The diagnostic value of serum pancreatic phospholipase A₂ (PLA₂) in pancreatic diseases

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Summary: The diagnostic significance of serum immunoreactive pancreatic phospholipase A₂ (PLA₂) was studied in 119 patients with pancreatic disease, 200 with various non-pancreatic disease, and 203 healthy controls using radioimmunoassay (RIA) specific to human pancreatic PLA₂. This newly developed RIA using monoclonal antibody was satisfactorily sensitive and reliable. Serum PLA₂ was elevated in all six patients with acute pancreatitis. Frequency of abnormal serum PLA₂ levels was 60% in chronic pancreatitis (n=52) and 67% in pancreatic cancer (n=61). Serum PLA₂ levels were low in chronic pancreatitis with severe exocrine insufficiency and advanced pancreatic cancer. In chronic pancreatitis, patients with low serum PLA₂ level showed lower enzyme output in secretin test than patients with normal or high serum PLA₂ level. Frequency of abnormal PLA₂ levels was 27% in non-pancreatic disease and, in particular, patients with renal failure showed high PLA₂ levels. Sensitivity (62%) and efficiency (69%) of serum PLA₂ assay in pancreatic disease were superior to those of amylase. In conclusion, serum PLA₂ determination using RIA was useful for the diagnosis of acute pancreatitis by high serum PLA₂ levels and the diagnosis of severe exocrine pancreatic insufficiency by low serum PLA₂ levels. Gastroenterol Jpn 1991;26:62-68

Key words: acute pancreatitis; chronic pancreatitis; pancreatic cancer; pancreatic phospholipase A₂; radioimmunoassay

Introduction
Phospholipase A₂ (PLA₂) has been thought to play an important role in the exacerbation of acute pancreatitis1-4. Many reports revealed that serum PLA₂ was elevated in acute pancreatitis and the determination of serum PLA₂ was useful for early recognition of severe acute pancreatitis5-7. Conventional enzymological methods for the measurement of serum pancreatic PLA₂ require very complicated procedures and long incubation time8,9, so it is difficult to measure many samples. Therefore, there have been few reports studying the diagnostic significance of serum PLA₂ in other pancreatic diseases or non-pancreatic diseases.

Recently a radioimmunoassay (RIA) for serum pancreatic PLA₂ has been developed10,11 and a convenient RIA kit has become available12,13. In present study, we measured serum immunoreactive pancreatic PLA₂ in healthy controls and patients with pancreatic and non-pancreatic disease, and evaluated the significance of the measurement of serum PLA₂ for the diagnosis of pancreatic diseases.

Subjects and Methods

Subjects
The subjects included 203 healthy controls (141 males and 62 females, 18-70 years old), 119 patients with pancreatic diseases and 200 patients with non-pancreatic diseases. The diagnosis of 6 patients with acute pancreatitis was made by
clinical presentation, imaging examinations and elevation of serum amylase and lipase. Chronic pancreatitis was diagnosed in 52 patients (28 patients with calcified pancreatitis and 24 with non-calcified pancreatitis) by clinical presentation, radiological findings, exocrine pancreatic function test, endoscopic retrograde cholangiopancreatography (ERCP), or pancreatic histology. The exocrine pancreatic function was evaluated by secretin test. This test was performed using a Bartelheimer’s double ballon tube. Collection of duodenal juice was obtained in 10-min samples for 60 min after intravenous administration of secretin (100 CHR units/body) (Eisai, Tokyo, Japan). The juice was collected into ice-chilled tubes and assayed immediately. Duodenal juice was analyzed for juice volume, amylase output and maximal bicarbonate concentration. Pancreatic cancer was diagnosed in 61 patients (2 patients with stage II, 18 with stage III and 41 with stage IV). Of 61 patients, 41 patients underwent laparotomy and histological diagnosis was obtained. In the other 20 patients diagnosis was made by clinical findings, ERCP, computed tomography, ultrasonography, and angiography. Staging of pancreatic cancer was performed according to the UICC TNM classification of malignant tumors.

Groups of non-pancreatic diseases consisted of cholelithiasis (40 patients), liver cirrhosis (28 patients), diabetes mellitus (30 patients), gastric ulcer (21 patients), colorectal cancer (24 patients), and chronic renal failure under hemodialysis (15 patients).

In all patients, serum amylase was also determined by Caraway’s method using the same specimens for serum PLA2. In some patients with chronic pancreatitis and pancreatic cancer, serum lipase activity (lipase UV, TOYO, Tokyo, Japan), immunoreactive elastase 1 (Elastase 1 RIA kit, Dinabot, Tokyo Japan) and immunoreactive trypsin (RIA-gnost Trypsin Kit, Hoechst, Tokyo, Japan) were also measured.

Radioimmunoassay of serum PLA2

Serum PLA2 was determined in duplicate with an RIA kit using monoclonal antibody (RIA kit S-0932) provided by Shionogi (Osaka, Japan). In this method, 50μl of serum samples per tube was needed for assay. Separation of bound and free forms of PLA2 was carried out by a single antibody method using 125I-labeled PLA2 and mouse monoclonal antibody for human pancreatic PLA2. Incubation of the serum sample, 125I-PLA2 and antibody required 2.5 hours. It took about 4 hours to complete this assay. The serum samples were coded and kept frozen at −20°C until assay. The assay was done in coded samples without knowledge of the diagnosis. Prolonged freezing up to 16 weeks and repetition of thawing and freezing up to 4 times did not alter the serum level of PLA2. The lowest concentration that could be reliably measured by this assay was 75 ng/dl. The coefficients of variation (CV=SD/mean) for interassay in ten different assays were 9.8% (13/133), 6.7% (28/421), 3.9% (18/459), 5.1% (68/1322) and 6.5% (537/8230). The CV for intrassay of each ten samples of five specimens were 5.7% (12/209), 6.2% (21/338), 4.5% (33/735), 1.5% (19/1266) and 4.1% (151/3673). The recovery of PLA2 added to sera ranged 92.8% to 103.3% (standard PLA2 solution of 100, 300, 1000 ng/dl added to serum samples with concentrations of 155, 308, 749 and 1200 ng/dl).

Statistical analysis

Data were presented as means±standard deviation (SD). For statistical comparison of means, a simultaneous significance test (Bonferroni’s method) was applied to the control group and each disease group. The correlation between two factors was tested by linear regression analysis. Statistical comparison of frequency was analyzed with the chi-square test. Frequencies of abnormal levels of PLA2 and amylase in an identical group were compared by sign test or McNemar test. P<0.05 was taken as statistically significant.

Results

Normal range of PLA2

The means (M) ± standard deviation (SD) of the serum PLA2 in healthy controls were 341±78 ng/dl for the total group. The values were not af-