Serum isoamylase activities in diabetes mellitus

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SUMMARY Serum pancreatic isoamylase activities were used to assess exocrine pancreatic function in 39 patients with diabetes mellitus (21 on insulin, 12 on sulphonylureas, and six on biguanides or diet), and the results were compared with serum immunoreactive trypsin concentrations. Thirteen patients had decreased pancreatic isoamylase activity, the insulin-dependent diabetics showing the highest incidence of abnormality. This incidence of abnormality is similar to that previously described for serum immunoreactive trypsin, and the two procedures gave excellent overall correlation ($r = 0.9$). Our observations offer further evidence that pancreatic exocrine function is impaired in diabetes mellitus. Serum isoamylase determination provides a convenient, inexpensive, and rapid procedure for its detection.

Patients with diabetes mellitus have been shown by duodenal enzyme studies¹⁻⁵ and by serum immunoreactive trypsin (IRT) measurement⁶ to have impaired pancreatic exocrine function, the incidence being highest in insulin-dependent patients. Serum amylase activity normally derives from both the pancreas and the salivary glands, but the pancreatic isoenzyme is reduced when pancreatic exocrine function is deficient,⁷⁻⁹ and pancreatic isoamylase determination provides a convenient non-invasive test for such deficiency. We have, therefore, measured serum pancreatic isoamylase activity in diabetics and have compared the results with serum IRT levels.

Material and methods

Sera from 39 patients aged 24 to 77 years (19 men, 20 women) with diabetes mellitus were studied. They were classified on the basis of treatment: 21 on insulin, 12 on sulphonylureas, and six on diet or biguanide therapy. Occasional patients on sulphonylureas had also received biguanides but no subjects classified as on biguanides had received sulphonylureas. Reference values were obtained from 100 apparently healthy blood donors (50 men and 50 women).

Samples were separated on Beckman cellulose acetate membrane using the Beckman Microzone System (Beckman Instrument Inc, Fullerton, California, USA) in a discontinuous barbitone/

Tris-EDTA-borate buffer system; 0.25-2.5 μ l of sample was applied near the anode end of the strip, the volume of sample depending on total amylase activity. For samples with serum amylase activity of less than 200 U/l, 2.5 μ l of sample was applied. With samples of higher activity the volume applied was reduced by 0.5 μ l for each 100 U/l of amylase activity. Control specimens of acute pancreatitis serum and saline-diluted saliva were included in each run. Electrophoresis was carried out at 4°C at a constant voltage of 250 V for 1½ hours, after which the cellulose acetate membrane was layered on to the surface of a dyed starch substrate incorporated into an agar gel and incubated at 37°C.^{10 11} The time of incubation was adjusted to give adequate staining, as judged by visual inspection, isoamylases showing as sharp blue-staining bands. The membrane was allowed to air dry, and the isoamylases were quantitated by reflectance densitometry with a Corning densitometer model 720 (Corning Medical, Medfield, Mass, USA), which gave the proportion of the pancreatic and salivary isoamylase fractions as percentages of the total amylase activity.

Serum total amylase activity (upper reference limit 312 U/l at 37°C) was determined by the Phadebas method at 37°C, and the pancreatic and salivary isoamylase activities were calculated from the total activity multiplied by the pancreatic or salivary isoamylase percentage respectively.

Serum immunoreactive trypsin (reference range 140-390 μ g/l) was measured by radioimmunoassay using a commercial kit (Hoechst UK Ltd, Hounslow, Middlesex, UK).

Results

Isoamylase staining was demonstrated solely in the gamma globulin region. Clear separation of pancreatic (P) and salivary (S) isoamylase and their subfractions was obtained, pancreatic isoamylase showing greater mobility towards the cathode (Fig. 1). The analytical precision of the separation and quantitation procedure was determined from 10 runs of eight identical samples, and yielded an average within-batch coefficient of variation of 5% and between-batch coefficient of variation of 6% at a mean pancreatic isoamylase activity of 46 U/l at 37°C.

Reference values for total amylase, pancreatic, and salivary isoamylase in the blood donor controls, calculated after exclusion of three outliers, are given in Table 1. The total serum amylase, pancreatic, and salivary isoamylase activities each showed a normal distribution with no sex difference detected in the total serum amylase and salivary isoamylase. The reference values for the total serum amylase and salivary isoamylase ranged from 106 to 312 U/l and 13 to 177 U/l at 37°C with mean values of 209 U/l and 95 U/l, respectively. Mean pancreatic isoamylase (108 U/l) was significantly lower ($P < 0.02$) in men than in women (122 U/l). Serum pancreatic isoamylase of less than 54 U/l in men or 72 U/l in women

Table 1 Reference values for serum amylase, pancreatic, and salivary isoamylase activities (mean $\pm$ SD)

	All subjects (97)	Men (48)	Women (49)
Total serum amylase (U/l)	209 $\pm$ 52	205 $\pm$ 41	213 $\pm$ 61
Pancreatic isoamylase (U/l)	115 $\pm$ 27	108 $\pm$ 27*	122 $\pm$ 25*
Salivary isoamylase	95 $\pm$ 41	97 $\pm$ 57	93 $\pm$ 44

*Male cf female $P < 0.02$.

was considered subnormal and regarded as indicative of impaired pancreatic exocrine function.

In the diabetics (Table 2), no significant difference was seen in mean total serum amylase activity between the three treatment groups, and no subnormal values were observed, though three diabetics on insulin and three on sulphonylureas had increased total activity. Mean pancreatic isoamylase activity was significantly reduced ($P < 0.001$) in the insulin-dependent diabetics and in the female patients treated with sulphonylureas. Ten out of 21 patients on insulin, 2/12 on sulphonylureas, and 1/6 on diet had subnormal serum pancreatic isoamylase activity (Fig. 2). Overall, serum pancreatic isoamylase was subnormal in 13/39 patients (33%). For comparison, serum IRT levels were low in 9/21, 3/12, and 1/6 of the respective treatment groups and in 13/39

Fig. 1 Isoamylase patterns in normal serum (No. 5), salivary and pancreatitis serum markers (Nos 1 and 2), and patient sera are shown. Clear separation of pancreatic (P_2P_3) and salivary (S_1S_2) fractions is seen.

Table 2 Mean total and pancreatic isoamylase activities in diabetics and controls

	No.	Total amylase (U/l)	Pancreatic isoamylase (U/l)	
			Men	Women
Insulin	21	235	77*	80*
Sulphonylureas	12	271	123	72*
Diet/biguanides	6	236	125	105
Controls	97	209	108	122

*Patients of controls $P < 0.001$.

patients (33%) overall. Salivary isoamylase was increased in 5/21 patients on insulin, in 4/12 on sulphonylureas, and in 9/39 patients overall.

Serum pancreatic isoamylase activity and serum IRT levels showed excellent overall correlation, with a coefficient of correlation on 36 patients of 0.9 (Fig. 3). Three patients showed widely discrepant values (see Discussion), and these were excluded from this calculation.

Fig. 2 Serum pancreatic isoamylase activities in patients with diabetes mellitus and in normal controls. To allow for sex differences, results are expressed as multiples of the appropriate lower reference limit.

Discussion

Our results confirm that subnormal pancreatic isoamylase activity indicative of pancreatic exocrine insufficiency is common in diabetics. Serum pancreatic isoamylase activities were found to be lowest, and subnormal values were more prevalent in the insulin-dependent diabetics, intermediate in patients treated with sulphonylureas, and no different from the controls in those milder diabetics treated with

Fig. 3 Correlation between serum pancreatic isoamylase and immunoreactive trypsin (IRT) levels. The correlation coefficient is 0.9.

diet or biguanides. These observations are similar to those obtained with serum IRT in diabetics,⁶ and offer further evidence that the degree of pancreatic exocrine deficit in diabetes parallels the degree of impairment of endocrine function.

In our patients, the incidence of subnormal pancreatic isoamylase values was similar to that of reduced serum IRT levels. However, we regard isoamylase determination as preferable with regard to time and cost factors. The total assay time for isoamylase for eight samples is 3½ hours, increasing to 6 hours for 48 samples, but the corresponding actual bench times are 1-3 hours. In comparison, the determination time for serum IRT assay of 43 samples is 28 hours, the actual bench time being 3 hours. For the isoamylase determination the result can be obtained on the same day, whereas IRT results take two to three days. The reagent cost per test for isoamylase is also much lower than for IRT, currently one-sixth of the price.

Serum pancreatic isoamylase activity and serum IRT levels gave excellent overall correlation. Three patients, however, revealed discrepant results with extremely high serum IRT and normal isoamylase. The significance of these elevated serum IRT levels is unknown, though unexpectedly high serum IRT levels have been observed in cystic fibrosis.¹² It is possible that in some diabetics there occurs an excessive secretion of zymogen granules from the pancreatic acini, as in cystic fibrosis,¹³ and that the immunoreactive enzyme contained in these granules is not biologically active.

Two diabetics (one on insulin and one on sul-

phonylureas) showed reduced pancreatic isoamylase and an increase in salivary isoamylase. Seven other diabetics also had elevated salivary isoamylase activity, and in five this was sufficient to result in increased total serum amylase activity. The reason for the increase in salivary isoamylase is unknown. We are currently investigating whether salivary glands secrete amylase at an enhanced rate in diabetic patients.

In conclusion, serum isoamylase determination provides a rapid, inexpensive, and convenient non-invasive test for the investigation of pancreatic exocrine function and correlates well with measurement of serum IRT levels. Our high incidence of subnormal isoamylase activity in the serum of diabetics provides further evidence that pancreatic exocrine function is frequently impaired in diabetes mellitus and demonstrates that serum isoamylase determination is valuable for its detection.

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