

# Role of Proteases and Antiprotease in the Etiology of Chronic Pancreatitis

Srimanjari Kavutharapu, Balakrishna Nagalla<sup>1</sup>, Vidyasagar Abbagani<sup>2</sup>, Shravan K. Porika<sup>3</sup>,  
Jyothy Akka, Pratibha Nallari<sup>3</sup>, Venkateshwari Ananthapur

Department of Cell Biology,  
Institute of Genetics and  
Hospital for Genetic Diseases,  
Osmania University,  
Begumpet, <sup>1</sup>Division of  
Biostatistics, National Institute  
of Nutrition, <sup>2</sup>Department  
of Gastroenterology, Gandhi  
Hospital, <sup>3</sup>Department of  
Genetics, Osmania University,  
Hyderabad, India

**Address for correspondence:**

Dr. Venkateshwari Ananthapur,  
Institute of Genetics and  
Hospital for Genetic Diseases,  
Osmania University, Begumpet,  
Hyderabad, India.  
E-mail: venkateshwari@yahoo.  
com

## ABSTRACT

**Background/Aim:** Chronic pancreatitis (CP) is the progressive and irreversible destruction of the pancreas characterized by the permanent loss of endocrine and exocrine function. Trypsin, the most important digestive enzyme plays a central role in the regulation of all other digestive enzymes. Chymotrypsin, an endopeptidase hydrolyzes peptides at amino acids with aromatic side chains. Alpha-1-antitrypsin is a principal antiprotease which protects the mucosal tissue from the proteolytic effects of trypsin and chymotrypsin by the formation of molar complexes. The present study is aimed at examining the role of proteases (trypsin and chymotrypsin) and anti-protease ( $\alpha$ 1-anti-trypsin) in the etiopathogenesis of chronic pancreatitis. **Patients and Methods:** A total of 90 CP patients and 110 age and sex matched controls were considered for the study. Serum trypsin, chymotrypsin and  $\alpha$ 1-anti-trypsin levels were determined prospectively in CP patients and compared to healthy controls as described previously. **Results:** The mean activity of trypsin were found to be increased in CP patients ( $X \pm SD = 0.82 \pm 0.838$ ) in comparison to normal control group ( $X \pm SD = 0.55 \pm 0.328$ ), ( $P = 0.001$ ). Chymotrypsin activity were also found to be elevated in CP patients ( $X \pm SD = 0.63 \pm 0.278$ ) in comparison to control group ( $X \pm SD = 0.39 \pm 0.295$ ), ( $P = 0.0001$ ). The mean  $\alpha$ 1-anti-trypsin activity were found to be lowered in CP patients ( $X \pm SD = 0.42 \pm 0.494$ ) in comparison to control group ( $X \pm SD = 0.67 \pm 0.465$ ), with the variation being significant ( $P = 0.0003$ ). **Conclusion:** The findings suggest an imbalance in the synthesis and degradation of proteolytic enzymes and antiprotease indicating an altered aggressive and defensive role in the pathogenesis of chronic pancreatitis.

**Key Words:** Alpha 1 anti-trypsin, chronic pancreatitis, chymotrypsin, trypsin

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Chronic pancreatitis is the progressive and irreversible destruction of the pancreas, characterized by permanent loss of endocrine and exocrine function.<sup>[1]</sup> The incidence of chronic pancreatitis (CP) ranges from two to ten per 100,000, and has an estimated prevalence of 25/100,000.<sup>[2]</sup> Disease characteristics include inflammation, glandular atrophy, ductal changes, and fibrosis.<sup>[3]</sup> It is presumed that exposure to toxins and oxidative stress, result in acute

pancreatitis,<sup>[4,5]</sup> which if continued leads to, early and late-phase inflammatory responses with the production of profibrotic cells and stellate cells, culminating in collagen deposition, periacinar fibrosis, and chronic pancreatitis.<sup>[6]</sup>

Pancreatic acinar cell secretion results in three major categories of enzymes: Amylolytic, lipolytic and proteolytic enzymes which are capable of using carbohydrates, fats and proteins respectively. Acinar cell secretion is primarily induced by the ingestion of food, which initiates activation of multiple endocrine, neurocrine and paracrine pathways, regulating the release of appropriate amounts of acinar digestive enzymes. The proteolytic class of pancreatic enzymes secreted as inactive precursors into the duodenum consists of trypsin, chymotrypsin and carboxypolypeptides wherein the trypsin, chymotrypsin and elastase are endopeptidases of the serine protease family of enzymes.

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Trypsin, unlike amylase and lipase, is only produced by the pancreas.<sup>[7]</sup> Trypsinogen is one of the most important digestive enzymes as it plays a central role in the regulation of all the other digestive enzymes. Trypsin makes up to 19% of the protein in pancreatic juice, and is the most abundant of all pancreatic digestive enzymes.<sup>[8]</sup> Chymotrypsinogen is the second most abundant serine protease in pancreatic secretion and makes up to 9% of total pancreatic juice protein.<sup>[9]</sup> Chymotrypsin is an endopeptidase that hydrolyzes peptides at amino acids with aromatic side chains, including phenylalanine, tyrosine, and tryptophan. Alpha-1-antitrypsin ( $\alpha$ -1AT) is a principal serum protease inhibitor which protects the mucosal tissue from proteolytic enzymes like trypsin and chymotrypsin by the formation of molar complexes.<sup>[10]</sup> The enzymes that are inhibited by alpha-1-antitrypsin are particularly the serine proteases, which are characterized by a serine residue at their active sites.<sup>[11]</sup> Thus, the present study is aimed at evaluating the role of proteases (trypsin and chymotrypsin) and anti-protease ( $\alpha$ -1AT) in the etiopathogenesis of chronic pancreatitis.

## PATIENTS AND METHODS

### Subjects

A total of 90 radiologically and endoscopically confirmed chronic pancreatitis patients referred to the Gastroenterology unit of Osmania General Hospital and Gandhi Hospital, Hyderabad are included in the present study. A total of 110 sex matched healthy control subjects referred to our Institute for regular health check were also considered for comparative studies.

Demographic details such as age, sex, habits like smoking and alcohol consumption, duration of the disease, were obtained with the help of a standard proforma from all the subjects. The study was also approved by the Institutional Ethical committee. Blood samples were collected in plain vacutainers from all the subjects. Serum was separated and stored frozen until further use.

### Trypsin activity

Estimation of serum trypsin activity was carried out in serum samples of all the subjects following the method of Varley, (1991).<sup>[12]</sup> The acid liberated due to the hydrolysis of p-Toluene-Sulfonyl-2-arginine-Methyl ester (TAME) by the action of trypsin was measured spectrophotometrically at 300nm against a blank. 2.6 ml of Tris-HCl buffer and 0.3ml of TAME are taken in a test tube. The tubes were incubated for 5 minutes, to which 100  $\mu$ l of serum was added and the OD was measured against a blank.

### Chymotrypsin activity

Estimation of serum chymotrypsin activity was carried out in serum samples of all the subjects according to the

method of Varley, (1991).<sup>[12]</sup> The acid liberated due to the hydrolysis of Acetyl-L-Tyrosine Ethyl Ester (ATEE) by action of chymotrypsin was measured spectrophotometrically at 300nm against a blank. To each tube 1.5ml of Tris-HCl and 1.4ml of ATEE solution were added. Later 100 $\mu$ l of serum was added to the test sample and 100 $\mu$ l of distilled water to the blank tube and the absorbance was read at 300nm against a reagent blank.

### Alpha-1-anti-trypsin activity

Serum Alpha-1-anti-trypsin activity was carried out in serum samples of all the subjects according to the method of Ahmed *et al.*, (1979).<sup>[13]</sup> The ( $\alpha$ -1AT) activity in the serum was measured spectrophotometrically by the amount of tyrosine liberated on action of trypsin with hemoglobin by measuring the trypsin inhibiting capacity. To the test tube labeled as test, 1ml of 0.05M phosphate buffer, 1ml of trypsin (0.08mg/1ml of 0.001N HCl) and 1 ml of hemoglobin solution were mixed to which 40 $\mu$ l of serum was added and incubated for one hour at 37°C. The control reaction was carried out in the same way with the addition of serum after incubation. This was followed by addition of 2ml of 20% Trichloro acetic acid to both test and control tubes which were incubated for 10 minutes at room temperature. The solution was centrifuged till no precipitate was left suspended. Later, 250 $\mu$ l of the clear supernatant was taken in separate tubes labeled as test and control to which 250 $\mu$ l of 0.6N Folin's Phenol reagent and 1.5 ml of sodium carbonate solution was added to both test and control tubes and incubated at room temperature for 20 minutes till the solution turned blue. The absorbance was then read against water blank at 680nm in a spectrophotometer.

### Statistical analysis

Statistical analysis was carried out to interpret the variation of serum levels of trypsin, chymotrypsin and alpha 1 antitrypsin

**Table 1: Distribution of demographic details of controls and chronic pancreatitis patients**

Attributes	Control (%)	Chronic pancreatitis (%)	$\chi^2$	P value
Age				
<35 years	96 (87.3)	35 (38.9)	51.3	0.000**
≥35 years	14 (12.7)	55 (61.1)		
Sex			0.96	0.328
Males	103 (93.6)	87 (96.7)		
Females	7 (6.4)	3 (3.3)		
Addictions			24.21	0.000**
Smokers	21 (19.1)	47 (52.2)		
Non-smokers	89 (80.9)	43 (47.8)		
Alcoholics	69 (62.75)	65 (72.2)		
Non alcoholics	41 (37.3)	25 (27.8)	2.018	0.155

\*P<0.05, \*\*P<0.001

with regard to demographic parameters using IBM, SPSS version 17 software. The Pearson's  $\chi^2$  test was also carried out to examine the differences between the case and the control groups with respect to age, sex, smoking, and alcoholism. Odds ratio (ORs) and 95% confidence intervals for the factors under consideration were computed by logistic regression analysis.

## RESULTS

A total of 110 controls and 90 chronic pancreatitis patients were included in the present study. The demographic data of the samples under study are presented in Table 1. The proportion of smokers in CP patients (52.2%) was significantly higher compared to healthy controls (19.1%) [ $\chi^2 = 24.21, P < 0.0001$ ]. Similarly, the proportion of patients above the age of 35 years in CP patients (61.1%) was significantly higher than that in healthy controls (12.7%) [ $\chi^2 = 51.3, P < 0.0001$ ]. However, no significant difference was found with respect to gender and alcoholism on comparison of chronic pancreatitis patients with healthy controls ( $P > 0.05$ ).

The mean activity of pancreatic enzymes in controls and chronic pancreatitis patients is presented in Table 2. The mean activity of trypsin was found to be increased in chronic pancreatitis patients ( $X \pm SD = 0.82 \pm 0.838$ ) in comparison to normal control group ( $X \pm SD = 0.55 \pm 0.328$ ), ( $t = 2.945, P = 0.001$ ), indicating its possible role in the tissue damage and fibrosis of the pancreas. Chymotrypsin activity was also found to be elevated in chronic pancreatitis patients ( $X \pm SD = 0.63 \pm 0.278$ ) in comparison to control group ( $X \pm SD = 0.39 \pm 0.295$ ), ( $t = 5.884, P < 0.0001$ ), suggesting its role in the tissue damage. The mean  $\alpha$ -1-anti-trypsin activity was found to be lowered in chronic pancreatitis patients ( $X \pm SD = 0.42 \pm 0.494$ ) in comparison to control group ( $X \pm SD = 0.67 \pm 0.465$ ), with the variation being significant ( $t = 3.619, P = 0.0003$ ). It indicates lowered defensive role of alpha-1-anti trypsin against proteolytic enzymes in the pancreas.

Correlation coefficients of the mean levels of trypsin, chymotrypsin and alpha 1 antitrypsin also revealed increased proteolytic enzymes and lowered alpha 1 antitrypsin which may further aggravate the pancreatic damage, and progression of the disease [Table 3].

Logistic regression analysis revealed a significant association of demographic variables like age, smoking and protease chymotrypsin and alpha 1 antitrypsin in the etiopathology of CP was observed, further strengthening the role of these factors [Table 4].

**Table 2: Mean levels of pancreatic enzymes (trypsin, chymotrypsin,  $\alpha$ 1-anti trypsin) in controls and chronic pancreatitis groups**

Enzymes	Control X $\pm$ SD (n)	Chronic pancreatitis X $\pm$ SD (n)	t	P value
Trypsin	0.55 $\pm$ 0.328 (110)	0.82 $\pm$ 0.838 (90)	-2.945	0.001*
Chymotrypsin	0.39 $\pm$ 0.295 (110)	0.63 $\pm$ 0.278 (90)	-5.884	0.0001**
Alpha-1- anti-trypsin	0.67 $\pm$ 0.465 (110)	0.42 $\pm$ 0.494 (90)	3.619	0.0003**

\* $P < 0.05$ , \*\* $P < 0.001$

**Table 3: Correlation coefficients of trypsin, chymotrypsin and  $\alpha$ 1-anti trypsin**

	Trypsin	Chymotrypsin	$\alpha$ 1-anti trypsin
Trypsin	1.0	0.009	0.028
Chymotrypsin	-	1.0	-0.145*
Alpha-1-anti trypsin	-	-	1.0

\* $P < 0.05$ , \*\* $P < 0.001$

## DISCUSSION

Chronic pancreatitis is an inflammatory disease of the pancreas. The digestive juice/enzymes secreted into the duodenum by the pancreatic duct include trypsin, chymotrypsin and  $\alpha$ -1-anti-trypsin which are zymogenic and get activated on reaching the small intestine. In an inflamed pancreas, the enzymes directly attack the pancreatic tissue, resulting in an imbalance in the synthesis of proteolytic enzymes and antiproteases and autodigestion of the pancreatic tissue culminating as CP.<sup>[14]</sup>

Trypsin activity is properly suppressed in the pancreatic acinar cells under normal conditions, as small amount of trypsinogen is converted to active trypsin and inactivated by pancreatic secretory trypsin inhibitor (PSTI), thereby preventing damage to pancreatic acinar cells as a first line of defense. Serum trypsin may be normal or subnormal in chronic pancreatitis (Elias *et al.*, 1977).<sup>[7]</sup> Previous studies have revealed that exocrine insufficiency is invariably associated with a low trypsin concentration,<sup>[15,16]</sup> but a normal concentration has been reported.<sup>[17]</sup> However, increased levels observed in the present study in CP compared to controls suggests that proteolytic activity of trypsin on pancreas may result in the cascade of events which further results in the activation of other proteases with subsequent cell damage. Chymotrypsin activity was also found to be significantly elevated in chronic pancreatitis patients when compared to controls. Elevated levels of chymotrypsin may result in further inflammation and damage of the pancreatic tissue.

**Table 4: Odds ratio of estimates of the variables in chronic pancreatitis compared to control subjects group**

Attributes	Odds ratio (95% CI)	P value
Age	14.89 (5.988-37.025)	0.000**
Sex	0.908 (0.131-6.301)	0.922
Smoking	8.509 (3.186-22.727)	0.000**
Alcohol	0.402 (0.159-1.018)	0.055
Trypsin	1.937 (0.836-4.487)	0.123
Chymotrypsin	21.087 (4.519-98.397)	0.000**
Alpha-1-anti trypsin	0.336 (0.147-0.770)	0.01*

\*P<0.05, \*\*P<0.001

Alpha-1 antitrypsin ( $\alpha$ -IAT) is a known protease inhibitor, of trypsin and chymotrypsin. These proteases when left unchecked by the protease inhibitor can cause destruction of normal tissues. Thus, the low serum activity of this glycoprotein results in an imbalance between circulating proteases as aggressive enzymes are thought to play a role in the development of chronic pancreatitis.<sup>[18]</sup> Studies by Haber *et al.*, (1991), have also shown that mortality of experimental pancreatitis is increased with reduced concentrations of circulating  $\alpha$ -IAT.<sup>[19]</sup> The decrease in the antiprotease may result in the activation of proteases that release into the blood stream leading to membrane damage.

An infiltration of inflammatory cells including neutrophils, monocytes, and macrophages, play a critical role in tipping the protease-antiprotease balance toward protease excess.<sup>[20]</sup> The  $\alpha$ -IAT protein is involved in processes which could be important for the maintenance of normal pancreatic function, like regulation of reactive oxygen species toxicity, cell-mediated immunity/tolerance, neutrophil and endotoxin mediated inflammation, endothelial function and apoptosis.<sup>[21]</sup> A deficiency of this enzyme may lead to an excess release of inflammatory cytokines and free enzyme that in turn drives inflammatory responses, which further activates the pancreatic stellate cells resulting in fibrosis.

When  $\alpha$ -IAT concentration and isozymes phenotyping are taken into account, the conflicting data revealed the involvement of the  $\alpha$ -IAT variants in chronic pancreatitis. Some findings indicated increased prevalence of PiS and PiZ in chronic alcoholic patients, while others indicated no difference.<sup>[18,22,23]</sup> Serum  $\alpha$ -IAT concentrations were greater in patients with pancreatitis who were sampled shortly after an acute attack of pancreatitis compared to pancreatitis patients sampled at other times, or to alcoholic controls.<sup>[19]</sup> Increased  $\alpha$ -IAT concentration was found in pancreatic juice of patients with chronic pancreatitis by Miszczuk-Jamska *et al.* (1983)<sup>[24]</sup> It was speculated that this could alter the balance in pancreatic protease-antiprotease system and

subsequently lead to chronic pancreatitis.

Correlation coefficient studies revealed heterogeneity of the disease with regard to protease and antiprotease. A significantly elevated level of trypsin, resulting in pancreatic tissue damage was observed in a section of subjects while the other group of individuals exhibited an increased levels of chymotrypsin and lowered levels of  $\alpha$ -I-antitrypsin resulting in an increased pancreatic damage with elevated proteolytic enzymes and lowered anti-proteases. The second group revealed lowered action of  $\alpha$ -I-antitrypsin on the regulation of proteolytic enzymes like trypsin and chymotrypsin in the patients. Thus, the present study highlights the possible genetic heterogeneity of chronic pancreatitis with respect to the levels of trypsin, chymotrypsin and  $\alpha$ -I-anti trypsin activity in the disease.

Overall, the aggressive proteolytic enzymes trypsin and chymotrypsin were found to be elevated while the defensive alpha-1 anti-trypsin was lowered, further confirming the tilt in homeostasis of proteolytic and anti-proteolytic enzyme secretion. If proteinase inhibitors like alpha-1-anti-trypsin alter their normal function, limiting of unwanted proteolysis and prevention of tissue damage cannot be achieved appropriately. The lowered alpha-1-anti-trypsin can justify the altered immune and defense in CP, resulting in an increase in trypsin and chymotrypsin which later promotes progression of the disease with a history of bad prognosis.

## CONCLUSION

In conclusion, our results indicate a significant increase in the activity of trypsin and chymotrypsin activity and a decrease in  $\alpha$ -I-anti trypsin activity which activates further and initiate subsequent cascade of events resulting in the pancreatic acinar cell destruction and chronic pancreatitis.

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