

Diagnostic Value of Pancreatic Elastase-1 in Human Acute Pancreatitis

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Abstract. The diagnosis of acute pancreatitis (AP) is usually confirmed by a significant increase of the serum amylase and/or lipase level. However, serum pancreatic elastase-1 (pEla-1) was found to be a more sensitive diagnostic marker in AP, when assayed by the radioimmunoassay procedure. We analyzed the serum concentration of pEla-1, measured by the ELISA technique in 46 patients with AP and in a control group of 12 healthy volunteers. On admission (day 1) we found significantly higher pEla-1 levels in patients with AP than in the controls. During the following days, the concentration of pEla-1 rapidly decreased to nearly undetectable values on the 3rd day. There was no significant difference between patients with mild and severe AP nor those of different etiology. We suggest that pEla-1 has little diagnostic value and does not provide additional information to that of the less expensive and more widely available serum amylase and lipase.

Key words: acute pancreatitis; pancreatic elastase-1; diagnosis; prognosis.

Introduction

Among the many inflammatory diseases within the abdominal cavity, acute pancreatitis (AP) is characterized by a large variety of clinical symptoms. Despite significant progress in diagnosis, its course is still unpredictable and the variety of clinical symptoms of the disease is the cause of numerous diagnostic mistakes. In 40% of the cases, an accurate diagnosis is first made during autopsy^{16, 22}. It should be stressed that early diagnosis combined with a prediction of the severity of AP, allows commencement of the proper therapy at the

appropriate moment, which improves patient survival. Such treatment is of a special importance when we consider that the death rate reaches 30% in the necrotic form of the disease and 50–80% when complicated by multiple organ dysfunction syndrome^{5, 12}.

The imperfection of the methods used has prompted researchers to seek a single, simple and inexpensive biochemical test which would allow the detection of AP with high sensitivity and specificity, in both the first and second week of the disease, in patients with hyperlipidemia, kidney failure and other co-existing diseases. The currently measured indicators, such as α -amylase

Abbreviations used: AP – acute pancreatitis, ARDS – acute respiratory distress syndrome, ELISA – enzyme-linked immunosorbent assay, α_2 -M – α_2 -macroglobulin, NF- κ B – nuclear factor κ B, pEla-1 – pancreatic elastase-1, α_1 -PI – α_1 -protease inhibitor, RIA – radioimmunoassay.

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activity in serum and urine, pancreatic isozyme of α -amylase and lipase activity in serum, do not comply with the above requirements^{3, 16, 18, 22}. Alternative diagnostic tests for AP, which are methodologically difficult and expensive, include the serum concentration of trypsin, procarbonylpeptidase B, pancreatic phospholipase A₂, and pancreatic elastase-1 (pEla-1)^{3, 18, 22}.

Pancreatic elastase-1 is a proteolytic enzyme produced by the acinar cells of the pancreas in the form of an inactive proenzyme which is secreted to the pancreatic duct and then to the duodenum, where is activated by trypsin. Two types of pancreatic elastase were first isolated from human pancreatic tissue: types 1 and 2. A few years later, Japanese researchers identified the genetic structure of the next isoenzyme, pEla-3¹⁹. Pancreatic elastase type 1 is a 25 kDa molecule and is inactivated by bonding with α_1 -protease inhibitor (α_1 -PI) or α_2 -macroglobulin (α_2 -M)^{11, 13}. This multi-aminoacid peptide not only exhibits proteolytic properties, but also hydrolyzes scleroprotein elastin, shows fibrinolytic activity, and increases oxidative activity of neutrophils^{11, 20}.

The early trypsinogen activation creates the conditions for the active form of pEla-1 and other proteolytic and lipolytic enzymes within the pancreatic tissue⁴. During the rapid increase of pEla-1 concentration, the inhibitor capacity of α_2 -M is depleted and its function is taken over by α_1 -PI, which binds and inactivates numerous molecules of the active enzyme¹¹. The most recent experimental studies show that pancreatic elastase plays a significant role as pathogenetic factor in the development of pancreatic necrosis and systemic complications in the course of AP, such as acute respiratory distress syndrome (ARDS)^{8, 23} and liver obstruction⁹. It is suggested that pancreatic elastase induces lung and liver injury by the activation of nuclear factor κ B (NF- κ B) with a consequent increase of cytokin synthesis by inflammatory cells^{8, 9}.

The purpose of this study was to estimate the diagnostic and prognostic value of pEla-1 concentration measured by enzyme-linked immunosorbent assay (ELISA) in serum of patients with AP.

Materials and Methods

The study was performed with 46 patients suffering from AP, comprising 23 women and 23 men at ages between 31 and 84 (median 54 years old). They were hospitalized within 48 h after onset of the disease in the Department of Gastroenterology and Department of Anesthesiology and Intensive Therapy of the Medical University of Białystok. The control group comprised

Table 1. The clinical characteristics of patients with acute pancreatitis (AP) and healthy control group

	Acute pancreatitis (n)	Control group (n)
Number	46	12
Man : woman	23 : 23	7 : 5
Age (median, range in parenthesis)	54 (31–84)	43 (22–72)
Severity		
• mild AP	27 (59%)	
Ranson's score	< 3 points	
Balthazar's grade	A – 13, B – 14	
• severe AP	19 (41%)	
Ranson's score	≥ 3 points	
Balthazar's grade	C – 6, D – 8, E – 5	
Etiology		
• alcoholic AP	16 (34.8%)	
• biliary AP	30 (65.2%)	

12 healthy volunteers; 5 women and 7 men, aged 22–72 years (median 43). Diagnosis was made on the basis of typical clinical symptoms and elevated serum α -amylase activity at least twice the upper limit of the reference range (20–90 U/l). In all patients, additional biochemical assays and imaging techniques (ultrasonography, and contrast-enhanced computed tomography) were done to confirm the diagnosis and also to determine the etiology and severity of AP according to Ranson's¹⁷, Balthazar's¹ and Atlanta's² criteria (Table 1). Patients were categorized into two groups: mild AP (n = 27) and severe AP (n = 19). The treatment of patients with AP included procedures according to the accepted rules, depending on the severity of disease. By etiology, 16 patients presented alcoholic pancreatitis and 30 biliary pancreatitis. During hospitalization, one patient died due to late septic complications.

The medical University of Białystok Ethical Committee, which controls research on humans and animals, granted approval for performing this study. All the patients signed their agreement for participation in the study.

At admission (day 1) and on days 2, 3, 5 and 10, the serum concentrations (ng/ml) of pEla-1 were measured with the ELISA kit using two monoclonal antibodies specific for human pEla-1 and were calculated photometrically (OD 405 nm) in comparison with a standard solution (from ScheBoTech, Germany). The cut-off value was 3.5 ng/ml. The α -amylase and the lipase activities were measured by kinetic determinations (test from bioMerieux, France). The α -amylase cut-offs were 90 IU (serum) and 490 IU (urine). The lipase cut-off was 60 IU.

Since our data for the measured enzymes were not

normally distributed, the results were reported as median value, 25th and 75th quartiles, and range. The Wilcoxon and Mann-Whitney U tests were used to assess differences in measured values. In all calculations, $p < 0.05$ was regarded as statistically significant. For correlation studies, the Spearman rank correlation coefficient was used. The relationship between correlated parameters was shown as a regression line.

Results

pEla-1 concentration in the serum of patients with AP

Serum concentration of pEla-1 was significantly elevated by 1448% on admission (day 1) compared with the controls (Table 2). On the second day of observation, the pEla-1 level was still higher by 642% than the control value, though the difference was not statistically significant. During the subsequent days, the median enzyme level was close or equal to 0 ng/ml. When we consider the lower limit of the diagnostic value greater than 3.5 ng/ml, in accord with the to test manufacturer's recommendations, it should be noted that the median pEla-1 level was lower than this value already on day 2. The enzyme concentration was lower than or equal to 3.5 ng/ml on admission and remained at the same level during the whole observation in the serum of 16 out of 46 patients (34.8%). Among these 16 patients, 9 presented a mild form of AP and 9 patients had biliary pancreatitis. However, on the second day the number of patients with lower than diagnostic

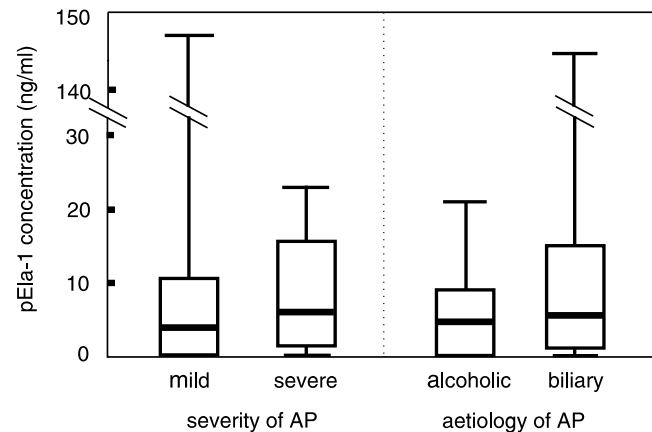


Fig. 1. Serum pEla-1 concentration (ng/ml) in patients with mild, severe, alcoholic and biliary acute pancreatitis. Minimum, maximum, 25th and 75th percentiles (box) and median (line) values are shown

serum pEla-1 level increased to 26 (56.5%). Only in 4 cases was an enzyme concentration above 3.5 ng/ml observed up to the 10th day. Analysis of pEla-1 level changes in relation to severity and etiology showed the lack of a significant difference between patients with mild and severe AP on admission (median 4.19 ng/ml vs 6.13 ng/ml), as well as between patients with biliary and alcoholic etiology (median 5.12 ng/ml vs 4.55 ng/ml; Fig. 1).

Lipase activity in the serum of patients with AP

In the studied group of patients, the median lipase activity in serum was the highest on day 1 and exceeded the reference value by 357%. Although lipase

Table 2. Median and range of serum pancreatic elastase (pEla-1) concentration, serum and urine α -amylase and serum lipase activity in patients with acute pancreatitis (AP) and control group

	Acute pancreatitis n = 46					Control group n = 12
	day 1	day 2	day 3	day 5	day 10	
Serum pEla-1 (ng/ml)	5.11 * (0.0–144)	2.45 • (0.0–17.55)	0.0 • (0.0–12.17)	0.01 • (0.0–7.64)	0.0 • (0.0–5.71)	0.33 (0.07–3.06)
mild AP n = 27	4.19 * (0.0–144)	2.44 (0.0–12.76)	0.0 (0.0–9.02)	0.0 (0.0–7.64)	0.0 (0.0–5.51)	
severe AP n = 19	6.13 * (0.0–22.8)	3.1 (0.0–17.55)	0.23 (0.0–12.17)	0.14 (0.0–6.98)	0.1 (0.0–5.71)	
Serum α -amylase (IU)	681 (99–3820)	260 • (12–2383)	85 • (12–714)	52 • (15–242)	59 • (20–124)	0–90
Urine α -amylase (IU)	6300 (320–48,400)	2164 • (90–20,485)	768 • (61–17,340)	278 • (54–1165)	240 • (50–1352)	0–490
Serum lipase (IU)	274 (6–2647)	237 (6–1746)	113 • (13–816)	86 • (22–438)	80 • (23–370)	0–60

* $p < 0.05$ in comparison with control group; • $p < 0.001$ in comparison with day 1.

activity in serum was elevated during the whole study, it was significantly lower on days 3, 5 and 10 than on day 1 (Table 2). Lipase activity did not differ significantly between patients with mild and severe forms of AP, nor between patients with alcoholic and biliary etiology of disease. On admission, serum lipase activity was two times higher than normal value in 33 patients (72%), on day 2 in 41 patients (89%), while on day 10 only in 9 cases of AP (19,5%).

α -Amylase activity in the serum of patients with AP

The activity of serum α -amylase was the highest on admission (day 1) and exceeded the normal value by 656.7% (Table 2). On the second day, enzyme activity was still elevated by 189% in comparison with the normal value, but was significantly lower than on admission ($p < 0.001$). During subsequent days, median enzyme activity was close to the reference value, although it was still elevated on day 3 in 20 patients (43.5%) and on day 10 in 5 patients (10.9%) with AP. No significant difference was found in enzyme activity between patients with the mild and severe forms of disease nor between patients with alcoholic and biliary AP.

α -Amylase activity in the urine of patients with AP

The highest α -amylase activity in urine was observed from the admission day until the 3rd day of hospitalization. Compared with the reference range, enzyme activity was increased by 1185.7, 441.6 and 56.7 on day 1, 2 and 3, respectively (Table 2). As in serum, α -amylase activity in urine did not differ significantly between patients with the mild and severe forms of disease nor was dependent on the etiology of AP.

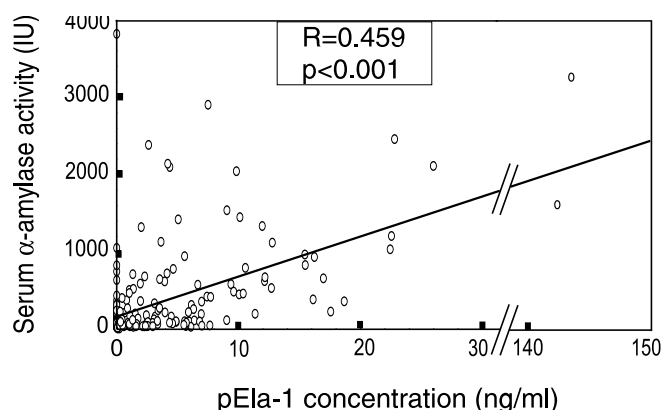


Fig. 2. Correlation of pEla-1 concentration with serum α -amylase activity. Spearman rank coefficient value is reported with its respective p value

Correlation calculations

Figure 2 shows significant correlation between serum pEla-1 concentration and serum α -amylase activity. Significant, positive correlation was also found between serum pEla-1 level and urine α -amylase activity ($R = 0.424$, $p < 0.001$) and between Ela-1 concentration and lipase activity in serum ($R = 0.364$, $p < 0.001$, data not shown).

Discussion

Among the many pancreatic enzymes which may be the key element for the differential diagnosis of AP, much attention has been paid to pEla-1 for the last ten years. Clinical studies of patients with AP showed elevated serum pEla-1 levels measured by radioimmunoassay (RIA), even up to the 10th day of disease^{6, 7, 21}. It was shown that a high serum concentration of this enzyme allows early diagnosis of AP with a high sensitivity and accuracy, even one week after clinical symptoms occur^{6, 7, 10, 21}. It should be stressed, however, that a technique employing radioisotopes demands special conditions, thus restricting test performance to large laboratories. Progress in diagnosis has allowed the development of the ELISA method, making pEla-1 concentration measurement less easier complicated and more available in small hospitals possessing their own laboratories.

In the present study we showed that serum pEla-1 level measured by the ELISA kit does not have an advantage over α -amylase or lipase activity in AP diagnosis. Among all the studied periods of the disease, only on admission (day 1) the median enzyme level was higher than the border value given by the manufacturer. It should be stressed that all the patients were hospitalized within 48 h after onset of AP, 16 of them within 24 h. An analysis of particular cases revealed that on admission the pEla-1 concentration did not reach the diagnostic value in 34.8% of patients; moreover, the percentage of such patients increased in the following days. Similar results were noted by MILLSON et al.¹⁴, who measured serum pEla-1 levels in 567 patients hospitalized due to different acute and chronic abdominal diseases, 27 of whom were with AP. In this group of 27 patients, increased values of enzyme concentration (median 4 ng/ml) were observed until the 3rd day of hospitalization, although the authors did not analyze the time of occurrence of clinical symptoms.

As in this study, MILLSON et al.¹⁴ showed normal pEla-1 levels in 1/3 of patients with AP on admission. Increased enzyme concentrations were found in 81 pa-

tients with other abdominal diseases, which considerably lowers its diagnostic value in AP. The authors assessed test sensitivity, specificity and accuracy as 66%, 85% and 0.84, respectively. The same parameters for α -amylase were: 81%, 98% and 0.97.

In another study of 14 patients with AP, a 3-fold increase in pEla-1 serum level was recorded on day 1, although after 3 days enzyme concentration was normal in 11 cases¹⁰. During the first 48 h of the disease, the sensitivity and specificity of the ELISA kit were around 92%, while these parameters did not reach even 40% between 48 and 96 h nor 10% after 4 days of AP. The authors found that the decrease in pEla-1 concentration during the first few days of AP parallels the changes in serum α -amylase and lipase activity.

Taking into account the analysis of serum pEla-1 concentrations in 253 patients hospitalized due to other abdominal diseases, it was suggested to decrease the lower limit of enzyme concentration to about 2 ng/ml, which increases test sensitivity up to 97% if the analysis is performed within the first 48 h¹⁰. The authors suggest that the low diagnostic value of the test is the consequence of the short half-life of pEla-1 as measured by the ELISA kit (0.4 days). This may be associated with the kind of antibodies used for measurements. Additionally, evaluation of serum pEla-1 concentration is difficult, as its active form is bound with α_2 -M and α_1 -PI. Multiclonal antibodies (RIA method) recognize enzymatic complex with α_1 -PI only¹⁵. It was not found until now whether a similar process occurs in the case of monoclonal antibody usage (ELISA method). In the course of AP both active and inactive proelastase may be present in serum and might be differently recognized, depending on the antibodies used. Authors speculate that in the ELISA method monoclonal antibodies are orientated against the active form of enzyme, while polyclonal antibodies detect inactive and active forms, which might explain the rapid decrease of pEla-1 concentration measured by the ELISA kit¹⁰.

Within the studied group of patients with AP, we showed that serum pEla-1 concentration on admission does not provide any information concerning etiology and is also not useful in the assessment of disease severity. This is in agreement with observations of other authors^{6, 14, 21}. As MILLSON¹⁴ et al., we also found a relationship between pEla-1 concentration and serum α -amylase activity. It seems that this relationship is a logical consequence of the premature activation of pancreatic enzymes and its penetration to organism fluids from the pancreas being injured by the inflammatory process.

Results presented in this study show that the pEla-1 concentration measured by the ELISA kit may be helpful in AP diagnosis only at a very early stage, i.e. up to 48 h after the onset of the disease. The determination of pEla-1 concentration in serum does not have an advantage over traditional biochemical indicators, such as α -amylase or lipase, in AP diagnosis nor does it allow a determination on either etiology or severity of the disease. Precise analysis and/or modification of antibodies used in the ELISA kit may improve this method and allow a higher diagnostic test value in AP.

The measurement of pancreatic enzyme activity/concentration in serum is one of three elements needed in AP diagnosis, together with the clinical picture and morphological changes of the pancreas as revealed by imaging techniques. In light of the latest studies on diagnosis management and biochemical test availability, the most recommended is the measurement of lipase activity in serum. This was perfectly formulated by DOMINIGUEZ-MUNOZ³: "Amylase or something new? Not amylase, but something old, lipase".

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