DOI 10.4149/BLL_2024_110

Bratisl Med J 2024; 125 (11):
724–727

CLINICAL STUDY

MMP9 and pancreatic cancer

Veronika ROSKOVICOVA[1], Jana KATUCHOVA[1], Ivana VECURKOVSKA[2], Jana MASLANKOVA[2], Maria MAREKOVA[2], Jozef RADONAK[1], Vladimir KATUCH[3]

1st Department of Surgery, Medical Faculty at Pavol Jozef Safarik University and University Hospital in Kosice, Kosice, Slovakia.
jana.katuchova@upjs.sk

ABSTRACT
BACKGROUND: Pancreatic carcinoma is one of the most severe oncological diseases of the gastrointestinal tract. At the time of diagnosis, up to 28% of patients have metastatic liver damage, and only 5% of patients survive five years. Scientific research focuses on non-invasive markers that could help screen for the disease and identify patients more quickly. Potential biomarkers also include matrix metalloproteinases, which play a role in oncogenesis.
MATERIAL AND METHODS: We prospectively followed 46 patients with pancreatic cancer and benign pancreatic diseases from September 2022 to March 2023. We determined the level of MMP9 in serum and tissue biopsied during surgeries.
RESULT: As a result, MMP9 levels were elevated from the T2 stage. The correlation between disease stage and MMP9 level was not confirmed in lower stages, possibly due to the small group of patients.
CONCLUSION: MMP9 seems suitable for detecting late stages of pancreatic cancer, possibly for secondary prevention. We could not confirm a correlation between MMP9 levels and the initial stages of the disease *(Tab. 1, Fig. 3, Ref. 21)*. Text in PDF www.elis.sk
KEY WORDS: pancreatic cancer, MMP9, marker, non-invasive, screening.

Introduction

Pancreatic carcinoma is an oncological severe disease of the digestive tract. Despite the attention paid to this disease, the diagnosis of pancreatic cancer is still late. The incidence of pancreatic cancer has been documented as rising in most countries, with mortality rates in the range of about 6–10/100 0000 per year (1). Approximately 5% of pancreatic cancer patients survive five years (2). Patients with unresectable pancreatic cancer or metastatic damage to the hepatobiliary system stay less than one year (3). Diagnosis is based on non-specific clinical symptoms, essential biochemical examination supplemented with oncological markers Ca19-9 and CEA, and imaging examinations. The basis of the treatment is surgical resection according to the anatomical location of the tumor, and an oncologist then manages the patient. The standardized non-invasive markers of pancreatic cancer are currently tumor markers Ca19-9 and CEA. However, they have a limitation in clinical application due to showing false positivity in diseases of the pancreaticobiliary system (4). Therefore, new markers that could be more sensitive and specific for pancreatic cancer are coming to the forefront of research.

Matrix metalloproteinases (MMPs) are one of them. MMPs are the main enzymes and endopeptidases responsible for the degradation and remodeling of the extracellular matrix, which is the basis of the pathological mechanism of oncogenesis (5) (Fig. 1). We currently know 28 subtypes of MMPs, which are divided into classes based on substrate specificity (6). MMP9 is also known as gelatinase B or collagenase IV. type catalyzes the degradation of sizeable extracellular matrix components such as elastin and collagen. It releases and activates extracellular signaling molecules (7). Based on these properties, the relationship of MMP9 with the stage of pancreatic cancer disease is assumed.

Material

The analyzed samples consisted of 46 patients admitted to our hospital for radical surgical treatment or biopsy of pancreatic tumor. All patients signed the instruction and informed consent of the participant in biomedical research according to §27 of Act no. 576/2004 Coll. The research sample consisted of 21 women (45%) and 25 men (55%), with an average age of 62.67 (26–81) years. Patients were histologically divided into two groups. The first group included a patient with a benign finding, which consisted of patients with chronic pancreatitis and benign pancreatic tumors (11 patients, 24%); this group of patients was used as a control group. The second group consisted of patients with malignant tumors of the pancreas

[1]1st Department of Surgery, Medical Faculty at Pavol Jozef Safarik University and University Hospital in Kosice, Slovakia, [2] Department of Medical and Clinical Biochemistry, Medical Faculty at Pavol Jozef Safarik University in Kosice, Slovakia, and [3]Department of Neurosurgery, Medical Faculty at Pavol Jozef Safarik University and University Hospital in Kosice, Slovakia

Address for correspondence: Jana KATUCHOVA, Prof, MD, PhD, MBA, 1st Department of Surgery, Medical Faculty at Pavol Jozef Safarik University and University Hospital in Kosice, Trieda SNP 1, SK-040 01 Kosice, Slovakia.
Phone: +421.55 640 3896

Acknowledgements: This publication was created thanks to grant support within the Internal Scientific Grant System of the University of Pavel Jozef Safarik in Košice (VVGS-2022-2198).

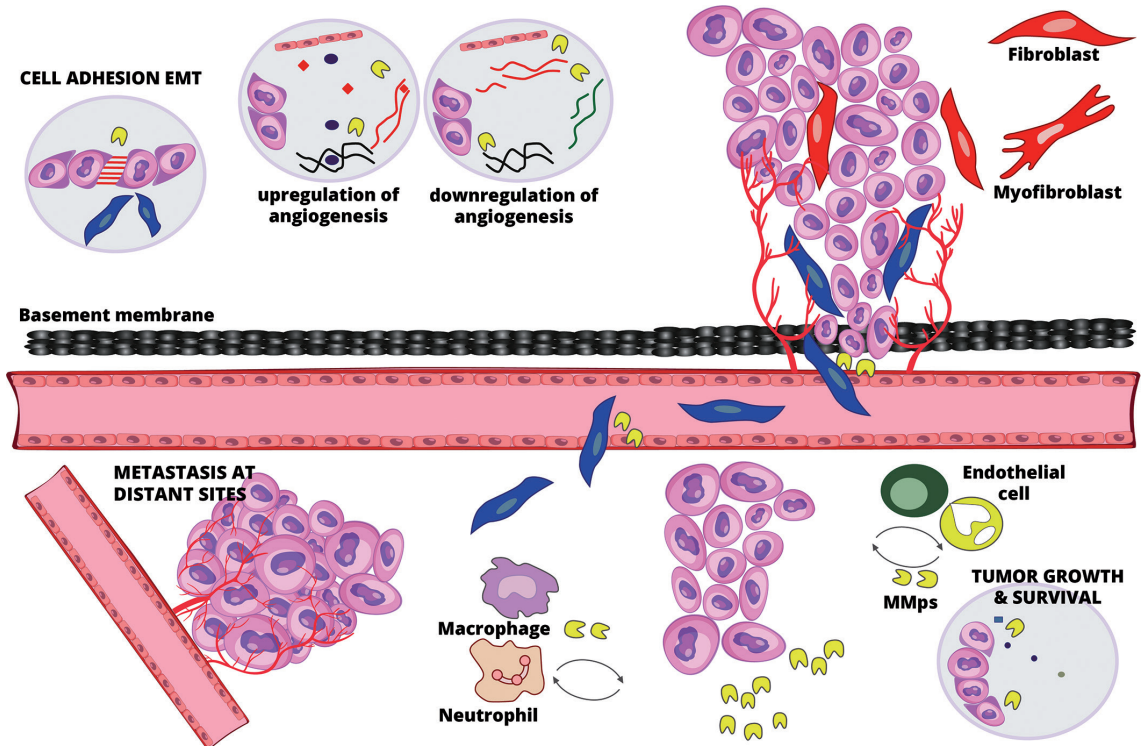

Fig. 1. Main processes of metalloproteinases at the cellular level illustrating oncogenesis.

(35 patients, 76%). This group of patients consisted of patients with adenocarcinoma of the pancreas, which was further divided into individual stages of pancreatic cancer (Tab. 1). Before the surgery in the morning, serum and whole blood samples were taken from the patients in BD Vacutainer tubes to speed up coagulation. These samples are then transported to the Department of Medical and Clinical Biochemistry and analyzed. Tissue samples were taken from the patients during surgery in saline solution with ice and then transported to the Department of Medical and Clinical Biochemistry.

Method

Tissue and serum samples were subsequently processed. Both serum and tissue were first processed by centrifugation, and the homogenized tissue was frozen at –80 °C. Later, we detected the expression of MMP9 in the tissue and the serum using the ELISA method (Human MMP9 Elisa Kit (ab246539). IBM SPSS Statistics 17.0 (SPSS Inc. Chicago, IL, USA) was used to create the research hypothesis. A p-value of less than 0.05 was considered statistically significant. Before surgery, oncological markers Ca19-9, CEA, and AFP were examined in patients' blood according to standardized protocols. These biomarkers will help us to compare MMP9 levels in the serum.

Results

When comparing groups of malignant and benign tumors, the level of MMP9 was measured using the ELISA method, and no significant difference was found between the groups. The reason may be that the level of MMP9 also occurs during tissue remodeling in chronic pancreatitis, as the control group consisted of patients with chronic pancreatitis and benign pancreatic tumors. If we compare the average MMP9 expression in the tissue and the serum of the group of benign tumors, it was 247.79 ng/ml in the serum and 72.61 ng/ml in the tissue. The level of MMP9 in the tissue and the serum in malignant findings was 64.194 ng/ml and 244.14 ng/ml, and no significant difference was proven here either. Between the genders, differences were noted. In men, an increased expression of MMP9 was pointed out in patients with malignant disease. The serum concentration was 312.33 ng/ml, and the tissue concentration was 170.97 ng/ml. In women in the group of patients with malignant disease, it was 311.09 ng/ml in serum and 69.30 ng/ml in tissue. A significant difference was confirmed between the groups. The level of MMP9 in the group of patients with benign disease was lower. In men, the serum concentration was 281.08 ng/ml, and the tissue concentration was 76.94 ng/ml. In women, the concentration in tissue was 275.83 ng/ml, and in serum, 63.9 ng/ml. The differences

Tab. 1. Division of patients based on TNM classification (Ueno et al, 2019).

Stage	T category	N category	M category	Number of patients
0	Tis	N0	M0	1 (2.8%)
I	T1/T2	N0	M0	2 (5.7%)
II	T2	N1	M0	5 (8.6%)
III	T2	N2	M0	4 (14.3%)
IV	Any T	Any N	M1	23 (68.6%)

in MMP-9 expression in tissues between groups were significant. The average concentration of MMP9 in patients in the control group, which consisted of patients with benign pancreatic tumors and chronic pancreatitis, was 247.792 ng/ml in serum and 72.612 ng/ml in tissue. We divided the patients with malignant disease into groups based on TNM classification stages. In stage T0, one patient had an MMP9 concentration in serum of 208.71 ng/ml and a tissue concentration of 22.19 ng/ml. In stage T1, we had two patients with an average concentration of MMP9 in the serum of 235.85 ng/ml and with a concentration of 45.21 ng/ml in the tissue of the patients. In stage T2, we recorded five patients with MMP concentrations in serum 166.60 ng/ml and in tissue 177.34 ng/ml. In stage T3, four patients had serum MMP9 concentration of 242.82 ng/ml and in tissue 59.17 ng/ml. In stage T4, we had the largest group of patients, which consisted of 23 patients. The average concentration of MMP9 in serum was 366.69 ng/ml; in tissue, it was 145.02 ng/ml. When comparing the stages in Figure 2, we noticed a significant elevation of the concentration of MMP9 in the tissues of patients T0 to T4. An observed elevation of the MMP9 level in serum is from stage T2. In the lower stages, the correlation between the stage of the disease and the level of MMP9 in the tissue was not confirmed.

Preoperatively, as part of the standardized diagnosis of the disease, we determined the levels of CA19-9, CEA, and AFP in patients, which are currently the most used biomarkers in the non-invasive diagnosis of pancreatic cancer. Currently, CA19-9 is the most specific and sensitive biomarker of pancreatic cancer used in clinical practice. The level of CA19-9 in the control group was 186.8 ng/ml; in stage T1, it was 89.35 ng/ml. In stage T2, it was 311.16 ng/ml; in stage T3, it was 623.33 ng/ml; and in stage T4, 670.85 ng/ml. The average concentration of CEA in the control group was 1.38 ng/ml, in stage T1 0.642 ng/ml, in stage T2 0.68 ng/ml, T3 6.10 ng/ml, and in stage T4 3.39 ng/ml. The average concentration of AFP in the control group was 1.2 ng/ml; in stage T1, it was 1.79 ng/ml; in stage T2, it was 0.31 ng/ml; in stage T3, it was 3.18 ng/ml, and in stage T4 it was 2. 324 ng/ml. The comparison of CA19-9 and MMP9 levels can be seen in Figure 3.

Discussion

So far, there is no screening program to detect early stages of pancreatic cancer. More than 90% of all pancreatic cancer diagnoses are diagnosed at a late stage (3). Patients have already present distant metastases in up to half of the diagnosed cases (9). We know risk groups, such as patients with chronic pancreatitis or diabetes mellitus II. type, but we do not yet know of a sufficiently sensitive and specific non-invasive biomarker that could help detect early forms.

Ca19-9 is currently the gold standard in the diagnosis of pancreatic cancer. Zhao et al (2022), summarized 79 studies with 20991 participants; the result was that Ca19-9 has a sensitivity of 72% and a specificity of 86% (10). The biggest problem is its false positivity in diseases of the pancreaticobiliary system, such as chronic pancreatitis, acute cholangitis, and benign bile duct tumors (4, 11). This is why new possibilities of non-invasive biomarkers, such as metalloproteinases, are coming to the forefront of research.

Since the basis of cell changes at the structural level, we decided to investigate an enzyme that directly affects the extracellular matrix's structure, which is the cell's central structural part. The primary function of MMP9 in oncogenesis is to support the dissemination of the process and angiogenesis through the breakdown of the extracellular matrix, especially type IV collagen (12). Many studies have confirmed the correlation between MMP9 levels and the stage of pancreatic cancer, as well as the difference between chronic pancreatitis and pancreatic cancer (13, 14, 15).

Our results demonstrate a significant elevation from stage T2. At this stage, pancreatic carcinoma invades the peripancreatic tissue, supporting the proposition that MMP9 levels are associated with disease dissemination and angiogenesis. Our T0 and T1 levels results are not significant; the reason may be the small cohort of patients. The most frequently diagnosed patients are patients with pancreatic cancer stage T3 and T4; this also confirms the statement that the diagnosis of pancreatic cancer is still late. After the diagnosis, the patient will undergo surgical resection, which, however, is not possible if the patient has metastatic liver damage or a tumor growing into the surrounding vascular structures (16). Only the histology of pancreatic cancer and subsequent oncological treatment remain in the management of the patient.

New non-invasive biomarkers would help us appropriately detect patients for neoadjuvant chemotherapy, which could subsequently increase the potential survival of patients (17). The problem in the treatment of pancreatic cancer is the frequent

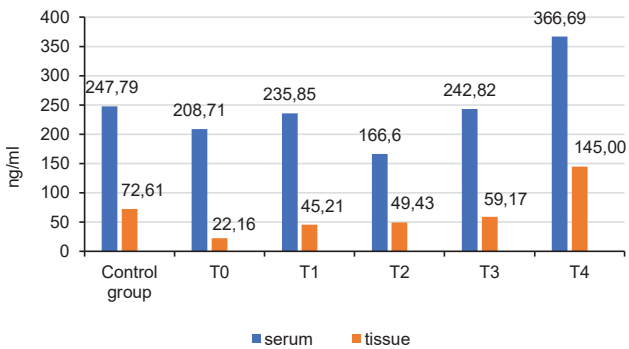

Fig. 2. Relationship between concentration of MMP9 in serum and tissue and TNM classification.

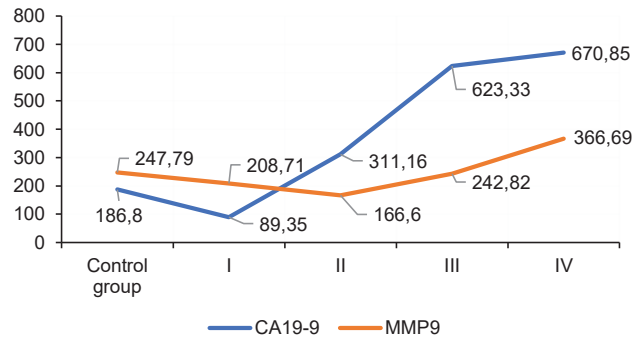

Fig. 3. CA19-9 and MMP9 levels depending on TNM stage and control group.

recurrence of the disease. Patients after surgical resection of the pancreas and subsequent oncological treatment must be monitored. However, the problem is again the low specificity of non-invasive oncological markers, which could serve in secondary prevention. Up to 20% of patients have a local recurrence within five years (18). In the future, according to our results, MMP9 could serve as a suitable marker for secondary prevention of the disease since its elevated levels were mainly in higher TNM stages.

The results of our study are very encouraging. For the possibility of their use in clinical practice, it will be necessary to perform multicenter studies on a larger sample of patients or to include in the examination other MMPs, such as MMP1,2,7, which could be more specific even for lower stages of the disease (19 ,20, 21).

Conclusion

Pancreatic cancer is a very aggressive disease with a low survival rate. Searching for a suitable non-invasive oncological marker can help doctors find patients at an early stage of disease, where the possibility of radical surgical treatment is relatively high. One possibility is the investigation of Matrix metalloproteinases MMP 9. For their accurate use in pancreatic cancer screening, multicenter studies with many patients will be needed.

References

1. Wolnarowska-Talar R, Gasiorowska A, Strzelczyk J et al. Prognostic factors in the operative and palliative treatment of pancreatic cancer. Neoplasma 2003; 50 (5): 383–387.

2. Klein AP. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. Nat Rev Gastroenterol Hepatol 2021; 18 (7): 493–502.

3. Siegel LR, Kimberley DM, Fuchs HE et al. Cancer statistics 2021. Cancer J Clin 2022;, 71 (1): 7–33.

4. Su J, Wang Y, Shao H. Value of multi-detector computed tomography combined with serum tumor markers in diagnosis, preoperative, and prognostic evaluation of pancreatic cancer. World J Surg Oncol 2022; 20 (323).

5. Weniger M, Honselmann KC, Liss AS. The Extracellular Matrix and Pancreatic Cancer: A Complex Relationship. Cancers 2018; 10 (9): 316.

6. Huang H. Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. Sensors (Basel) 2018; 18 (10): 3249.

7. Augoff K, Hryniewicz-Jankowska A, Tabola R, Stach K. MMP9: A Tough Target for Targeted Therapy for Cancer. Cancers 2022; 14 (7): 1847.

8. Ueno M, Morizane CH, Ikeda M et al. A review of changes to and clinical implications of the eighth TNM classification of hepatobiliary and pancreatic cancers. Jap J Clin Oncol 2019; 49 (12): 1072–1082.

9. Kommalapati A, Tella SH, Goyal G et al. Contemporary Management of Localized Resectable Pancreatic Cancer. Cancers 2018; 10 (1): 24.

10. Zhao B, Zhao B, Chen F. Diagnostic value of serum carbohydrate antigen 19-9 in pancreatic cancer: a systematic review and meta-analysis. Eur J Gatroenterol Hepatol 2022; 34 (9): 891–904.

11. Boyd LNC, Ali M, Comandatore A et al. Prediction Model for Early-Stage Pancreatic Cancer Using Routinely Measured Blood Biomarkers. JAMA Netw Open 2023; 6 (8).

12. Mehner C, Hockla A, Miller E et al. Tumor cell-produced matrix metalloproteinases 9 (MMP-9) drives malignant progression and basal-like triple negative breast cancer metastasis. Oncotarget 2014; 5 (9): 2736–2749.

13. Zeng Y, Gao M, Lin D et al. Prognostic and Immunological Roles of MMP-9 in Pan-Cancer. Biomed Research International 2022. DOI: 10.1155/2022/2592962.

14. Grünwald B, Vandooren J, Locatelli E et al. Matrix metalloproteinase-9 (MMP-9) as an activator of nanosystems for targeted drug delivery in pancreatic cancer. J Controll Release 2016; 239: 39–48.

15. Ren B, Cui M, Yang G et al. Tumor microenvironment participates in metastasis of pancreatic cancer. Molecular Cancer 2018; 17 (108).

16. Zhang Z, Shunrong J, Zhang B et al. Role of angiogenesis in pancreatic cancer biology and therapy. Biomed Pharmacist 2018; 1135–1140.

17. Klaiber U, Leonhardt CS, Strobel O et al. Neoadjuvant and adjuvant chemotherapy in pancreatic cancer, Langenbeck´s Arch Surg 2018; 403: 917–932.

18. Tanaka M, Mihaljevic AL, Probst P et al. Meta-analysis of recurrence pattern after resection for pancreatic cancer. Brit J Surg 2019; 106 (12): 1590–1601.

19. Hrabák P, Šoupal J, Kalousová M et al. Novel biochemical markers for non-invasive detection of pancreatic cancer. Neoplasma 2022; 69 (2): 474–483.

20. Xu X, Ji H, Guo Z et al. Elevated serum MMP-1 associated with advanced disease stage and lymph node metastasis in patients with pancreatic carcinoma. Am J Cancer Res 2023; 13 (11): 5405–5417.

21. Slapak EJ, Duitman JW, Tekin C et al. Matrix Metalloproteases in Pancreatic Ductal Adenocarcinoma: Key Drivers of Disease Progression? Biology 2020; 9 (4): 80.

Received April 11, 2024.
Accepted June 11, 2024.