Relationship between pancreatic cancer and Maresin 1

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ABSTRACT

Aims: Pancreatic cancer is the 4th most common cause of death from cancer. In addition, pancreatic cancer is the most common primary malignant tumor of the pancreas. There are many risk factors for pancreatic cancer, including age, certain genetic syndromes, smoking, diabetes, alcohol abuse and obesity. In our study, we aimed to evaluate the role of Maresin 1 (MaR1), which is responsible for the resolution of inflammation, in the pathogenesis of pancreatic cancer and its differential power between chronic pancreatitis and pancreatic cancer.

Methods: The study included 47 patients diagnosed with pancreatic cancer, 32 patients diagnosed with chronic pancreatitis, and 30 volunteers without any additional disease who applied to the internal medicine polyclinic for routine control, who applied to our clinic since October 2021 and accepted to participate in the study. MaR1 levels were measured using the ELISA technique from blood samples obtained from these volunteers.

Results: In our study, MaR1 level was 374.42 (97.27-74.129) pg/ml in the pancreatic cancer group, 491.39 (252.66-949.28) pg/ml in the chronic pancreatitis group and 558.53 (286.94) in the healthy control group. -886,68) pg/ml was measured. There was a significant difference between the pancreatic cancer patients group and the chronic pancreatitis patients and healthy control group. In the pancreatic cancer patient group, MaR1 level was found to be lower than the chronic pancreatitis group (p=0.01).

Our ROC analysis, the discriminative performance of MaR1 (AUC=0.651; [95%CI: 0.535-0.754]; p=0.0169 values) was found to be high in predicting patients with pancreatic cancer according to the patient group with chronic pancreatitis. For MaR1 ≤315.52 cut-off point sensitivity was 36.17% and selectivity was 90.62%.

Conclusion: Proinflammatory cytokines, which have a role in the pathogenesis of pancreatic cancer, increase as a result of the decrease in lipid mediators involved in resolution pathways. Reduction of MaR1 may trigger chronic inflammation and pancreatic carcinogenesis. MaR1 may make an important diagnostic contribution in clinical practice in predicting the progression of patients with chronic pancreatitis to pancreatic cancer.

Keywords: Pancreatic cancer, chronic pancreatitis, inflammation, resolution, Maresin 1

INTRODUCTION

Among the causes of death worldwide, malignancies take second place after cardiovascular system diseases.1 Pancreatic ductal adenocarcinomas (PDAC) are among the cancers with an aggressive course compared to other cancers.2 Pancreatic cancer, which is ninth in frequency in our country, ranks fourth in terms of mortality1 with a survival rate of 10-15% in a 5-year period.3 The incidence of pancreatic cancer is higher in men than in women.4 The disease is frequently seen in the age range of 65-69 in men and 75-79 in women.5

At the mention of pancreatic cancer, the pancreas ductal adenocarcinoma (PDAC) and its variants come to mind. PDAC accounts for approximately 85% of all pancreatic neoplasms. As a result of incomplete resolution of acute inflammation, the inflammatory response may become chronic and progress to fibrosis. If complete resolution is achieved, the spread of inflammation can be prevented and as a result, the tissues can be restored functionally and structurally.6 Mediators produced from omega-3 and omega-6 play an active role in inflammation resolution. Deficiencies in polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and omega-3 fatty acid derivatives, which have immunomodulatory effects, inhibit resolution of inflammation leading to chronic inflammation. Maresins are DHA derivatives secreted from macrophages involved in the resolution of inflammation. Maresin 1 (MaR1) is synthesized in macrophages after several enzymatic reactions with 14-lipoxygenation (LOX) of DHA. An increase in pro-inflammatory cytokines can cause chronic inflammation, cancer formation, and proliferation of cancer.
Maresins are involved in the resolution of inflammation as they decrease proinflammatory cytokines and suppress the communication between molecules involved in proliferation. Thus, they can prevent the formation of cancer as well as suppress inflammation.7–9

This study aimed to evaluate the role of MaR1, a mediator of inflammation resolution, in the pathogenesis of pancreatic cancer and its potential to differentiate between chronic pancreatitis and pancreatic cancer. This is the first clinical study to show the relationship between pancreatic cancer and MaR1.

**METHODS**

**Patient Selection and Study Method**

The study included 47 patients diagnosed with pancreatic cancer and 32 patients diagnosed with chronic pancreatitis at the Gastroenterology Outpatient Clinic of the Faculty of Medicine of Kirikkale University, and 30 volunteers without any disease who applied to the Internal Medicine Polyclinic for routine check-up.

This thesis study was carried out with the permission of Kirikkale University Faculty of Medicine Clinical Researches Ethics Committee (Date: 05.10.2021, Decision No: 16/01). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

**Inclusion Criteria for the Study**

1. Patients over the age of 18,
2. Patients with newly diagnosed pancreatic cancer,
3. For the control group, patients with a new and old diagnosis of chronic pancreatitis,
4. Another control group with individuals without health problems,
5. Patients and healthy individuals who accepted and signed the informed consent form.

Blood was drawn from patients who had taken omega-3 supplements in the last 1 week, from patients using non-steroidal anti-inflammatory and acetylsalicylic acid 1 week after drug discontinuation, and from patients who had not consumed fish in the last week.

**Exclusion Criteria for the Study**

1. Being under the age of 18,
2. Patients with any other pancreatic disease besides pancreatic cancer and chronic pancreatitis,
3. Patients with pancreatic metastases or other malignancies,
4. Patients and healthy individuals who did not accept and sign the informed consent form,
5. For the healthy control group; Patients with A. Diabetes mellitus
   B. Chronic kidney disease
   C. Obesity
   D. Collagen tissue diseases
   E. Thyroid diseases
   F. Anemia
   G. Acute infection
   H. Hyperlipidaemia
   I. Those with a diagnosis of inflammatory bowel disease

J. Those with pancreatic disease
K. Individuals with a history of using fish, omega-3 supplements, and non-steroidal drugs at least once in the last week.

**Laboratory Analysis Methods**

Blood samples were taken from the patients included in the study after at least 12 hours of fasting. The blood taken for routine examinations was centrifuged in 2 cc tubes (13×100, 5 mL plastic tube with Vacutainer gel) at 5000 rpm for 5 minutes, and the separated serum samples were transferred to sterile Eppendorf tubes. Serum samples were stored in a deep freezer at −80°C. All samples were studied concurrently and those with hemolysis were excluded from the study. C-reactive protein was studied with the Cobas C 501 particle surface expanded immunoturbidimetric test using the Roche cobas® c501 brand device and original Roche diagnostic kits (Roche Diagnostic Gmbh, Sandhofer Strasse 116, D-68305 Mannheim). Hemogram parameters (hemoglobin, white blood cell, platelet, neutrophil, lymphocyte) were studied with the flow cytometric impedance method in an automatic complete blood count device (Mindray BC 6800, Shenzhen, China). Glucose (GLUC Hk Gen.3,800 tests Cobas C kit with UV test hexokinase method), aspartate transaminase (Cobas Integra ASTL 500 tests kit with UV absorbance method), alanine transaminase (Cobas Integra ALT 500 tests kit with UV absorbance method), alkaline phosphatase (Cobas Integra ALP 500 tests kit with UV absorbance method), gammaglutamyl transferase (Cobas Integra GGT 500 tests kit with UV absorbance method), total bilirubin, direct bilirubin (Cobas Integra ALP 500 tests kit with the colorimetric method), albumin (Cobas Integra ALB Gen.2 300 tests kit by colorimetric method), creatinine (CREAJ Gen.2, 700 tests, Cobas C, Integra kit using photometric method), sodium (Na, Gen.2 300 tests, Cobas c, Integra kit using colorimetric method) were studied using the Roche cobas® c501 brand device and original Roche diagnostic kits (Roche Diagnostic Gmbh, Sandhofer Strasse 116, D-68305 Mannheim). CA 19.9 and CEA levels were studied by chemoluminescence method using cobas® e601 brand device and original Roche diagnostic kits.

The Maresin 1 Elisa kit was prepared as follows:
- 50 μl of standard diluent was added to each tube.
- 100 μl of standard (1350 pg/ml) was added to the 5th tube.
- 100 μl was transferred from the 5th tube to the 4th tube.
- 50 μl was transferred from the 4th tube to the 3rd tube and the dilution process was continued as in 6th tube
- Undiluted standard is high standard (1350 pg/ml), diluent (empty well) was accepted as zero standard.

![Figure 1. Preparation of standard solution with Elisa kit](image)

**Statistical Analysis**

Descriptive statistics are presented as frequency (n) and percentage (%) for categorical variables, and mean ± standard deviation (SD) or median (IQR) values for continuous variables. The assumption of normality was checked with the Shapiro-Wilk test. Pearson chi-square test was used to analyze
#### RESULTS

A total of 109 participants, including 47 newly diagnosed pancreatic cancer patients, 32 patients with chronic pancreatitis, and 30 healthy control group, were included in the study. Demographic characteristics of the whole population are presented in Table 1 in detail. The mean age was 69.40±9.77 years for the pancreatic cancer group, 58.06±8.01 years for the chronic pancreatitis group and 61.46±8.74 years for the control group. While the mean age did not differ significantly between the chronic pancreatitis group and the healthy group (p=0.420), it was higher in the pancreatic cancer group than the other groups (p<0.05). The rate of male was lower in the pancreatic cancer group than the other groups (Pancreatic cancer group: 63.8% vs. Chronic pancreatitis group: 75% vs. Control group: 73.3%, p=0.040), while the gender distribution did not differ significantly between the chronic pancreatitis and control groups (p>0.05).

The rate of smoking user was higher in the chronic pancreatitis group than the other groups (Pancreatic cancer group: 38.3% vs. Chronic pancreatitis group: 62.5% vs. Control group: 33.3%, p=0.040), while it did not differ significantly between the pancreatic cancer and control groups (p>0.05). The rate of alcohol user was higher in the chronic pancreatitis group than the other groups, while it was higher in the pancreatic cancer than the control groups (Pancreatic cancer group: 38.3% vs. Chronic pancreatitis group: 62.5% vs. Control group: 33.3%, p=0.040), while the gender distribution did not differ significantly between the pancreatic cancer and control groups (p>0.05) (**Table 1**).

The rate of patients with diabetes mellitus was higher in the pancreatic cancer than the chronic pancreatitis group (25.5% vs. 9.4%, p < 0.001). The rate of patients with hypertension were higher in the pancreatic cancer than the chronic pancreatitis group (31.9% vs. 12.5%, p<0.001). The proportion of patients with coexistence of diabetes mellitus and hypertension were lower in the pancreatic cancer than the chronic pancreatitis group (29.8% vs. 37.5%, p<0.001) (**Table 1**).

Median FPG, CRP, AST, ALT, ALP, GGT, total bilirubin, and direct bilirubin values were higher in the pancreatic cancer group compared to the chronic pancreatitis and control groups (p<0.05 for each parameter). While median albumin value was higher in the control group compared to the other groups, it was lower in the pancreatic cancer group compared to the other groups (p<0.001). Median WBC and PLT values were higher in the pancreatic cancer and chronic pancreatitis group than in the control group (p<0.001 for each parameter). While median LDH value was lower in the control group compared to the other groups, it was higher in the pancreatic cancer group compared to the other groups (p<0.001). Median MaR1 value was 374.42 (97.27-74.129) pg/mL in the pancreatic cancer patient group, 491.39 (252.66-949.28) pg/mL in the chronic pancreatitis group, and 558.53 (286.94-886.68) pg/mL in the control group. MaR1 value was lower in pancreatic cancer patients compared to chronic pancreatitis and control groups (p=0.001). MaR1 levels did not differ significantly between chronic pancreatitis and control groups (p=0.370).
The distribution of patients diagnosed with pancreatic cancer by stage was as follows: 3 patients (6.4%) stage 1A, 2 patients (4.3%) stage 1B, 1 patient (2.1%) stage 2A, 5 patients (% 10.6) stage 2B, 12 patients (25.5%) stage 3, 24 patients (51.1%) stage 4 (Table 3).

The patients were grouped according to their stage as Stage 1-2 (group 1) and Stage 3-4 (group 2). MaR1 (p=0.725), CEA (p=0.142), CA19-9 (p=0.440), total bilirubin (p=0.580) values did not differ significantly between group 1 and group 2. The values of MaR1, CEA, CA19-9, and total bilirubin according to tumor stage of patients diagnosed with pancreatic cancer are summarized in Table 4.

MaR1 values showed high diagnostic performance in predicting patients with pancreatic cancer compared to the control group (AUC=0.752, 95% CI=0.641-0.844, p<0.001). The threshold value of MaR1 in predicting pancreatic cancer was ≤372.72 with 85.11% sensitivity and 88.66% specificity. Total bilirubin values showed high diagnostic performance in predicting patients with pancreatic cancer compared to the control group (AUC=0.752, 95% CI=0.641-0.844, p<0.001). The threshold value of TBIL in predicting pancreatic cancer was ≤0.416 with 85.11% sensitivity and 96.76% selectivity. GGT values showed high diagnostic performance in predicting patients with pancreatic cancer compared to the control group (AUC=0.899, 95% CI=0.809-

![Figure 4. Maresin 1 values by pancreatic cancer stage](Image)
was reduced as a result of adenosine monophosphate-
replacement was given to mice on a fatty diet, hepatic steatosis
to the Caurelein group. Chronic pancreatitis group given 2 ng/dl MaR1 compared
of the pancreas in mice developed acute pancreatitis.
MaR1 showed reduced edema, inflammation, and necrosis
caurelein+10 ng/mouse. Mice with acute pancreatitis given
days a week for 4 weeks. 5 groups were formed as control,
induced by Caurelein injection. The chronic pancreatitis
model was obtained with 50 μg/kg caerulein injection 5
of stress in the endoplasmic reticulum (ER).
In a study by Morris et al. including 73 pancreatic
patients, 115 patients with chronic pancreatitis, and 19 patients with cholangiocellular carcinoma, CA19-9
levels were reported to be significantly higher in malignant
diseases than in benign diseases. Median values were
calculated as 653 U/ml for pancreatic cancer, 19 U/ml for
chronic pancreatitis, and 408 U/ml for cholangiocellular carcinoma. In the differentiation of benign and malignant
pathologies, a cut-off point of 70.5 U/ml was determined,
with a sensitivity of 82.1% and a specificity of 81.3%. In
our study, CEA, CA 19-9 and MaR1 levels were compared in
patients diagnosed with pancreatic cancer and chronic
pancreatitis. CEA and CA 19-9 levels were found to be
significantly higher in patients with pancreatic cancer. In
ROC analysis, there was a significant difference between
the areas under the curve. The cutoff point for the CA
19-9 value was determined as 100 U/ml, the sensitivity was
determined as 82.98% and the specificity as 100%.
In a study conducted by Gu et al. through developing
sepsis models on mice, it was determined that after MaR1 replacement with the obstruction method in the cecum,
the cytokine levels involved in inflammation decreased
through the lipoxin A4 receptor/cyclic adenosine
monophosphate/ reactive oxygen species (ALX/cAMP/
ROS) activation, and a significant regression was observed
in developing lung damage.
To the best of our knowledge, this is the first clinical
study in the literature investigating the relationship
between serum MaR1 levels in patients with pancreatic
cancer. In our study, 47 patients with pancreatic cancer, 32
patients with a diagnosis of chronic pancreatitis, and 30
healthy individuals as a control group were included. The
mean age of pancreatic cancer patients was higher than
that of chronic pancreatitis and healthy controls, this was
consistent with the occurrence of the disease in advanced
age. However, age distributions were similar between
chronic pancreatitis and the healthy group.
The diabetes rate in patients with pancreatic cancer was
found to be higher when compared to patients with chronic
pancreatitis, which is consistent with the literature.
MaR1 level was significantly lower in patients
diagnosed with pancreatic cancer compared to chronic
pancreatitis and healthy control groups. However, MaR1
levels of patients did not differ according to tumor stage.
ROC analysis showed that MaR1 has high sensitivity and
selectivity in differentiating pancreatic cancer patients
from chronic pancreatitis and healthy controls (85.11% for
sensitivity, %60 for specificity, and MaR1 ≤492.08 for
cutoff point). Although the MaR1 level was observed to
be low in chronic pancreatitis patients compared to
the control group, it was not significant. These results
suggested that MaR1 could be used as a biomarker to
predict inflammation in patients with pancreatic cancer
and chronic pancreatitis.
The strengths of our study are the exclusion of patients
who had used a food supplement containing omega-3
PUFA, NSAIDs or consumed fish in the last 1 week prior
to the study. An important limitation of this study was
the inability to measure MaR1 measurements at the tissue
level and the low sample size.
CONCLUSION

In our study, we evaluated the role of MaR1 in the pathogenesis of pancreatic cancer. We compared it with the tumor markers CA 19-9 and CEA. According to our literature review, this is the first clinical study showing the relationship between pancreatic cancer and MaR1. MaR1 can be a marker for distinguishing patients with pancreatic cancer from chronic pancreatitis and healthy control groups. It has been shown that it can predict inflammation in patients with chronic pancreatitis. Prospective studies with more patients and longer follow-ups are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: This thesis study was carried out with the permission of Kırıkkale University Faculty of Medicine Clinical Researches Ethics Committee (Date: 05.10.2021, Decision No: 16/01).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

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