DIAGNOSTIC EFFICIENCY OF AMYLASE AND TYPE IV COLLAGEN IN PREDICTING CHRONIC PANCREATITIS

Subir Kumar Das, Sowmya Varadhan, L Dhanya, Sukhes Mukherjee, S Mohana*, V Balakrishnan** and D M Vasudevan

Departments of Biochemistry, *Pathology and **Gastroenterology, Amrita Institute of Medical Sciences, Elamakkara P.O. Cochin 682 026, India

ABSTRACT

Chronic pancreatitis, an irreversible inflammatory disease of the pancreas, is associated with the replacement of the destroyed parenchyma by extended development of fibrosis. Despite marked progress in diagnostic tools, no consensus has been reached in diagnosis of chronic pancreatitis. In this study we examined the hematological and biochemical parameters among 40 chronic pancreatitis patients within 18 to 67 yrs. ESR level and ALP activity was elevated in 40% cases. Serum amylase activity increased in 32 patients and it showed significant correlation with ALP ($r=0.458$, $p=0.003$), CA-19.9 ($r=0.556$, $p<0.001$), and calcium level ($r=-0.472$, $p=0.002$). Type IV collagen level in chronic pancreatitis also elevated (164.4 ± 55.5 ng/ml) and showed negative significant correlation with calcium level ($r=-0.505$, $p=0.001$). However, no significant correlation was observed between amylase activity and type IV collagen ($r=0.289$, $p=0.07$).

KEY WORDS

Amylase, Chronic pancreatitis, Fibrosis, Type IV Collagen.

INTRODUCTION

Pancreatitis is the most common disease of the pancreas. Acute pancreatitis is a potentially lethal disease and mortality depends on severity (1). Chronic pancreatitis is most often a result of recurrent attacks of acute pancreatitis (2). Chronic pancreatitis, an irreversible inflammatory disease of the pancreas, is characterized by chronic inflammatory cell infiltration, acinar cell degeneration (3), associated with the replacement of the destroyed parenchyma by extended development of fibrosis (4) (Fig. I & II), which lead to impairment of pancreatic exocrine and endocrine function (3).

Tissue fibrosis is one of the characteristics of chronic pancreatitis (5). The biological cause of fibrosis is the accumulation of excessive amounts of extracellular matrix

(ECM), which leads to tissue dysfunction and organ failure. There is growing evidence that pancreatic fibrosis represents a dysregulation of the normal repair processes after injury (6). Early-stage chronic pancreatitis may be undetected as a clinical entity. However, it may carry a definite risk for subsequent secondary damage, depending on the etiology of the disease (7). The severity of pancreatic fibrosis can be assessed only by direct histologic analysis of pancreatic tissue (8). The anatomic position and relationships of the pancreas make direct observation of pancreatic pathology difficult, so the diagnosis of pancreatic disease is typically based on the measurement of biochemical markers in serum and imaging studies (9). Therefore, in this study we examined the hematological and biochemical parameters commonly done in chronic pancreatitis patients and analyzed their association in particular with serum amylase and type IV collagen levels.

Address for Correspondence :

Dr. Subir Kumar Das

Department of Biochemistry,
Agartala Govt. Medical College,
Kunjaban P.O., Agartala-799006
Phone: 91-381-2356072
E-mail: drsubirkdas@yahoo.co.in

MATERIALS AND METHODS

Laboratory routine test kits from Roche diagnostics, Meylan, France and Type IV collagen from Daiichi Pure Chemicals, Japan were used. The study protocol was in keeping with the ethical guidelines of the 1975 Declaration of Helsinki and all the patients gave written informed consent to the study. Forty

(40) chronic pancreatitis patients (32 males, 8 females) from 18 to 67 yrs (38.6 ± 13.8 yrs) were selected on the basis of laboratory findings, clinical investigations and imaging analysis from those who had visited Gastroenterology Department, Amrita Institute of Medical Sciences, Cochin. Institutional ethics Committee approved the procedures.

A complete clinical history, including anthropometric measurements was obtained. Haematological and biochemical investigations including renal function tests, liver function tests and lipid profile were performed by conventional methods. Type IV collagen was measured by latex turbidometric immunoassay (10).

Results are expressed as mean $\pm$ SD (standard deviation). All data were analyzed using the statistical package SPSS (version 11.0, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Chronic pancreatitis is a de novo disease with a silent but progressive restructure of the pancreas (11) that causes a long-lasting inflammatory disease (12). Despite marked progress in diagnostic tools and, especially, imaging methods, no consensus has been reached in diagnosis of chronic pancreatitis (3). Routine haematological examinations in

chronic pancreatitis patients (Table 1) indicated that there were marginal deviations from normal level in certain parameters of a few patients. For example, ESR (erythrocyte sedimentation rate), which is a nonspecific screening test to measure inflammation in the body, elevated in 16 patients (40%), and hematocrit level decreased in 19 patients (Table 1).

Diabetes mellitus is a common complication of chronic pancreatitis (13) and was reported in 20-30% of patients above forty years old in one study (14). In another study the prevalence of impaired or diabetic glucose tolerance were reported in 40-70% cases, and half of these patients suffered from an insulin-dependent diabetes mellitus (15). However, hyperglycemia was observed in 18 (45%) patients in this study (Table 2). Exocrine pancreatic insufficiency is strongly associated with anatomical changes of the pancreas, and also depends on endocrine function (14). Onset and severity of the endocrine dysfunction is believed to be depending on parenchymal destruction of the pancreas. Pathological findings in the endocrine pancreas are a loss of β -cells with decrease in secretion of insulin but also a loss of β -cell responsiveness to glucose by impaired perisinusoidal diffusion. In addition, an impaired α -cell function may be important (15).

Chronic pancreatitis patients did not show any apparent changes in renal function tests or electrolyte disturbances

Table 1. Hematological profile of chronic pancreatitis patients

	Mean	Normal Level	95% CI	Deviation from normal
WBC (K/uL)	8.34 ± 3.48	4.4-11.3	7.22-9.45	6 $\uparrow$
RBC counts $\times 10^6$ (cells / cu.mm.)	4.48 ± 0.59	4.04-6.13	4.29-4.66	-
Hb (gm%)	13.07 ± 1.79	12.2-18.1	12.5-13.65	2 $\downarrow$
HCT (%)	36.76 ± 4.85	37.7-53.7	35.2-38.31	19 $\downarrow$
MCV (fl)	83.04 ± 3.36	80-97	81.97-84.11	4 $\downarrow$
MCH (pg)	29.58 ± 1.84	27-31.2	28.99-30.17	2 $\downarrow$
MCHC (g/ dl)	35.55 ± 1.31	31.8-35.4	35.13-35.97	6 $\uparrow$
RDW (%)	14.25 ± 1.08	11.6-14.8	13.9-14.59	6 $\uparrow$
MPV (fl)	7.81 ± 0.66	6.8-10	7.59-8.02	-
Neutrophil (%)	55.22 ± 13.04	37-80	51.05-59.39	2 $\uparrow$
Lymphocyte (%)	29.68 ± 11.18	10-50	26.1-33.26	2 $\uparrow$
Monocyte (%)	6.96 ± 2.1	0-12	6.29-7.63	-
Eosinophil (%)	3.14 ± 2.39	0-7	2.38-3.91	-
Basophil (%)	0.51 ± 0.45	0-2.5	0.37-0.66	-
ESR (mm/h)	20.85 ± 12.52	8-20	16.85-24.85	16 $\uparrow$
Platelet count (K/uL)	277.6 ± 96.07	150-450	246.8-308.3	4 $\uparrow$

Values are mean $\pm$ SD of number of observations (n). $\uparrow$: indicates number of subjects reported above normal level, $\downarrow$: indicates number of subjects reported below normal level.

Table 2. Biochemical parameters of chronic pancreatitis patients

	Mean	Normal Level	95% CI	Deviation from normal
Glucose fasting (mg%)	155.7 ± 101.35	<110	123.29-188.11	18 ↑
Urea (mg%)	21.15 ± 10.6	10-50	17.76-24.54	2 ↑
Creatinine (mg%)	0.8 ± 0.22	0.5-1.4	0.729-0.871	-
Sodium (mmol/L)	135.95 ± 2.98	136-145	135-136.9	-
Potassium (mmol/L)	4.07 ± 0.41	3.5-5.1	3.94-4.2	-
Uric acid (mg%)	4.4 ± 1.04	2.4-7	4.07-4.75	-
Total cholesterol (mg%)	159.13 ± 25.45	150-250	150.98-167.27	-
HDL-cholesterol (mg%)	36.65 ± 9.08	35-65	33.75-39.55	15 ↓
LDL-cholesterol (mg%)	103.05 ± 23.41	90-149	95.56-110.54	2 ↑
VLDL-cholesterol (mg%)	20.35 ± 5.62	0-39	18.55-22.15	-
Triglyceride (mg%)	98.85 ± 26.97	50-190	90.22-107.48	-
Amylase (IU/L)	292.85 ± 427.17	<100	163.4-322.2	32 ↑
AST (IU/L)	28.7 ± 9.66	<38	25.61-31.79	6 ↑
ALT (IU/L)	28.33 ± 14.25	<41	23.77-32.88	6 ↑
ALP (IU/L)	154.15 ± 104.8	39-117	120.63-187.67	16 ↑
Total protein (g %)	7.37 ± 0.73	6.6-8.7	7.14-7.61	-
Albumin (g %)	4.07 ± 0.64	3.5-5	3.87-4.28	-
Globulin (g %)	3.3 ± 0.68	2.5-4	3.08-3.52	1 ↓
Total bilirubin (mg %)	0.93 ± 0.8	<1	0.674-1.186	7 ↑
Direct bilirubin (mg %)	0.27 ± 0.25	<0.2	0.188-0.352	1 ↑
Calcium (mg%)	8.88 ± 0.49	8.6-10.2	8.72-9.03	10 ↓
CA-19.9 (IU/ ml)	35.8 ± 59.6	<37	16.74-54.86	4 ↑
PT INR	1.17 ± 0.35	1.05-1.28		
IV-Collagen (ng/ ml)	164.4 ± 55.5	146.6-182.1		

Values are mean ± SD of number of observations (n). ↑: indicates number of subjects reported above normal level, ↓: indicates number of subjects reported below normal level.

(Table 2). However, alkaline phosphatase (ALP) activity was elevated in 16 patients (40%), while AST and ALT activities were elevated in 6 chronic pancreatitis patients (15%) (Table 2). Elevation of alkaline phosphatase level was also observed in other study (16). In another study chronic pancreatitis patients showed high triglycerides, chylomicrons, VLDL, LDL-Cholesterol concentrations, and low-diglycerides, free fatty acids, HDL-Cholesterol concentrations in serum (17). Though no apparent change in lipid profile was observed in this study, HDL-cholesterol level decreased in 15 patients (37.5%) (Table 2). The carbohydrate antigen 19-9 (CA 19-9) level in serum higher than the upper limit of range in reference group was noted in 10% of patients with chronic pancreatitis in this study (Table 2). In another study, CA 19-9 level in serum higher than the upper limit of range in reference group was noted in 46.9% of patients with chronic pancreatitis and in 90.6% in the group of patients with pancreatic carcinoma and CA 19-9 concentration in patients with pancreatic carcinoma was

significantly higher in comparison to the level in patients with chronic pancreatitis (18). CA 19-9 assay is a useful adjunct in the diagnosis of pancreatic carcinoma (19). In chronic pancreatitis, CA 19-9 did not show any diagnostic sensitivity.

Chronic pancreatitis manifestations include changes in the calcium metabolism (12). Hyperamylasemia is associated with development of pancreatitis (20), but its sensitivity can be reduced by late presentation (21). Interestingly, calcium level decreased in 10 patients (25%) and serum amylase activity increased in 32 patients (80%) (Table 2). Hyperamylasemia in chronic pancreatitis is generally due to high pancreatic type isoamylase (P-amylase) activity in acute exacerbation, sometimes accompanied by a transient elevation in salivary type isoamylase (S-amylase) (22). Amylase activity showed positive significant correlation with ALP and CA-19-9, whereas negative significant correlation with calcium and hematocrit (HCT) level (Table 3).

Table 3: Correlation of amylase activity and type IV collagen with different parameters

		Glucose	HDL-Chol	ALP	Calcium	CA19.9	ESR	HCT
Amylase	r	0.255	-0.014	0.458	-0.472	0.556	-0.118	-0.484
	p	0.113	0.933	0.003*	0.002*	<0.001*	0.467	0.002*
IV-Col	r	-0.1	-0.052	0.152	-0.505	0.289	-0.205	-0.309
	p	0.538	0.748	0.35	0.001*	0.07	0.205	0.053

*Correlation is significant at <0.01 (2-tailed).

Pancreatic fibrosis (Fig 1 and 2) is a key pathological feature of chronic pancreatitis (23). The imbalance between synthesis and degradation of extracellular matrix (ECM) proteins is a characteristic feature in chronic pancreatitis (24). The matrix metalloproteinase (MMP) plays important roles in extracellular matrix turnover, and is involved in the regeneration of type IV collagen structure in the basement membrane (24). The type IV collagen (Col-IV) consists of 3 α chains. Six different α chains [α 1(IV), α 2(IV), α 3(IV), α 4(IV), α 5(IV), and α 6(IV)] have been identified, and their combination is considered to be organ specific (25). In chronic pancreatitis interstitial

collagens are extensively disorganized and fibrosis possibly is relatively labile (26). In this study, type IV collagen level elevated in 17 patients when compared with our recommended cut off value (154 ng/ ml) (10) and 28 patients (70%) when compared with manufacturer's recommended cut off value (124 ng/ ml). Type IV collagen level showed negative significant correlation with calcium level (Table 3). Interestingly, no significant correlation was observed between amylase activity and type IV collagen ($r=0.289$, $p=0.07$).

In conclusion, chronic pancreatitis causes pancreatic fibrosis and sometimes results in diabetes mellitus. The blood parameters such as amylase activity and type IV collagen level, and their correlations with hematocrit, calcium, alkaline phosphatase activity and CA-19-9 may be monitored in these patients. More study is necessary to identify the biomarker or combination of biomarkers in these patients.

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Fig 1: Dense fibrocollagenous stroma with focal lymphoid collection separates the pancreatic acinar lobules

Fig 2: Dilated pancreatic duct and acinar atrophy surrounded by dense fibrocollagenous stroma.

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