Serum Protease Activity As a Marker in Patients of Acute Pancreatitis- A Hospital Based Retrospective Study

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Abstract
Introduction: Acute pancreatitis being a frequent casuality in the surgical department with griever consequences need an early diagnosis and treatment. In a developing country it is utmost important to diagnose such case and refer it to the tertiary care hospital for further evaluation and treatment to decrease the mortality rate. With this background the present study was carried out to examine the possibility of an easily conclusive test for acute pancreatitis which can be performed at the side laboratory of any hospital. Acute pancreatitis due to various etiology was diagnosed with the classical test, depedent on amylase and lipase activity. In this study the effect of protease fraction of pancreatic enzyme (mainly trypsinogen) which produce free amino acids and small peptides (2-8 amino acids prod, protease activated product PAP) was evaluated. During a phase of acute pancreatitis these fractions are expected to reflect the effect of circulating protease activity either as a relatively direct observable parameter or as an index derived from computation of some functional test.
Material and Method:- The present study was a retrospective study conducted in Guwahati Medical College and Hospital within a period of one year (from 2001-2002) . The target population was divided into two main groups of control and experimental , each comprising 20 individuals. The experimental group was assayed for serum amylase,lipase,total protein and PAP on admission and on discharge.In the control group serum amylase,lipase, total protein and PAP was estimated. As they were not admitted the estimation was done once. The serum PAP was estimated by Biuret test using semiautomatic Boehringer 4020 photometer. Result:-A highly significant PAP (P<0.001) was seen between control and patient on admission, control and patient on discharge and patient on admission and discharge.
Conclusion: From the present study it could be concluded that the PAP may be regarded as a reliable index of pancreatic function, though further study with the inclusion of large no of patients is advisable to draw a positive conclusion.
Keywords: Acute pancreatitis, protease activated product (PAP), trypsinogen, amylase,   lipase, total protein.

I.   Introduction

Acute pancreatitis (AP) is a potentially life threatening disease with varying severity of presentation [1,2]. Nearly 60%-80% of all cases of AP in developed countries are attributable to either gallstone disease or alcohol abuse [3,4]. The incidence is similar in both sexes, although alcohol abuse is the more common cause in men and gallstones is the more common cause in women[5,6].

Though clinical signs and symptoms are sufficient to direct the physician to think about acute pancreatitis still in most cases it is difficult to arrive on conclusion because of diversity of symptom without laboratory investigations.

The pancreatic enzymes derived from pancreatic acinar cells [amylase, lipase and the proenzyme trypsinogen] are the cornerstone in the laboratory diagnosis of AP[7]. Serum lipase is a more sensitive and specific biochemical marker of AP than the more frequently used amylase [7,8].

Additional biomarkers under evaluation for diagnosis of AP include pancreatic isoamylase, pancreatic elastase, serum trypsin, urinary trypsinogen activated peptide (TAP), Phospholipase A_2 and Carboxypeptidase B (CAPB) [9,10]

Trypsinogeb is the zymogen of the pancreatic enzyme trypsin which is cleaved by duodenal enterokinase to produce the active enzyme trypsin and trypsinogen activated peptide (TAP) [7,11]. Normally trypsinogen (trypsinogen-1 and trypsinogen-2) is secreted into the pancreatic fluid by the acinar cells, of which a small amount enters into the circulation and is excreted in urine. In pancreatitis large amounts of this enzyme enter the systemic circulation due to increased vascular permeability and there is a consequent increased clearance in urine. This forms the basis of the use of trypsinogen in the diagnosis and severity assessment of AP[12].

The premature activation of trypsin in pancreatic parenchyma acting as the central step in the initiation of autodigestion of pancreatic tissue and subsequent local and systemic inflammation is presently the most accepted theory [13,14,15,16].

C-Reactive Protein (CRP) is an acute phase reactant synthesized by the hepatocytes and is usually elevated in inflammatory conditions [17]. It takes nearly 72 hours for the serum level of CRP to peak after the onset of symptoms [18]. It is the most frequently used single biomarker for assessment of severity in AP today [17].

The delay in adoption of protease based test for evaluation of pancreatic function is due to their heterogeneity in activation, substrate specificity and product character. The difficulty in their utilization is finally over come by introduction of substrate independent electrophoretic , chromatographic and immunological separation of these substances in serum.

The combined effect of the protease-fraction of the pancreatic enzymes result in the formation of free amino acids and small peptides (2-8 amino acids)[19]. During a phase of acute pancreatitis the serum level of these fractions are expected to reflect the effect of circulating protease activity.

II.   Aim And Objective
The present study was proposed with the aim to establish serum protease activity in acute pancreatitis and with an objective to probe the reliability of findings against reference pancreatic function test.

III.   Materials And Methods
The present study was conducted in a group of forty subjects irrespective of age and sex taken randomly from different socio-economic status as per plan of the study; the target population was divided into two main groups.

Control group:
Inclusion criteria
Control group consisted of normal healthy individuals without any history of pancreatic disorder. These individuals were taken after taking proper history about not having pain abdomen and serum amylase and lipase values were assayed which were within normal limit. This group comprised 20 individuals.

Exclusion criteria
Individuals with absence of history of pain abdomen but borderline serum amylase and lipase values were excluded from the control group.

Experimental group:
Inclusion criteria
Experimental or the test group was composed of patients admitted in surgical units, suffering from acute pancreatitis based on clinical diagnosis and investigative procedure. This group comprised of 20 individuals.

Exclusion criteria
Patients admitted in surgical ward with pain abdomen but serum amylase and lipase level within normal limit, were excluded from the experimental group.

The experimental group was further divided into

Patients on admission which comprised 20 individuals

Patients on discharge the same 20 individuals

The blood was collected for estimation of total protein and protease activated product (PAP). The estimations were done by using semiautomatic Boehringer 4020 photometer.

The total protein and PAP were estimated by biuret test. The alkaline copper sulphate present in the biuret reagent reacts with protein and their derivatives containing more than one peptide linkage and give a violet coloured complex which was compared against blank and standard [21].

After estimation of protein, a protein free filtrate was made and estimation of PAP was done using the biuret test [20].

In addition to serum amylase and lipase the other pancreatic parameters are serum and urinary trypsinogen-2 levels, Interleukin 6 and 8, C-reactive protein [22].

Carboxypeptidase B (CAPB) and urinary Trypsinogen activated peptide (TAP) both were excellent prognostic markers but within the first day of admission urinary TAP was superior [23].

Statistical analysis
The data was analysed by unpaired student's t test using the Statistical Package for the Social sciences (SPSS) version 20. In this study p value <0.001 was highly significant.

ETHICAL APPROVAL
All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Before proceeding for laboratory investigation written consent of the study population was taken.

IV. Results

The study consisted of 40 individuals which were grouped into two i.e. control and experimental(patients suffering from acute pancreatitis). Both the groups comprised of 20 individuals with varying age and sex.

The normal control group comprised 20 individuals, the age varied within the range of 16 to 80 years, with mean age of 43.28 ± 3.89 yrs. There were 18 males and 2 females.

The experimental group comprised 20 individuals, the age varied within the range of 16-60 yrs with mean age of 40 ± 2.79yrs. There were 15 males and 5 females.

In the control group the serum PAP mean value was 0.7570 ± 0.1241g/dl and in the patient group the mean value was 3.145 ± 0.17g/dl on admission and 2.080 ± 0.13g/dl on discharge.

The mean serum amylase value in control group was 65.65 ± 2.19. U/L , patient on admission was 492 ± 55.27U/L and on discharge was 87.90 ± 1.38U/L.
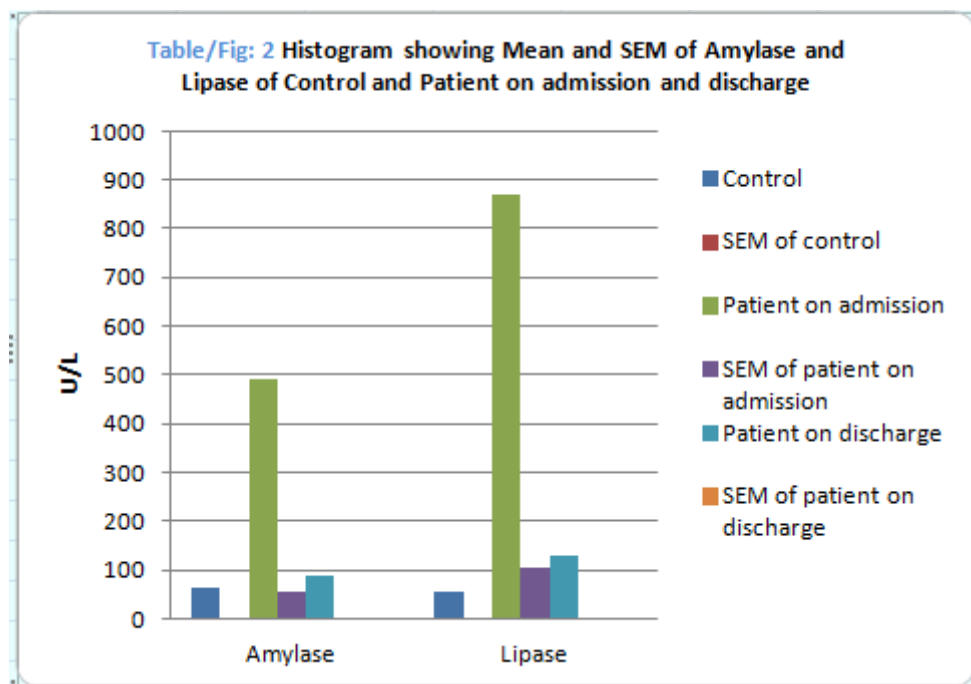
The mean serum lipase value in control was 57.44 ± 2.43U/L , patient on admission was 869.1 ± 104.77U/L and on discharge was 129.7 ± 1.39.U/L.
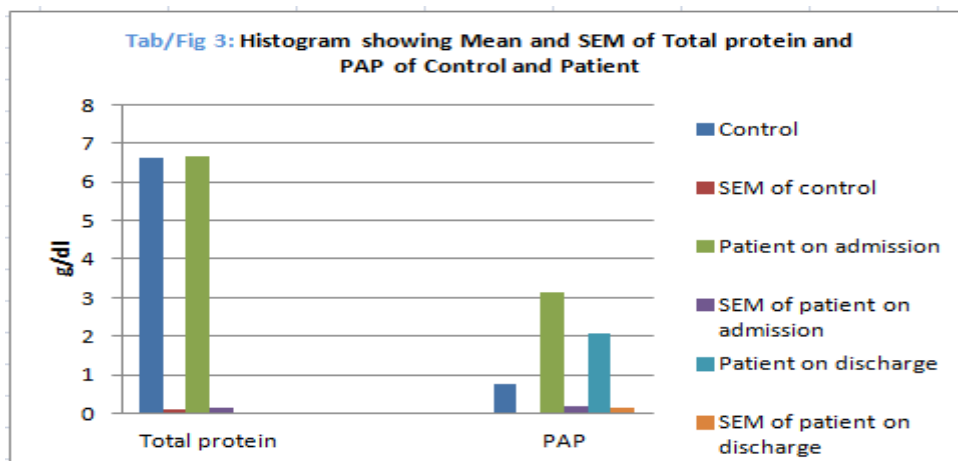
The mean value of total protein in control group was 6.64 ± 0.09g/dl and patient on admission was 6.675 ± 0.15g/dl.
PAP concentration in serum is well correlated with coefficient of correlation more than 0.7 with the distribution of serum amylase and lipase during acute pancreatitis.

Table/figure : 1Statistical analysis and p value of Serum Amylase , Serum Lipase, Total Protein and Protease Activated Product						
Parameters	Control	Experimental		p value		
		On Admission	On Discharge	Control/admission	Control/Discharge	Admission/Discharge
Amylase U/L	65.65±2.19	492±55.27	87.90 ±1.38	<0.001***	>0.05ns	<0.001***
Lipase U/L	57.44.±2.43.	869.1 ± 104.77.	129.7±1.39.	<0.001***	>0.05ns	<0.001***
Total protein g/dl	6.64±0.09	6.675±0.15		>0.999ns		
Protease activated product g/dl	0.7570±0.03	3.145±0.17	2.080±0.13	<0.001***	<0.001***	<0.001***
Significance of p value , p value < 0.001 was highly significant *** p value > 0.05 was non significant**						

Table/Fig: 2 Histogram showing Mean and SEM of Amylase and Lipase of Control and Patient on admission and discharge

Tab/Fig 3: Histogram showing Mean and SEM of Total protein and PAP of Control and Patient

Table /Figure 4 Coefficient of co-relation			
Parameters	Control	Patient on Admission	Patient on Discharge
Serum Amylase/Serum protease activated product	0.293	0.723	0.136
Serum Lipase/Serum protease activated product	0.229	0.792	0.046
Serum Amylase/Serum Lipase	0.416	0.857	0.341
Serum Total Protein/Serum protease activated product		0.25	

V.   Discussion

Introduction of protein activity based tests in serum into the existing area of pancreatic function tests commonly represented by serum lipase and amylase activity is the present day trend in evaluation of pancreatic function and integrity[19]. In the evaluationary history of devising serum biochemistry based methods to evaluate and monitor functional integrity of the pancreas, the proteolytic activity of the pancreatic enzymes in circulation was initially tried to be exploited as an index for functional integrity of pancreas[19]. Pancreatic protease activator, trypsinogen activated peptide (TAP) and carboxypeptidase precursor activation peptide (CAPAP) is a very early event in acute pancreatitis with high levels of serum markers available for analysis within the first 48hrs of the disease. The protease activation then declines about 48-72 hour of disease[26]. Acute pancreatitis increases the catabolism and proteolysis of skeletal muscle by as much as 80% in comparison with healthy controls. Further nitrogen losses increase to as much as 20-40g/day. Decreased concentrations of total plasma proteins, rapid turnover of proteins and a marked decrease of the ratio of branched chain to aromatic amino acid further characterized this hyper catabolic state [27]. With advancement in analytical techniques the once rejected protease based tests have been developed to a high degree of reliability and specificity [22,23]. This has added an array of high resolution pancreatic function tests to the already existing and time tested lipase and amylase dependent pancreatic function test. These tests require a fairly high degree of sophistication in analytical procedure which may not be accessible to any laboratory with facilities only for written serum bio-chemistry.

Within the diversity of substrate – product combinations of the pancreatic proteases, one unified criteria is that the proteases as a group acts on the substrate protein as a group and produces a group of oligopeptides reacting positively with the classical biuret reagent[24,25]. On the basis of these unified interactions of the proteases on the unified products detectable by a common reaction, the present study was aimed to probe the relationship between serum proteins and protease activity products in acute pancreatitis with reference to a group of normal subjects taken as control.

In the present study the mean serum amylase and lipase activity was found to be elevated in the patients with pancreatitis to a very highly significant level ($p< 0.001$). No significant differences in the mean serum total protein was observed between the normal control and the pancreatitis patients.

The mean serum PAP concentration of 0.7570±0.1241gm/dl in the normal control group is elevated to a very highly significant level($p<0.001$) of mean protease activated product of 3.145±0.7681gm/dl in the group of patients with acute pancreatitis.

The increase in the PAP concentration in serum under condition of study was well correlated with coefficient of correlation more than 0.7 with the distribution of serum amylase and lipase during acute pancreatitis. The similarities in both types and extent of the distributions, probabilities and correlations of PAP , the experimental variable with serum lipase and amylase the reference indices for pancreatic function logically lead us to opine the protease activated product may be regarded as a reliable index of pancreatic function.

LIMITATION OF STUDY

There are several limitations of the present study. The sample size of the study was a bit small. The causality role of PAP and Total Protein in acute pancreatitis however requires to be investigated further to come to a definite conclusion.

VI.   Conclusion

The consistent and well defined correlation of PAP with serum amylase and lipase during pancreatitis observed in the present study strongly suggested that serum PAP estimation may be utilized as an additional monitoring factor for diagnosis and prognosis of acute pancreatitis.

CONFLICT OF INTEREST: No conflict of interest was associated with this work.

CONTRIBUTION OF AUTHORS: We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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