Before applying the secondary antibody, the samples were washed twice with PBS. The slides were incubated for 45 minutes with the biotinylated secondary antibody, followed by a wash and a 50-minute incubation in an avidin-biotinylated peroxidase complex reagent (Vectastain Rabbit ABC Elite Kit; Vector Laboratories, Burlingame, CA). Expressions were visualized with a 5-minute diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) treatment. The slides were counterstained with Mayer's hematoxylin, dehydrated, and mounted with DePex (BDH Ltd, Poole, United Kingdom). A routinely processed colorectal cancer section without the primary antibody served as a negative control in each staining series. Placenta samples served as positive controls.

Positive staining for MMP-2 and MMP-9 was detected as cytoplasmic brown staining. The staining was evaluated in two sites: a- stromal cells: as positive or negative. b- Tumor cells: they were semi quantitatively evaluated using the pathologist microscope to a standard for the intensity and the percentage of stained cells as follows:

The intensity of staining was graded from 0 to 3:

a) No staining 0
b) Mild staining 1
c) Moderate staining 2
d) Strong staining 3.

The percentage of positive cells was classified into four grades (23):

1) Less than or equal 15%....grade1 (G1).
2) 16-30%.....grade 2 (G2).
3) 31-60%.....grade 3 (G3).
4) More than 60% …….grade 4 (G4)

The statistical analysis was done using the Chi-square test to detect the probability factor (p) value and correlate them with different clinicopathological factors. Probability values of 0.05 were considered significant in the analyses.

RESULTS

The study included 55 patients with histopathologically proved colorectal cancer. There were 32(58.2%) males and 23 (41.8%) females, their ages ranged from 20+73 years, mean age was 41 years.

Clinicopathological study: The histological types of 55 cases were 45 (81.8%) conventional adenocarcinomas and 10 (18.2%) mucinous carcinomas. The ages of patients with conventional adenocarcinoma ranged between 20 and 73 years (mean was 44.9+/-2.5) with male predominance 64.4 % (29/45), while in mucinous carcinoma, it was 23-48 years (mean was 33.4+/-2.67) with predominance of females (7/10) Table 1.

As regard the gross appearance; the tumor acquired annular, polypoid or ulcerative shape. Conventional adenocarcinomas were predominantly ulcerative (21/45) (46.6%) with tumor size ranging between 1.5 and 10 cm (mean+/-SEM was 4.83+/- 0.31).

Most of these cases 64.4% (29/45) involved the distal colon and the remainder were located in the proximal colon 35.6% (16/45). In mucinous carcinoma, there was more predominance of the polypoid shape (5/10) (50%) with tumor size ranging between 2 and 12 cm (mean+/-SEM was 6.1+/- 1.04). They were equally distributed in both distal and proximal colon Table 1. The growing border of the tumor was found to be infiltrating in 73.3 % (33/45) of conventional adenocarcinomas and 40 % (4/10) of mucinous carcinomas. Others revealed pushing borders Table 1.

Immunohistochemical study of MMP-2 expression:

MMP-2 cytoplasmic staining was detected in the few glandular cells in the lower 1/3 of normal mucosal crypts. Positive faint expression of MMP-2 was observed in the stromal cells and matrix present between tumor cells in all studied cases with no significant difference in positivity in different grades and stages of colorectal adenocarcinoma cases. However, 81.8% (45/55) of the cases showed positive staining with MMP-2 in the malignant cells. All cases of mucinous carcinoma (100%), and 77.8% of conventional adenocarcinoma cases were positive Table 2.

Moderate intensity of staining was predominant in conventional adenocarcinomas (13/35) (37%), while in 50% of cases of mucinous carcinoma the intensity was strong (Figs.1,3). Regarding the percentage of positive cells, there were predominance of both grades G1 and G3 in cases of conventional adenocarcinoma. In mucinous carcinoma, there were equal distribution in grades G2, G3 and G4 and only one cases in G1 Table 2. Table 2 showed that the difference in staining intensity and percentage of positive cells for MMP-2 between different histological types of colonic carcinoma was statistically significant (p value = 0.05 and 0.009 respectively).

Table 3 shows that all cases of stage C (100%) were positive for MMP-2, followed by stage B (90.9%) and stages A (74.2%). There was predominance of moderate intensity of stain in stage C, strong intensity in stage A In stage B, there was equal distribution of both weak and strong intensity of stain and only two cases showed moderate intensity. The percentage of positive cells was equally distributed in grades G1, G3 and G4 in stage C cases. While in stage A cases, there was predominance in grade G3. However in stage B cases the positive cells were predominant in grade G1. The difference in staining intensity and percentage of positive cells for MMP-2 between different stages was statistically significant (p value = 0.024 and 0.044 respectively).

In the present study, immunoreactivity for MMP-2 was detected in 94.1 % of grade III, 87.5% of grade II and only 41.6 % of grade I of conventional adenocarcinoma cases. Table 4 demonstrates that the moderate intensity of staining was predominant in grade I cases, weak or high in grade II, and moderate or high in grade III (Figs. 1,2). The percentage of positive cells in grade I and grade II was predominant in grades G1 and G3, while in grade III, there were predominance in grades G3 and G4. The difference in staining intensity and percentage of positive cells for MMP-2 between different grades was statistically significant (p value < 0.0001 and 0.05 respectively).

Immunohistochemical study of MMP-9 expression:

MMP-9 cytoplasmic staining was detected in the glandular cells up to 1/3 of the length of normal mucosal crypts. There was weak positive expression of MMP-9 in the stromal cells and matrix present between tumor cells in all studied cases with no significant difference in positivity in different grades and stage of colorectal adenocarcinoma cases, but only 78.2% (43/55) of the cases showed positive

staining with MMP-9 in the malignant cells which were analyzed in this study. Half of the cases of mucinous carcinoma (50%), and 84.4% of conventional adenocarcinoma cases were positive Table 5. High intensity of staining was predominant in conventional adenocarcinomas (37%), while in 40% of cases of mucinous carcinoma the intensity was moderate. Regarding the percentage of positive cells, there was predominance of grade G4 in cases of conventional adenocarcinoma. In mucinous adenocarcinoma, there were equal distribution in grades G1, G3 and G4 Table 5. Table 5 showed that the difference in staining intensity and percentage of positive cells for MMP-9 between different histological types of colonic carcinoma was statistically significant (p value was 0.001 and 0.05 respectively).

Table 6 shows that all cases of stage C (100%) were positive for MMP-9, followed by stage B (81.8%) and stage A (71.4%). There was predominance of moderate intensity of stain in stage A and B, strong intensity in stage C. All cases from different stages showed predominance in grade G4. The difference in staining intensity and percentage of positive cells for MMP-9 between different stages was statistically significant for the intensity (p value = 0.000) and statistically insignificant for the percentages of positive cells (p value = 0.670) (Figs. 4,5).

As regarding the grade, immunoreactivity for MMP-9 was detected in 91.1 % of grade III, 87.5% of grade II and 70.8% of grade I of conventional adenocarcinoma cases. High intensity of staining was predominant in grade III cases, weak or moderate in grade II. The percentage of positive cells in grade II and grade III was predominant in grade G4, while in grade I, there was predominance in grade G1. The difference in staining intensity and percentage of positive cells for MMP-9 between different grades was statistically insignificant (p value = 0.22 and 0.14 respectively).

We found that 100% of cases in age group ≤ 25 years and more than 50 years were positive for MMP-2 while only 72.7 % of cases in age group 25-50 years showed positivity. Regarding the MMP-9 expression, we found that the number of positive cases was increased in age group less than 50 years.

Table 1. Clinicopathological parameters in studied cases of colorectal carcinoma.

Clinicopathological parameters	Conventional adenocarcinoma	Mucinous carcinoma
Number of cases:	45 (81.8 %)	10 (18.2 %)
Age:		
Range	20-73	23-48
Mean+/-	44.9+/-2.5	33.4+/-2.67
Male : Female	29: 16	3: 7
Tumor site:		
Proximal colon	16 (35.6 %)	5 (50 %)
Distal colon	29 (64.4 %)	5 (50 %)
Tumor shape:		
Annular	8	2
Polypoid	16	5
Ulcerative	21	3
Tumor size:		
Range	1.5-10 cm	2-12 cm
Mean+/-SEM	4.83+/-0.306	6.1+/-1.04
Tumor border:		
Infiltrating border	33 (73.3 %)	4 (40 %)
Pushing border	12 (26.7 %)	6 (60 %)
Tumor grade:		
Grade I	16 (35.5 %)	
Grade II	17 (37.7 %)	
Grade III	12 (26.6 %)	
Tumor stage:		
A	35 (0.64%)	
B	11 (0.20%)	
C	9 (0.16%)	

Table 2. Relationship between pattern of MMP-2 immunostaining and the two histological subtypes of colorectal carcinoma.

Tumor type	No. of cases	No. of +ve cases	Intensity				Sig	% of +ve cells				Sig
			0	+1	+2	+3		≤15	16-30	31-60	>60	
Conventional adenocarcinoma	45	35 (77.8%)	10	10	13	12	0.05	12	2	12	9	0.009
Mucinous carcinoma	10	10 (100%)	-	2	3	5		1	3	3	3	

Table 3. Relationship between pattern of MMP-2 immunostaining and different tumor stages.

Tumor type	No. of cases	No. of +ve cases	Intensity				Sig	% of +ve cells				Sig
			0	+1	+2	+3		≤15	16-30	31-60	>60	
A	35	26 (74.2%)	9	5	10	11		5	4	11	6	
B	11	10 (90.9%)	1	4	2	4	0.024	5	1	1	3	0.044
C	9	9 (100%)	-	3	4	2		3	-	3	3	

Table 4. Relationship between pattern of MMP-2 immunostaining and different grades of conventional colorectal adenocarcinoma cases.

Tumor type	No. of cases	No. of +ve cases	Intensity				Sig	% of +ve cells				Sig
			0	+1	+2	+3		≤15	16-30	31-60	>60	
Grade I	12	5 (41.6%)	7	1	3	1		2	1	2	-	
Grade II	16	14 (87.5%)	2	5	4	5	0.000	6	1	4	3	0.05
Grade III	17	16 (94.1)	1	4	6	6		4	-	6	6	

Table 5. Relationship between pattern of MMP-9 immunostaining and the two histological subtypes of colorectal carcinoma.

Tumor type	No. of cases	No. of +ve cases	Intensity				Sig	% of +ve cells				Sig
			0	+1	+2	+3		≤15	16-30	31-60	>60	
Conventional adenocarcinoma	45	38 (84.4%)	7	9	14	15	0.001	10	5	4	19	0.05
Mucinous carcinoma	10	5 (50%)	5	1	4	-		1	2	1	1	

Table 6. Relationship between pattern of MMP-9 immunostaining and different tumor stages.

Tumor type	No. of cases	No. of +ve cases	Intensity				Sig	% of +ve cells				Sig
			0	+1	+2	+3		≤15	16-30	31-60	>60	
A	35	25 (71.4%)	10	8	10	7		5	4	3	18	
B	11	9 (81.8%)	2	1	6	2	0.000	1	2	1	10	0.670
C	9	9 (100%)	0	1	2	6		1	1	1	6	

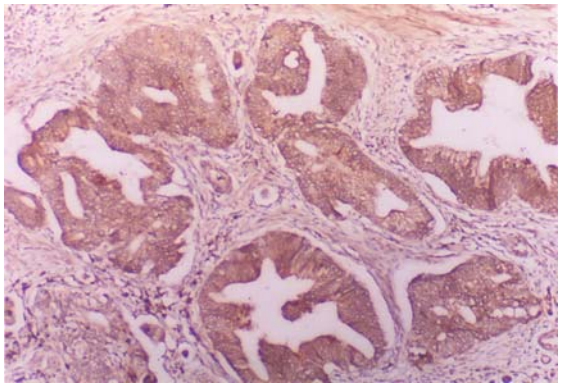

Fig 1. Conventional adenocarcinoma grade I showing moderate intensity of cytoplasmic staining of the glands (immunostaining with MMP-2 X400).

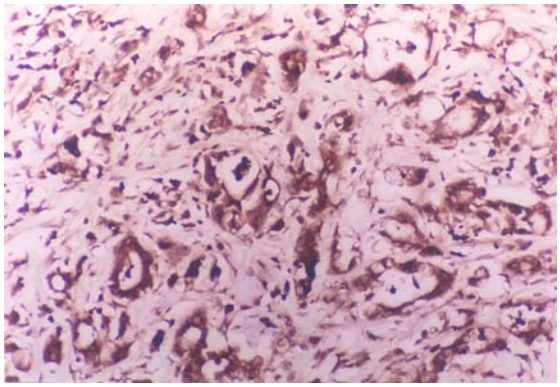

Fig 2. Conventional adenocarcinoma grade III showing strong cytoplasmic positive staining of the tumor cells (immunostaining with MMP-2 X400).

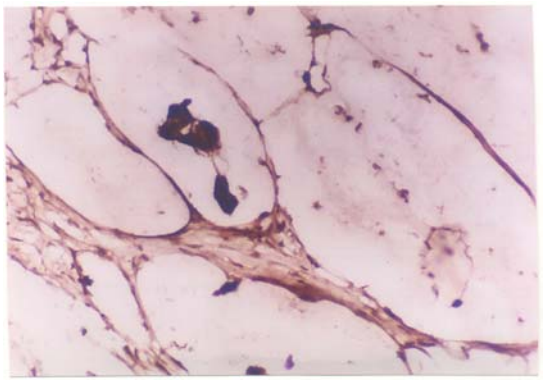

Fig 3. Mucinous carcinoma showing strong intensity of cytoplasmic staining of the glands (immunostaining with MMP-2 X400).

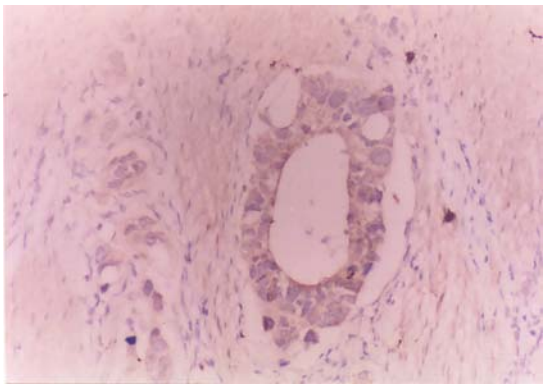

Fig 4. Conventional adenocarcinoma grade I showing weak intensity of cytoplasmic staining of the malignant glands in the lamina propria (immunostaining with MMP-9 X400).

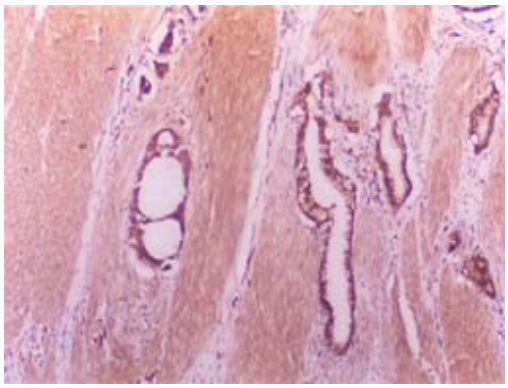

Fig 5. Conventional adenocarcinoma grade I showing strong intensity of cytoplasmic staining of the malignant glands among the muscle bundles (immunostaining with MMP-9 X200).

DISCUSSION

In Egypt, there is an increasing incidence of colorectal cancer.[2] Most of these patients are in age group equal or less than 40 years and usually have advanced disease at presentation and a high incidence of treatment failure caused by a delay in the diagnosis and an aggressive pattern of the disease.[3] To decrease treatment failure and improve patient survival, pathological assessment of resection specimens for the prognostic factors such as tumor type, histological grade, tumor stage and metastasis must be performed.[24] Tumor metastasis is the main cause of death in cancer. Assessment of the metastatic potentials of the tumor determines the need for adjuvant therapy after an apparently curative surgery.[25] Metastatic potential of the tumor is associated with alteration in the expression of molecules such as MMP-2 and MMP-9.

In the present study, the histological examination of sections prepared from 55 cases of colorectal carcinomas revealed that 81.8% of them were conventional adenocarcinomas and only 18.2 % were mucinous carcinomas, in agreement with some authors who found that conventional adenocarcinoma represented about 78.9% and 92.1% respectively and only 21.2% and 7.9% respectively of the cases were mucinous carcinomas.[26,27] We found that 81.8% of colorectal cancer patients were under the age of 51 years and 47.3% were under 40 years, others also found that 59% of colorectal cancer patients were under the age of 50 years and 35% were under the age of 40 years.[28]

Regarding sex distribution, there was male predominance in cases of conventional adenocarcinoma (64.4%). While female predominance (70%) was detected in cases of mucinous carcinoma. These findings are in accordance with author who stated that most of mucinous carcinomas occurred in females, in contrast to conventional adenocarcinoma which occurred commonly in males.[29] However, it has been proved that predominance of male cases was found in both conventional adenocarcinoma and mucinous carcinoma (57.7% and 71.4% respectively).[30] In our study we found that 61.8% of the colonic carcinomas were located in the distal colon versus 38.2% in the proximal colon. These findings are in agreement with some authors who found 75% and 62.5% of the colonic carcinomas in the distal colon versus 25% and 37.5% in the proximal colon respectively.[2,30]

There were predominance of both ulcerative and polypoid gross appearance in our cases, 43.6% and 38.2% respectively but annular lesions was 18.2%. These findings are in agreement with author, who found that 74.6% of the colonic carcinomas had ulcerative shape and 25.4% had polypid shape.[27] The border of the tumors showed predominance of infiltrating borders in cases of conventional adenocarcinoma (73.3%), while in cases of mucinous adenocarcinoma, there were predominance of the pushing borders (60%). These findings are in agreement with others who found that mucinous carcinomas grow in pushing pattern more than in non mucinous carcinomas at percentage of 66% versus 39% respectively.[31]

Most of the conventional adenocarcinoma cases (64.4%) were located in the distal colon but cases located in the proximal colon represented 35.6%. However, in mucinous carcinoma there was equal distribution of cases between proximal and distal colon. These findings are in agreement with those who found that 58% of colorectal cancers occur in the rectosigmoid region.[32]

Currently Dukes' staging of colorectal carcinoma is the most important prognostic indicator. However recent advances in molecular biology suggest great need to develop a prognostic indicator in human cancers such as Matrix metalloproteinase.[33] In this work, we studied the expression of MMP-2 and MMP-9 in colorectal carcinoma specimens in an attempt to elucidate their role in tumor progression. Our results demonstrated that MMP-2 expression was much higher in colorectal carcinoma tissues than in normal tissues. Moreover, there was a significant positive correlation between the MMP-2 expression and tumor invasion depth, and tumor Duke's stage, indicating that MMP-2 expression was not only associated with the development of colorectal carcinoma, but also played an important role in the process of invasion and metastasis. Immunohistochemical staining for MMP-2 identified that MMP-2 positive staining mainly occurred in the cytoplasm of colorectal carcinoma cells and matrix around them and frequently occurred in stromal cells. This suggests that the major cell source of MMP-2 in colorectal carcinoma is heterogeneous. Others demonstrated more immunohistochemical localization of MMP-2 and MMP-9 to regions where tumor cells invaded the muscle layer of the colon compared to malignant cells in more superficial areas.[19] They also showed significant increase of MMP-9 in colorectal cancer tissue, predominantly in the tumor stroma.

Our study also showed that there was an increase in the MMP-9 in colorectal carcinoma tissues compared to that in normal colorectal tissues. Furthermore, with the progression of tumor Duke's stage, the MMP-9 was gradually increased. We think that it is related to the increased MMP-2 expression in the late stage. Correlation was found between both MMP-2 and MMP-9 with tumor differentiation, and Dukes' staging. These findings are in agreement with others who found that MMP-2 concentration positively correlated with tumor differentiation, lymphatic invasion and Dukes' staging.[34] Others also demonstrated that MMP-2 and MMP-9

expression levels in adenocarcinoma tissue correlated with the histological grade and invasion/metastasis trend.[35] In contrast, some authors reported that the depth of submucosal invasion was not significantly correlated with MMP-2 and MMP-9 expression.[36]

Concerning age, We found that 100% of cases in age group ≤ 25 years and more than 50 years were positive for MMP-2 while only 72.7 % of cases in age group 25-50 years showed positivity. Regarding the MMP-9 expression, we found that the number of positive cases was increased in age group less than 50 years. These findings confirm the concept that colorectal carcinoma in young patients was known to be biologically aggressive.[37]

In order to expand the growing space, colorectal carcinoma needs to enhance the MMP-2 expression and keep the ECM environment suitable for tumor cells, which must depend on MMP-9 as a cofactor. Extracellular matrix degradation not only enhance tumor invasion but also affects tumor cell behavior and leads to cancer progression.[38] Further characterization of the expression and utilization of MMPs in the progression of colorectal carcinoma would lead to development of anticancer therapy. In conclusion; MMP-2 and MMP-9 are widely expressed in colorectal carcinoma, and their increased expression correlated with poor prognostic variables suggesting their role in proteolysis facilitating invasion and metastasis and warrants further investigations.

REFERENCES

1. Sinicrope F, Ruan S, Cleary K, Lee J, and Levin B. relationship of polyps to cancer of the large intestine. Cancer Res. 1995;55:237-41.

2. Abou-Zeid AA, Khafagy W, Marzouk DM, Alaa A, Mostafa I, Ela MA. Colorectal cancer in Egypt. Dis Colon Rectum. 2002;45:1255-60.

3. El-Hennawy MM, Moussa ME, El-Saeidy MK, Shawky AM, Bessa SS, Badour NM. Rectal carcinoma in Egyptian patients less than 40 years of age. Int Surg 2003;88:137-44.

4. Cooper H. Intestinal neoplasms. Sternberg diagnostic surgical pathology, third edition, volume 3, chapter. 1999;34:1441.

5. Curran S, Murray GI. Matrix metalloproteinases in tumour invasion and metastasis. J Pathol. 1999;189:300-8.

6. Curran S, Murray GI. Matrix metalloproteinases. molecular aspects of their roles in tumour invasion and metastasis. Eur J Cancer. 2000;36:1621-30.

7. Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases. a tail of a frog that became a prince. Nat Rev Mol Cell Biol. 2002;3:207-14.

8. Yana I, Seiki M. MT-MMPs play pivotal roles in cancer dissemination. Clin Exp Metastasis. 2002;19:209-15.

9. Jiang Y, Goldberg ID, Shi YE. Complex roles of tissue inhibitors of metalloproteinases in cancer. Oncogene. 2002;21:2245-52.

10. Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. Curr Opin Cell Biol. 2004;16:558–564.

11. Talvensaari-Mattila A, Paakko P, Turpeenniemi-Hujanen T. Matrix metalloproteinase-2 (MMP-2) is associated with survival in breast carcinoma. Br J Cancer. 2003;89:1270-5.

12. Talvensaari-Mattila A, Paakko P, Blanco-Sequeiros G, Turpeenniemi-Hujanen T. Matrix metalloproteinase-2 (MMP-2) is associated with the risk for a relapse in postmenopausal patients with node-positive breast carcinoma treated with antiestrogen adjuvant therapy. Breast Cancer Res Treat. 2001;65:55-61.

13. Zeng ZS, Huang Y, Cohen AM, Guillem JG. Prediction of colorectal cancer relapse and survival via tissue RNA levels of matrix metalloproteinase-9. J Clin Oncol. 1996;14:3133-40.

14. Zeng Z-S, Cohen AM, Zhang ZF, Stetler-Stevenson W, Guillem JG. Elevated tissue inhibitor of metalloproteinase 1 RNA in colorectal cancer stroma correlates with lymph node and distant metastases. Clin Cancer Res. 1995;1:899-906.

15. Ring P, Johansson K, Hoyhtya M, Rubin K, Lindmark G. Expression of tissue inhibitor of metalloproteinases TIMP-2 in human colorectal cancer-a predictor of tumour stage. Br J Cancer. 1997;76:805-11.

16. Mook O, Frederiks W, Van Noorden C. The role of gelatinases in colorectal cancer progression and metastasis. Biochim Biophys Acta. 2004;1705:69–89.

17. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer. 2002;2:161-74.

18. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol. 2001;17:463-516.

19. Tutton MG, George ML, Eccles SA, Burton S, Swift RI, Abulafi AM. Use of plasma MMP-2 and MMP-9 levels as a surrogate for tumour expression in colorectal cancer patients. Int J Cancer. 2003;107:541-50.

20. Kim TD, Song KS, Li G, Choi H, Park HD, Lim K, Hwang BD, Yoon WH. Activity and expression of urokinase-type plasminogen activator and matrix metalloproteinases in human colorectal cancer. BMC Cancer. 2006;18;6:211.

21. Broders AC. The grading of carcinoma. Minn Med. 1925;8:726.

22. Dukes CE. The surgical pathology of rectal cancer. J clin. Pathol. 1949;2:95-9.

23. Lipponen P, Altoma S, Kosma VM, Alaopas M, Eskelinen M. Expretion of CD44s and V6 proteins in transitional cell bladder tumors and their relation to prognosis during a long term follow up. J. Pathol. 1998;186:157-64.

24. Carolyn AC and Compton C. Colorectal carcinoma: Diagnostic, prognostic, and molecular features. Mod. Pathol. 2003;16:376-88.

25. Wielenga VJ, Heider KH, Offerhaus GJ, Adolf GR, van den Berg FM, Ponta H, Herrlich P, Pals ST. Expression of CD44 variant proteins in human colorectal cancer is related to tumor progression. Cancer Res. 1993;53:4754-6.

26. Imazeki F, Yokosuka O, Yamaguchi T, Ohto M, Isono K, Omata M. Expression of variant CD44-messenger RNA in colorectal adenocarcinomas and adenomatous polyps in humans.Gastroenterology. 1996;110:362-8.

27. Ishida T. Immunohistochemical expression of the CD44 variant 6 in colorectal adenocarcinoma. Surg Today. 2000;30:28-32.

28. Soliman AS, Bondy ML, Levin B, El-Badawy S, Khaled H, Hablas A, et al. Familial aggregation of colorectal cancer in Egypt. Int J Cancer. 1998;77:811-6.

29. Wynder EL. Large bowel cancer: prospects for control. Cancer Detect Prev. 1985;8:413-20.

30. Fernandez JC, Vizoso FJ, Corte MD, Gava RR, Corte MG, Suarez JP, Garcia-Muniz JL, Garcia-Moran M. CD44s expression in resectable colorectal carcinomas and surrounding mucosa.. Cancer Invest. 2004;22:878-85.

31. Zhang H, Evertsson S, Sun X. Clinicopathological and genetic characteristics of mucinous carcinomas in the colorectum. Int J Oncol. 1999;14:1057-61.

32. Levi F, Randimbison L, La Vecchia C. Incidence of colorectal cancer following adenomatous polyps of the large intestine. Int J Cancer. 1993;55:415-8.

33. Bhatavdekar JM, Patel DD, Chikhlikar PR, Shah NG, Vora HH, Ghosh N, Trivedi TI. Molecular markers are predictors of recurrence and survival in patients with Dukes B and Dukes C colorectal adenocarcinoma. Dis Colon Rectum. 2001;44:523-33.

34. Baker EA, Bergin FG, Leaper DJ.Matrix metalloproteinases, their tissue inhibitors and colorectal cancer staging. Br J Surg. 2000;87:1215-21.

35. Guo W, Chen G, Zhu C, Wang H. Expression of matrix metalloproteinase-2, 9 and it's tissue inhibitor-1, 2 in endometrial carcinoma. Zhonghua Fu Chan Ke Za Zhi. 2002;37:604-7.

36. Jung SA, Yang SK, Kim JS, Shim KN, Im SA, Myung SJ, et al. The expression of matrix metalloproteinases (MMPs), tissue inhibitor of metalloproteinases (TIMPs) and angiogenesis in relation to the depth of tumor invasion and lymph node metastasis in submucosally invasive colorectal carcinoma. Korean J Gastroenterol. 2005;45:401-8.

37. Deen KI, Kumar D, Williams JG, Olliff J, Keighley MR. The prevalence of anal sphincter defects in faecal incontinence: a prospective endosonic study. Gut. 1993;34:685-8.

38. Roeb E, Matern S. Matrix metalloproteinases and colorectal cancer. Med Klin (Munich). 2003;98:763-70.