Abnormal pancreolauryl tests in coeliac disease: lack of correlation with the degree of intestinal mucosal damage

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Abstract

Aims—To determine the frequency of abnormal pancreolauryl tests in untreated and treated adults with coeliac disease and to see whether abnormalities in treated coeliac patients correlate with the degree of recovery of intestinal morphology or brush border enzyme activity.

Methods—Pancreolauryl tests were performed in a study population of 57 adult coeliac patients (25 on gluten containing diets and 32 on gluten free diets), 59 symptomatic controls, and eight patients with pancreatic disease. Brush border enzyme activity and morphological assessment were performed on small intestinal biopsies in 27 of the treated coeliac patients.

Results—Forty per cent of untreated coeliac patients and 18% of treated coeliac patients had abnormal tests. In treated coeliac patients, no significant correlation was detected between the pancreolauryl test result and either brush border enzyme activity or morphological parameters.

Conclusion—Abnormal pancreolauryl test results are common in untreated and treated adult coeliac disease patients. Abnormalities in treated coeliac patients do not correlate with the degree of recovery of small intestinal morphology or brush border enzymes.

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Some individuals with treated coeliac disease have co-existent exocrine pancreatic insufficiency.¹⁻⁶ Only a small proportion of these patients continue to have steatorrhoea when on a strict gluten free diet and persistent severe exocrine pancreatic dysfunction has been demonstrated in these individuals.¹⁻⁴ The majority of cases are thought to result from a disturbance of the afferent limb of the enteropancreatic axis secondary to incomplete histological and biochemical recovery of the small intestinal mucosa.^{5,6} Suboptimal pancreatic function may be clinically significant because it potentiates malabsorption of nutrients as a result of small intestinal mucosal damage.

We have studied the prevalence and severity of dysfunction of the enteropancreatic axis and its relation to parameters of small mucosal his-

tology and brush border enzyme activity in adults with untreated and treated coeliac disease. We have used the pancreolauryl test that examines the ratio of 10 hour urinary excretion of fluorescein following the ingestion of fluorescein dilaurate (test) or fluorescein sodium (control).⁷ Fluorescein is cleaved from the dilaurate complex by a cholesterol ester hydrolase produced by the pancreas, the secretion of which reflects the production of digestive enzymes. Because the fluorescein dilaurate/sodium is taken with food, the test assesses physiological stimulation of the pancreas.

Materials and methods

The coeliac study population consisted of 57 patients (male:female ratio, 15:42; mean age, 48.9 years; range, 16-78 years). At the time of diagnosis, the small bowel biopsies of all these patients showed subtotal or total villus atrophy with crypt hypertrophy and an intense infiltrate of chronic inflammatory cells in the lamina propria and the epithelial cell layer compatible with untreated coeliac disease (type 3).⁸ In all patients, clinical remission or amelioration of some symptoms followed the exclusion of gluten from the diet. Architectural improvement, as measured by increased villus height and decreased inflammatory cell infiltrate, has been demonstrated in 22 of 27 patients in whom a repeat small intestinal biopsy has been performed. Twenty five of the coeliac patients (male:female ratio, 5:20; mean (SD) age, 47.6 (16.4)) were studied while on normal gluten containing diets. Thirty two treated coeliac patients (male:female ratio, 10:22; mean (SD) age, 49.2 (16.1)) had been on gluten free diets for between six months and 18 years.

Pancreolauryl test results were available from two groups of non-coeliac individuals. Firstly, a group of 59 symptomatic controls who presented with diarrhoea and/or steatorrhoea (male:female ratio, 31:28; mean (SD) age, 39.0 (8.4)) and in whom coeliac disease and/or pancreatic disease was excluded; the final diagnoses included irritable bowel disease, amyloidosis, intestinal tuberculosis, and diabetic diarrhoea. Inflammatory bowel disease was excluded in most control patients. Fifty seven of these subjects had tall villi (type 0)⁸ on small intestinal biopsy, precluding a diagnosis of covert coeliac disease. The remaining two patients, with final diagnoses of intestinal amyloidosis and tuberculosis, had intestinal

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Figure 1 T:K ratio in patients with untreated coeliac disease (UCD), patients with treated coeliac disease (TCD), symptomatic controls (SC), and patients with pancreatic disease (PD).

biopsies showing partial villus atrophy (type 2) and subtotal villus atrophy (type 3), respectively.⁸ The second group consisted of eight patients (male:female ratio, 4:4; mean (SD) age, 48.8 (11.4)) in whom pancreatic disease was previously or eventually confirmed by surgery, x ray, or response to pancreatic supplements. Normal villi were found on biopsy of the small intestine and the diagnoses included chronic calcific pancreatitis secondary to alcohol or possible viral infections and carcinoma of the head of the pancreas.

At the time of study the patients were taking neither pancreatic enzyme supplements nor any drug known to interfere with the assay of fluorescein and no patient had previously undergone upper intestinal, biliary, or pancreatic surgery.⁹ The excretion volume and/or urinary creatinine output indicated a complete urinary collection in all subjects.

PANCREOLAURYL TEST

The manufacturer's protocol⁷ was modified to incorporate a washout day between the test day (T) and the control day (K) and aliquots (0.5 ml) of urine were incubated with 4.5 ml of 0.1 N sodium hydroxide for 15 minutes at 80°C. A T:K ratio < 20% was considered to be indicative of exocrine pancreatic deficiency, > 30% was considered to be normal, and between 20% and 30% was thought to be equivocal.⁹

Morphological and biochemical data were examined from 27 of the treated coeliac patients who had a further small intestinal biopsy either contemporaneous with the pancreolauryl test or, in the case of patients who had been maintained on a gluten free diet for many years, a biopsy within one year of the pancreolauryl test. The biopsies were obtained with suction capsules from the distal duodenum/jejunum or by pinch biopsies from the descending duodenum at endoscopy. Half of the suction biopsy or three endoscopic biop-

Table 1 Matrix of pairwise comparison probabilities of T:K ratio results

	UCD	TCD	SC	PD
UCD	1.00			
TCD	0.2	1.00		
SC	< 0.004	0.3	1.00	
PD	< 0.03	< 0.001	< 0.001	1.00

p values derived by χ^2 analysis.

UCD, untreated coeliac disease; TCD, treated coeliac disease; SC, symptomatic control; PD, pancreatic disease.

sies were examined with a dissecting microscope and processed for routine histology. Coded 5 μ m sections were examined, graded,⁸ and intraepithelial lymphocytes were counted on the sides of well orientated villi or the luminal surface of biopsies with partial villous atrophy.¹⁰ The remainder of the suction biopsy or two endoscopic biopsies were wrapped in Parafilm and frozen at -20°C until the brush border enzymes—alkaline phosphatase, lactase, and sucrase—were assayed.¹¹ As the activity of the brush border disaccharidases and alkaline phosphatase rises rapidly in an aboral direction,¹² each individual result has been designated low or normal for the appropriate site, because this is more meaningful than analysis of numerical results.

Statistical analyses were performed with the Systat statistical software package (Systat Inc, Evanston, Illinois, USA). One-way analysis of variance with Tukey HSD post hoc test of pairwise mean differences,¹³ the Kruskal-Wallis test, and regression analysis were used for continuous variables and the χ^2 statistic was used for discrete variables. A p value of < 0.05 was considered to be statistically significant.

This project had been approved by the Hospital Ethics Committee and informed consent was obtained from each patient before study.

Results

The T:K ratios of the untreated coeliac patients, treated coeliac patients, symptomatic controls, and pancreatic disease patients are shown in fig 1. Ten untreated coeliac patients (40%), six treated coeliac patients (18%), five symptomatic controls (8.5%), and seven pancreatic disease patients (87.5%) had T:K ratios that were below 20% and therefore compatible with exocrine pancreatic deficiency. Fourteen untreated coeliac patients (56%), 21 treated coeliac patients (65%), and 45 (78%) symptomatic controls had normal tests but none of the subjects with pancreatic disease had a T:K ratio > 30%. One untreated coeliac disease patient, five treated coeliac disease patients, eight symptomatic controls, and one pancreatic disease patient had equivocal test results.

Multiple comparison testing revealed a significant difference between the four groups, $F = 10.975$; $p = < 0.05$. The T:K ratio of the untreated coeliacs (mean (SD), 30.5% (16.6); 95% confidence interval (CI) 23.6–37.3) was significantly lower than the symptomatic controls (mean (SD), 46.5% (20.7); 95% CI 43.7–53.9; $p = 0.004$), whereas the T:K ratio of the treated coeliac patients (mean (SD) 40.8% (20.6); 95% CI 33.3–48.2) did not vary significantly from either the untreated coeliac

Figure 2 Percentage excretion of administered fluorescein on test day in patients with untreated coeliac disease (UCD), patients with treated coeliac disease (TCD), symptomatic controls (SC), and patients with pancreatic disease (PD).

or symptomatic control groups, $p = 0.2$ and 0.54 , respectively. The T:K ratio of the pancreatic disease group (mean (SD), 9.1% (7.6); 95% CI 2.5 – 15.6) was significantly lower than in all other groups (untreated coeliac group, $p < 0.04$; treated coeliac disease group, $p < 0.001$; symptomatic controls, $p < 0.001$). If only those subjects in whom the T:K ratio is normal or abnormal are considered (that is, excluding those with a T:K ratio of 20 – 30%), when all four groups are analysed together there is a highly significant difference in distribution, $\chi^2 = 30.6$; $p < 0.001$. The distribution differences between the groups are shown in table 1. As would be expected, pancreatic dysfunction is significantly more common in the

Figure 3 Percentage excretion of administered fluorescein on control day in patients with untreated coeliac disease (UCD), patients with treated coeliac disease (TCD), symptomatic controls (SC), and patients with pancreatic disease (PD).

Table 2 T:K ratio and mucosal brush borders enzymes, morphological grade, and intraepithelial lymphocytes (IEL) in treated coeliac patients

	<i>n</i>	<i>r</i> ²	<i>p</i> value
Lactase	27	0.060	0.22
Sucrase	27	0.030	0.39
AP	27	0.013	0.58
IEL/1000E	23	0.029	0.43
Grade	25	0.018	0.53

AP, alkaline phosphatase.

pancreatic disease group than either of the coeliac groups; however, there were significantly more abnormal tests in the untreated coeliac disease group than in the symptomatic controls.

The excretion of fluorescein from the fluorescein dilaurate on the test day is shown in fig 2. A lower mean value was found in untreated coeliac patients (mean (SD), 16.3% (8.8); 95% CI 12.7 – 19.9) than either treated coeliac patients (mean (SD), 19.1% (9.2); 95% CI 15.7 – 22.4), or symptomatic controls (mean (SD), 21.93% (10.4); 95% CI 19.3 – 24.7), but these three groups were significantly higher than the pancreatic disease group (mean (SD), 3.8% (3.6); 95% CI 0.8 – 6.97). The variance between the groups, $F = 9.36$; $p < 0.001$ is due to statistical differences between the pancreatic disease group and the other groups.

On the control day (fig 3), significantly higher amounts of fluorescein were excreted by the untreated coeliac patient group (mean (SD), 55.9% (16.0); 95% CI 49.3 – 62.5) than all other groups: treated coeliac disease patients (mean (SD), 47.6% (10.1); 95% CI 43.5 – 50.8 ; $p < 0.04$); symptomatic controls (mean (SD), 47.7% (9.4); 95% CI 42.3 – 47.2 ; $p < 0.02$); and pancreatic disease (mean (SD), 39.7% (5.9); 95% CI 34.8 – 44.7 ; $p < 0.004$). There is no statistical difference between the excretion of the treated coeliac, symptomatic control and pancreatic disease groups. The overall variance between the four groups is statistically significant, $F = 5.51$; $p < 0.001$.

Analysis of the relation of T:K ratio to the intestinal brush border enzyme activity (lactase, sucrase, and alkaline phosphatase), intraepithelial lymphocyte count, and morphological grading in small bowel biopsies from treated coeliac patients failed to reveal any statistically significant associations (table 2).

Discussion

In the current study, 16 coeliac patients were found to have abnormal pancreolauryl tests. High or normal excretion of fluorescein on the control day in the two groups demonstrates the ability of the coeliac intestine to absorb the dye. Six of the 32 treated coeliac disease patients had abnormal tests, confirming previous findings in smaller groups of patients using the pancreolauryl or para-aminobenzoic acid (PABA) tests.^{5 6} Studies of pancreatic enzyme output following administration of IV secretagogues in coeliac disease rarely demonstrate significant abnormalities.^{2 3 14}

The pancreolauryl and PABA tests examine the function of the entire enteropancreatic axis by simulating normal physiological pathways,

whereas tests that use pharmacological doses of secretagogue measure only pancreatic acinar capacity or reserve, because the afferent loop of the axis is bypassed. Therefore, because only pancreolauryl and PABA tests are abnormal, it would appear that the pancreas itself is normal and the site of the disturbance is in the proximal part of the axis. However, the T:K ratio does not correlate with histological and brush border enzyme parameters, suggesting that the abnormality is more complex than simple small intestinal epithelial damage. In this study the T:K ratio in coeliac patients was not influenced by age or the duration of symptoms before diagnosis of coeliac disease.

It is interesting to note that the two non-coeliac patients with intestinal mucosal disease both have evidence of pancreatic dysfunction, the T:K ratios being 19% (amyloidosis) and 1.4% (intestinal tuberculosis), suggesting that parameters of mucosal damage other than those studied here could be operative in producing the abnormalities in treated coeliac disease. There are other mechanisms that are implicated in exocrine pancreatic dysfunction in coeliac disease. Duodenal acidification is an important stimulus for the release of secretin and impaired gastric acid production,¹⁵ possibly due to lymphocytic gastritis,¹⁶ has been described in some cases of coeliac disease. Also, in some coeliac patients with a good histological response to gluten withdrawal there is enhanced food stimulated release of somatostatin,¹⁷ which is an inhibitor of gastrin, cholecystokinin, and secretin. Both secretin and cholecystokinin are stimulants of the exocrine pancreas.¹⁸ Further delineation of the mechanism responsible for the disturbance of pancreatic function will require investigation of the treated coeliac disease patients with abnormal T:K ratios; parallel studies examining small intestinal biopsy structure and function (including APUD cell status), food

stimulated release of pancreatic secretagogues, Lundh test meals, and stimulation of the pancreatic acinar cells with exogenous IV secretagogues¹⁸ would be useful.

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