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Expression of matrix metalloproteinases 2, 7 and 9 in patients with colorectal cancer

Ekspresija matriks metaloproteinaza 2, 7 i 9 kod bolesnika sa kolorektalnim karcinomom

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Abstract

Background/Aim. Matrix metalloproteinases (MMPs) are perceived to play a key role in tumor invasion and metastasis by their capacity to degrade basement membranes and extracellular matrix proteins. The aim of this study was to investigate the expressions of MMP-2, MMP-7 and MMP-9 in tumor 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. The results of immunohistochemical expression of MMPs were correlated with some clinical and pathologic parameters. **Results.** Immunohistochemical expression of MMP-2 was more frequent in the patients with higher preoperative serum levels of carcinoembryonic antigen (CEA) (p = 0.047), MMP-2 (p = 0.018), MMP-9 (p = 0.036) and in those with lymph node metastasis (p=0.018) and the advanced stage of the disease (p = 0.046). Expression of MMP-7 was more frequent in the patients with elevated preoperative serum levels of: CEA (p = 0.012), MMP-7 (p = 0.036), MMP-9 (p = 0.023) and with deeply invasive neoplasms (p = 0.027). MMP-9 cell expression was in a positive correlation with elevated preoperative serum levels of: CEA (p = 0.013), MMP-2 (p = 0.012), MMP-9 (p = 0.018) and depth of CRC invasion, *ie* T-parameter (p = 0.027). **Conclusion.** Immunohistochemical expression of MMPs is a useful indicator of the disease development and progression in patients with colorectal cancer.

Key words:
colorectal neoplasms; matrix metalloproteinases; neoplasm staging; immunohistochemistry; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Smatra se da matriks metaloproteinaze (MMPs) igraju ključnu ulogu u procesima tumorske invazije i metastaziranja preko njihove sposobnosti za degradaciju bazalnih membrana i proteina ekstracelularnog matriksa. Cilj rada bio je ispitati ekspresiju MMP-2, MMP-7 i MMP-9 u tumorskom tkivu i njihovu povezanost sa kliničkopatološkim karakteristikama bolesnika sa kolorektalnim karcinomom. **Metode.** Tkivni uzorci reseciranih kolorektalnih karcinoma i opkružujuće normalno tkivo 82 bolesnika sa kolorektralnim karcinomom bili su imunohistohemijski obojeni za MMP-2, MMP-7 i MMP-9. Rezultati imunohistohemijske ekspresije MMPs korelirani su sa pojedinim kliničkim i patološkim parametrima. **Rezultati.** Imunohistohemijska ekspresija MMP-2 bila je mnogo češća kod bolesnika sa većim preoperatvinim nivoima CEA u serumu (p = 0.047), predoperativnim nivoima MMP-2 (p = 0.018) i MMP-9 (p = 0.036) u serumu, kod bolesnika sa metastazama u limfnim žlezdama (p = 0.018) i sa uznapredovalim stadijumom bolesti (p = 0.046). Ekspresija MMP-7 bila je mnogo češća kod bolesnika sa povišenim preoperativnim serumskim nivoima CEA (p = 0.012), MMP-7 (p = 0.036) i MMP-9 (p = 0.023) i kod bolesnika sa dubokom invazijom neoplazme (p = 0.027). Ćelijska ekspresija MMP-9 bila je u pozitivnoj korelaciji sa povišenim preoperativnim serumskim nivoima CEA (p = 0.013), MMP-2 (p = 0.012), MMP-9 (p = 0.018) i sa dubinom invazije kolorektalnog karcinoma, tj. parametra T (p = 0.027). **Zaključak.** Imunohistohemijska ekspresija MMPs korisni je indikator razvoja i napredovanja bolesti kod bolesnika sa kolorektalnim karcinomom.

Ključne reči:
kolorektalne neoplazme; matriks metaloproteinaze; neoplazme, određivanje stadijuma; imunohistohemija; osetljivost i specifičnost.

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Introduction

Colorectal cancer (CRC) is one of the most common malignant neoplasms in developed countries. Being the third most common malignant disease CRC accounts for an estimated 570,000 new cases per year and it is the second most common cause of death in the Western-European countries and eight in the developing countries [1]. CRC prognosis predominantly depends on the disease stage, but new prognostic factors are being investigated in order to determine disease progression and outcome in patients, as well as postsurgical pharmacology treatment [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 tumor 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 tumor 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 have 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 tumor 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 [6–8].

Among MMPs, matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9), as members of gelatinases, play important roles in the migration of malignant cells, because of their ability to 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 extracellular matrix proteins and is intensively expressed by tumor and stromal components of cancer [10].

Matrix metalloproteinase 7 (MMP-7) or matrilysin, as a member of stromelysins is able to induce cell apoptotic impairment. Matrilysin can regulate angiogenesis either by inducing a direct proliferative effect on vascular endothelial cells or producing angiogenesis inhibitors (angiostatin, endostatin and neostatin-7) or enriching the variety of angiogenesis mediators, such as the soluble vascular endothelial growth factor (sVEGF)[11]. It degrades type IV and X collagen, elastin, fibronectin, laminin, osteopontin, proteoglycans, as well as numerous others substrates [12].

Increased levels of MMPs 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 of CRC occurring and its progression [13, 14].

The aim of this study was to investigate the expressions of MMP-2, MMP-7 and MMP-9 and their relation to clinicopathologic 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%) males, ages ranging from 43 to 75 years, the 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 (2007–2009).

Blood samples from all the patients were drawn before surgical treatment in order to examine 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 sandwich enzyme linked immuno sorbent assay (ELISA, R&D Systems, USA) according to the manufacturer's instructions. MMP-2, MMP-7 and MMP-9 technique can detect both 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 a calibrator, to produce samples with a 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 Tumor Nodes Metastasis (TNM) classification of American Joint Committee on Cancer (AJCC) (2010).

Immunohistochemical staining

Tissue sections for immunohistochemistry were taken from tumor tissue of the invasive neoplasm front and of the peritumoral 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 monoclonal antihuman 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 were used.

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 nonspecific 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 tumor epithelial cells were stained, and staining was considered to be positive when > 10% of tumor epithelial cells were stained.

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

Stromal cells positivity 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 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

Statistical analysis was done by applying the Pearson's χ^2test, Fisher's exact test, Mann-Whitney's *U*-test, analysis of variance and Kolmogorov-Smirnov test. Differences were considered statistically significant for *p* values < 0.05.

Results

The localization of analyzed cancers and number of cases are listed in Table 1.

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 find in 57 (69.51%) patients with different pT category (Table 2). The majority of patients were with pT3N0M0 (26.82%), *ie* patients in stage II A of the disease, and the smallest number of patients were with pT4aN1M0 (4.87%), *ie* patients in stage III B of the disease.

Immunohistochemical staining with anti-MMP-2

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

Table 1

Localization and the number of colorectal cancer cases

Localization of cancers	Cancer cases	
	n	%
Rectum	13	15.85
Rectosigmoideum	17	20.7
Sigmoideum	14	17.07
Colon descendens	10	12.19
Colon transversum	3	3.65
Flexura lienalis	7	8.53
Flexura hepatica	3	3.65
Colon ascendens	3	3.65
Coecum	12	14.63
Total	82	100

Table 2

Tumor nodes metastasis (TNM) staging of the disease in colorectal cancer patients according to the American Joint Committee on Cancer

TNM stage	pTNM	Patients	
		n	%
I	pT1 N0 M0	8	20.73
	pT2 N0 M0	9	
II	pT3 N0 M0	22	48.78
	pT4a N0 M0	18	
III	pT3 N1b M0	7	30.48
	pT3 N2a M0	9	
	pT4a N1b M0	4	
	pT4a N2b M0	5	

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

The signal intensity of stained stromal cells in all the 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 tumor neighboring tissue showed no immunoreactivity with anti-MMP-2 antibody either in the mucosal epithelial cells or in lamina propria.

Immunohistochemical staining with anti-MMP-7

Staining with anti-MMP-7 antigen showed a weak cytoplasmic reactivity (+) with tumor epithelial cells in 32 (39.02%) cases whereas positive stromal staining was detected in 4 (4.86%) cases alone in the regions of inflammatory reaction.

Sections from the tumor neighboring tissue showed no immunoreactivity with anti-MMP-7 antibody neither in the mucosal epithelial cells nor in lamina propria (Figure 1).

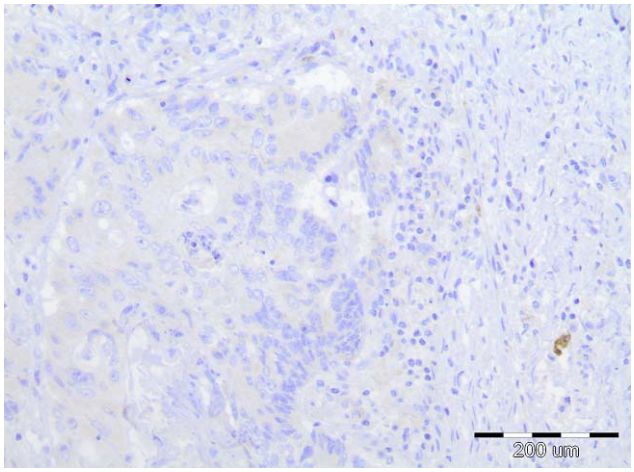

Fig. 1 – Weak staining with anti-MMP-7 for tumor cell components (+) and negative staining for stromal cells (-) with exception of 2 stromal cells (< 10%) (MMP-7 IHH, 10 × 40).

Immunohistochemical staining with anti-MMP-9

Staining with anti-MMP-9 showed positive immunoreactivity of weak intensity in tumor 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 tumor stroma. Macrophages showed the most intense staining (Figure 2).

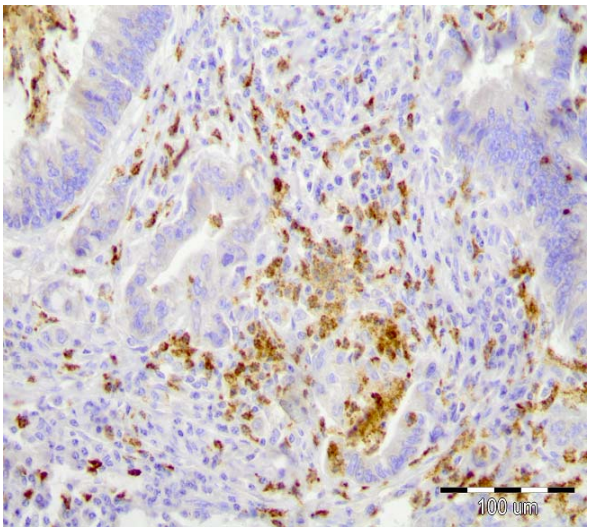

Fig. 2 – Immunohistochemical staining with MMP-9. A weak signal of neoplastic cells (+) and strong signal of stromal inflammatory cells (+++); (MMP-9 IHH, 10 × 20).

Sections from the tumor neighboring tissue showed no immunoreactivity with anti-MMP-9 antibody either in the mucosal epithelial cells nor in lamina propria, except in the inflammatory cells if present in the specimens (Figures 3 and 4).

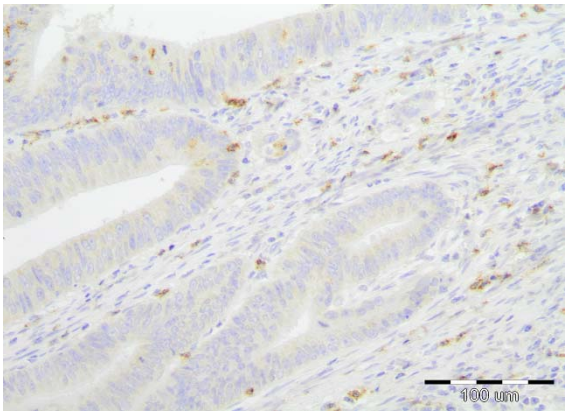

Fig. 3 – Immunohistochemical staining with MMP-9. A weak signal of all epithelial cells (+++) and a strong signal of stromal inflammatory cells (+++); (MMP-9 IHH, 10 × 20).

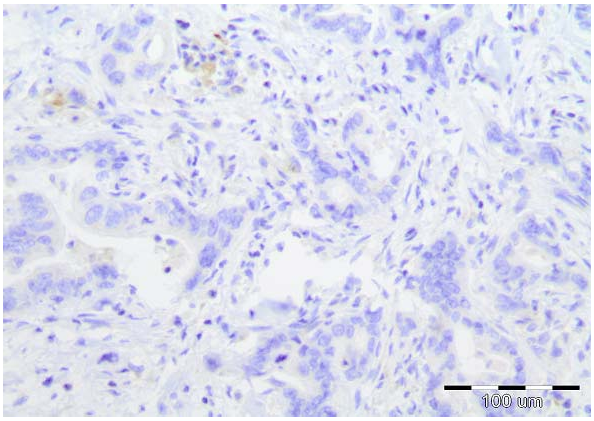

Fig. 4 – Immunohistochemical staining with MMP-9. Negative staining of neoplastic epithelium and negative staining of stromal inflammatory cells (< 10%) (MMP-9 IHH, 10 × 20).

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 ($p < 0.001$).

The correlations between clinicopathologic parameters and expression of MMPs are presented in Table 3.

Table 3

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

MMPs expression	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 (ng/mL)												
< 5	4		19		27		3		21		59	
> 5	15	0.047	8	ns	7	0.012	1	ns	17	0.013	20	ns
CA19.9 (U/mL)												
< 37	5		17		21	ns	2	ns	19	ns	57	ns
> 37	14	ns	10	ns	11		2		18		22	
MMP-2 (ng/mL)												
< 200	2		19		18		3		22		45	
> 200	17	0.018	8	0.021	14	ns	1	ns	16	ns	32	0.012
MMP-7 (ng/mL)												
< 2,3	6		13		21		3		18		41	
> 2,3	13	ns	14	ns	11	0.036	1	0.047	19	ns	38	ns
MMP-9 (ng/mL)												
< 400	3		16		16		4		29		43	
> 400	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	
Limph nodes												
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; 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 the 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 tumor invasion. No significant correlations were obtained between stromal expression 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, *ie* T-parameter. Stromal positivity to MMP-9 was in a significant positive correlation with serum levels of MMP-9 and depth of tumor invasion.

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

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 3).

Discussion

Prognosis of newly diagnosed CRC cases is predominantly based on the disease stage defined according to the 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 the presence or absence of distant metastasis.

In spite of the advancement in surgical techniques and pharmacological strategies of adjuvant and neoadjuvant therapy, the 5-year survival in CRC patients is estimated to be from 90% to 10% depending on tumour progression [15].

However, the already accepted fact that CRC is a heterogenic, multifactorial disease has been shown by the fact that histologically identical tumors might have different prognosis and different therapy response [16].

New methods and possibilities are being investigated that might find practical application in anticipation of the disease course and outcome [17–20].

Invasion and metastasis are major biological features of malignant neoplasms and they are main cause for morbidity and mortality related to malignant diseases [21, 22].

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 tumor 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 tumor 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 [23,24].

Later, *in situ* hybridization supported the findings that stromal tumor cells *in vivo* create mRNA for MMP-2 [25]. It was further confirmed that MMP-9 is produced both by cancer cells and stromal cells and that stromal cells matrix proteins, such as laminin, have an impact on MMPs secretion [26].

Contemporary researches more often emphasize MMPs to be prognostic factors for several types of malignant tumors, including CRC [27].

Diverse results have been obtained from numerous examinations performed to determine the significance of MMPs in the diagnosing of malignancies and to determine their influence on the outcome [6].

In 1998, in order to determine active and inactive MMP-2 and MMP-9 expression, Pearsons et al. [28] examined on 53 colorectal carcinomas, 15 colorectal adenomas and 15 gastric carcinomas upon which they determined that in both colorectal and gastric carcinomas there was overexpression of the two enzymes [28].

Tutton et al. [29] made an examination in order to determine MMP-2 and MMP-9 distribution in CRC patients in comparison to the levels of the two enzymes in patients plasma and the changes that occur in plasma after resection, in order to determine whether plasma levels were the consequence of clinical staging and the development of the disease. That examination determined that the MMP-2 plasma levels were considerably elevated in patients with CRC, that they considerably decreased after surgical resection of the tumor, and that the MMP-9 serum levels were considerably elevated in all stages of the diseased in patients with CRC, while they decrease after the surgical resection of neoplasm.

On the contrary, in the Ruokolainen's [30] investigation for the prognostic role of MMP-2 and MMP-9 and their tissue inhibitors (TIMP-1 and TIMP-2) in squamous head and neck cancer, was shown that serum MMP-2 immunoreactive protein levels in check-ups of healthy patients were higher than in the patients with cancer, while MMP-9 and TIMP-1 levels were considerably higher in patients with squamous carcinoma. The authors determined an important correlation between the serum levels of MMP-9 and TIMP-1 with immunohistochemical expression of MMP-9 and TIMP-1 from the tumor tissue.

Dragutinović et al. [31] 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 was an important correlation of the MMP values with staging, but not with CEA and CA 19-9 serum values. They concluded that the serum MMP-2 and MMP-9 detection can be a useful tool for identification of the patients with CRC.

Maurel et al. [32], during the investigation of 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 MMP-7 levels are in important correlation also with the shorter survival time, which leads them to the conclusion that elevated MMP-7 serum levels are independent prognostic factor for survival in patients with advanced CRC.

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 Kim [33] 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 tumor cell cytoplasm at the invasive front of the tumor and they found out that the intensity and distribution of staining were well correlated with the modified Astler-Coller classification. Positive staining for MMP-9 was restricted to cytoplasm of tumor 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. Tumor microvascular density was higher in CRC patients with MMP-2 expression than in those patients where the tumor showed no MMP-9 expression.

An immunohistochemical investigation conducted in 2005 by Li et al. [34] at specimens of colorectal cancer showed that the expression level of MMP-2 was higher in CRC tumor 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 tumor tissues was lower than that in normal tissues. They also presented that with the progression of tumor 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 MMP-7 expression in tumor tissues was associated with lymph node metastasis and a poor five-year survival, and MMP-9 expression was related to the depth of tumor invasion [35].

The aim of the Schwandner et al. [36] study was to determine the prognostic role of MMPs in CRC. They presented positive staining for MMP-2 both in tumor 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 tumor 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 the above reports in our study we found out that positive tissue expression of all 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 correlation with the presence of nodal metastasis, and MMP-7 was in a significant correlation with disease stage.

Conclusion

Our investigation confirmed the presence of MMP-2, MMP-7 and MMP-9 in tumor cells and tumor stroma with significantly more common immunoreactive expression compared to that in normal tissue.

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

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