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ORIGINAL ARTICLE

Role of matrix metalloproteinase 3 gene promoter polymorphism in chronic pancreatitis

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Abstract
Aim To study the role of 5A/6A polymorphism of matrix metalloproteinase (MMP-3) and their levels in the pathogenesis of chronic pancreatitis (CP).
Methods One hundred and twenty CP patients and an equal number of age and sex-matched healthy controls were included in the study. Genotypes were determined for 5A/6A allele of MMP-3 gene by allele specific PCR (AS-PCR). The serum MMP-3 levels were estimated using sandwich ELISA method.
Results The distribution of the genotypes of the 5A/6A polymorphism in both control and study patients was similar (p=0.523). Within the disease group, patients with older age, early onset of the disease, and addictions such as smoking and alcohol consumption had higher levels as compared to those who did not have these features.
Conclusion We conclude that functional polymorphism of MMP-3 (5A/6A) is not associated with CP. However, the higher levels within the disease group indicate its possible role in the disease process.

Keywords Extracellular matrix · Fibrosis · Pancreatic stellate cells · Stromelysin

Introduction

Chronic pancreatitis (CP) is an inflammatory disease of the pancreas characterized by the irreversible destruction of exocrine and endocrine parenchyma and fibrosis [1, 2]. Although the etiologic factors and morphologic changes in CP have been more clearly identified in the past decade, the pathogenetic mechanism remains obscure. Recent studies suggest that CP has a strong genetic basis, and increasing knowledge of gene-environment interactions has provided new insights into the pathophysiology of CP that is increasingly being thought of as a multifactorial disorder, in which multiple risk factors operate together during disease initiation and progression [3–5].

Pancreatic fibrosis is a key pathological feature of alcohol induced CP. Pancreatic stellate cells (PSCs) play a major role in pancreatic fibrogenesis. Studies have also shown that PSCs have the capacity to synthesize and secrete a number of matrix degrading enzymes, which alter the homeostatic balance between extracellular matrix (ECM) protein synthesis and degradation culminating to pathological increases of ECM and fibrotic tissue [6].

MMPs are a family of zinc-dependent enzymes that degrade the components of the ECM and are essential for tissue remodeling and repair during development and inflammation [7]. MMP-3 (stromelysin 1) is the main metalloproteinase that is secreted by fibroblasts, synovial cells and chondrocytes. It degrades proteoglycans, fibro-

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nectin, laminin and type IV collagens and activates other MMPs that synergistically regulate the accumulation of ECM [1]. MMP-3 expression is regulated in response to stimulation of growth factors and cytokines [8].

A common polymorphism in the promoter region of the human MMP-3 gene has been identified in which one allele has a run of six adenosines (6A) and the other five (5A). This polymorphism was reported to be involved in the regulation of MMP-3 gene expression with the 5A allele having two-fold activity compared to the 6A allele product [9]. Earlier studies have shown the association of MMP-3 gene polymorphism with rapid progression of aneurysm, rheumatic diseases and primary sclerosing cholangitis [10]. To the best of our knowledge there are no reports on association of MMP-3 polymorphism with CP in Indian population. The present study is aimed at evaluating the qualitative and quantitative role of MMP-3 in CP.

Methods

One hundred and twenty CP patients clinically evaluated by computed tomography scan, referred to the Gastroenterology Unit of Gandhi Hospital, Secunderabad and Osmania General Hospital, Hyderabad were included in the study. An equal number of age and sex matched healthy controls, not related to the patients, were also analyzed for comparative purposes. Those who had complications like dilated pancreatic duct or strictures with dilatations of the duct, pancreatic calcification, pancreatic atrophy or pseudocyst were confirmed for CP. Informed consent was obtained from all subjects. The study was also approved by the Institutional Ethical Committee. Information on the epidemiological factors such as sex, age, duration of the disease, familial incidence, smoking and alcohol consumption was obtained from all the subjects based on a standard proforma. 5 mL of blood was collected in vacutainers with and without ethylenediamine tetra acetic acid (EDTA) for the separation of plasma and serum respectively and stored at −70°C until use.

Genomic DNA was extracted from the whole blood by salting out method [11]. The 5A/6A polymorphism in the promoter of the human MMP-3 gene was determined by the polymerase chain reaction (PCR) with allele-specific primers (AS-PCR). The promoter sequences of the human MMP-3 gene were obtained by reference to GenBank entries HSU43511 and HSU56422. The allele-specific primers are as follows: forward primer, 5′-GAT TAC AGA CAT GGG TCA CGG CAC-3′, and reverse primer, 5′-AAT CAG GACAAG ACA TGG TTT TTC-3′ for the 5A allele and 5′-AAT CAG GAC AAG ACA TGG TTT TTT-3′ for the 6A allele. Hot-start PCR was performed with the annealing temperatures being 65°C for the 5A allele and 62°C for the 6A allele, and 30 cycles of amplification were carried out. The MMP-3 activity was measured and determined by sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions [The Calbiochem® MMP-3 ELISA Kit Merck KGaA,Darmstadt, Germany].

The allelic frequencies were estimated by gene counting and genotypes were scored. Odds ratios were calculated for the possible association of genotypes with the disease. The mean MMP-3 levels were compared within different attributes of the disease followed by student's t test for the statistical significance.

Results

Table 1 gives the genotype distribution of MMP-3 gene in control subjects and CP patients with CP. Among the 120 controls the genotype distribution was 5% of 5A/5A, 88.3% of 5A/6A and 6.7% of 6A/6A. Among the 120 CP patients 6.67% were of 5A/5A, 91.6% of 5A/6A, and 1.67% of 6A/6A genotype, with no significant variation [OR- 0.875 (CI, 0.61–1.25)] with respect to the genotypic and allelic distribution in patient group as compared to control group.

MMP-3 levels were slightly elevated in patients (mean[SD]: 79.20 [28.43] ng/mL) compared to controls (mean[SD]: 73.54 [24.89] ng/mL; p=0.1021. Comparison was also made within the disease group in different attributes and is presented in Table 2. There was no difference between the MMP-3 levels with respect to gender. Patients below 35 years of age had significantly higher levels of MMP-3 than those above 35 years of age. Patients were further categorized into two groups based on the age of onset by subtracting the duration of the disease with the actual age at the time of sampling. Thus, the patients were divided into two groups; one group with younger patients and early onset of the disease (<25 years) and the other with elderly patients (≥25 years) with late onset. Smokers had significantly higher levels of MMP-3 than non-smokers. Similarly, alcoholics showed significantly higher levels than non-

Table 1 Distribution of MMP-3 genotypes in patients and control subjects

	Controls		Chronic pancreatitis	
Genotypes	*n*	%	*n*	%
5A/5A	6	5	8	6.67
5A/6A	106	88.3	110	91.6
6A/6A	8	6.7	2	1.67
Total	120	–	120	–
Allele				
5A	118	0.49	126	0.52
6A	122	0.51	114	0.48

p=0.523, OR=0.875, (95% CI, 0.61–1.25)

Table 2 Mean serum MMP-3 levels in chronic pancreatitis patients with different attributes

Attributes	*n*	%	Mean (SD)	t- test
Sex				
Males	116	96.7	80.0 (23.86)	0.16
Females	4	3.3	78.0 (24.83)	
Age <35	54	45	86.70 (28.19)	2.67**
≥35	66	55	73.70 (25.12)	
Age of onset <25 y	30	25	82.81 (28.37)	5.52**
≥25 y	90	75	61.58 (40.77)	
Alcoholics	86	71.67	84.15 (21.58)	2.47*
Non-alcoholics	34	28.33	72.30 (28.45)	
Smokers	68	56.67	85.73 (19.28)	3.06*
Non-smokers	52	43.33	73.50 (24.54)	

*$p<0.005$, **$p<0.0001$

alcoholics. Intra group comparison revealed significant variation between the attributes, thus pinpointing the role of these variables in the etiology of CP.

Discussion

Chronic pancreatitis is a long standing inflammation and fibrosis, leading eventually to destruction of pancreatic parenchyma and loss of exocrine and endocrine functions, that alter the normal structure and function of the pancreas. PSCs are thought to play a role in extracellular matrix turnover via their ability to synthesize extracellular matrix proteins as well as matrix-degrading enzymes or matrix metalloproteinases (MMPs).

MMP-3 is a secreted metalloproteinase mainly found in the connective tissue, with an ability to degrade a variety of extracellular matrix components including proteoglycan, fibrinogen, laminin and type IV collagen. MMP-3, also known as stromelysin-1 is up-regulated in a variety of tumors and has been shown to influence tumor initiation [12]. There have been a number of genetic and epidemiological studies on the MMP-3 5A/6A polymorphism, which provide evidence indicating that this polymorphism is associated with various cardiovascular conditions [13].

Transient transfection experiments have indicated that the 5A-allele expresses a two-fold higher activity of the reporter gene than does the 6A allele, a finding suggesting that carriers of the 5A-allele exhibit a higher MMP-3 promoter activity [14]. The NFkB p50 homodimer functions as a transcriptional repressor for several genes, by inhibiting transactivation of the p50/p65 heterodimer. The higher promoter activity of the 5A allele is a result of reduced binding of the transcriptional repressor NFkB p50/p50 homodimer to the 5A allele compared to the 6A allele. In this study, there is no association of MMP-3 gene polymorphism with the disease as the distribution of genotypes and alleles in controls and disease group did not seem to vary.

Several epidemiological variables such as sex, age, duration, smoking, and alcoholism were examined within the patient group to understand the role of these factors in the disease onset. Male predominance was observed in the patient group, which may be attributed to the role of environmental triggering factors like smoking and alcohol. This is also in consistent with the earlier study of Balakrishnan et al. [15] wherein an increased susceptibility of the males to the disease was reported.

Elevated levels of serum MMP-3 was observed in CP patients compared to control subjects. When serum levels were examined within the patient group with respect to different attributes, a higher level of serum MMP-3 was found among patients belonging to age group below 35 years, which could be attributed to increased inflammatory changes and progression in the patients at an early age. Similarly, higher levels of MMP-3 were observed in younger patients with early onset of the disease than in elderly patients suggesting the role of MMP-3 in the early stage of the disease onset. These findings are in agreement with earlier reports wherein an increased level of MMP-3 was found in the earlier stages of the disease in rat model of cerulein-induced pancreatitis compared to later stage onset [6]. When comparison was made between patients with and without addictions, a significantly increased MMP-3 levels were observed in smokers, and alcoholics compared to non-smokers and non-alcoholics. Such an increase may result in pancreatic injury by increasing the fibrosis through the activation of PSCs via production of profibrogenic growth factors, pro-inflammatory cytokines which play an important role in the ECM remodeling [16].

In conclusion, no significant difference in the distribution of MMP-3 5A/6A polymorphism between patients and control subjects was observed. However, cases with early age and early onset, addictions such as smoking, alcohol consumption have shown significantly elevated MMP-3 levels compared to their counterparts indicating the inflammatory changes in the disease process. Thus, further analysis on a larger sample size is required to confirm the possible association of MMP-3 in CP.

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