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Serum immunoreactive trypsin in tropical pancreatic diabetes syndrome

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SUMMARY. Fifteen patients with tropical pancreatic diabetes syndrome (TPDS), 16 insulin-dependent diabetics (IDD), 27 non-insulin-dependent diabetics (NIDD) and 14 normal subjects, all from India, were investigated for markers of β-cell (C-peptide) and exocrine (immunoreactive trypsin; IRT) reserve. IRT and C-peptide concentrations were the lowest in TPDS, lower than normal in IDD, and not significantly different from normal in NIDDs. There was a highly significant correlation ($r_s = 0{\cdot}93$; $P < 0{\cdot}0001$) between IRT and C-peptide (measured in 50% of patients and controls) concentrations when all diabetic groups were combined. Such a correlation was absent when TPDS patients were considered in isolation, largely because of the markedly low IRT concentration. Fourteen of 15 patients (93%) with TPDS had subnormal IRT concentrations, of which 11 had IRT values of less than 50 μg/L. These IRT values are similar to those previously reported in cystic fibrosis. Only 6 of 16 IDDs (38%) had subnormal IRT concentrations, of which only one was below 50 μg/L. These data suggest that exocrine pancreatic reserve is markedly diminished in TPDS and that a subnormal IRT concentration may be a useful biochemical marker for this form of diabetes.

Tropical pancreatic diabetes syndrome (TPDS) is a cause of diabetes in a significant proportion of young diabetics in tropical countries.[1,2] The hallmark of TPDS is the presence of pancreatic calculi demonstrable on abdominal X-ray.[2] It has been suggested that subclinical chronic pancreatitis is fairly common in tropical countries, and that TPDS represents the final stage of evolution of this process. There have hitherto been no attempts to determine the prevalence of such pancreatitis in normal subjects or diabetics in tropical countries. This is to some extent due to the lack of a simple biochemical marker. Loss of β-cell reserve in TPDS is variable and is responsible for the marked heterogeneity in clinical presentation and course.[2] The relationship between exocrine and endocrine functions of the pancreas in TPDS is largely unknown.

We have previously demonstrated that the serum concentration of immunoreactive trypsin (IRT) is significantly diminished in diabetics.[3] This diminution is more marked in insulin-dependent diabetics (IDD) than in non-insulin-dependent diabetics (NIDD). We have made similar observations for serum pancreatic isoamylase (PIA) and lipase activity.[4–6] Exocrine pancreatic reserve has also been shown to be diminished by intraluminal tests of pancreatic function in IDD.[7] We have recently demonstrated that there is also a significant correlation between exocrine and endocrine pancreatic reserve as reflected in IRT and PIA concentrations on the one hand and C-peptide concentration on the other.[6] Related to this is the fact that the size of the pancreas has been found to be markedly diminished in IDD and moderately but significantly diminished in NIDD.[8]

We therefore undertook a study to investigate whether IRT concentration could be used as a marker for TPDS and to determine the relationship between pancreatic endocrine reserve, as indicated by fasting C-peptide concentrations, and IRT concentrations. The latter has been shown to be a useful marker for exocrine pancreatic damage/reserve in various clinical conditions including cystic fibrosis,[9] β thalassaemia and iron overload,[10] the very elderly,[11] the 'toxic' effect of large doses of steroids,[12] primary biliary cirrhosis[13] and Sjögren's syndrome.[14]

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TABLE 1. Clinical features of subjects studied. Values are shown as median and (range)

	Controls ($n=14$)	IDDs ($n=12$)	NIDDs ($n=27$)	TPDS ($n=15$)
Age (years)	34 (23–53)	23 (12–32)	42 (25–72)	22 (11–36)
Sex	11 M, 3 F	9 M, 7 F	19 M, 8 F	9 M, 6F
Body mass index (kg/m^2)	21·9 (15·4–28·9)	17·4 (13·3–20·8)	21·7 (16·5–34·6)	16·2 (11·2–24·2)
Duration of DM (years)	—	0·5 (0·5–3)	1 (0·5–16)	1·5 (0·5–7)

Patients and methods

Three types of Indian diabetic patients were investigated: IDD ($n=16$), NIDD ($n=27$) and patients with TPDS ($n=15$). The following criteria were used to classify diabetic subjects: IDDs were young subjects who had onset of diabetes before 30 years of age, were severely hyperglycaemic and symptomatic at diagnosis, with substantial weight loss. Some, but not all, were ketotic at presentation. None of these patients showed evidence of pancreatic calculi on abdominal X-ray. All of them required insulin treatment for symptomatic control and improvement in body weight. NIDDs were older (over 25 years; range 25 to 65 years) and could be controlled on diet with or without oral hypoglycaemic agents.

The diagnosis of TPDS was established by the following criteria: onset of diabetes during youth (usually in the second decade) usually associated with chronic abdominal pain and by the presence of pancreatic calcification on X-ray in the absence of previous history of alcoholism or gallstones.

The clinical features of patients with TPDS, IDD, NIDD and controls are summarised in Table 1. The control population comprised 14 normal Indian subjects (age range 23–55 years; 8 of the control subjects were aged less than 40 years) with no family history of diabetes or symptoms of pancreatitis and a fasting blood glucose concentration of less than 5 mmol/L. Data from IDDs and cystic fibrosis patients in the UK are included for comparison (Fig. 1 and Table 2). Fasting blood samples were obtained from these patients in Pune, India, centrifuged and the serum frozen at −20°C. These samples were transported in the frozen state to the Metabolic Unit at The Royal Free Hospital, London, where they were assayed for IRT and C-peptide without prior knowledge of the diagnosis of the patients from whom they were obtained.

IRT concentration was measured by a specific radioimmunoassay using a kit (Hoechst, Hounslow, UK), as described previously.[3] C-peptide concentration was also measured by a specific radioimmunoassay using a kit (Novo, Copenhagen, Denmark) following the method of Heding[15] as described previously. Fasting C-peptide concentrations were measured in 12 patients with TPDS, 7 patients with IDD, 7 patients with NIDD and 6 controls.

The results are expressed as medians and ranges. Statistical comparisons were carried out

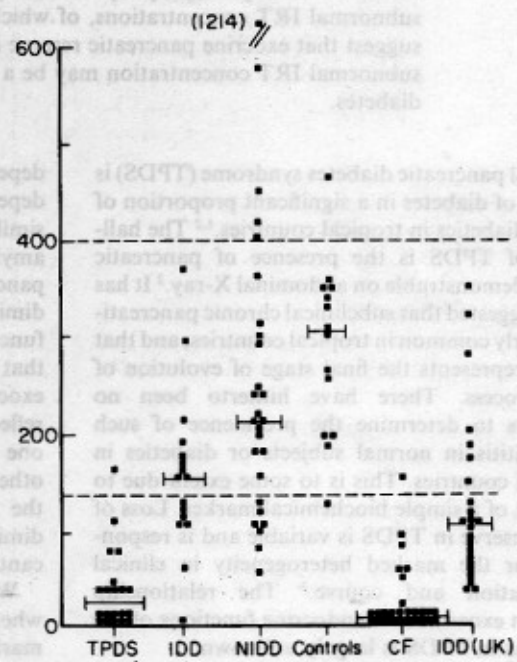

FIG. 1. Serum IRT concentrations in TPDS, Indian IDD and Indian NIDD patients, and Indian controls. IRT values for Caucasian patients with CF and IDD have been added for comparison. Amongst the diabetic groups, IRTs were the lowest in the TPDS patients and were similar to those in CF patients. Horizontal bars show medians in each group. Broken horizontal lines indicate the upper and lower limits of the reference range in normal subjects in the UK.

TABLE 2. IRT concentrations, shown as medians and (ranges) and the frequency of abnormal IRT concentrations in various patient groups

	n	IRT (μg/L)	Subnormal IRT			Supranormal IRT
			< 140 μg/L	< 50 μg/L	Non-detectable	> 400 μg/L
TPDS	15	0 (0–160)	14 (93%)	11 (73%)	10 (67%)	0 (0%)
IDD (Indian	16	155 (0–385)	6 (38%)	1 (0%)	0 (0%)	0 (0%)
NIDD (Indian)	27	231 (51–1214)	7 (26%)	0 (0%)	0 (0%)	5 (19%)
Controls (Indian)	12	305 (136–465)	1 (8%)	0 (0%)	0 (0%)	1 (8%)
CF (UK)	27	0 (0–156)	26 (97%)	22 (81%)	20 (74%)	0 (0%)
IDD (UK)	23	102 (46–350)	16 (70%)	4 (18%)	0 (0%)	0 (0%)

using Mann-Whitney U test for non-parametric data. Correlations were analysed by Spearman's rank sum correlation (r_s). Multiple regression analysis was also carried out to determine whether the correlation of a C-peptide with IRT was independent of other factors like age, body mass index and the duration of diabetes.

Results

IRT concentrations (Fig. 1) were lowest in TPDS patients and were significantly lower than those in IDDs. IRT concentrations in IDDs in turn were significantly lower than those in NIDDs and controls. IRTs in NIDDs were lower than those in controls, but this difference was not significant (see Fig. 1). Fourteen of 15 TPDS patients (93%) had subnormal IRT, with the concentration being less than 50 μg/L in 11 of these. Six of 16 IDDs (38%) had subnormal IRT concentrations, of which only one was below 50 μg/L. Seven of 27 NIDDs had subnormal IRT concentrations, none of which was less than 50 μg/L. Five of 27 NIDDs (19%) had supranormal IRT values. None of the IDDs or TPDS subjects had supranormal IRT concentrations. IRT concentrations in patients with TPDS were similar to those in patients with cystic fibrosis from the UK over the age of 12 years. IRT concentrations in Indian controls over the age of 40 years ($n=6$) were similar to those in younger patients ($n=8$). IRT values in Indian controls were similar to those in controls in the UK.[3] Our previous data and those contained in this paper show that in controls there is no variation in IRT concentrations between the ages of 10 and 65 years.[3,6,11] Only one patient with NIDD in this series was more than 65 years old.

C-peptide concentrations in IDDs were significantly lower than those in controls and NIDDs. There was no significant difference between C-peptide concentrations in NIDDs and controls; those in TPDS patients were the lowest. C-peptide concentrations in IDD and NIDD patients were comparable to those found in Caucasian patients in the UK. Thus, the clinical diagnosis of IDD and NIDD in Indian patients was validated by biochemical criteria.

There was a highly significant correlation between C-peptide and IRT concentration when all investigative groups were combined ($r_s=0{\cdot}93$; $P<0{\cdot}0001$; Fig. 2). The correlation was also

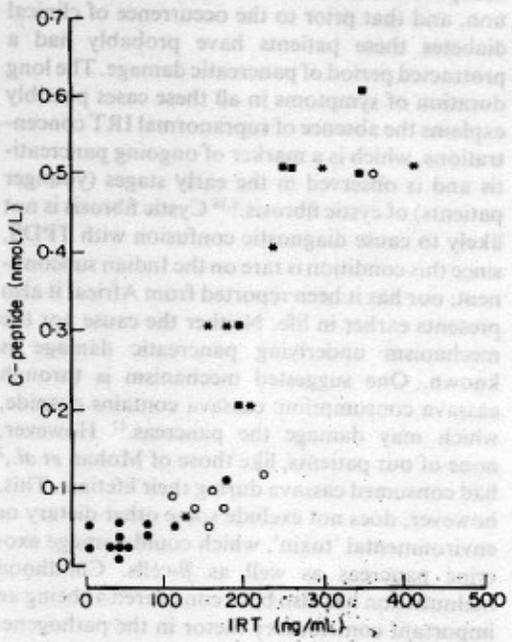

FIG. 2. The correlation of fasting C-peptide and IRT concentrations in serum. There were highly significant correlations between IRT and C-peptide in all patients and controls ($r=0{\cdot}90$; $P<0{\cdot}0001$); all diabetic patients ($r=0{\cdot}93$; $P<0{\cdot}0001$), TPDS and IDD ($r=0{\cdot}78$; $P<0{\cdot}001$). There was no significant correlation between IRT and C-peptide in TPDS (● TPDS; ○ IDD; • NIDD; ■ controls).

observed when TPDS and IDD were examined in isolation from the other two groups ($r_s = 0.78$, $P < 0.001$), but not when TPDS was considered by itself. This was largely due to the fact that IRT concentrations in TPDS were extremely low.

Multiple linear regression analysis revealed a highly significant correlation between C-peptide and IRT concentrations independently of age, body mass index, and the duration and type of diabetes.

Discussion

Our data show that both endocrine reserve, as reflected in C-peptide concentrations, and exocrine reserve, as reflected in IRT concentrations, are markedly diminished in TPDS patients. IRT concentrations in this group are the lowest amongst various groups of diabetics investigated so far. Since 14 of 15 patients with TPDS had subnormal IRTs, and 11 of 15 had markedly low IRT concentrations (< 50 μg/L), the latter can be used as a marker for this condition in young diabetic patients who have pancreatic calcification.

Our observations are consistent with the fact that patients with TPD have pancreatic calcification, and that prior to the occurrence of clinical diabetes these patients have probably had a protracted period of pancreatic damage. The long duration of symptoms in all these cases possibly explains the absence of supranormal IRT concentrations, which is a marker of ongoing pancreatitis and is observed in the early stages (younger patients) of cystic fibrosis.[9,16] Cystic fibrosis is not likely to cause diagnostic confusion with TPDS, since this condition is rare on the Indian subcontinent; nor has it been reported from Africa; it also presents earlier in life. Neither the cause nor the mechanism underlying pancreatic damage is known. One suggested mechanism is through cassava consumption: cassava contains cyanide, which may damage the pancreas.[17] However, none of our patients, like those of Mohan *et al.*,[2] had consumed cassava during their lifetime. This, however, does not exclude some other dietary or environmental 'toxin', which could damage exocrine pancreas as well as β-cells. Childhood malnutrition has also been considered as being an important contributory factor in the pathogenesis of TPDS. It is relevant, therefore, that our preliminary studies with IRT in patients with malnutrition due to anorexia nervosa show abnormal IRT concentrations (unpublished observations).

We have previously demonstrated that in diabetics in the UK, both endocrine and exocrine reserve are the lowest amongst IDDs, intermediate amongst NIDDs controlled with sulphonylureas, and comparable to control values in diet- and biguanide-controlled diabetics.[3,6] However, such a correlation was not evident in TPDS patients, largely because of the extremely low IRT and C-peptide concentrations. This feature isolated them from the rest of the diabetic patients (Fig. 2). Thus, the degree of diminution in exocrine pancreatic function in TPDS is greater than that in IDD and is similar to that observed in some patients with cystic fibrosis.[9] The highly significant correlation between C-peptide and IRT concentrations confirms our previous data on the existence of such a correlation in patients with diabetes mellitus[6] and suggests that the loss of β-cell reserve and exocrine pancreatic function is concomitant.

We were surprised by the presence of supranormal IRT concentrations in some NIDD patients. We have previously not found elevated IRTs in NIDDs. Since supranormal IRTs indicate active pancreatic damage, it is possible that they reflect an ongoing pancreatitis in some NIDD patients in our series. If indeed exposure to an environmental 'toxin' is the cause of pancreatic damage in TPDS, it is possible that this toxin may be responsible for exocrine pancreatic damage in NIDDs in the Indian setting. On the other hand, it is possible that NIDD patients with increased IRT concentrations represent a milder variant of TPDS. The occurrence of supranormal IRT concentrations in NIDDs also argues against the exocrine depletion and the consequent loss of 'trophic' effect of insulin on exocrine pancreatic tissue.

In conclusion, our data suggest that TPDS is associated with a marked loss of β-cell and pancreatic acinar cell reserve. IRT concentration and other newer tests of exocrine pancreatic function (e.g. bentorimide test) may be used as an additional marker for this form of diabetes.

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