PAPERS AND SHORT REPORTS

Clinical value of serum immunoreactive trypsin concentration

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Abstract

The clinical value of estimation of serum concentrations of immunoreactive trypsin was evaluated by studying 46 healthy controls, 23 controls in hospital, 44 patients with chronic pancreatic disease, and 184 patients with non-pancreatic conditions in which pancreatic disease commonly enters into the differential diagnosis. Serum trypsin concentration had a log normal distribution in the controls, and the calculated normal range was considerably wider than that previously reported. The concentration was abnormal in only 13 out of 27 patients with chronic pancreatitis and was extremely variable in patients with pancreatic cancer. Abnormal results occurred in 11% of the patients with non-pancreatic disease. Eighteen patients had a subnormal trypsin concentration, of whom six did not have pancreatic disease and 12 had either chronic pancreatitis or pancreatic cancer. There was no correlation between serum trypsin concentration and mean tryptic activity as measured by the Lundh test. Of 11 patients with pancreatic steatorrhoea, only seven had subnormal trypsin concentrations.

These results suggest that the serum concentrations of immunoreactive trypsin has a low specificity and sensitivity for pancreatic disease and does not reflect the degree of exocrine insufficiency in patients with proved chronic pancreatitis.

Introduction

The recent development of specific radioimmunoassays of serum trypsin concentration¹⁻³ has led to the suggestion that the test might be used in screening for pancreatic disease,⁴ but its value in clinical practice has not been adequately studied. Concentrations of immunoreactive trypsin are raised in acute pancreatitis² and chronic renal failure,⁵ presumably because of impaired renal clearance, but little attention has been paid to the importance of high concentrations in other conditions. Serum trypsin concentrations are said to reflect the degree of pancreatic insufficiency in patients with chronic pancreatic disease.^{4,6} In pancreatic cancer and chronic pancreatitis without exocrine insufficiency, however, the concentrations may be normal or even high.^{2,4}

A simple non-invasive test for chronic pancreatic disease is still required, particularly to evaluate patients with chronic abdominal pain or jaundice. We assessed the clinical contribution of a random measurement of serum immunoreactive trypsin concentration in 297 subjects, comprising healthy and hospital controls, patients with chronic pancreatitis and other painful abdominal conditions, and patients with liver disease or non-pancreatic steatorrhoea in whom a definite diagnosis was known. The relation between serum concentrations of immunoreactive trypsin and pancreatic exocrine function was studied in patients with proved chronic pancreatitis.

Patients and methods

A random blood sample was taken from 297 subjects with normal renal function. Serum was separated the same day and stored frozen until assayed within three months using the RIA-gnost radioimmunoassay kit for trypsin (Hoechst). All samples were assayed in duplicate; the coefficient of variation was 6.2%. Lundh tests were performed by a standard technique⁷ and mean tryptic activity determined by the method of Wiggins.⁸ The lower limit of normal for Lundh tests in our laboratory is 9.5 $\mu\text{EqH}^+/\text{ml}/\text{min}$. Faecal fats were estimated by the method of van de Kamer *et al.*⁹ An oral pancreatic function test was performed as described by Mitchell *et al.*¹⁰ Endoscopic retrograde pancreatograms included as evidence of chronic pancreatitis showed gross abnormalities of both the main duct and side branches.

The subjects studied included 46 normal controls, consisting of healthy laboratory and medical staff without a history of abdominal

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pain or excess alcohol intake, and 23 hospital controls, consisting of inpatients with various non-abdominal conditions.

Twenty-seven patients had chronic pancreatitis, the diagnosis having been established by the following criteria: 13 patients had an abnormal Lundh test and endoscopic retrograde pancreatogram, plus pancreatic calcification (five), steatorrhoea responsive to pancreatic enzyme supplements (five), calcification and responsive steatorrhoea (one), or an abnormal pancreas at laparotomy (two); seven patients had an abnormal pancreatogram and pancreatic calcification, four also having an abnormal pancreas at laparotomy; three patients had an abnormal Lundh test and responsive steatorrhoea; two patients had an abnormal oral pancreatic function test and abnormal pancreas at laparotomy plus either responsive steatorrhoea (one) or an abnormal pancreatogram (one); one patient had an abnormal Lundh test and pancreatic calcification; and one patient had an abnormal pancreatogram and responsive steatorrhoea. Thus 19 patients had evidence of exocrine insufficiency on function testing, of whom 11 had steatorrhoea responsive to pancreatic enzyme supplements. No patient was suffering an exacerbation of pain at the time of study.

Seventeen patients had histologically proved pancreatic cancer. Of 33 patients with chronic liver disease, 20 were jaundiced. Forty-one patients had duodenal ulcer and 20 gastric ulcer. Fifteen patients had gastric and four colonic cancer.

Twenty-five patients had Crohn's disease diagnosed by accepted clinical, radiological, and histological criteria. Twenty-five had symptomatic gall stones, of whom 10 had additional common duct stones; none had a history of pancreatitis and all had either a normal pancreatogram or a normal pancreas at laparotomy. Eleven patients with a characteristic history were considered to be suffering from the irritable bowel syndrome after extensive investigations. Ten patients had non-pancreatic steatorrhoea due to coeliac disease (four), bacterial overgrowth due to blind loop (two), jejunal diverticulosis (one), small-bowel resection for Crohn's disease (one), radiation enteritis (one), and hypobetalipoproteinaemia (one).

In most patients with non-pancreatic disease and abnormal serum trypsin concentrations estimations of total serum amylase and pancreatic isoamylase activity (Phadebas)¹¹ were performed on the same serum samples. These results are given only for interest since the pancreatic isoamylase test has yet to be adequately evaluated in clinical practice.

Results

Serum concentrations of immunoreactive trypsin in the 46 healthy controls showed a log normal distribution and after logarithmic transformation showed a mean of 273 $\mu\text{g/l}$ and a 95% range of 117–637 $\mu\text{g/l}$ (mean ± 2 SD). Three hospital controls (convalescing after a stroke, breast cancer, or asthma) had concentrations outside this normal range, but serum total amylase and pancreatic isoamylase activities were normal.

FIG 1—Serum immunoreactive trypsin concentrations in controls and patients with pancreatic disease.

The 27 patients with chronic pancreatitis had serum trypsin concentrations ranging from zero to 1090 $\mu\text{g/l}$ (fig 1). Lundh tests were carried out in two of the six patients with raised concentrations and were grossly subnormal in both cases. Seven patients with documented pancreatic exocrine insufficiency had a low trypsin concentration. Among the 14 patients with chronic pancreatitis and normal concentrations, Lundh or oral pancreatic function tests were severely subnormal in all 10 patients tested.

Among the 17 patients with pancreatic cancer trypsin concentration was normal in six, raised in six, and low in five (fig 1). Of the 33 patients with chronic liver disease, 28 had a normal and four a raised concentration (fig 2). The diagnoses in those with raised concentrations were primary biliary cirrhosis, chronic active hepatitis, chronic lobular hepatitis, and cirrhosis, possibly of alcoholic aetiology. One patient with chronic active hepatitis had a subnormal trypsin concentration of 65 $\mu\text{g/l}$, even though a Lundh test, endoscopic retrograde pancreatography, and pancreatic ultrasonography all yielded normal results. The presence or absence of jaundice per se did not appear to influence the trypsin concentration.

FIG 2—Serum immunoreactive trypsin concentrations in liver disease, non-pancreatic painful abdominal conditions, and non-pancreatic steatorrhoea.

Fifty-six of the 61 patients with peptic ulcer had a normal trypsin concentration (fig 2). Four patients (two with gastric and two with duodenal ulcer) had a raised concentration but normal total amylase and pancreatic isoamylase activities. One patient with a gastric ulcer had a subnormal concentration (64 $\mu\text{g/l}$) but a normal pancreas at laparotomy.

Twenty-three of the 25 patients with Crohn's disease had normal serum trypsin concentrations (fig 2). Two had raised concentrations, one of whom also had raised total and pancreatic isoamylase activities, suggesting coexistent pancreatic disease.

Of the 19 patients with gastrointestinal cancer, 15 had a normal trypsin concentration (fig 2). Two patients with gastric cancer had a raised concentration, of whom one also had marginally raised total serum amylase activity, but the pancreas was normal at laparotomy in both cases. Two patients with gastric cancer had a subnormal trypsin concentration, and in one of these laparotomy showed pancreatic invasion.

Twenty-one of the 25 patients with gall stones had a normal trypsin concentration (fig 2). Three patients with common duct stones had a raised concentration but only one had raised total and pancreatic isoamylase activities. One patient with gall-bladder stones had a raised concentration but total and pancreatic isoamylase activities were normal. No patient with gall stones had a history of pancreatitis, and in all endoscopic retrograde pancreatography or laparotomy was normal.

All patients with the irritable bowel syndrome and non-pancreatic steatorrhoea had a normal serum trypsin concentration (fig 2).

Relation between serum trypsin concentration and pancreatic exocrine function.—Formal tests of pancreatic exocrine function were carried out in 19 of the 27 patients with chronic pancreatitis. The oral pancreatic function test was grossly abnormal in two patients, but the serum immunoreactive trypsin concentration was normal in both. In the remaining 17 patients a Lundh test was subnormal (mean tryptic activity $< 7 \mu\text{EqH}^+/\text{ml}/\text{min}$). Figure 3 shows the relation between serum trypsin concentrations and mean tryptic activity in these 17 patients; there was no significant correlation. Eleven patients had severe exocrine insufficiency (mean tryptic activity $< 2.5 \mu\text{EqH}^+/\text{ml}$).

min); of these, six had a low trypsin concentration, three a normal concentration, and two a high concentration. The remaining six patients all had normal trypsin concentrations despite appreciable exocrine insufficiency. Thus only 35% of patients with documented exocrine insufficiency had low trypsin concentrations. Furthermore, three out of nine patients with documented pancreatic steatorrhoea had a normal concentration (fig 3). A further two patients with severe

FIG 3—Serum immunoreactive trypsin concentrations and mean tryptic activity in 17 patients with chronic pancreatitis.

steatorrhoea plus either a subnormal result on oral pancreatic function testing or a grossly abnormal endoscopic retrograde pancreatogram had a normal concentration. Thus the trypsin concentration was abnormally low in only seven out of 11 patients with pancreatic steatorrhoea.

Discussion

These results do not support the use of serum immunoreactive trypsin concentration as a screening test for pancreatic disease. Our normal range for healthy controls (117–637 µg/l) is appreciably wider than ranges found in previous studies using the same radioimmunoassay,^{2,4,12} but only one study² investigated a comparable number of subjects. There are several possible reasons for this discrepancy. Most studies have assumed a normal distribution of serum immunoreactive trypsin concentration in healthy controls, though this study and one other¹³ have shown a log normal distribution. Two previous studies^{2,12} combined healthy and hospital controls to determine a normal range which may not be valid, since three of the 23 hospital controls in our study had concentrations outside the range calculated for healthy controls. All hospital controls studied by Koop *et al*¹² gave normal results to a secretin-pancreozymin test, which effectively excludes subjects with occult pancreatic disease but reflects a highly selected control group of doubtful clinical relevance.

When our data for healthy and hospital controls are pooled the calculated normal range for serum trypsin concentrations widens to 112–794 µg/l, but our conclusions would still pertain if previously published normal ranges were used. In subsequent discussion the “normal range” referred to is that for healthy controls—namely, 117–637 µg/l.

The results in patients with chronic pancreatitis were particularly disappointing; in all patients the disease had been diagnosed by standard criteria, and none were studied during a

relapse. Only 13 of the 27 patients with chronic pancreatitis (48%) had a serum trypsin concentration outside the normal range, of whom only seven (26%) had subnormal concentrations. Furthermore, only six of the 19 patients (32%) with exocrine insufficiency had a subnormal concentration. Previous studies have found that exocrine insufficiency is invariably associated with a low trypsin concentration,^{4,6} but a normal concentration has been reported.^{12,14} Ninety-five per cent of functioning pancreatic tissue has to be destroyed before steatorrhoea results,¹⁵ and it would seem reasonable to expect a low serum trypsin concentration in patients with pancreatic steatorrhoea if the concentration reflects pancreatic acinar cell mass.^{5,6} Our finding of a normal concentration in 36% of patients with pancreatic steatorrhoea, however, suggests that the serum trypsin concentration is unreliable as a screening test even in these extreme circumstances. Serum trypsin concentration will not distinguish cancer from chronic pancreatitis, nor is it of value in pancreatic cancer as nearly half of the patients with this disease had a normal concentration.

An abnormal serum trypsin concentration was found in 15% of patients with chronic liver disease. Since pancreatic disease frequently enters the differential diagnosis of patients with jaundice, measuring trypsin concentration was not helpful in these circumstances. Abnormal concentrations were found in 15 out of 141 patients (11%) with other gastrointestinal disease in which pancreatic disease may enter into the differential diagnosis. Although one of the three patients with a low concentration showed pancreatic invasion by gastric cancer and three of the 12 patients with raised concentrations had slightly raised amylase activities, there was no clinical evidence of pancreatic disease in any of these patients.

Gamble *et al*¹³ found a raised serum trypsin concentration in 7% of patients with acute viral infections and suggested that this was due to silent pancreatic involvement. This seems an unlikely explanation for the many raised concentrations that we found in various clearly defined clinical entities, and we suggest that serum trypsin concentration is not as specific to the pancreas as has been thought. An alternative explanation is that mild sub-clinical pancreatitis is extremely common in patients who are ill from a wide variety of complaints, although this seems less likely because most of the patients with a raised trypsin concentration had normal total and pancreatic isoamylase activities. Our findings indicate that raised trypsin concentration outside the clinical context of acute pancreatitis is a highly non-specific finding.

The importance of a low serum trypsin concentration is less easy to evaluate. In this study 18 patients had a low concentration but only 12 had pancreatic disease. If serum trypsin concentration is used as a screening test for pancreatic disease only a small proportion of patients screened will actually have pancreatic disease and the specificity of the test will drop even further. Serum trypsin concentration is also unlikely to be of value in the differential diagnosis of steatorrhoea because, although all 10 patients with non-pancreatic steatorrhoea had a normal concentration, 36% of patients with pancreatic steatorrhoea had a normal concentration. The low specificity and sensitivity of serum immunoreactive trypsin concentration does not justify its use as a screening test for pancreatic disease, and the concentration does not correlate with exocrine function in patients with documented chronic pancreatitis.

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Tamoxifen versus aminoglutethimide in advanced breast carcinoma: a randomised cross-over trial

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Abstract

Altogether 117 patients with advanced breast cancer were treated with either tamoxifen 10 mg by mouth twice daily or aminoglutethimide 250 mg by mouth four times daily with hydrocortisone 20 mg twice daily in a randomised cross-over trial in which patients who failed to respond to the first treatment or relapsed while receiving it were switched to the other. Eighteen (30%) out of 60 patients initially treated with tamoxifen achieved an objective response and 11 (18%) showed stable disease. Seventeen (30%) out of 57 patients treated initially with aminoglutethimide achieved an objective response and 13 (23%) achieved stable disease. Objective responses in bone metastases were achieved more commonly with aminoglutethimide (11 patients (35%)) than with tamoxifen (five (17%)). The predicted median duration of response for tamoxifen was 15 months and for aminoglutethimide over 15 months (no significant difference). Five (15%) out of 34 patients who failed to respond to tamoxifen and four out of six patients who relapsed after responding to tamoxifen subsequently responded to aminoglutethimide. In contrast, only two (6%) out of 31 patients who failed to respond to aminoglutethimide and none out of

four patients who relapsed while receiving aminoglutethimide subsequently responded to tamoxifen. The main side effects occurring in the 97 patients who received aminoglutethimide as first- or second-line treatment were lethargy and drowsiness (36 patients) and rash (29); seven patients had to stop treatment because of side effects. In contrast, side effects were rare and mild with tamoxifen and no patient had to stop treatment because of them.

Both tamoxifen and aminoglutethimide appear from this study to be equally effective in the medical endocrine treatment of advanced breast cancer.

Introduction

In the past few years traditional approaches to the endocrine management of patients with advanced breast cancer have been challenged by two important new forms of medical endocrine treatment. The first, tamoxifen, is an anti-oestrogen already in widespread clinical use. The second, aminoglutethimide, inhibits adrenal steroid synthesis and therefore may act as a "medical adrenalectomy," though it also has other actions including inhibition of the aromatase enzyme involved in the conversion of androgens to oestrogens in peripheral tissues.² At present aminoglutethimide is still a trial drug and is less widely used than tamoxifen, but both appear to be as effective as the more traditional forms of endocrine treatment in achieving and maintaining tumour regression.³⁻⁵ In addition both drugs are easily administered even to ill patients, and their effects are readily reversible if treatment proves ineffective.

So far no randomised comparative trial of these two agents has been reported, and thus their relative efficacies and the extent to which they show cross-resistance have not yet been properly established. We therefore carried out a randomised cross-over trial of tamoxifen and aminoglutethimide in 117 patients with advanced breast cancer. The trial was started in January 1979 and we report our first results here.

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