Cellobiose/mannitol sugar test – a sensitive tubeless test for coeliac disease: results on 1010 unselected patients

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SUMMARY   The cellobiose/mannitol (Ce/Ma) test is a non-invasive technique for investigating intestinal permeability. In coeliac disease there is a decreased absorption of small molecules and paradoxically increased absorption of large molecules. The simultaneous administration of cellobiose and mannitol as two probe molecules allows the permeability of the small bowel mucosa to be studied, eliminating extraneous factors such as gastric emptying, and incomplete urine collection. One thousand and ten patients presenting to a gastroenterology clinic with symptoms, signs, or biochemical indices compatible with coeliac disease had a Ce/Ma test. Eight hundred and seventeen had a normal test and of these 197 had a jejunal biopsy showing 148 normal mucosa, two coeliac disease, 43 non-specific abnormalities, four giardiasis. One hundred and ninety three had an abnormal test; of these 132 had a jejunal biopsy showing 62 normal mucosa, 48 coeliac disease, and 22 other abnormalities. Considering those who had jejunal biopsies, the sensitivity of the test for coeliac disease is 96%, specificity 70%, the predictive value of the positive 36%, and predictive value of the negative 99%. Eleven per cent of the patients with a 'false positive' test had abnormalities in the jejunal biopsy or a diagnosis which could explain the abnormal permeability.

The Ce/Ma test is a non-invasive investigation of small bowel permeability.[1 2] Cellobiose, a disaccharide (molecular radius 0·50 nm) and mannitol, a polyhydric alcohol (molecular radius 0·40 nm) are taken orally in hypertonic solution and a five hour urine collection is analysed for both probe molecules, the results being expressed as a ratio of the percentage recoveries.[1] In coeliac disease more cellobiose and less mannitol is absorbed than in normal subjects.[1 3] By expressing the results as a ratio there is good separation of coeliacs from normal subjects and the influence of certain non-intestinal factors acting similarly on both molecules such as renal impairment, gastric and bladder emptying and are eliminated.[4]

Although results of the Ce/Ma test have been reported previously they relate to small numbers of

selected groups of patients.[1 3 5–9] This paper describes our experience in 1010 patients presenting to a general gastroenterology clinic with symptoms compatible with coeliac disease.

Methods

Ce/Ma TEST

The test solution comprises 5 g cellobiose (Ce) and 2 g mannitol (Ma), lactose 20 g and sucrose 20 g are added as osmotic fillers and the solution is made up to 100 ml with water producing osmolality of 1500 mOsmol/l. The solution is taken orally after a six hour fast (normally at 6·00 am). A baseline urine specimen is obtained and urine collected for five hours into 1% thiomersal as a preservative. In nearly all cases this procedure was done as an outpatient at home, only occasionally as an inpatient.

Cellobiose absorption was measured by quantitative paper chromatography[10] and mannitol by spectrophotometry as previously described.[11] The

results are expressed as a ratio of percentage Ce recovery to percentage Ma recovery. (Normal range 0·004–0·028 calculated in 50 patients with normal jejunal biopsies.)

JEJUNAL BIOPSY

Jejunal biopsies were performed with a Watson biopsy capsule under *x*-ray control (263 patients) or through oral gastroduodenoscopy with large biopsy forceps (66 patients). Coeliac disease was defined as the finding of partial villous atrophy on jejunal biopsy with clinical and in some cases biopsy response to a gluten free diet.

PATIENTS

Records of 1010 new patients who had undertaken the Ce/Ma test between 1978 and 1985 were reviewed (age 18–85 years). These had presented to a general gastroenterology clinic with symptoms or biochemical findings compatible with coeliac disease.

Results

Eight hundred and seventeen patients had a normal Ce/Ma test and of these 197 had a jejunal biopsy (24·11%). One hundred and ninety three patients had an abnormal test of whom 132 had a jejunal biopsy (68·39%). The overall biopsy rate was
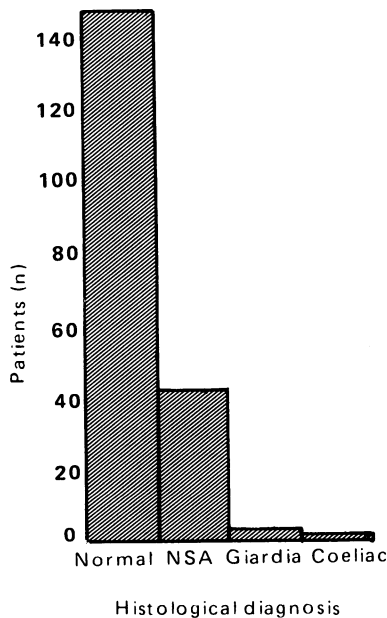
Fig. 1 *Jejunal biopsy result in normal Ce/Ma test group. NSA=non-specific abnormality, presence of chronic inflammatory cells±minor changes in architecture.*
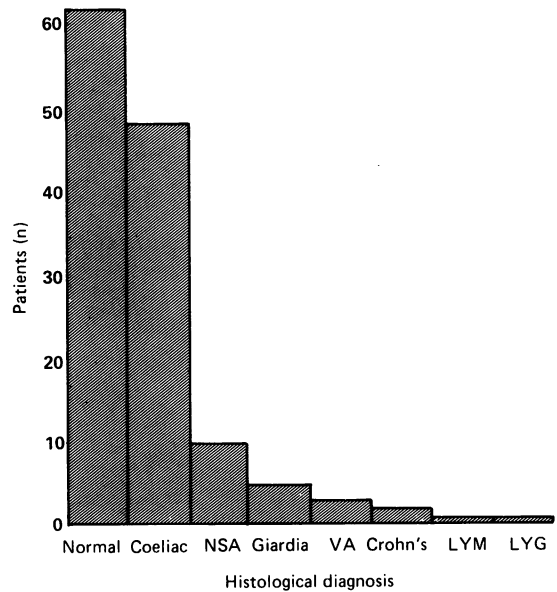
Fig. 2 *Jejunal biopsy result in abnormal Ce/Ma test group. NSA=non-specific abnormality, presence of chronic inflammatory cells±minor changes in architecture. VA=villous atrophy not coeliac disease. LYM=lymphoma. LYG=lymphangiectasia.*

32·57%. In the normal test group there were 148 normal biopsies (75·12%), two coeliac disease (1·01%), and 47 (23·85%) with abnormalities other than coeliac disease (Fig. 1). In the abnormal test group 62 (46·96%) had a normal jejunal biopsy, 48 (36·36%) had coeliac disease, and 22 (16·66%) abnormalities other than coeliac disease (Fig. 2). Those with non-specific abnormalities on duodenal biopsy underwent a jejunal biopsy using the Watson capsule to exclude coeliac disease. In the group that had a gastroscopic biopsy 50% of biopsies were abnormal and in the jejunal biopsy group 13% abnormal. This reflects the proportion of abnormal sugar tests in each group (jejunal biopsy 13%, GB 47%) rather than the type of biopsy performed.

The Table shows the diagnoses made in the patients with an abnormal Ce/Ma test.

SENSITIVITY AND SPECIFICITY OF THE Ce/Ma TEST

The sensitivity of a test measures the ability to identify those patients who have the disease, and the specificity, those that do not have the disease.[12] For those patients who had a jejunal biopsy the sensitivity of the Ce/Ma test for coeliac disease is 96% and specificity 70% with the predictive value of the positive 36% and negative 99%.

There is a bias in these results because a greater

Table   *Diagnosis in the abnormal Ce/Ma test group*

Diagnosis	n	N	NSA	NP	Other
Coeliac disease	48	–	–	–	48 Villous atrophy
Irritable bowel syndrome	21	16	1	4	
No diagnosis but became asymptomatic	20	13	1	6	
Radiation enteritis	2	2	–	–	
Crohn's disease	9	2	–	5	2 Changes consistent with Crohn's of small bowel
Metastatic bone disease	1	–	–	1	
Myxoedema	1	–	–	1	
Iron deficiency anaemia ? cause	5	5	–	–	
Primary osteoporosis	3	–	1	2	
Died before investigated further	2	–	–	2	
? Sprue	1	–	–	–	1 VA
Giardiasis	5	–	–	–	5 Giardiasis
Non-steroidal anti-inflammatory drugs	3	3	–	–	
Alcoholic	3	2	–	1	
Gall stones	1	–	–	1	
Crohn's disease causing oral aphthae	1	1	–	–	
Malabsorption in chronic renal failure	1	–	–	–	1 VA
Thyrotoxic	1	–	–	–	1 VA
Duodenitis	3	–	3	–	
Bacterial overgrowth	3	2	1	–	
Small bowel lymphoma	1	–	–	–	Lymphoma
Eczema	1	–	–	1	
Gastric ulcer	1	1	–	–	
Dietary anaemia	3	2	1	–	
Dermatitis herpetiformis	1	–	–	1	
Anorexia nervosa	2	2	–	–	
Carcinoma pancreas	1	–	1	–	
Anaemia due to uterine fibroids	1	1	–	–	
Tuberculosis (respiratory)	1	1	–	–	
Abdominal migraine	1	1	–	–	
Lymphangiectasia	2	–	1	–	1 Lymphangiectasia
Allergic to eggs	1	1	–	–	
Laxative abuse	1	1	–	–	
Iron deficiency secondary to gastric surgery	1	–	–	1	
Pancreatitis	1	–	–	1	
Non-specific colitis	1	1	–	–	
Diverticular disease	1	1	–	–	
Sarcoidosis	1	–	–	1	
Discoid lupus	1	–	–	1	
Idiopathic oral aphthous ulceration	1	1	–	–	
Failed to attend for follow up	35	3	–	32	
Total	193	62	10	61	60

n=number tests; N=normal JB; NSA=non-specific abnormalities; NP=not performed; VA=villous atrophy not coeliac disease.

number of biopsies were done on those patients who had an abnormal test. If the patients who were not biopsied were assumed to have the same distribution as those who were biopsied, the sensitivity would be 91%, specificity 88%, predictive value of the positive 43% and negative 99%. Considering detection of any small bowel disease diagnosed by jejunal biopsy or other means the sensitivity is 91%, specificity 73%, predictive value positive 45% and negative 97%.

Discussion

In coeliac disease there is a reduced absorption of small molecules – for example, low molecular weight PEG,[13][14] monosaccharides,[5][15] mannitol,[1-3][6] and increased absorption of relatively larger molecules – for example, CrEDTA,[16] lactulose and cellobiose[17][18] which is enhanced by hypertonicity of the solute.[17-19] The absorption of small molecules of less than 0·5 nM diameter is 10–200-fold that of molecules greater than 0·50 nM suggesting, in the absence of active transport, that whilst small molecules are absorbed through classical aqueous pores[20] large molecules are excluded by their size. The paradoxical changes in absorption of different sized molecules which occurs in coeliac disease and in experimental animals[21] also suggests different routes for absorption. The absorption route of large molecules may be through intercellular tight junctions[22] or cell extrusion zones at the villous tips,[18] thus the increased disaccharide absorption in coeliac disease may reflect epithelial injury, cell shedding or changes in the tight junction.[23] The decreased absorption of small molecules may result from a reduction in the number of aqueous pores secondary to a reduced surface area.[24]

The Ce/Ma sugar test is highly sensitive for coeliac disease (sensitivity 96%). Only two coeliac patients were identified in this study with a normal sugar test. The first, a woman of 40 complained of indigestion. A barium meal showed gastric erosions and a routine full blood count revealed a high mean cell volume caused by folate deficiency. She therefore had a Ce/Ma test and jejunal biopsy which showed changes typical of coeliac disease, she had no symptoms of coeliac disease at presentation. The second patient, a woman of 50 years, presented with diarrhoea and weight loss. An investigation plan for diarrhoea included a Ce/Ma test, jejunal biopsy, and thyroid function tests, the latter showing her to be thyrotoxic. She was treated for the thyrotoxicosis and the diarrhoea ceased. The jejunal biopsy was then reported as coeliac disease, her presenting symptoms were caused by thyrotoxicosis, coeliac disease being an incidental finding.

Earlier work from our laboratory[2] compared the Ce/Ma test with the xylose test, serum and red cell folate, and reticulin antibodies in a group of patients comprising 24 coeliacs, 54 with normal jejunal biopsy, and 50 disease controls. The results showed sensitivities and specificities of 96 and 99% for the Ce/Ma test, 67 and 67% for the xylose test, 62 and

78% for serum folate, 46 and 95% for red cell folate, and 44 and 97% for reticulin antibodies. Negative predictive values were 99%, 76%, 82%, 82%, and 82% respectively. The Ce/Ma test, therefore, appears to be an excellent screen for coeliac disease when compared with other investigations. In this study 25% of the patients with abnormal tests had coeliac disease giving a specificity of 70%. The Table shows the Ce/Ma test was abnormal in other small bowel diseases where a change in permeability is known to occur – for example, Crohn's disease,[7 8 25] alcoholics,[26] patients taking non-steroidal anti-inflammatory agents,[9 27] eczema,[28 29] food allergy,[30] and dermatitis herpetiformis[31] (9·3%). A change in permeability might be expected in certain other conditions – for example, radiation enteritis, giardiasis, sprue, small bowel lymphoma, lymphangiectasia and duodenitis (7·30%) when there is a change in the morphology of the jejunum which might disrupt normal absorptive pathways. Permeability in other disease such as thyroid disease, primary osteoporosis, metastatic disease, cholelithiasis, renal failure, peptic ulcer disease, anorexia nervosa, pancreatic inflammation and carcinoma, gastric surgery, sarcoidosis and discoid lupus has not been fully studied and at present these are either isolated cases or falsely positive tests.

In the five cases of iron deficiency anaemia the gastrointestinal tract was fully investigated and no cause for blood loss found, the iron deficiency may have been caused by iron malabsorption reflected by the abnormal permeability, or it is possible that iron deficiency affects intestinal permeability.

There was only one proven case of food allergy in this study and it would be interesting to study permeability changes in a larger group of these.

Fifty nine per cent of tests appear to be false positives, however, it may be that there is a change in permeability but not in jejunal morphology which might account for some of the abnormal Ce/Ma tests with normal jejunal biopsies. Recent studies have shown that although the jejunal biopsy may be normal on routine microscopy there is often a change in morphometry when analysed using a computerised measuring facility.[32]

In conclusion the Ce/Ma test is a sensitive and specific test for coeliac disease. The low predictive value of a positive test reflects the low prevalence of the disease in the population studied whilst the high predictive value of a negative test means it can be relied on to miss only a few patients with coeliac disease.

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