Is the sugar intestinal permeability test a reliable investigation for coeliac disease screening?

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Abstract

Background—The lactulose/mannitol (L/M) intestinal permeability test is a simple, non-invasive screening test for coeliac disease. The reliability of the L/M test has so far only been tested in selected groups of patients with coeliac disease.

Aim—To evaluate the reliability of the L/M test in a group of patients with coeliac disease who had been diagnosed during mass serological screening of the general population.

Patients and methods—Twenty nine patients with coeliac disease detected by screening and 54 age matched coeliac disease free controls aged 11–15 years underwent an L/M test with 5 g lactulose and 2 g mannitol in isotonic aqueous solution. Urinary sugars were measured by high performance liquid chromatography.

Results—The median % urinary recovery of lactulose (lactulose UR) was significantly higher in patients with coeliac disease than in controls (0.63 v 0.18, $p < 0.001$). The mean mannitol % UR was lower in patients with coeliac disease than in controls (17.6 v 18.5) but the difference was not significant. The median urinary L%/M% ratio was significantly higher in patients with coeliac disease than in controls (0.038 v 0.014, $p < 0.001$). However, 16 of the 29 patients with coeliac disease showed an L%/M% ratio within normal limits (< 0.044).

Conclusions—The L/M intestinal permeability test is not a valuable tool for screening of coeliac disease in the general population. The pattern of the urinary probe recovery suggests that many patients with coeliac disease could remain symptomless because the extent of their intestinal mucosal damage is small ("short" coeliac disease).

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Keywords: intestinal permeability, coeliac disease, mass screening, lactulose, mannitol.

The lactulose/mannitol (L/M) intestinal permeability test is a simple investigative tool currently used in the diagnostic investigation of patients with suspected coeliac disease. After an orally administered dose of sugar probes, patients with active coeliac disease usually show higher lactulose and lower mannitol urinary recovery (UR), with a pronounced increase in the L/M UR. These changes have mostly been found in selected groups of symptomatic patients.^{1 2}

In recent years it has become clear that many patients with coeliac disease escape diagnosis.³ Despite typical coeliac damage at jejunal biopsy, many patients often show only minor symptoms or are apparently healthy. Treatment of these patients with a gluten-free diet is nevertheless recommended to prevent long term complications of coeliac disease, such as infertility or malignancy.⁴ Many patients with coeliac disease can be identified through serological screening of the general population using markers of coeliac disease such as the antigliadin antibodies (AGAs) as the screening test.⁵ The reliability of the sugar intestinal permeability test as a screening tool for undiagnosed coeliac disease has not yet been determined.

We recently screened a general population sample of teenagers for coeliac disease by performing a serum IgG and IgA-AGA assay as the first level screening test.⁵ Most of the patients with coeliac disease detected by screening also had an L/M test at the diagnosis. The results of the intestinal permeability study on 29 coeliac patients detected by screening and on 54 age matched controls without coeliac disease are reported here.

Patients and methods

Both patients and controls took part in a coeliac disease screening project, the design of which has previously been published.⁵ Briefly, 7293 teenagers attending compulsory secondary school were screened using the serum IgG-AGA test and the IgA-AGA test, with the informed consent of their parents. Positive cases underwent a second step evaluation including a repeat of the AGA test, the IgA class antiendomysium antibody (AEA) test, and serum immunoglobulin measurement followed by a jejunal biopsy in those who showed at least one of the following: (a) a positive IgA-AGA test, (b) a positive AEA test, (c) a positive IgG-AGA test and selective IgA deficiency. This diagnostic algorithm allowed the detection of 38 patients with coeliac disease in this population sample.

PATIENTS

The intestinal permeability study was performed on 29 of the 38 patients with coeliac disease detected by screening. The other nine patients did not undergo the L/M test because of contingent problems, such as no fasting or incomplete sugar ingestion. There were 11 male and 18 female patients, with a mean (SD) age of 12.9 (1.0) (range 11.6–14.1) years. In each patient the diagnosis of coeliac disease was

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confirmed by intestinal biopsy according to currently accepted criteria.⁶ All the patients with coeliac disease in this study were either free from major symptoms or showed a low grade intensity illness. The most common clinical findings were iron deficiency, recurrent abdominal pain, and behavioural disturbances.

CONTROLS

These were 54 randomly selected age matched students participating in the coeliac screening. They were considered to be free of coeliac disease because of normal results in the IgG-AGA test, the IgA-AGA test, the AEA test, and the serum immunoglobulin measurements. In a large multicentre study the negative predictive value of this serological pattern was as high as 99.3%.⁷ Controls comprised 23 male and 31 female subjects with a mean age of 12.7 (SD 0.9) (range 11.3–14.0) years.

METHODS

The L/M test was performed after an overnight fast and bladder emptying. A total of 5 g lactulose and 2 g mannitol in 150 ml water was given orally (isotonic load). All urine passed in the subsequent five hours was collected, the volume recorded, and an aliquot preserved at -20°C with sodium azide. The urinary concentration of each sugar was measured by high performance liquid chromatography as previously described⁸ and the ratio of recovered to ingested sugar was calculated as a percentage (lactulose% UR and mannitol% UR). The ratio of the lactulose% UR to the mannitol% UR (L%/M%) was evaluated.

Intestinal biopsy specimens were taken at the Traitz ligament by a Watson capsule under fluoroscopic control.

STATISTICAL ANALYSIS

Whereas the mannitol% UR values showed a normal distribution, the lactulose% UR and the L%/M% distributions were positively skewed. A normal plot showed that both the lactulose% UR and the L%/M% values fitted a Gaussian distribution after a square root ($\sqrt{}$)

transformation. The results of the L/M test in control subjects were used to fix the normal limits of the permeability indexes (one tail 95% tolerance interval). The cut off values were established as follows: (a) mean $+1.65$ SD of the $\sqrt{\text{lactulose\% UR}}$; (b) mean -1.65 SD of mannitol% UR; (c) mean $+1.65$ SD of the $\sqrt{\text{L\%/M\%}}$. The results of the intestinal permeability study in controls and patients with coeliac disease were compared by either the Mann-Whitney *U* test (median lactulose% UR and median L%/M%) or the Student's *t* test for independent samples (mean mannitol% UR).

The difference between the result of the haematological investigations in patients with coeliac disease with either a normal or a pathological value of the L%/M% urinary ratio has been analysed by a Student's *t* test for independent samples.

Results

Figures 1 and 2 show the results of the intestinal permeability study. The median lactulose% UR was significantly higher in patients with coeliac disease than in controls (0.63 v 0.18 , $p < 0.001$); the mean mannitol% UR was lower in patients with coeliac disease than in controls (17.6 v 18.5) but the difference was not significant. The median L%/M% UR was significantly higher in patients with coeliac disease than in controls (0.038 v 0.014 , $p < 0.001$). However, 16, 15, and 27 of the 29 patients with coeliac disease respectively showed an L%/M% ratio, a lactulose% UR, and a mannitol% UR within normal limits. Based on the L%/M% UR, regarded as the most discriminating permeability index between controls and patients with coeliac disease,⁹ the sensitivity of the intestinal permeability test (subjects with a positive test $\times 100$ /number of patients with coeliac disease) was 45%.

The comparison between patients with coeliac disease with a normal L%/M% urinary ratio and those with an L%/M% UR higher than the cut off level (0.044) showed no significant difference in clinical presentation, the serological coeliac disease marker pattern, the histological picture, and the routine haematological investigations. The mean serum iron

Figure 1: L%/M% urinary ratio ($\times 10^3$) in patients with coeliac disease (CD) and in the control (C) group. The dotted line indicates the upper limit of normal (0.044).

Figure 2: $\sqrt{\text{L\% UR}}$ and M% UR in patients with coeliac disease (CD) and in controls (C). Data are expressed as mean (SEM).

concentration was 51 (SD 17) $\mu\text{g/dl}$ in patients with coeliac disease with an abnormal L%/M% UR and 71 (SD 31) in patients with coeliac disease with a normal permeability test, but the difference was not significant ($p=0.09$).

Discussion

A unique feature characterised the patients with coeliac disease investigated in this study; they were apparently healthy students who had been diagnosed through a mass serological screening. Although a typical gluten sensitive enteropathy was found at the intestinal biopsy in all cases, most of these patients with coeliac disease were either clinically silent or reported only minimal complaints. Could these patients have been suspected of having the disease after less invasive tests (not requiring a blood sample to be taken) such as the sugar intestinal permeability test? This hypothesis seemed plausible, as previous studies had suggested that the sensitivity of the L/M test in diagnosing coeliac disease could be as high as 96–100%.^{10–11}

Patients with coeliac disease detected by screening tended to have abnormal results in the intestinal permeability test with monodisaccharides, as has been shown in patients referred to hospital.¹ However, neither the L%/M% UR nor the other permeability indexes provided clear discrimination between controls and patients with coeliac disease. This finding is at variance with our previous results showing that the sugar intestinal permeability test is a valuable screening investigation in children referred for clinically suspected coeliac disease.⁸ We would argue that the sugar intestinal permeability test cannot be considered as a useful tool for coeliac disease screening on the general population as high sensitivity (a low number of false negative results) is a critical requirement for a first level screening test.¹² This is especially true when the disease is not fully expressed on a clinical basis, as is often the case in coeliac disease.

It remains to be seen whether better diagnostic discrimination between patients with coeliac disease detected by screening and control subjects might be achieved by giving the sugar probes in hyperosmolar rather than isotonic solution. It is well known that hyperosmolar stress (test solution 1500 mmol/l) increases the permeability of abnormal epithelia to the disaccharide probes.¹³ However, most workers have now abandoned the use of hyperosmolar stress in favour of the more physiological iso-osmolar tests.¹⁴

The median lactulose% UR was significantly increased in our patients with coeliac disease detected by screening, as usually happens in patients with active coeliac disease. This is likely to be a consequence of their enteropathy, which was morphologically undistinguishable from that of clinically typical patients with coeliac disease. The mucosal damage might in turn lead to increased lactulose absorption by

several mechanisms, such as the disruption of membrane integrity, changes in the tight junctions, and increased accessibility of the crypts to luminal contents.^{9–14} On the other hand, mannitol% UR is usually reduced in patients with active coeliac disease. Although it is still unclear whether the main route of intestinal mannitol permeation is transcellular or paracellular, it is commonly assumed that reduced mannitol% UR reflects the reduction in the total surface area of the small intestine caused by villous atrophy.¹⁵ In our patients with coeliac disease the mean mannitol UR was not significantly lower than in the control group. This might indicate that the absorptive surface was not grossly reduced in these patients. It can therefore be speculated that those detected by screening showed a clinically silent or mild form of coeliac disease because of a small extent of the mucosal damage ("short" coeliac disease). This hypothesis should be confirmed by the morphometric analysis of biopsy samples taken at different levels in the jejunum.

In conclusion, the results of this study indicate that the isotonic L/M intestinal permeability test is not a valuable tool for coeliac disease screening of the general population. The pattern of the urinary recovery suggests that many patients with coeliac disease remain symptomless because they have limited damage to the mucosa of the small intestine.

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