Is an intestinal permeability test a valid marker for slight dietary transgressions in adolescents with coeliac disease?

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Abstract

Adolescents with coeliac disease often fail to adhere to a strict gluten free diet. The effectiveness of intestinal permeability to sugars as a marker of slight dietary transgressions by such adolescents was assessed. Severe dietary transgressions were excluded from the study. Subjects were divided into two groups according to whether they committed slight dietary transgressions or adhered to a strict gluten free diet. A reference group of preadolescents with coeliac disease was also included in the study. Intestinal permeability and antigliadin antibody tests were performed on all patients. The diagnostic marker of intestinal permeability was excellent in the reference group. Neither the intestinal permeability test nor antigliadin antibody tests, however, succeeded in discriminating between the two groups of adolescents considered in this study. In conclusion the intestinal permeability test is not a valid marker for slight dietary transgression in such patients.

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The specific treatment of coeliac disease is strict adherence to a gluten free diet.¹ Such diets are not always properly followed by patients. Good dietary control is crucial for remission of the disease as well as for prevention of the development of malignant pathology of the small intestine in adults.^{2,3}

Because of their psychosociological make up, adolescents present the highest dietary transgression rate of all age groups.^{4,5} Dietary transgressions may be too minor to produce clinical or biochemical effects. Small transgressions do not, however, prevent future complications,^{2,5-7} hence it would be useful to find a sensitive marker capable of detecting slight dietary transgressions.

Antigliadin, antiendomysial, and human jejunum antibodies have been proposed as such markers,^{8,9} but they are of little value in adolescents with coeliac disease, in whom, through an as yet unidentified mechanism, sensitivity is low.^{10,11} Also measurements of antiendomysial or human jejunum antibodies is not feasible at all laboratories.

Intestinal permeability has also been proposed as a reliable test for the detection of villous atrophy in coeliac disease.¹²⁻¹⁷

Certain workers have suggested this method as a screening test in gluten challenge or in the detection of dietary non-compliance.^{18,19} Our

study attempts to assess intestinal permeability as a marker of slight dietary transgressions in a population of adolescents with coeliac disease.

Patients and methods

STUDY SUBJECTS

Subjects were selected from among adolescents with coeliac disease who attended the gastroenterology output service of the hospital.

Inclusion criteria were: Confirmed diagnosis of coeliac disease according to the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN)²⁰ and voluntary consent to participate in the study.

Exclusion criteria were severe intestinal involvement suggesting major dietary transgressions assessed by clinical or biochemical alterations (total faecal fat >6 g/24 h as described by Van de Kamer,²¹ faecal nitrogen >1.5 g/24 h as described by Lowry *et al*,²² and faecal chymotrypsin activity <18 IU/g of dry stool (test combination Boehringer-Mannheim, Mannheim, Germany).

The selection requirements were fulfilled by 29 patients (15 males and 14 females) ranging from 14 to 20 years of age.

Selected patients were divided into subgroups on the basis of a comprehensive dietary questionnaire: subgroup I, nine patients (four males and five females) who admitted to occasional transgressions in the preceding six months; subgroup II; 20 patients (11 males and nine females) who asserted that they had strictly adhered to a gluten free diet.

REFERENCE GROUP

Previously we analysed a group of 30 patients who were undergoing diagnostic testing for coeliac disease. The aim of this part of the study was to establish the exact level of confidence in the ability of the intestinal permeability test to identify gluten induced villous atrophy. These patients were also divided into two subgroups: subgroup A, 19 patients from three to 10 years of age (12 males and seven females). All were on a gluten free diet and their intestinal biopsy results were normal (the second biopsy in their diagnostic series). On conclusion of the study, diagnoses of coeliac disease were confirmed for all these patients; Subgroup B, nine patients (six males and three females) aged between three and 13. All were in the gluten challenge period and their third biopsy showed severe villous atrophy.

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METHODS

The intestinal permeability test was performed within a maximum of two days after the dietary questionnaire was given. In the reference group, the test was performed three days after intestinal biopsy.

After an overnight fast (that continued until the end of the test) and emptying of the bladder, a mixture of 5 g lactulose and 1 g L-rhamnose diluted in 60 ml water was given. Urine was collected for a five hour period and patients were allowed to drink (water only) after three hours.

The volume of urine excreted during the five hour period was recorded and an aliquot of 25 ml for each subject was stored in a plastic tube with merthiolate (10% w/v) as preservative at -30°C until analysis.

Sugar concentration in the urine was determined by high performance liquid chromatography (HPLC) with the method described by Delahunty and Hollander²³ modified slightly by replacing the last two centrifugation steps with a single filtration procedure. The HPLC equipment (Waters, Division of Millipore, Waterford, MA, USA) included a model 6000A pump, an automatic model WISP 712 injector, and a model 410 refraction index detector. The column was a Sugar-Pack I calcium form resin column and the mobile phase was grade I water/0.001 mM disodium calcium ethylenediaminetetra-acetic acid at a flow rate of 0.5 ml/min. Calibration was performed with an internal standard of cellobiose. Peak areas were integrated by computer (NEC Power Mate I, Nec Co, USA). Accuracy of the method was: intrabatch: 4.26% for lactulose and 4.82% for L-rhamnose; between day: 6.46% and 5.7%. Recovery of both sugars was 84%. Sensitivity was 1064 mvol/mmol/l for lactulose and 356 mvol/mmol/l for L-rhamnose. The slope ranged from 0.49 to 1.46 mmol/l ($y = -0.027 + 0.72x$, $r^2 = 0.999$) for lactulose and 1.94 $\mu\text{mol/l}$ to 3.04 mmol/l ($y = -0.88 + 0.81x$, $r^2 = 0.999$) for L-rhamnose. The only interference was produced by galactose and D-xylose, which coeluted with L-rhamnose.

Simultaneous IgA and IgG analyses of anti gliadin antibodies were performed by enzyme linked immunosorbent assay microtitre (Pharmacia Diagnostic, Sweden) with 25 U/ml as the upper limit to the reference range.

A breath hydrogen test was given to all patients during the intestinal permeability test at basal metabolism, at 15 minutes, and then every 30 minutes for 210 minutes, and the results were quantified on an exhaled hydrogen monitor (GMI Medical LTd, Renfrew, England). The aim of this test was to assess whether altered oro-caecal transit times or colonisation of the upper intestine might interfere with the reliability of

the intestinal permeability test compared with intestinal biopsy. Sustained increases of up to 20 ppm above the basal value recorded in the first 30 minutes were considered suggestive of intestinal overgrowth. Any peak >20 ppm above the basal value occurring outside the reference range (65–120 minutes) was defined as indicative of altered oro-caecal transit times. Results of the intestinal permeability test were expressed as the percentage ratio of ingested lactulose recovered in the urine to the percentage of ingested L-rhamnose recovered in the urine.

STATISTICAL TREATMENT

Statistical analysis was carried out with a statistical package²⁴ that included the Mann-Whitney test, one and two factor analysis of variance, a diagnostic performance study (sensitivity, specificity), and the Youden index, which is a calculation of the total diagnostic value of a probe. Its formula is expressed as: $1 - (\text{sensitivity} + \text{specificity})$ and it ranges from 0 to 1.

Receiver operating characteristic (ROC) curves and the areas below the curves were also calculated. This procedure is based on the calculation of the area below the curve formed by the graphical representation of sensitivity (ordinate) and 1-specificity (abscissa). It represents the discriminating power of the diagnostic probe for every cut value of the test.

Results

REFERENCE GROUP

Table I shows descriptive statistics for the reference group. The difference between the intestinal permeability test values for subgroups A and B was statistically significant. One factor analysis of variance gave significant differences for the intestinal mucosal state and the test values ($F = 57.87$, $p < 0.05$).

The figure shows the diagnostic performance characteristics of the permeability test related to the histological state of the mucosa in the diagnosis protocol for coeliac disease. It also shows the excellent discriminating power of the test, with a cut off for the maximum Youden index at 0.92.

IgA and IgG anti gliadin antibodies were outside the reference range in all patients in subgroup B and below the upper limit of normality in all subjects in subgroup A.

When two factor analysis of variance was applied (intestinal permeability and breath hydrogen test), the results indicated that neither oro-caecal transit time nor the probability of

TABLE I Descriptive statistics for the reference group

	Group A	Group B
Lactulose excreted in urine (%)	0.261 (0.03)	0.657 (0.15)*
L-Rhamnose excreted in urine (%)	5.611 (0.60)	3.703 (1.05)
Lactulose:L-Rhamnose ratio	0.048 (0.005)	0.159 (0.03)**
Antigliadin antibodies above normal range (%)	0	100
Altered breath hydrogen test (%)	39.2	21.4

All results are expressed as mean (SEM). * $p < 0.05$; ** $p < 0.01$; Mann-Whitney test.

TABLE II Descriptive statistics for the adolescent group

	Group A	Group B
Lactulose excreted in urine (%)	0.231 (0.04)	0.257 (0.02)
L-Rhamnose excreted in urine (%)	5.899 (0.59)	7.268 (0.49)
Lactulose:L-Rhamnose ratio	0.039 (0.008)	0.041 (0.005)
Antigliadin antibodies above normal range (%)	0	0
Altered breath hydrogen test (%)	10.25	20.5

All results are expressed as mean (SEM).

Figure: The area below the receiver operating characteristic curve delimited by symbols represents the good diagnostic performance of the intestinal permeability test, approaching 1 – that is, the optimum value of the area. The arrow represents the cut off test value that renders the better diagnostic discrimination (maximum Youden Index) and is equal to a value of 0.092.

intestinal overgrowth influenced the intestinal permeability results ($F=1.34$). Severe histological damage was the only factor that affected intestinal permeability values. Results of the breath hydrogen test did not influence the relation between intestinal biopsy and intestinal permeability, and the variance for the two factor interaction was non-significant ($F=1.0009$).

STUDY GROUP

Table II shows results for the adolescents with coeliac disease. No differences in intestinal permeability values were established between subgroups I and II. None of the patients showed IgG or IgA anti gliadin antibody concentrations above the upper limit for the reference range.

Discussion

Adequate therapeutic control is the main problem currently encountered in the treatment of coeliac disease. It is a well known fact that major dietary transgressions are normally accompanied by relapses, which are thus easy to recognise. Minor dietary regressions, which are difficult to detect, are factors that affect the future morbidity and mortality of patients with coeliac disease. Holmes *et al* studied development of coeliac disease in 210 patients over 11 years.² They found that the patients who adhered strictly to a gluten free diet presented an excess morbidity similar to that for the general population (1.2); the excess mortality increased significantly in the patients who committed dietary transgressions, rising to 10.7 in the patients who followed a normal diet.

Adolescents with coeliac disease are able to evade parental dietary control and thus are more likely to commit dietary transgressions.⁴ As a standard for dietetic follow up testing, the ESPGAN has proposed the performance of anti gliadin,²⁵ antiendomysial, or antireticulin antibody determinations. Because of their low sensitivity, these tests are of questionable value in adolescents.^{10,11} As a result in these patients verification of gluten induced villous atrophy can

only be confirmed by means of intestinal biopsy. This involves subjecting patients to invasive techniques, with the consequent risk of rejection by patients.

It is therefore important to find a marker for slight dietary transgressions other than dietary questionnaires that is sensitive, non-invasive, and objective.

The intestinal permeability test is simple and is readily tolerated by patients. It has proved to be highly sensitive and specific in detecting severe villous atrophy. Our results for the reference group were in agreement with the results reported by other workers.²⁶ They were normal in all the patients in subgroup A (no histological damage) and abnormal in all the patients in subgroup B (severe atrophy). This excellent diagnostic performance supports the use of intestinal permeability as a screening test for secondary relapses due to severe dietary transgressions, as proposed by various researchers.^{27,28}

Results on the sensitivity of intestinal permeability in cases of moderate histological changes in the intestinal mucosa have been contradictory, and its value in such cases remains unclear.^{18,19,29-31}

All patients who presented evidence of severe dietary transgressions were excluded from this study. Our findings produced no differences in the results of intestinal permeability tests between patients who admitted having committed slight dietary transgressions and those who denied having committed any. Likewise, no differences were found in the behaviour of anti gliadin antibodies in the two groups. Altered oro-caecal transit times or intestinal overgrowth did not decrease the diagnostic confidence of intestinal permeability tests.

In conclusion intestinal permeability tests can be useful in providing confirmation of diagnoses of coeliac disease and as a screening test for performing both the second and third confirmatory biopsies. Our results, however, confirm that intestinal permeability is not a valid marker for slight dietary transgressions. Three possible explanations for this are: (1) intestinal permeability is not sensitive enough to detect mild villous damage; (2) histological changes have not yet appeared in adolescents with coeliac disease; (3) after a period of meticulous compliance with a therapeutic diet, adolescents are able to tolerate variable amounts of gluten without exhibiting overt symptoms.³²

More studies aimed at finding other markers of transgression are needed to enable clinicians to take steps to persuade patients to adhere to a gluten free diet and thus avert future malignant complications.

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