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Prognostic significance of plasma matrix metalloprotease-2 in pancreatic cancer patients

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***Background & objectives*: Pancreatic cancer has a propensity for wide stromal invasion. Matrix metalloprotease-2 (MMP-2) is a protease that degrades the peri-tumoural tissue and helps in tumour dissemination. Thus, this study was aimed to assess any association of plasma MMP-2 levels with clinicopathological parameters and survival of patients with pancreatic cancer.**

***Methods*: Plasma samples from 127 pancreatic cancer patients were analyzed for MMP-2 levels by ELISA. Survival and other clinicopathological parameters of patients were analyzed for any correlation with plasma MMP-2 levels.**

***Results*: The mean MMP-2 levels in pancreatic cancer patients were 560.3±222.0 ng/ml which were significantly elevated compared to chronic pancreatitis patients (P<0.001) and healthy individuals (P<0.05). The plasma levels of MMP-2 significantly correlated with tissue expression of this protease (P=0.004). However, MMP-2 levels did not exhibit any association either with clinicopathological parameters or with survival.**

***Interpretation & conclusions*: Elevated MMP-2 levels were observed in blood of pancreatic cancer patients which correlated with its tissue expression. However, these levels did not associate with survival or any clinicopathological parameters of patients. Further studies need to be done to confirm the prognostic/ clinical significance of MMP-2 in cancer patients before and after surgery.**

Key words Matrix metalloprotease-2 - pancreatic cancer - prognosis

Pancreatic cancer is one of the leading causes of cancer-related deaths with a five-year survival rate of only five per cent[1,2]. At present, surgical resection is the only choice of treatment for pancreatic cancer, but post-resection recurrence is common in 80-90 per cent of patients who undergo surgery[3]. Pancreatic cancer is a highly invasive and metastatic neoplasm, and this behaviour accounts for its poor prognosis. The local microenvironment contributes significantly to the progression of cancer with tumour-associated proteolytic enzymes playing a key role in invasion and metastasis of solid tumors[4]. Invasive tumour cells have a marked ability to degrade extracellular matrix via activation of matrix metalloproteinase (MMP)-2. MMP-2 is secreted as an inactive zymogen and requires distinct activation processes to be converted into an active MMP-2[5].

MMP-2 has been studied in various cancers in tissue as well as blood. Co-expression of MMP-2/MMP-9 in breast tumour cells has been considered to be an independent risk factor for patient survival[6]. Dong *et al*[7] reported MMP-2 overexpression as an independent prognostic indicator for survival of patients with colorectal cancer. However, MMP-2 was found to be of limited prognostic value in breast cancer tissue[8], ovarian cancer tissue[9] and colorectal tumour tissue[10]. Though Hong *et al*[10] observed MMP-2 to be an insignificant predictor of prognosis in colorectal cancer, MMP-2 overexpression correlated with T-stage indicating some role of MMP-2 in progression of the disease. Immunohistochemical studies on pancreatic cancer have shown that MMP-2 expression correlates with local advancement of tumour, vascular encasement by tumour, lymph node involvement, metastasis and recurrence rate[11-14]. In a study on oral squamous cancer cell lines, active MMP-2 was noted to contribute to lymph node invasion[15]. Vasala *et al*[16] did not find any correlation between MMP-2 expression and survival or with any of the clinicopathological parameters in bladder cancer patients. In another study on pancreatic tumour tissue, MMP-2 was not found to be an independent prognostic marker, but its epithelial expression correlated with tumour stage and grade[17].

Increased levels of MMP-2 protein in serum have been observed in breast cancer[18,19]. Sheen-Chen *et al*[18] found higher MMP-2 levels correlating with advanced tumour staging and advanced lymph node status. However, Patel *et al*[19] observed that raised MMP-2 levels did not correlate with clinicopathological factors in breast cancer. Acar *et al*[20] demonstrated that serum MMP-2 levels were significantly lower in patients with ovarian malignancies than those in the controls. MMP-2 has been proposed to be a putative biomarker for gastric cancer in serum[21] and body fluids[22]. In pancreatic cancer, only one study[23] reported raised levels of serum MMP-2 in a subgroup of pancreatic cancer patients with unresectable disease. Therefore, the aim of this study was to evaluate the plasma levels of MMP-2 protein in pancreatic cancer patients and to look for any clinical and/or prognostic significance of this protease in pancreatic cancer.

Material & Methods

The study was performed on 127 consecutive confirmed pancreatic cancer patients who came to the departments of Gastroenterology and Gastrointestinal Surgery, All India Institute of Medical Sciences, New Delhi, India, between 2007 and 2011. The diagnosis was confirmed on the basis of computed tomographic scan and/or histopathological evidence. Patients who were found to have periampullary carcinoma, neuroendocrine tumour or insulinoma were excluded. The study was approved by the Institute's Ethics Committee and informed written consent was taken from all patients. Blood samples (10 ml) were drawn and collected in vacutainers. The plasma was separated by centrifuging at 2000 ×*g* for 10 min at room temperature, within two hours of blood collection. The separated plasma was divided into 200 µl aliquots and stored at −80°C till further use. The plasma samples of 25 healthy individuals (relatives or attendants of patients) and 25 chronic pancreatitis patients were also collected as controls.

Of the 127 pancreatic cancer patients, 25 underwent surgical resection of the tumour. The remaining patients were unresectable and were offered other palliative or chemotherapeutic procedures. Three-month post-operative blood samples were collected from patients who underwent surgical tumour resection and were processed and stored; their clinical data were recorded to see for any recurrence of the disease.

ELISA for matrix metalloprotease-2 (MMP-2) protein: The concentration of MMP-2 in the plasma was determined using an ELISA kit (Calbiochem, Merck, USA) as per the manufacturer's instructions. Plasma samples were diluted 1:20 (v/v) with sample diluent. A standard curve for MMP-2 was plotted, and concentration of MMP-2 in plasma samples of patients was extrapolated from this curve. Carbohydrate antigen (CA) 19-9 levels were also measured by ELISA.

Statistical analysis: Student's *t* test, Mann–Whitney U-test and Chi-square test were used for comparing variables between groups. For correlating MMP-2 levels in tissue with that of blood, McNemar Chi-square test was used. For assessing survival, Kaplan–Meier method[24] and log-rank test[25] were used. The software SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

The mean age of the patients in the study group was 55±10.2 yr. Of the 127 patients, 88 were males and 39 were females. Clinical investigations describing the patient disease status are given in Table I. Serum levels of CA19-9 were recorded for 96 (75.6%) patients and the mean level was 2772.8±981 U/ml.

Table I. Summary of clinical parameters of the patients with carcinoma of pancreas (n=127)

Clinical parameters	Number of patients (%)
Pain	93 (73.2)
Jaundice	83 (65.4)
Anorexia	106 (83.5)
Weight loss	107 (84.3)
Diabetes	38 (30.0)
Chronic pancreatitis history	17 (13.4)
Common bile duct dilated	84 (66.0)
Main pancreatic duct dilated	98 (77.2)
Locally advanced disease	87 (68.5)
Lymphatic invasion	50 (39.4)
Vascular encasement	59 (46.5)
Metastasis	52 (41.0)
Resectability	25 (19.6)
Chemotherapy	59 (46.5)

On the basis of imaging parameters, tumour was found to be at resectable stage in 40 (31.4%) patients. However, after biopsy or fine needle aspiration cytology, and during surgery, the number of patients who actually had resectable tumour was 25 (19.6%). After surgery, 14 patients were followed up and their post-operative samples were collected after three months of surgery. The other nine patients who underwent surgery either died before three months or were not available for sample collection.

Survival data: Of the 127 patients, 101 patients died due to the disease, eight patients were alive until the last follow up (three years) was done, six patients died due to reason other than pancreatic cancer while 12 patients were lost to follow up. Overall survival time of the patients was considered as the period between the day on which sample was taken and the day of death of the patients. The median overall survival time of all the patients taken together was five months (range 1-36 months). Patients who were lost to follow up were considered alive and their survival time was recorded until the last follow up.

Matrix metalloprotease-2 levels in plasma: The mean value for MMP-2 levels in plasma of 127 pancreatic cancer patients was 560.3±222.0 ng/ml. This was significantly higher than that of chronic pancreatitis patients (245±68.3 ng/ml; n=25) (P<0.001) as well as healthy individuals (250±140 ng/ml; n=25) (P<0.05). No significant association was observed between plasma MMP-2 levels and clinicopathological parameters (such as tumour size, vascular encasement, lymphatic invasion, stage of the disease and metastasis) (Table II). However, a positive correlation was observed between MMP-2 and CA 19-9 levels in plasma (r=0.243; P=0.012) of cancer patients (n=96).

Table II. Association of plasma matrix metalloproteinase-2 (MMP-2) levels with clinicopathological parameters of patients with carcinoma of the pancreas (n=127)

Clinicopathological parameters	n	MMP-2 levels (ng/ml)
Age (yr)		
<55	57	599.4±101.3
≥55	70	528.8±180.2
Sex		
Male	88	564.7±214.2
Female	39	550.2±240.5
Vascular encasement		
No	65	548.17±120.3
Yes	62	587.6±190
Mass >2 cm		
No	26	569.6±98.9
Yes	101	562±88.7
Stage		
I + II	48	574.9±132.0
III + IV	79	564.0±60.8
Lymphatic invasion		
No	73	562.3±120.2
Yes	54	566.7±180.1
Metastasis		
No	74	569.0±218.7
Yes	53	553.0±209.0
Resectability		
No	102	573.6±176.0
Yes	25	548.9±176.0

Prognostic significance of MMP-2 in plasma: Receiver operating curve (ROC) analysis was used to calculate the cut-off for the survival analysis. The ROC curve was drawn for MMP-2 and the area under the curve was 0.702 (Fig. 1A). The strength of the association of MMP-2 levels in plasma with prognosis was assessed by Kaplan–Meier survival analysis. A cut-off level of MMP-2 at 500 ng/ml was decided for MMP-2 protein using ROC curve where optimal sensitivity and specificity were 66.31 and 65.3 per cent, respectively.

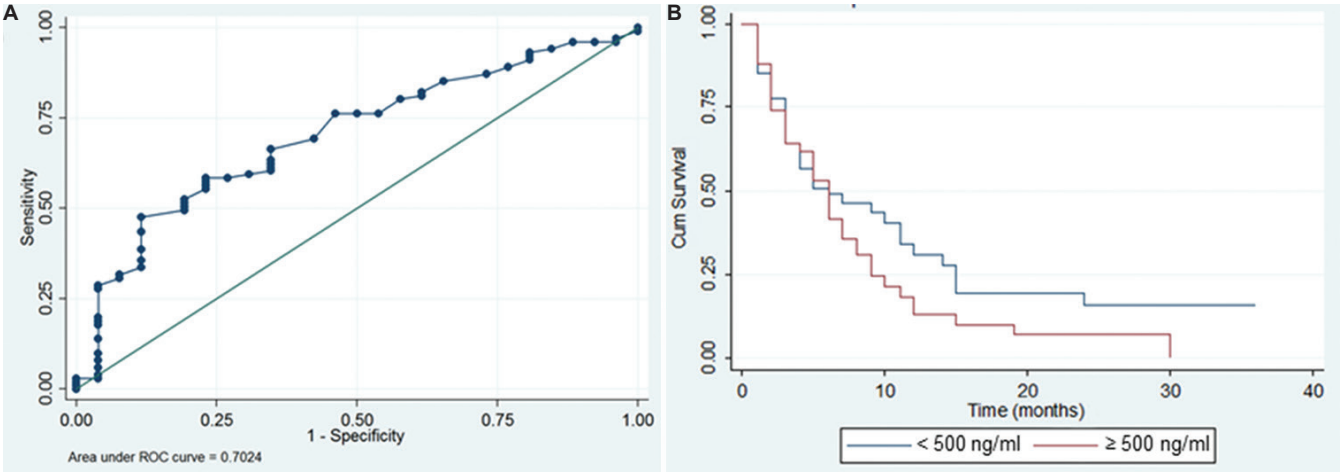

Fig. 1. (**A**) Receiver operating characteristic curve for matrix metalloprotease-2 (ng/ml). (**B**) Kaplan-Meier survival curve for pancreatic cancer patients with low levels of matrix metalloprotease-2 (<500 ng/ml) (blue) and patients with high levels of matrix metalloprotease-2 (≥500 ng/ml) (red) in plasma (log-rank test, *P*=0.134).

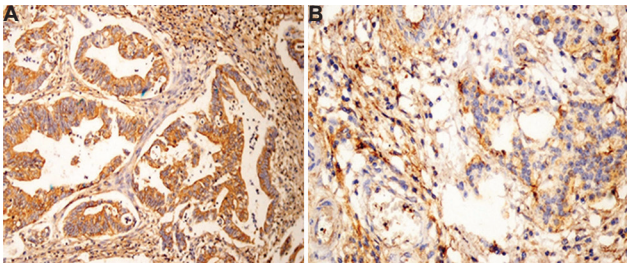

Fig. 2. (**A**) Photomicrograph showing strong immunoreactivity of pancreatic tumour tissue with matrix metalloprotease-2 in ducts (IHC ×100). (**B**) Photomicrograph showing mild matrix metalloprotease-2 staining in stroma of pancreatic tumour tissue (IHC ×200).

The median overall survival time for patients with low (<500 ng/ml) levels of MMP-2 (median survival time: six months) was not significantly different from that of patients with high (≥500 ng/ml) levels of MMP-2 (median survival time: five months) (Table III). Kaplan–Meier survival curve showing cumulative survival as a function of time for low and high levels of MMP-2 is shown in Fig. 1B.

Among the other clinicopathological parameters, shorter survival correlated with patients having vascular encasement (*P*=0.003), metastasis (*P*=0.001), locally advanced disease (*P*=0.008) and advanced stage (*P*=0.004) (Table III).

Further, parameters such as metastasis, stage of the disease and MMP-2 levels were taken in multivariate analysis and only metastasis was found to be independent prognostic marker (*P*=0.01; hazard's ratio=1.86, 95% confidence interval=1.1, 3.0). Parameters such as vascular encasement and locally advanced disease though found to associate with survival in univariate analysis, but could not adjusted in multivariate analysis because as clinically known, these parameters are responsible for giving rise to metastatic disease and influence survival.

Correlation with carbohydrate antigen (CA) 19-9: Of the 14 patients who were followed up after resection, 10 showed recurrence of disease. Patients with distant recurrence (n=3) after surgery had higher levels of both MMP-2 (525±213 ng/ml) and CA19-9 (3546±1210 U/ml) compared to MMP-2 (362±120 ng/ml) and CA19-9 (457±213 U/ml) in patients with local recurrence (n=7). The four patients who did not have recurrence after three months had low MMP-2 (230±110 ng/ml) and CA19-9 (342±212 U/ml) levels.

Correlation between blood and tissue levels of MMP-2: To see the concordance between tissue and blood levels of MMP-2, its expression in tumour tissue of the 25 patients who underwent surgical tumour resection was compared with the levels in their blood samples[26]. The levels of MMP-2 in tissue as well as in blood were individually divided into low expression (0-4 scores) and high expression (5-8 scores) categories and then analyzed for correlation. The immunoreactivity pattern of MMP-2 in pancreatic tumour tissue is shown in Fig. 2 A, B. Table IV shows the comparative account of the MMP-2 levels in plasma with the immunoreactivity of MMP-2 in pancreatic tumour tissue. Taken together, there was a significant correlation between blood

Table III. Comparison of survival between groups in patients with carcinoma of pancreas (n=127)

Parameters	Number of cases	Event (%)	Median survival in months (95% CI)	*P* (log rank test)	Univariate analysis	
					Unadjusted hazard ratio (95% CI)	*P*
Age (yr)						
<55	58	46 (80.0)	5 (3.1-6.8)	0.313	1.0	0.337
≥55	69	54 (78.2)	7 (4.9-9.0)		0.8 (0.5-1.2)	
Sex						
Male	88	70 (80.4)	6 (4.9-7.0)	0.682	1.0	0.720
Female	39	30 (77.0)	5 (2.1-7.9)		0.9 (0.6-1.4)	
Tumor mass (cm)						
<2	26	18 (72.0)	5 (2.1-7.8)	0.202	1.0	0.234
≥2	101	82 (81.0)	6 (4.5-7.4)		1.3 (0.8-2.2)	
Vascular encasement						
No	65	46 (71.8)	6 (3.7-8.3)	0.003	1.0	0.007
Yes	62	53 (86.8)	4 (2.4-5.5)		1.7 (1.2-2.6)	
Locally advanced						
No	39	28 (73.6)	8 (5.1-10.8)	0.008	1.0	0.014
Yes	88	72 (81.8)	5 (3.3-6.6)		1.7 (1.1-2.7)	
Lymphatic invasion						
No	73	55 (76.3)	6 (4.5-7.5)	0.231	1.0	0.232
Yes	54	45 (85.0)	6 (4-8)		1.3 (0.8-1.9)	
Metastasis						
No	74	55 (74.3)	9 (7-11)	0.001	1.0	0.001
Yes	53	45 (86.5)	3 (2-4)		2.1 (1.4-3.1)	
Surgery						
No	102	81 (79.4)	6 (4.7-7.2)	0.349	1.0	0.380
Yes	25	20 (80.0)	6 (4.4-7.5)		0.8 (0.5-1.3)	
Stage						
I + II	48	35 (72.9)	9 (5.5-12.4)	0.004	1.0	0.008
III + IV	79	66 (83.5)	5 (3.3-6.6)		1.7 (1.1-2.7)	
MMP-2 (ng/ml)						
Low (<500)	53	36 (68.0)	5 (1-10)	0.134	1.0	0.141
High (≥500)	74	64 (87.6)	6 (4-7)		1.4 (0.90-2.0)	

CI, confidence interval; MMP, matrix metalloproteinase

Table IV. Correlation of matrix metalloproteinase-2 (MMP-2) in tissue and blood (n=25)

MMP-2 in tissue	MMP-2 in blood		*P*
	Low (<500 ng/ml)	High (≥500 ng/ml)	
Low (0-4 scores)	1	0	0.004
High (5-8 scores)	8	16	

and tissue expression of MMP-2 protein (*P*=0.004) (Table IV).

Discussion

We analyzed plasma MMP-2 concentration in patients with pancreatic cancer and found it to be significantly higher than those of normal healthy individuals and chronic pancreatitis patients. Another study focussing on MMPs and their inhibitors in pancreatic cancer patients also reported elevated levels of MMP-2[23]. This was in agreement with the observations of Sheen-Chen *et al*[18] in breast cancer patients. Angulo *et al*[27] reported increased MMP-2 mRNA levels in

blood of patients with bladder cancer as compared to that of healthy controls. Acar *et al*[20] demonstrated that serum MMP-2 levels were significantly lower in patients with ovarian malignancies than those in the control subjects.

There are studies that have assessed not only concentration of total MMP-2 in the blood but also measured the activity of MMP-2 in serum or plasma with conflicting results. Decock *et al*[28] reported that the total MMP-2 concentration in plasma of patients with primary breast cancer was not significantly higher as compared to controls, but the MMP-2 activity was observed to be significantly increased in cancer patients than control. Contrary to this, Somiari *et al*[29] found that total MMP-2 concentration was higher in patients with breast cancer than controls, but concentration of active MMP-2 was found to be higher in control group as compared to cancer patients.

In our study, no correlation of MMP-2 concentrations in plasma was found with survival or any of the clinicopathological parameters. This could be because we measured total MMP-2 and not the active form of this enzyme. Vasala *et al*[30] found that low circulating pro-MMP-2 as well as low tissue inhibitor of metalloproteinases 2 levels were associated with poor prognosis in patients with bladder cancer. Kuvaja *et al*[31] showed that serum concentration of pro-MMP-2 correlated inversely with node positivity and advanced stage of the disease and high nuclear grade of the tumour while elevated levels of the active form were found to be associated with survival in patients with breast carcinoma. Thus, the effect of MMP-2 on clinical and pathological parameters, progression of disease and survival of patients may be due to interplay of pro-form, active-form and inhibitor of MMP-2.

Limitations of this study were that only total MMP-2 levels were measured and small sample size involved.

In conclusion, elevated levels of MMP-2 were observed in patients with carcinoma of the pancreas as compared to healthy control, but these levels did not show any association with survival or any clinicopathological parameters. However, blood levels of this protein correlated with tissue expression of the protein. Further studies with a large sample need to be done to confirm these findings.

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***Conflicts of Interest*:** None.

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