Journal of Clinical Pathology, 1979, **32**, 897-901

Serum immunoreactive trypsin concentrations in infectious and non-infectious illnesses and in juvenile diabetes

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SUMMARY Serum immunoreactive trypsin (SIT) concentrations were measured in 244 patients with infectious illnesses and in 281 children with diabetes of recent onset. Results were compared with reference ranges established in 107 patients with non-infectious, non-diabetic illnesses, in whom SIT concentrations were found to increase with advancing age.

Reduced or undetectable concentrations of SIT were associated with diabetes in children and with a few cases of severe childhood infection. Increased SIT concentrations were associated with virologically confirmed cases of infection with mumps and Coxsackie B virus infection, and with clinical diagnoses of mumps, PUO, and meningitis in children, and with Bornholm disease, cardiac infection, and respiratory infection in adults. It is suggested that silent invasion of the exocrine pancreas with elevation of the SIT concentration may accompany infection by Coxsackie B, mumps, and, possibly, other viruses.

Trypsin, or more properly its inactive precursor trypsinogen, is, in contrast to other pancreatic enzymes, produced solely by the pancreas. It has therefore been suggested that a recently developed radioimmunoassay for measuring its concentration in serum might provide a specific test of pancreatic exocrine functions (Elias *et al.*, 1977). However, non-pancreatic factors such as hormones, antibodies, and the rate or extent of its reabsorption from the gut may also affect serum immunoreactive trypsin (SIT) concentrations, and a full understanding of these interactions will require new experimental data.

Published studies suggest that the SIT concentration may be high in cases of acute pancreatitis or chronic renal failure (Temler and Felber, 1976; Elias *et al.*, 1977) and high, normal, or low in cases of chronic pancreatitis and pancreatic carcinoma, depending perhaps on the stage of the disease (Bambule-Dick *et al.*, 1978; Adrian *et al.*, 1979; Minaire *et al.*, 1979). In diabetes, low levels have been reported in patients treated with insulin or sulphonylureas but not in those treated with biguanides (Dandona *et al.*, 1978). In the present study we have assayed SIT concentrations in three groups of subjects: patients with non-infectious illnesses, patients with a variety of infections, and patients with juvenile diabetes.

Received for publication 13 February 1979

Patients and methods

All specimens tested were surplus from samples submitted for microbiological investigation. Almost all were sent through the post at ambient temperature as unfrozen whole blood or serum, and bacterial contamination was sometimes present. Sera were stored at $-20°C$ on receipt but were thawed and refrozen a number of times for a variety of other tests.

Preliminary results showed that SIT levels were often raised in patients with certain infectious illnesses and depressed in diabetics. Specimens from normal children were not obtainable, so a 'control' group was assembled from 107 patients comprising 55 children aged 0-14 years and 52 adults with illnesses unlikely to be due to infection, diabetes, or other pancreatic disease. The diagnoses in these 'control' patients were: psychiatric illness (46) and ophthalmological (14), arthritic (13), neurological (7), gastrointestinal (7), and other (20) diseases.

Altogether 244 patients with putative infectious illnesses were tested. These comprised 132 children

and 112 adults, selected to include a range of clinical diagnoses (Table 1), but particularly those associated with infectious agents known to cause pancreatitis (mumps virus, Coxsackie B virus, and *Mycoplasma pneumoniae*). In 63 of these patients a specific infectious agent was implicated by microbiological tests (Table 2).

The diabetes group comprised 281 children notified to the British Diabetic Association Register of Newly Diagnosed Diabetic Children (Bloom *et al.*, 1975). The duration of diabetic symptoms at the time of blood collection was up to eight years, but was less than one year in 260 (92%).

SIT concentrations were measured by radioimmunoassay using a double antibody technique (Elias *et al.*, 1977). Highly purified human trypsin was used as immunogen and for radioiodination. Antibodies were produced in rabbits. Radioiodination was performed by the chloramine-T method using ^{125}I-labelled sodium iodide. Standards used in the assay were made from a semipurified preparation of human trypsin, and they ranged from

Table 1 *Serum immunoreactive trypsin levels by clinical diagnosis*

Diagnosis	*Number tested*	*Patients outside 'reference' ranges (see text)*					
		Serum trypsin	*Age (yr)*	*Above normal*	*Significance**	*Below normal*	*Significance**
Lymphadenopathy	31	—	—	0/31	NS	0/31	NS
Pyrexia of uncertain origin	24	760	6	5/24	<0·0005	1/24	NS
		460	5				
		400	7				
		390	13				
		360	1				
		70	36				
Lower respiratory infection	21	790	16	2/21	<0·05	2/21	NS
		470	42				
		0	11				
		0	2				
Upper respiratory infection	31	510	53	1/31	NS	5/31	0·025
		70	7				
		60	13				
		60	10				
		50	2				
		20	12				
Meningitis	23	420	7	1/23	NS	4/23	<0·025
		60	6				
		50	4				
		40	11				
Bornholm disease	13	820	11	3/13	<0·0005	0/13	NS
		820	25				
		480	50				
Myocarditis or pericarditis	33	960	20	3/33	<0·025	3/33	NS
		490	16				
		440	13				
		70	31				
		70	55				
		40	50				
Other infections:	68						
Rash		1250	11	3/68	NS	4/68	NS
Mumps		420	6				
Mumps		380	7				
Septic arthritis		70	10				
Convulsions		50	6				
Rash		15	7				
Encephalitis		0	4				
Infections—total	244			18/244	<0·01	19/244	NS
Insulin dependent diabetes	281			2/281	NS	110/281	<0·0001
Non-infectious illness in non-diabetic patients	107			0/107		3/107	

*Significance estimates: Chi square test of comparison with non-infection, non-diabetic group.
NS = not significant at 0·05 probability level.

Table 2 *Serum immunoreactive trypsin levels by virological diagnosis**

Virus	*Number tested*	*Patients outside 'reference' ranges (see text)*							
		Serum trypsin	*Age (yr)*	*Clinical diagnosis*	*Virus type*	*Above normal*	*Significance†*	*Below normal*	*Significance†*
Coxsackie B virus	18	960	20	Myocarditis and encephalitis	B4	4/18	<0·0005	2/18	NS
		820	11	Bornholm	B2				
		820	25	Bornholm	B3				
		480	50	Bornholm	B4				
		70	31	Pericarditis	B1				
		70	36	Neuralgic pain	B4				
Mumps	17	420	6	Malaise		3/17	<0·0005	2/17	NS
		380	7	Mumps					
		420	7	Meningitis					
		50	4	Meningitis					
		60	6	Meningitis					
Mycoplasma pneumoniae	13	790	16	Pneumonia		1/13	NS	2/13	NS
		70	7	Asthma					
		0	11	Pneumonia					
Influenza A	9	40	11	Meningitis		0/9	NS	2/9	NS
		60	10	Mumps					
Respiratory syncytial virus	2	0	2	Pneumonia		0/2	NS	1/2	NS
Other:	4	—	—	—		0/4	NS	0/4	NS
Measles 2									
CMV 2									
Adenovirus 1									

*Virological diagnostic criteria—see methods.
†Significance estimates—Chi square test of comparison with non-diabetic, non-infection group (see Table 1).
NS = not significant at 0·05 probability level.

80 to 1280 μg/l. Kits for the assays were supplied by Behring Institute, Hoechst UK Ltd. All samples were assayed in duplicate. Within-assay coefficient of variation was 6·6% for a low concentration control serum and 7·9% for a high concentration control serum. Between-assay coefficient of variation was 14% for a low concentration control serum and 15% for a high concentration control serum.

Tests for viral and mycoplasmal antibodies employed conventional virological techniques. Coxsackie B virus antibodies were assayed by neutralisation test in tissue culture, and other antibodies by complement fixation tests. A virological diagnosis of recent infection was based on any of the following criteria: the isolation of a virus, or a fourfold or greater rise in antibody titre in consecutive samples of serum, or the attainment of a minimum titre in a single specimen of serum. These minimum titres were: Coxsackie B virus 1:1024, cytomegalovirus 1:512, mumps 'V' 1:256, mumps 'S' 1:40, others 1:256.

Results

In the non-infection, non-diabetic 'control' group, SIT levels ranged from 70 to 440 μg/l and showed an approximately log-normal distribution (Fig. 1). Levels were clearly lower in children (geometric mean 167 μg/l) than in adults (geometric mean 213 μg/l); there was no apparent age effect within these two subgroups but numbers were small. Geometric mean concentrations were slightly higher in males (186 μg/l) than females (166 μg/l) but the difference was not significant. Using these data, provisional reference ranges of ± 2 standard deviations were established for children (79-340 μg/l) and adults (98-460 μg/l) (Fig. 1), and these were used for the evaluation of results from other patients.

In virologically identified infections (Table 2) high SIT levels were associated with mumps virus and Coxsackie B virus infection, and although pancreatitis was not diagnosed clinically in any of these cases, the results suggest that subclinical pancreatitis may sometimes accompany these infections. In patients classified by clinical diagnosis, high SIT concentrations were associated with pyrexia of uncertain origin (PUO), lower respiratory infection, Bornholm disease, and myocarditis or pericarditis (Table 1). All three patients with Bornholm disease and one of those with myocarditis had evidence of recent Coxsackie B virus infection. Apart from one

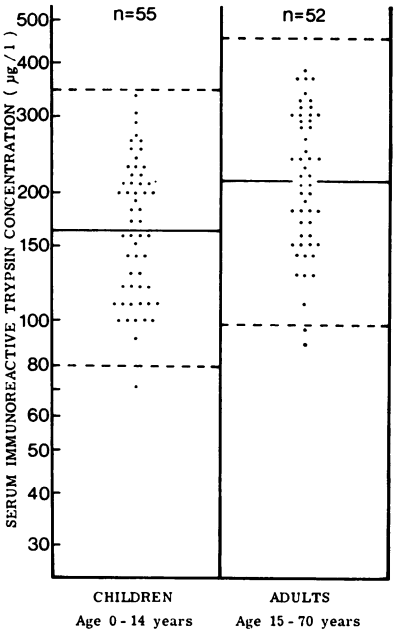

Fig. 1 *Serum immunoreactive trypsin concentrations in patients with non-infective, non-diabetic illnesses by age group. Horizontal lines show geometric mean concentrations ± 2 SD. The mean concentrations in children and adults differ significantly (P<0·005* (t *test)).*

case of lower respiratory infection due to *M. pneumoniae* a microbiological basis was not established for the other clinical diagnoses associated with high SIT concentration. In children, high SIT concentrations were most frequently associated with mumps, PUO, or meningitis, while in adults they were mainly associated with Bornholm disease, cardiac infection, or respiratory infection.

Low levels of SIT were most strikingly evident in diabetic children, of whom 110/281 (39%) were subnormal and 32/281 (12%) had no detectable SIT by the assay used. Low levels of SIT were more frequent in older children and in those with diabetes of longer duration. This group is being studied in greater detail, and the results will be reported elsewhere.

Low SIT levels were also found in a few non-diabetic patients, and an association with upper respiratory infection and meningitis was of marginal statistical significance ($P < 0{\cdot}025$). An absence of detectable SIT was found in only three non-diabetic patients: in two children with pneumonia and one with encephalitis.

SIT concentration is clearly altered in a number of infectious diseases, and since infections are common in all age groups, and particularly in children, it is difficult to identify a population in which to establish normal values in relation to age. The numbers available for the compilation of Fig. 1 were insufficient for detailed analysis by age. A further analysis was, therefore, undertaken using data from all the patients tested with the exception of those with diabetes and infected patients with SIT levels outside the provisional reference range. The result (Fig. 2) suggests that the SIT concentration changes little throughout childhood, rises sharply to the adult level at about age 15 to 19, and then increases gradually during adult life, particularly after the age of 40 years.

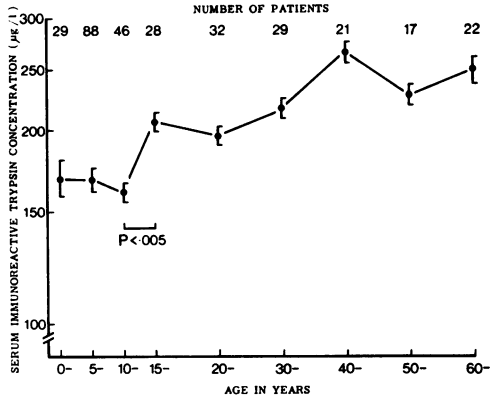

Fig. 2 *Estimated 'normal' (see text) serum immunoreactive trypsin concentrations (geometric mean ± SEM) by age group. The only significant difference between adjacent points was between those aged 10-14 years and those aged 15-19 years (P < 0·005).*

Discussion

Other investigators have assumed that SIT concentrations in healthy subjects follow a normal distribution with a mean of 272 μg/l and a normal range of 138-406 μg/l (Elias *et al.*, 1977). Our results in adults suggest a log-normal distribution with a lower mean (213 μg/l) and a wider range (98-460 μg/l). The reason for this discrepancy is not clear, but the increasing concentration with age (Figs 1 and 2) suggests that age differences may be a factor. The effects of the less than ideal conditions of transmission and storage of specimens in our study may also have affected our results.

Hypertrypsinaemia has been reported in cases of pancreatitis and chronic renal failure (Temler and Felber, 1976; Elias *et al.*, 1977). None of the patients in the present study had renal failure, and although pancreatitis was not suspected clinically, it remains the most likely cause of the high SIT levels observed.

This conclusion is supported by the strong association found between increased SIT concentration and infections with viruses known to be pancreatropic. Acute pancreatitis is a rare but well recognised complication of Coxsackie virus (Murphy and Simmul, 1964), mumps virus (Wesselhoeft, 1941), and *M. pneumonia* (Leinikki *et al.*, 1973) infections, and it is of interest that our results suggest that subclinical pancreatitis may not infrequently accompany these infections. A similar conclusion was reached by (Nakao 1971), who reported a transient depression of amylase and lipase concentrations in the duodenal fluid of 14/22 (64%) cases of mumps, and high levels of serum or urinary amylase in about 30% of 87 patients infected with Coxsackie B3 or A9 viruses. In view of the recent interest in the possible role of viruses in the aetiology of diabetes, it is of some interest that several seem to produce a silent invasion of the exocrine pancreas.

Apart from the diabetic children, no clear pattern in the distribution of low SIT concentrations emerged. In most cases levels were only just below the reference range and of uncertain significance, but in two children with lower respiratory tract infection and one with encephalitis SIT was undetectable. Low levels of SIT have been reported in patients with chronic pancreatitis or pancreatic carcinoma (Bambule-Dick *et al.*, 1978; Adrian *et al.*, 1979; Minaire *et al.*, 1979) and are presumed to be due to extensive and irreversible destruction of pancreatic acinar tissue. In children in the UK, severe exocrine pancreatic damage is rare and is usually associated with fibrocystic disease, in which a predisposition to infection is usual; it is possible therefore that, in children with low SIT concentrations, infection may be the result rather than the cause of pancreatic dysfunction. This explanation seems unlikely in the children concerned since advanced pancreatic disease was not suspected, and three cases would represent a surprisingly high incidence among the 132 children tested. More probably, the absence of detectable SIT was a result of their infectious illnesses and, since we found no increase in prevalence of low SIT concentrations with advancing age, it must be assumed that it reflects a transient phenomenon. The assay procedure used in this study detects both inhibited and uninhibited trypsin as well as its biologically inactive precursor, trypsinogen. Consequently, the absence of SIT presumably reflects a transient suppression of enzyme synthesis, secretion, or reabsorption from the gut. The effect of fasting on the SIT concentration has not been studied but a decrease might be expected, and in severe infections such as pneumonia or encephalitis the food intake may be very low. Hence reduced pancreatic secretion would provide an obvious explanation for the low SIT concentrations in some severe infections, but further studies will be necessary to elucidate this problem.

We are grateful to Dr M. Redshaw, of Behring Institute, Hoechst UK, for the gift of the RIA-ghost Trypsin Radioimmunoassay Kits, and to Mr S. Archibald, who prepared the illustrations. The support of the British Diabetic Association is also gratefully acknowledged.

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