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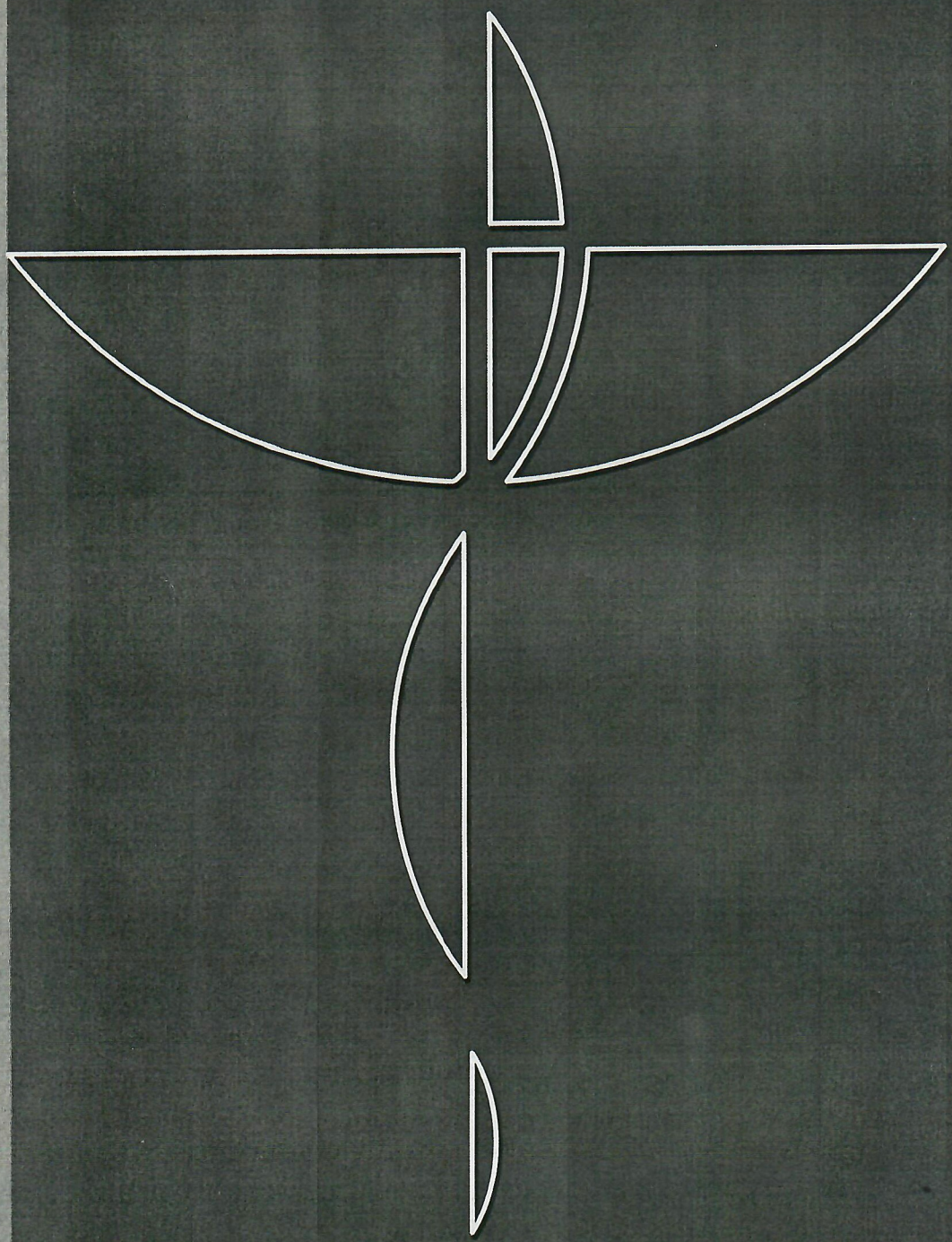

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CORRELATION BETWEEN EXPRESSION OF MATRIX METALLOPROTEINASE AND CLINICOPATHOLOGIC FEATURES IN PATIENTS WITH COLORECTAL CANCER

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Abstract

Background. Matrix metalloproteinases (MMP) are a large family of zinc-dependent endoproteases that have been implicated in many physiologic and pathologic processes, such as tumour invasion and metastasis by their capacity to degrade basement membranes and extracellular matrix proteins.
Objectives. The aim of this study was to investigate the expressions of MMP-2, MMP-7 and MMP-9 in tumour tissue and their relation to clinicopathologic features in patients with colorectal cancer.
Methods. Specimens of resected colorectal cancer and surrounding normal tissue of 82 patients were immunohistochemically stained for MMP-2, MMP-7 and MMP-9. Results of immunohistochemical expression of MMPs were correlated with some clinical and pathologic parameters.
Results. Immunohistochemical expression of MMP-2 was more frequent in patients with higher preoperative serum levels of: CEA, MMP-2, MMP-9 and in patients with lymph node metastasis and advanced stage of the disease. Expression of MMP-7 was more frequent in patients with elevated preoperative serum levels of: CEA, MMP-7, MMP-9 and with deeply invasive neoplasms. MMP-9 cell expression was in a positive correlation with elevated preoperative serum levels of: CEA, MMP-2, MMP-9 and depth of CRC invasion, i.e. T-parameter. According Kaplan-Meier survival curve, 2,65% of the followed CRC patients survive more than 60 months; 52% of the CRC patients in Stadium II survive more than 48 or 60 months and 28% of patients in Stadium III survive more than 55 or 60 months.
Conclusion. Immunohistochemical expression of MMPs is a useful indicator for disease development and progression in patients with colorectal cancer.

Key words: matrix metalloproteinases; expression; colorectal cancer

ПОВРЗАНОСТА ПОМЕЃУ ЕКСПРЕСИЈАТА НА МАТРИКС МЕТАЛОПРОТЕИНАЗИТЕ И КЛИНИЧКО-ПАТОЛОШКИТЕ КАРАКТЕРИСТИКИ КАЈ ПАЦИЕНТИ СО КОЛОРЕКТАЛЕН КАРЦИНОМ

Апстракт

Вовед. Се смета дека матрикс металопротеиназите (ММПи), како голема фамилија на цинк-зависни ендопротезаи, преку деградација на базалните мембрани и екстраклеточните протеини на матриксот играат клучна улога во процесите на туморската инвазија и метастазирање.
Цел. Да се испита експресијата на ММП-2, ММП-7 и ММП-9 во туморското ткиво, како и нивната поврзаност со клиничко-патолошките карактеристики на пациентите со колоректален карцином.
Методи. Од 82 пациенти беа добиени ресецирани примероци од колоректален карцином и околно нормално ткиво, и истите беа имунохистохемиски боени за ММП-2, ММП-7 и ММП-9. Резултатите од имунохистохемиската експресија на испитуваните ММПи беа корелирани со некои клинички и патолошки параметри.
Резултати. Имунохистохемиската експресија за ММП-2 беше многу почеста кај пациентите со повисоки предоперативни нивоа на КЕА (карциноембрионален антиген) во серум, со предоперативните нивоа на ММП-2 и ММП-9 во серум, со метастазираните лимфни јазли и со напреднатиот стадиум на болеста. Експресијата за ММП-7 беше многу почеста кај пациентите со повисоки серумски нивоа на КЕА, ММП-7, ММП-9, како и кај подлабоките инвазивни неоплазми. Клеточната експресија за ММП-9 беше во позитивна корелација со зголемените предоперативни нивоа на КЕА, ММП-2, ММП-9 и со длабочината на инвазијата на колоректалниот карцином, односно Т-параметарот.
Според Kaplan-Meier кривата на преживување, 2,65% од следените пациенти со КРК преживеале повеќе од 60 месеци, 52% од пациентите во Стадиум II преживеале повеќе од 48 до 60 месеци и 28% од иследуваните пациенти во Стадиум III преживеале повеќе од 55 до 60 месеци.
Заклучок. Имунохистоехемиската експресија на ММПи е корисен индикатор за развојот и прогресијата на болеста кај пациентите со колоректален карцином.
Клучни зборови: матрикс металопротеинази, експресија, колоректален карцином

Introduction

Colorectal cancer (CRC) is a common disease and it is one of the leading causes of cancer related deaths in developed countries (1). Despite improvements in surgical techniques, adjuvant and neo-adjuvant chemotherapy, the 5-year survival rate in patients with CRC ranges from 5-90% with tumour progression (stage I: 90-95%, II: 75-85%, III: 50-60% and IV: 0-10%). The prognosis in patients without distant metastasis varies from 50-95% depending on the tumour stage (2).

Matrix metalloproteinases (MMPs) play an important role in several physiological and pathologic processes such as tissue remodeling, wound healing, angiogenesis, morphogenesis of organs, embryonic development, leukocyte migration and tumour invasion and metastasis. MMPs are a multigene family of structurally similar proteolytic enzymes, that is, zinc-dependent endopeptidases, which have the capacity to degrade virtually every component of the extracellular matrix. It is thought that tumour cells overexpress proteases and induce expression of enzymes in the neighboring stromal cells in order to degrade the basement membrane and invade the surrounding tissue (3).

Expression of MMPs in tumour tissue is regulated by the growth factor and cytokines that are secreted by tumour cells, stromal cells and tumour infiltrating inflammatory cells.

An elevated MMPs activity and their overexpression has been determined in several malignant neoplasms such as lung cancer, pancreatic cancer, ovarian cancer, prostate cancer, breast cancer, and brain cancer and a correlation with the tumour aggressiveness and its

malignancy potential has been detected (4,5). Earlier studies presented contradictory results related to the expression of the most commonly associated MMP-2 (gelatinase A), MMP-7 (matrilysin) and MMP-9 (gelatinase B) with prognosis in CRC patients, which was the motive to conduct our study (6,7,8).

Among the MMPs, matrixmetalloproteinase 2 (MMP-2) and matrix metalloproteinase9 (MMP-9), as members of gelatinases, plays important roles in themigration of malignant cells, because of their abilityto degrade type IV collagen (9). The mechanisms of activation of these enzymes are different. MMP-9 modulates permeability of the vascular endothelium,whereas MMP-2 promotes cleavage of extracellularmatrix proteins and is intensively expressed by tumor and stromal components of cancer (10).

Matrix metalloproteinase 7 (MMP-7) or matrilysin, as a member of stromelysinsis able to induce cell apoptotic impairment. Matrilysin can regulate angiogenesis eitherby inducing a direct proliferative effect on vascular endothelialcells or producing angiogenesis inhibitors (angiostatin, endostatinand neostatin-7) or enriching the variety of angiogenesismediators, such as the soluble vascular endothelial growth factor(sVEGF) (11).

It degrades type IV and X collagen, the elastin, the fibronectin, the laminin, the osteopontin, the proteoglycans, as well as numerous others substrates (12).

Increased levels of matrix metalloproteinase in tumor tissues or in blood circulation have been found to correlate with many cancers, including colorectal cancer (CRC). Several previous studies have shown that MMPs may play an important role as an indicator for the occurring of CRC and its progression (13).

The aim of this study was to investigate the expressions of MMP-2, MMP-7 and MMP-9 andtheirrelationtoclinicopathologic parameters in CRC patients.

Methods

The study included a total of 82 previously untreated CRC patients, 30 (36.58%) were females and 52 (63.41%) were males, ages ranging from 43 to 75 years, mean age of 67.85 years (SD±9.67) with operable CRC, without detectable distant metastases, who respected the medical instructions and were available for follow-up. All the patients underwent surgical resection of the primary neoplasm at the University Clinic for Abdominal Surgery in Skopje in the period of 2 years.

Blood samples from all the patients were drawn before surgical treatment in order to examine the CEA, CA 19.9, MMP-2, MMP-7 and MMP-9 serum levels. None of the CRC patients had received chemotherapy before blood sample collection. To standardize clotting conditions, all sera were separated within 1 h after blood collection, aliquoted and stored at −80°C until assayed.

Serum levels of CEA and CA 19.9 were determined using an enzyme immunoassay (EIA) (Monobind Inc., USA) according to the manufacturer's instructions.Serum levels of MMP-2, MMP-7 and MMP-9 were determined using a quantitative solid phase sandwichenzyme linked immuno sorbent assay (ELISA) (R&D Systems,USA) according to the manufacturer's instructions. MMP-2, MMP-7 and MMP-9 technique can detectboth pro- and active forms of recombinant human MMP-2, MMP-7 and MMP-9. High concentrations of MMP-2, MMP-7 and MMP-9 were diluted with calibrator, to producesamples with values within the dynamic range of the assay.

The resected specimens were sent to the Institute of Pathology, Medical Faculty in Skopje, where the pathologic stage of the disease in each and every patient was determined according to the TNM classification of AJCC (2010).

Tissue sections for immunohistochemistry were taken from tumour tissue of the invasive neoplasm front and of the peritumoural tissue without obvious macroscopic changes; they

were fixed in formalin, embedded in paraffin and cut at 5-7μ and were primarily stained with hematoxylin eosin.

For immunohistochemical staining were used monoclonal anti-human MMP antibodies 2, 7 and 9; mouse IgG, clone 36006.211, 6A4 and 36020.111, respectively, Cat.No MAB902, MAB907, MAB936, R&D Systems, Inc. and Polyclonal rabbit Anti-Human Matrix Metaloproteinase 9, code AO150 Dako.

Then the sections were deparaffinized in xylene, rehydrated through a series of graded alcohol solutions and pretreated for antigen retrieval in 10 mM citrate buffer (pH 6.0) in a microwave oven for 15 min at 700 W, and left in the buffer to cool at room temperature.

Endogenous peroxidase activity was suppressed with a solution of peroxidase-blocking reagent (DakoCytomatin, Germany) for 10 min, and non-specific antibody binding was blocked with protein block serum-free (DakoCytomation, Germany) incubation for 10 min. The sections were incubated with the primary antibodies, diluted with antibody diluents (DakoCytomation) (MMP-2 1:200, MMP-7 1:30, MMP-9 1:100) for two hours in a wet chamber at room temperature. For subsequent staining EnVision+two step visualization technique (Dako, Germany) was used with diaminobenzidine (DAB) as a chromogene, incubated for 10 min, and hematoxylin for counterstaining. Omission of the primary antibody served as negative control and carcinoma tissue with high expression of relevant proteins served as positive control.

Immunohistochemical expression of MMP-2, MMP-7 and MMP-9 was determined semi-quantitatively by defining the signal intensity and quantity of immunohistochemically stained cells.

Staining was considered to be negative when 0-10% of tumour epithelial cells were stained, and staining was considered to be positive when >10% of tumour epithelial cells were stained.

The intensity of the staining pattern was scored to be weak (+), moderate (++) and strong (+++).

Stromal cellspositivity was considered to be weak (+) if 1-2 stromal cells were stained, moderate (++) if groups of 3-5 cells were stained and strong (+++) if there were diffusely stained cells or groups of more than 5 cells. The intensity of the staining was determined in the same manner as in the epithelial cells.

The cell quantity was determined in the invasive front of the neoplasm.

All specimens were independently evaluated by two pathologists.

Statistical analysis

Descriptive statistics (mean) are given according to normality of the distribution. Normality of the distribution was determined by Kolmogorow-Smirnov's test. Analysis of variance with Kruskall-Wallis test was first used in the analysis of different sample types. In the case of significant results, the analyses were continued by pairing the variables and analyzing them with Mann-Whitney's U-test. Fisher's exact probability test and Pearson's Chi-Square test (r) were used for testing the association (linearity of the correlation of serum concentrations) between MMPs and major prognostic variables in CRC, such as grade and stage. P-values less than 0.05 ($p<0.05$) were considered as statistically significant. Survival curves were generated by the Kaplan-Meier method, and the log rank test was utilised to compare survival curves. A value of $p< 0.05$ was considered statistically significant. 95% confidence interval (95%CI) was calculated for variables including gender, age, and delay in diagnosis, tumour site and stage.

Results

There have been 17 (20.73%) patients in stage I of the disease, 40 (48.78%) patients in stage II and 25 (30.48%) patients in stage III. Lymph node metastases were substantiated in 25 (30.48%) patients and were not found in 57 (69.51%) patients with different pT category (Table 1).

Table 1 Staging of the disease in CRC patients according to AJCC

Stage	pTNM	Number of patients	Percent (%)
I	pT1 N0 M0 pT2 N0 M0	8 9	20.73
IIA IIB	pT3 N0 M0 pT4a N0 M0	22 18	48.78
IIIB IIIB IIIB IIIC	pT3 N1b M0 pT3 N2a M0 pT4a N1b M0 pT4a N2b M0	7 9 4 5	30.48

The majority of patients were with pT3N0M0 (26.82%), i.e. patients in stage II A of the disease, and the smallest number of patients were with pT4aN1M0 (4.87%), i.e. patients in stage III B of the disease.

Positive immunoreactivity in tumour cells for MMP-2 was detected in 19/82 cases (23.17%), and in stromal cells in 27/82 cases (32.92%).

Cytoplasmic positive immunoreactivity in tumour cells of weak intensity (+) was detected in 16 cases (84.21%), and of moderate intensity (++) in 3 cases (15.78%).

Thesignal intensity of stained stromal cells in all evaluated cases was assessed to be strong (+++).

Elongated fibroblastoid types of cells were positively stained in the stroma. There was no specific arrangement of stromal positive cells, except for the number of positively stained cells that was semi-quantitatively evaluated, which was larger in the invasive front of the neoplasm (+++) than in the neoplastic stroma in other regions. There was also a larger number of MMP-2 positive cells in the regions with more distinct inflammatory reaction to the neoplastic process.

Sections from the tumour neighboring tissue showed no immunoreactivity with anti-MMP-2 antibody either in the mucosal epithelial cells or in lamina propria.

eak cytoplasmic reactivity (+) with tumour epithelial cells in 32 cases (39.02%) whereas positive stromal staining was detected in 4 cases alone (4.86%) in the regions of inflammatory reaction.

Sections from the tumour neighboring tissue showed no immunoreactivity with anti-MMP-7 antibody either in the mucosal epithelial cells or in lamina propria.

Staining with anti-MMP-9 showed positive immunoreactivity of weak intensity in tumour epithelial cells in 37 cases (45.12%), stromal positivity in fibroblastoid cells in 1 case (1.21%) and positivity of inflammatory cells in 79 cases (96.34%). There was a strong staining intensity of inflammatory cells in all specimens. Inflammatory cells were grouped in the invasive front of the neoplasm, while there were few and scattered in the other regions of the tumour stroma. Macrophages showed the most intense staining.

Sections from the tumour neighboring tissue showed no immunoreactivity with anti-MMP-9 antibody either in the mucosal epithelial cells or in lamina propria, except in the inflammatory cells if present in the specimens.

Immunohistochemical staining using antibodies against MMP-2, MMP-7 and MMP-9 showed a statistical overexpression in the neoplastic tissue when compared with the neighboring normal tissue ($p<0.001$).

Correlations between clinicopathologic parameters and expression of MMPs are presented in Table 2.

Table 2 Correlations between clinicopathological features and the expression of MMP-2, MMP-7 and MMP-9

	MMP-2 Cell		MMP-2 Stromal		MMP-7 Cell		MMP-7 Stromal		MMP-9 Cell		MMP-9 Stromal	
	Positive n=19	P	Positive n=27	P	Positive n=32	p	Positive n=4	P	Positive n=37	p	Positive n=79	p
CEA												
<5	4		19		27		3		21		59	
>5 ng mL	15	0.047	8	NS	7	0.012	1	NS	17	0.013	20	NS
CA19.9												
<37	5		17		21	NS	2	NS	19	NS	57	NS
>37 U mL	14	NS	10	NS	11		2		18		22	
MMP-2												
<200	2		19		18		3		22		45	
>200 ng mL	17	0,018	8	0.021	14	NS	1	NS	16	NS	32	0,012
MMP-7												
<2,3	6		13		21		3		18		41	
>2,3 ng mL	13	NS	14	NS	11	0.036	1	0.047	19	NS	38	NS
MMP-9												
<400	3		16		16		4		29		43	
>400 ng mL	12	0.036	11	NS	18	0.023	4	NS	8	0.018	36	NS
pT1	2		9		7		0		6		14	
pT2	5	NS	8	NS	11	0.039	2	NS	10	0.027	24	0,027
pT3	12		10		14		2		21		41	
N												
present	15	0.018	17	NS	17	NS	2	NS	17	NS	43	NS
Absent	4		10		15		2		21		36	
Stage I	4		6		9		0		9		18	
Stage II	5	NS	6	0.046	10	NS	2	NS	11	NS	29	NS
StageIII	9		15		13		2		17		32	

NS-Statistically not significant, **n**-number of cases, **pT**-pathological Tumor (from pTNM classification), **N**-Limph nodes (from pTNM classification)

MMP-2 cell expression was in a significant positive correlation with serum levels of CEA and MMP-2 preoperatively in CRC patients, and serum levels of MMP-9 and metastasis in the lymph nodes. Stromal positivity to MMP-2 was in a positive correlation with serum levels of MMP-2 and disease stage.

MMP-7 cell expression was in a significant positive correlation with serum levels of CEA, MMP-7 and MMP-9 and depth of tumour invasion. No significant correlations were obtained between stromalexpression in MMP-7 and any of the examined parameters.

MMP-9 cell expression was in a positive correlation with serum levels of CEA and MMP-9 and depth of CRC invasion, i.e. T-parameter. Stromal positivity to MMP-9 was in a significant positive correlation with serum levels of MMP-9 and depth of tumour invasion.

Stromal expression of MMP-2 was also in a significant positive correlation with depth of invasion.

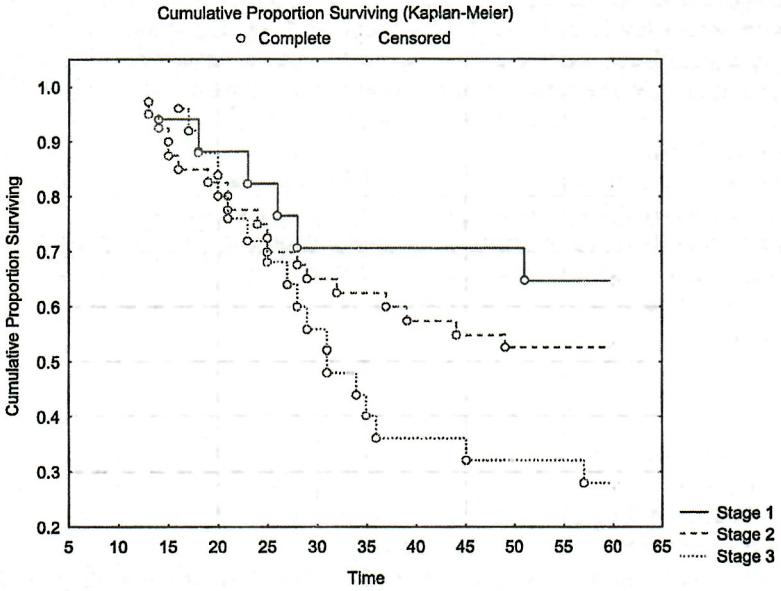

Figure 1 Kaplan-Meier survival curves for colorectal cancer patients in Stadium I, II and III

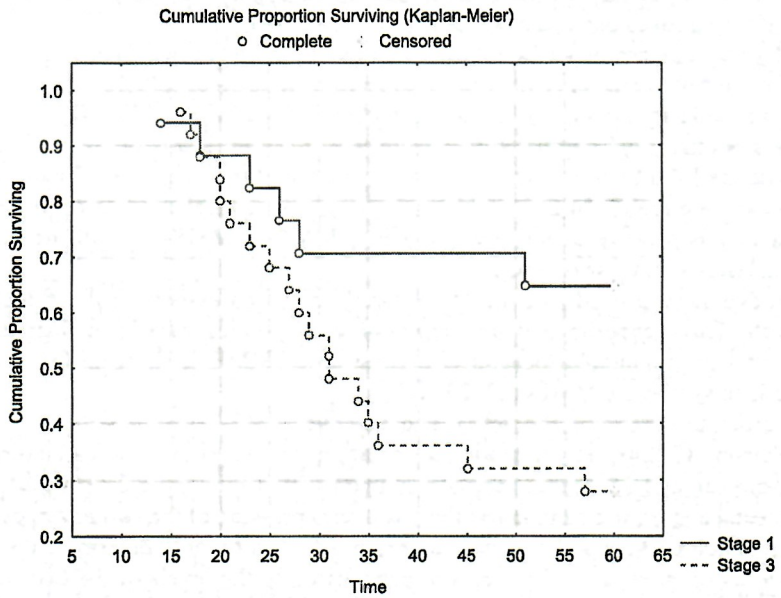

Figure 2 Kaplan-Meier survival curves for colorectal cancer patients in Stadium I and III

Cell expression of the examined enzymes, MMP-2, MMP-7 and MMP-9 was in a positive correlation with serum levels of CEA, MMP-2 and MMP-9. Expression of MMP-7 and MMP-9 was in a significant positive correlation with depth of invasion, MMP-2 was in a significant correlation with the presence of metastasis in the lymph nodes and MMP-7 was in a significant correlation with stage of the disease (Table 2).
According Kaplan-Meier survival curve shown in Figure 1, 65% of the followed CRC patients survive more than 60 months; 52% of the CRC patients in Stadium II survive more than 48 or 60 months and 28% of patients in Stadium III survive more than 55 or 60 months. We found significant differences in terms of the poor outcome in the CRC patients between stage I and stage II B ($p<0,05$), between stage I and stage III ($p<0,01$) (Figure 2. Log-Rank Test: WW = -5.041, Sum = 22.863, Var =5.6428, Test statistic =-2.12226, p = .03382), as well as between stage II A and stage III ($p<0,01$).

Discussion

Prognosis of newly diagnosed CRC cases is predominantly based on the disease stage defined according to International Union Against Cancer (UICC-TNM) and American Joint Committee on Cancer (AJCC, 2010), that is, on the local spread of the disease, lymph nodal status and presence or absence of distant metastasis.
In spite of the advancement in surgical techniques and pharmacological strategies of adjuvant and neo-adjuvant therapy, the 5-year survival in CRC patients is estimated to be from 90% to 10% depending on tumour progression (14).
However, the already accepted fact that CRC is a heterogenic, multifactorial disease has been shown by the fact that histologically identical tumours might have different prognosis and different therapy response (15).
New methods and possibilities are being investigated that might find practical application in anticipation of the disease course and outcome (16, 17, 18, 19).
Invasion and metastasis are major biological features of malignant neoplasms and they are main cause for morbidity and mortality related to malignant diseases (20, 21).
In our study we made an analysis of immunohistochemical staining of MMP-2, -7 and -9 in cancer tissue specimens and correlated the findings with serum levels of CEA, MMP-2,-7 and -9 and with the local tumour invasion and the presence of metastases in 82 patients with colorectal cancer. We found out that positive immunohistochemical staining of MMPs is in correlation with preoperative serum level of CEA, MMP-2, MMP-9, depth of invasion, limph node metastasis and disease stage.
Numerous studies have proved that the MMP family plays an essential role in malignant tumour growth. Early experimental and morphological studies have demonstrated that carcinoma cells have immunohistochemical expression of MMP-2, showing the ability of carcinoma cells to synthesize MMPs (22, 23, 24).
In 1998, in order to determine the active and inactive MMP-2 and MMP-9 expression, Pearsons, Warson, Collins, et al. made examination on 53 colorectal carcinomas, 15 colorectal adenomas and 15 gastric carcinomas upon which they have determined that in both the colorectal and gastric carcinomas there is overexpression of the two enzymes (25).
Tuton, George, Eccles SA, et al. have made an examination in order to determine the MMP-2 and MMP-9 distribution in CRC patients in comparison to the levels of the two enzymes in the patients plasma and the changes that occur in the plasma after the resection, in order to determine whether the plasma levels are a reflection of the clinical staging and the development of the disease. In this examination it is being determined that the MMP-2

plasma levels are considerably elevated in patients with CRC, that they considerably decrease after the surgical resection of the tumor, and that the MMP-9 serum levels are considerably elevated in all the stages of the disease in the patients with CRC, while they decrease after the surgical resection of the neoplasm (26).

Dragutinovic, Radonjic, Petronijevic, et al, in their study of 32 CRC patients and another 11 controls using immunohistochemistry and CEA serum values, CA 19-9 and MMP-2 and 9 determined that there is an important correlation of the MMP values with the staging, but not with the CEA and CA 19-9 serum values. They concluded that the serum MMP-2 and MMP-9 detection can be useful tool for identification of the patients with CRC (27).

Maurel, Nadal, Garcia-Albeniz et al, during the investigation of the MMP-7 serum levels in 87 check-ups of healthy patients and in 120 patients with CRC in order to determine the serum level prognostic significance of this enzyme report that the patients with advanced cancer have considerably higher average MMP-7 values in comparison to those without metastases and in comparison to the healthy patients check-ups. They have determined that the MMP-7 levels are in important correlation also with the shorter survival time, which leads them to the conclusion that the elevated MMP-7 serum levels are independent prognostic factor for survival in patients with advanced CRC (28).

Serum measurements of total MMP-2, MMP-7 and MMP-9 can be considered as an indirect estimation of tumor MMP-2, MMP-7 and MMP-9 expression.

There are studies in which immunohistochemical expression of MMPs are correlated to clinical and pathological parameters with different results.

Kim and Young (29) in their study from 1999 showed the correlation between the expression of MMP-2 and MMP-9 and angiogenesis in CRC. They presented a positive immunohistochemical staining pattern of strong intensity for MMP-2 in tumour cell cytoplasm at the invasive front of the tumour and they found out that the intensity and distribution of the staining were well correlated with modified Astler-Coller classification. The positive staining for MMP-9 was restricted to cytoplasm of tumour cells and isolated stromal cells. The intensity of cytoplasm staining was not in agreement with the modified Astler-Coller classification, lymph nodal status or depth of invasion. Tumour microvascular density was higher in CRC patients with MMP-2 expression than in those patients where the tumour showed no MMP-9 expression.

An immunohistochemical investigation conducted in 2005 by Li et al. (30) at specimens of colorectal cancer showed thatthe expression level of MMP-2 was higher in CRC tumour tissues than in normal tissues. The authors showed that the expression level of MMPs was related to depth of invasion, lymph node metastasis and Duke's stage and that the expression of TIMP-2 in tumour tissues was lower than that in normal tissues. They also presented that with the progression of tumour invasion, lymph node metastasis and stage, the expression of TIMP-2 increased, but it never reached the expression of the normal colorectal tissue.

Another similar investigation conducted in the same year showed that the MMP-7 expression in tumour tissues was associated with lymph node metastasis and poor five-year survival, and the MMP-9 expression was related to depth of tumour invasion (31).

The aim of the Schwandner et al. study (32) was to determine the prognostic role of MMPs in CRC. They presented positive staining for MMP-2 both in tumour tissue and in stroma, 35% and 77% respectively, where stormal staining pattern was correlated with the depth of invasion, MMP-7 and TIMP-2 expression. Cytoplasmic staining of neoplastic cells was in correlation with MT1-MMP (membrane-type 1 matrix metalloproteinase) and TIMP-2 expression. The authors of this study found no correlation between immunohistochemical staining pattern for MMP-2 and gender, age, grading, stage, nodal status and preoperative serum CEA level. Regarding staining with anti-MMP-7 the authors found positive

expression in tumour epithelial cells, but not in stromal cells in 51% of cases. This staining was in correlation with depth of invasion and TIMP-2 expression.
In comparison to above reports in our study we found that positive tissue expression of all examined enzymes, MMP-2, MMP-7 and MMP-9, was in a positive correlation with serum levels of CEA, MMP-2 and MMP-9. Expression of MMP-7 and MMP-9 was in a significant correlation with presence of nodal metastasis, and MMP-7 was in a significant correlation with disease stage.

Conclusion

In conclusion, our investigation confirmed the presence of MMP-2, MMP-7 and MMP-9 in tumour cells and tumour stroma with significantly more common immunoreactive expression compared to that in normal tissue.
We found positive significant correlation of MMP-7 and MMP-9 tissue expression with depth of invasion, positive correlation of MMP-2 tissue expression with presence of nodal metastasis, as well as a positive correlation of tissue expression of MMPs with the serum levels of CEA, MMP-2 and MMP-9. These correlations indicate that tissue expression of MMPs is a useful indicator on the disease spreading and might be used as a prognostic factor for CRC.

Conclusion

In conclusion, a positive significant correlation of MMP-7 and MMP-9 with depth of invasion, the positive correlation of MMP-2 with presence of nodal metastasis, as well as a positive correlation of tissue expression of MMPs with the serum levels of CEA and MMPs indicate that tissue expression of MMPs is a useful indicator on the disease spreading and might be used as a prognostic factor for CRC.

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