Differentiation of cows' milk intolerance and gastro-oesophageal reflux

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Abstract

The aim of this study was to compare a non-invasive test of small bowel permeability with a more invasive approach involving endoscopy, mucosal biopsy, and oesophageal pH monitoring for rapidly differentiating gastro-oesophageal reflux (GOR) and cows' milk intolerance in 25 infants with persistent vomiting. Each subject underwent a cellobiose/mannitol permeability study, upper gastrointestinal endoscopy with oesophageal and small bowel biopsies, and a 24 hour pH study.

Reflux disease and/or cows' milk intolerance was responsible for vomiting in 24 (96%) of the subjects. Sixteen (64%) of the infants had GOR alone, four (16%) had GOR and cows' milk intolerance, and four (16%) had cows' milk intolerance alone. Morphometric analysis of small bowel biopsies was abnormal in 19% of the patients with GOR alone and in 67% with cows' milk intolerance with or without GOR. The permeability test was abnormal in only 6% of the patients with GOR but in 100% with GOR and cows' milk intolerance and in 100% with cows' milk intolerance alone.

The non-invasive permeability study aimed at rapid determination of cows' milk intolerance should pre-empt a more invasive approach in the evaluation of infants with persistent vomiting.

(Arch Dis Child 1995; 73: 439–442)

Keywords: cows' milk intolerance, gastro-oesophageal reflux.

Cows' milk intolerance and gastro-oesophageal reflux (GOR) disease are the most common causes of persistent vomiting in infants, and the two can be difficult to distinguish on clinical grounds.^{1–3} Distinction between the two is of obvious importance in management planning. Vomiting and other symptoms improve with specific antireflux treatment in most children with GOR, whereas children with cows' milk intolerance respond only to a diet without cows' milk protein.^{1 2}

The correct diagnosis generally is established by therapeutic trial, but in some children with persistent vomiting a more direct and prompt diagnostic method is desired. No specific laboratory test apart from histological evaluation of small bowel biopsy at present is useful in diagnosing cows' milk intolerance, but the clinical value of biopsy remains debated.^{4 5} GOR may be accurately diagnosed

by 24 hour pH monitoring and upper endoscopy with oesophageal biopsy. Although prompt in making the diagnosis, these tests, like small bowel biopsy, are considered quite invasive in this age group. Tests of intestinal permeability are relatively simple and non-invasive, and are abnormal in disorders that affect small bowel integrity.⁶ The purpose of this study was to compare a test of small bowel permeability aimed at detecting cows' milk intolerance with a more invasive approach incorporating endoscopy with oesophageal and duodenal biopsies in conjunction with 24 hour pH monitoring aimed at diagnosing GOR and cows' milk intolerance to see which could most effectively lead to the correct management plan.

Patients and methods

PATIENTS

Twenty five consecutive infants with persistent vomiting (mean (SD) age 9.3 (3.8) months, range 2–13 months) were referred from January 1993 to January 1994 to our unit, a tertiary referral centre for paediatric gastroenterology. Each had been consuming a diet which included cows' milk, in some cases with gluten and in others without. None had undergone a trial of dietary milk elimination before referral. Lactose intolerance, enteric or other infections, and immune deficiency had been excluded in all subjects. In those infants who were receiving gluten, serum antigliadin and antiendomysium antibodies were measured and were not present.

Each subject underwent a cellobiose/mannitol permeability study (CMPS), upper endoscopy with oesophageal and duodenal biopsy, and 24 hour oesophageal pH study.

This investigation was approved by the local institutional review board. In each case, informed written consent for participating in the study was obtained from the parents.

PERMEABILITY TEST

The intestinal permeability test was performed after an overnight fast. Each subject drank a solution containing 2 g mannitol, 5 g cellobiose, and water to make 100 ml (osmolality 270 mmol/l). Urine was collected for the next five hours and stored at –20°C. The subjects went without food during the test but were allowed to drink water after the first hour. Mannitol in urine was measured by the method of Corcoran and Page whereby it is oxidised to formaldehyde by periodic acid.⁶ The formaldehyde reacts with chromotropic

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Accepted 26 June 1995

acid to form a purple complex which is then measurable at 570 nm absorbance. Urine cellobiose was measured using the method described by Strobel *et al* in which the compound is digested by D-glucosidase to glucose.⁷ Glucose was then quantified using the hexokinase procedure with NADPH generation measured at 340 nm.⁷ The final ratio of percentage recovery of cellobiose to percentage recovery of mannitol was calculated. A cellobiose:mannitol ratio >0.022 was considered abnormal, as the value exceeded 2 SD over the mean derived from 50 age and sex matched normal children.

ENDOSCOPY AND HISTOLOGY

Endoscopy was performed using a paediatric fibre endoscope (GIF PQ20, Olympus Corporation) after administration of intravenous diazepam (0.3 mg/kg) and pethidine (1 mg/kg). Two mucosal specimens were taken 3–4 cm above the squamocolumnar junction and a further two specimens near the ligament of Treitz for histological examination. Oesophagitis was classified as mild when one to 19 intraepithelial eosinophils and/or four to 19 neutrophils were seen per high power field, as moderate when ≥ 20 eosinophils or ≥ 20 neutrophils were observed per high power field, and severe if evidence of mucosal ulceration was also present.⁸ Duodenal tissue sections were cut perpendicular to the luminal surface with thickness of 4 μ m and were mounted on glass slides. Haematoxylin and eosin stained slides were used for morphometric studies, the following parameters being measured: (a) mean villus:crypt ratio determined from the linear measurement of five villus heights divided by the corresponding crypt depths, and (b) number of intraepithelial lymphocytes per 100 enterocytes counted in the upper two thirds of the villus. Reference values were obtained by analysing biopsy specimens from 36 subjects with normal jejunal histology whose clinical diagnoses were short stature, failure to thrive, urinary tract infection, anorexia, and Down's syndrome. The 10th–90th centile values for villus:crypt ratio and intraepithelial lymphocytes derived from these subjects were 1.5–3.0 and 7–22, respectively. A villus:crypt ratio falling below this range was considered abnormal. All histological studies were carried out blind to the clinical data.

pH STUDY

The 24 hour oesophageal pH study was performed using a previously reported method.⁹ In brief, a small, flexible electrode (Ingold) was placed at 87% of the length from nose to lower oesophageal sphincter, as determined from subject height.¹⁰ Reflux episodes were defined as decreases in the intraoesophageal pH to <4.0 for at least 20 seconds each. Oesophageal pH was recorded on a modified computer monitor (Proxima) and analysed using personal computer software. A total oesophageal acid exposure time which exceeded 4% was

considered abnormal, as this value was more than 2 SD above the mean value in a control population from our laboratory.¹¹

DEFINITIONS AND MANAGEMENT

A final diagnosis of GOR was made if histological evidence of oesophagitis was detected.¹² Cows' milk intolerance was defined by resolution of vomiting on an exclusion diet for 4–6 weeks and reappearance of the symptom with cows' milk challenge, using a milk with cows' milk protein and without lactose.

Infants with GOR were treated initially with positional therapy, cisapride (0.2 mg/kg three times a day), and ranitidine (4 mg/kg twice a day) for 12 weeks. Those subjects with GOR who did not improve after two weeks of treatment and in whom the CMPS and/or small bowel biopsy was abnormal underwent a trial during which cows' milk protein were eliminated from the diet.

In subjects who underwent the dietary trial the CMPS was repeated before and after the challenge. Endoscopy with small bowel biopsy was repeated in three infants with cows' milk intolerance after 12 weeks of a milk free diet.

STATISTICS

Data are reported as mean (SD) throughout. Normal/abnormal comparisons across all diagnosis groups were made using χ^2 analyses; Fisher's exact test was used for similar comparisons between any two groups. Comparisons of raw data between diagnosis groups were made using Wilcoxon's rank sum test considering the non-parametric nature to the data. A p value <0.05 was required for statistical significance in all cases.

Results

Using our diagnostic criteria, 16 (64%) of the infants with vomiting had GOR alone, four (16%) had GOR and cows' milk intolerance, four (16%) had cows' milk intolerance alone, and one (4%) remained undiagnosed. No infants were excluded for the study because of the diagnosis of lactose intolerance, enteric or other infections, immune deficiency, and coeliac disease. Clinical features of the 24 infants with a diagnosis of GOR and/or cows' milk intolerance are shown in table 1, and these infants will be the subject of the remainder of this report.

Table 1 Clinical features of the 24 patients with vomiting

	Infants with GOR (n=16)	Infants with CMI* (n=4)	Infants with GOR and CMI* (n=4)
Mean (SD) age in months	11.5 (7.5)	6.3 (2.9)	4.5 (1.3)
Male/female	10/6	2/2	3/1
No (%) with:			
Atopic dermatitis	0	1 (25)	1 (25)
Failure to thrive	5 (31)	0	2 (50)
Diarrhoea	0	0	1 (25)
Anaemia	3 (19)	1 (25)	1 (25)
Respiratory complaints	3 (19)	1 (25)	3 (75)

*CMI=cows' milk intolerance.

Oesophagitis was mild in 12 subjects (50%), moderate in six (25%), and severe in two (8%), whereas the remaining four infants (17%) had normal oesophageal histology. Using oesophageal histology as the standard, 24 hour pH monitoring was 83% accurate in correctly determining the presence or absence of GOR and detected 16 of the 20 (80%) diagnosed infants. In three of the subjects with GOR, total acid exposure time was normal, irrespective of the presence of moderate or severe oesophagitis. In one subject the 24 hour pH study was inadequate for analysis.

Results of the CMPS were significantly lower for infants with GOR alone (0.013 (0.007), range 0.003–0.057) than for infants with cows' milk intolerance with GOR or cows' milk intolerance alone (0.047 (0.010), range 0.037–0.062, and 0.052 (0.044), range 0.027–0.108, respectively; $p < 0.01$ for each comparison using Wilcoxon rank sum analysis). Using a test cut off of 0.022, the CMPS was abnormal in 100% of subjects with cows' milk intolerance with or without GOR compared with only 6% of subjects with GOR alone (table 2). Morphometric analysis of small bowel biopsies was completed in 22 infants, as the specimens were inadequate in two subjects. In contrast to the CMPS, the villus:crypt ratio was not satisfactory in identifying all subjects with cows' milk intolerance (table 2), although total villus atrophy was present in three of six patients with cows' milk intolerance (50%). No differences were found in the number of intraepithelial lymphocytes between any two groups (GOR 13.7 (10.4), range 6.4–42.0; cows' milk intolerance with GOR 13.9 (1.5), range 12.8–15.0; and cows' milk intolerance alone 10.5 (2.8), range 7.3–12.4). Those infants with GOR alone improved with antireflux treatment, whereas patients with GOR and cows' milk intolerance responded only to treatment for cows' milk intolerance. CMPS normalised in all subjects with cows' milk intolerance after elimination of cows' milk (figure). Small bowel mucosa normalised by morphometry in two of three subjects with total villus atrophy after 12 weeks of the exclusion diet. The third patient, despite the disappearance of vomiting, still showed an abnormal villus:crypt ratio, possibly because of the incomplete exclusion of milk protein from the diet.

Discussion

In this investigation we made three observations. Nearly all infants with persistent vomiting who were referred for gastroenterological investigation and had undergone a reasonable evaluation for other causes of vomiting had

GOR, cows' milk intolerance, or both as the final diagnosis. In fact we found that a single, non-invasive test of intestinal permeability was >95% accurate in identifying cows' milk intolerance in this study group. Additionally, we found that restriction of cows' milk from the diet was the correct management approach in subjects with cows' milk intolerance or the combination of cows' milk intolerance and GOR.

The invasive studies employed in this investigation were aimed at diagnosing both GOR and cows' milk intolerance. Endoscopy was fully accurate in establishing the presence of GOR because of the diagnostic convention used, but 24 hour pH monitoring was insensitive to more than 20% of patients with oesophagitis. Choosing between these tests was not important in the final analysis, however, as diagnosing GOR did not prove as essential for two reasons. First, nearly all the subjects who were derived from a referral practice were diagnosed as having GOR, cows' milk intolerance or both, and the combination occurred in a small minority. Consequently, diagnosing or excluding either one of the two conditions would be sufficient to direct an initial management approach in most subjects. Second, when the conditions were present together, the subjects improved only after elimination of cows' milk protein from the diet. Making the diagnosis of cows' milk intolerance is, thereby, of principal importance, and, in infants with both disorders, GOR appears to be a result of vomiting from milk intolerance. This finding is consistent with the study by Forget and Arends who showed that obstructive lesions of the upper gut and food allergies should be included when considering potential causes of reflux disease. These authors recommended that cows' milk allergy should be suspected in cause of 'intractable' reflux and investigated accordingly.³

The diagnosis of cows' milk intolerance is typically based on the resolution of symptoms on an exclusion diet and their reappearance with cows' milk challenge. At times, a more prompt diagnosis is desired or there remains a

Table 2 Sensitivity and specificity of intestinal permeability test (CMPS) and of morphometric analysis of small bowel biopsy in relation to diagnosis of cows' milk intolerance with or without GOR

	Sensitivity (%)	Specificity (%)
Cellobiose:mannitol ratio	100	93.7
Villus:crypt ratio	66.6	81.2

Cellobiose:mannitol ratio at diagnosis in patients with GOR, cows' milk intolerance (CMI) and GOR associated with CMI and after dietary exclusion in patients with CMI and GOR associated with CMI.

question of the diagnosis after an attempted dietary trial – for example, because of questionable compliance with dietary instructions. An intestinal biopsy can be helpful in diagnosis, as most children with cows' milk allergy have an enteropathy.⁴ The enteropathy, however, is non-specific and could be a result of other causes.⁵ Rosekrans *et al* have suggested that a single jejunal biopsy is useful in confirming the diagnosis,¹³ but others feel that the risk of biopsy is not justified for diagnosing this disorder.⁵ Consequently, the role of biopsy remains unsettled. An increased number of intraepithelial lymphocytes has been found in some children with cows' milk intolerance or with an abnormal permeability test, irrespective of the diagnosis.^{6,13} Our failure to make this observation may be related to a more chronic form of milk intolerance in our infants who are older than subjects in other reported series. Conflicting results have also been obtained concerning the value of measuring mucosal IgE plasmacytes in cows' milk intolerance.^{14,15} The controversy could relate to the relative non-specificity of conventional antisera.

Intestinal permeability tests are not commonly employed in the diagnosis of cows' milk intolerance, although the enteropathy associated with this disorder would be expected to have an effect on such studies. Our results confirm that the majority of infants with persistent vomiting from cows' milk intolerance have a functional derangement of intestinal permeability in which the mucosa is leaky to large molecules. In fact, abnormal permeability to cellobiose was found in all of our subjects with cows' milk intolerance, even though histopathological evaluation of small bowel biopsy was normal in a third. This finding could be related, in part, to patchy distribution of enteropathy, but our observations are in agreement with Strobel *et al* who previously demonstrated that abnormal sugar permeability can occur in the presence of unequivocally normal small bowel histology.⁷ An enteropathy with abnormal intestinal permeability has been reported in children with GOR,¹⁶ but this is probably an uncommon confounder. In our study, only 6% of infants with GOR had an abnormal intestinal permeability test and 19% an abnormal villus:crypt ratio.

The more invasive approach outlined in this study favoured identification of GOR, but

oesophageal mucosal biopsy and quantitation of reflux episodes will not distinguish 'primary' from 'secondary' GOR. Our results indicate that the simple permeability study is a good initial diagnostic test for rapid determination of cows' milk intolerance and should pre-empt more invasive studies in the evaluation of infants with persistent vomiting. Although our observations have been performed in a selected group of patients, we recommend the permeability test as the first line management in all children with persisting vomiting, before embarking on dietary exclusion trials. This recommendation is related to the non-invasive nature of the permeability test and to the long diagnostic procedure, including a challenge, which is initiated by the decision of excluding cows' milk proteins from the diet.

The authors wish to thank Dr Ray E Clouse, Washington University School of Medicine, St Louis, for his assistance in the critical review and preparation of this manuscript.

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