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ORIGINAL ARTICLE

Role of Plasma MMP 9 levels in the Pathogenesis of Chronic Pancreatitis

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Abstract Pancreatic fibrosis is a key pathological feature in the etiology of chronic pancreatitis that leads to obliteration of exocrine and endocrine pancreatic tissues and its replacement by fibrous tissue resulting in clinical manifestations. Matrix metalloproteinase 9 is a member of the MMP family that is also known as gelatinase B, degrades type IV collagen of extracellular matrix and basal membrane. The present study is aimed at evaluating the clinical significance of plasma concentration of MMP-9 in chronic pancreatitis. The samples were obtained from 112 chronic pancreatitis patients and an equal number of age and sex matched healthy controls. MMP-9 levels were quantitatively measured by ELISA assay. Statistical analysis was applied to test the significance of results. The present study revealed a significant increase of plasma MMP 9 levels in chronic pancreatitis patients compared to control subjects. Elevated levels were also observed in all the patient groups compared to control subjects with regard to sex, age, addictions etc. MMP-9 degrades the type IV collagens in normal basement membrane, which in turn activates the pancreatic stellate cells which promote the development of pancreatitic fibrosis. Thus, elevated plasma levels of MMP-9 may act as a susceptibility factor for the development of chronic pancreatitis.

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Introduction

Chronic pancreatitis is an inflammatory disease causing structural and functional damage resulting in exocrine and endocrine deficit [1]. Pancreatic fibrosis is a characteristic feature of chronic pancreatic injury and is thought to result from a change in the balance between synthesis and degradation of extracellular matrix proteins [2].

Matrix metalloproteinases are a family of zinc-containing zymogen endopeptidases which have an important role in extracellular matrix synthesis and degradation [3]. MMP 9 (gelatinase B, 92 kDa collagenase) is structurally related endopeptidase and is a Zn^{2+} containing enzyme that degrades wide range of ECM components, including collagen types IV and V, different types of gelatin, proteoglycan core protein, fibronectin and elastin [4, 5]. Earlier studies on rats suggest that activated pancreatic stellate cells play a central role in pancreatic fibrogenesis via increased synthesis of ECM proteins [6]. Therefore the present study has been carried out with an aim to estimate the levels of MMP 9 to evaluate its role in the pathogenesis of chronic pancreatitis.

Materials and Methods

Study Subjects

A total of 112 chronic pancreatitis patients attending the Gastroenterology unit of Gandhi Hospital and Osmania General Hospital during the last 2 years were included in the present study. The patients were confirmed for chronic

pancreatitis based on the clinical diagnosis, biochemical findings and imaging analysis. An equal number of asymptomatic control subjects were included in the present study. Informed consent was obtained from all the subjects after explaining the nature of the study. The study was approved by Institutional Ethics Committee. The demographic characteristics such as sex, age, duration, familial incidence, addictions like smoking, and alcohol consumption were noted in a standard proforma.

Sample Collection

Five millilitre of blood was obtained from all the subjects in vacutainers with and without anticoagulant, for plasma and serum analysis. The samples were stored at −70°C until further use.

Enzyme-Linked Immunosorbent Assay (ELISA)

Concentrations of MMP-9 in plasma were measured in duplicate using a commercial ELISA kit according to the manufacturer's protocols (Calbiochem®). In brief, 50 µl detector antibody was added to different wells designated as samples and standards. 50 µl of standard or control samples were added to each well. After 2 h of incubation at room temperature on a constant shaker (500 ± 50 rpm), the reaction solution was aspirated and the wells were washed 4 times with wash buffer; 100 µl of MMP-9 conjugate was then added to each well and incubated for another 30 min on the shaker at room temperature. Then the aspiration/wash steps were repeated as mentioned above, followed by adding 100 µl of substrate solution to each well. The microplate was allowed to stand for 30 min at room temperature in the dark. After adding 100 µl of stop solution, the optical density at 450 nm of each sample was determined and represented in ng/ml. The MMP-9 concentrations for each sample were calculated from the standard curve obtained.

Statistical Analysis

Mean and standard deviation were calculated for all the groups. Student's *t*-test was applied to test the significance of the data at 5 and 1% level.

Results

The demographic characteristics of the chronic pancreatitis patients and control subjects are presented in Table 1. A total of 112 chronic pancreatitis patients and an equal number of control subjects were included in the present study. Among the patients, gender wise distribution of patients showed 93% of males and 7% of females respectively. Age wise classification of the patients below and above 35 years of age showed 41 and 59% respectively. 59% of the patients are smokers and 77% were found to be alcoholics, indicating addictions as one of the risk factor for the disease.

Table 1 Demographic features of chronic pancreatitis patients and control subjects

Variables	Patients		Control subjects		Odds ratio	*P* value
	n	%	*n*	%		
Total	112	112				
Gender						
Males	104	93	92	82		
Females	8	7	20	18	2.826(0.870–9.093)	0.151
Age						
<35 years	46	41	70	63		
≥35 years	66	59	42	37	0.418(0.197–0.889)	0.037
Addictions						
Smokers	66	59	30	27		
Non-smokers	46	41	82	73	3.922(1.780–8.638)	0.001
Alcoholics	86	77	44	39		
Non-alcoholics	26	23	68	61	5.112(2.266–11.523)	0.001
Familial	6	5	–	–		
Non-familial	106	95	–		–	0.243

Table 2 gives the mean levels of plasma MMP 9 in chronic pancreatitis patients and control subjects. The mean MMP-9 levels in CP patients and control subjects were 18.325 ± 3.023 and 13.621 ± 0.5978 ng/ml, respectively. The levels were found to be significantly elevated in the disease group compared to the control subjects ($t = 16.1549$ with $P < 0.001$). A general increase in mean MMP 9 level was apparent irrespective of the gender, age, addictions such as smoking and alcoholism etc.

Table 3 gives the mean MMP 9 levels in different attributes within the disease group. Female patients revealed an elevated activity compared to male patients with no significant difference. Intragroup comparisons indicated the general enhanced activity of MMP 9 in all the groups as a consequence of the disease. Further familial cases showed slightly elevated levels of MMP 9 compared to the non-familial cases.

Discussion

Chronic pancreatitis is a progressive destructive and inflammatory disease of multifactorial etiology that leads to irreversible obliteration of the exocrine and endocrine

Table 2 Mean levels of plasma MMP-9 in chronic pancreatitis patients and control subjects

	CP Mean ± S.D (*n*)	Controls Mean ± S.D (*n*)	*t*-value
Total	18.325 ± 3.023 (112)	13.621 ± 0.598(112)	16.1549**
Sex			
Males	18.271 ± 3.024(104)	12.043 ± 1.006 (92)	18.8484**
Females	21.250 ± 0.606(8)	13.990 ± 1.576 (20)	12.5445**
Age			
<35 years	18.273 ± 3.298 (46)	12.087 ± 0.659 (70)	15.2687**
≥35 years	18.361 ± 2.867 (66)	12.087 ± 0.659 (40)	13.6003**
Alcoholics	18.289 ± 2.835 (86)	13.001 ± 0.560 (44)	12.2291**
Non-alcoholics	18.442 ± 3.703 (26)	12.988 ± 1.656 (68)	9.8871**
Smokers	18.161 ± 2.821 (66)	13.064 ± 1.046 (30)	9.5783**
Non-smokers	18.561 ± 3.342 (46)	11.769 ± 1.503 (82)	15.8064**

**P value < 0.0001

Table 3 Mean levels of plasma MMP-9 within the disease group

Attribute	Mean ± S.D (*n*)	Attribute	Mean ± S.D (*n*)	*t* value	*P* value
Males	18.271 ± 3.024 (104)	Females	21.251 ± 0.606 (8)	2.7719	0.0065
<35 years	18.273 ± 3.298 (46)	≥35 years	18.361 ± 2.867 (66)	0.1502	0.8809
Alcoholics	18.289 ± 2.835 (86)	Non-alcoholics	18.442 ± 3.703 (26)	0.2238	0.8233
Smokers	18.161 ± 2.821 (66)	Non-smokers	18.561 ± 3.342 (46)	0.6840	0.4954
Familial	20.683 ± 1.719 (6)	Non-familial	18.192 ± 3.035 (106)	1.9868	0.0494

pancreatic tissues and its replacement by fibrous tissue resulting in clinical manifestations [7].

The matrix metalloproteinases (MMPs) are a family of calcium-dependent proteinases, which have an important role in extracellular matrix degradation. The accumulation of extracellular matrix may result not only from increased synthesis but also from changes in the pattern of repair and degradation [8]. MMP-9 (gelatinase B) may be particularly important in regulating fibrogenesis and scar degradation. They degrade partially degraded collagens I and III (gelatins) and basement membrane collagen (collagen IV) [9].

The present study revealed a significant increase of plasma MMP 9 levels in chronic pancreatitis patients compared to control subjects. Elevated levels of MMP 9 might result in degradation of type IV collagens in normal basement membrane which is harmful to the environment of many cells thus, results in the activation of pancreatic stellate cells. It is also evident from the earlier studies that activated PSCs play a pivotal role in the development of pancreatic fibrosis and result in tissue inflammation [10]. In response to pancreatic injury or inflammation, quiescent PSCs undergo morphological and functional changes to become myofibroblast cells which express α-smooth muscle actin (alpha-SMA). Activated PSCs actively proliferate, migrate and produce extracellular matrix components such as type 1 collagen and express cytokines and chemokines in the pancreas leading to the development of pancreatic fibrosis [11, 12].

A significant increase of MMP 9 in all the patient groups compared to control subjects with regard to sex, age, addictions was observed indicating it as a risk factor in the disease process. Elevated levels might result in enhanced degradation of ECM proteins in PSCs thereby causing tissue damage. The study is also in consistent with the report of Bhanot and Moller [13], wherein activated PSCs produce pro and anti-inflammatory cytokines, growth factors (TGF beta and PDGF), angiotensin II, and reactive oxygen species which further result in the destruction of neighboring cells and degradation of extracellular matrix proteins thereby disturbing the normal architecture of the pancreas.

In conclusion, patients with chronic pancreatitis presented with higher plasma concentrations of MMP 9 than healthy individuals. These concentrations were related to sex, age, addictions such as smoking, alcoholism etc. Hence, estimation of plasma MMP 9 levels appears to be a sensitive test for the detection of chronic pancreatitis patients.

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