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Helicobacter pylori-Related Gastroduodenal Disease in Children

Diagnostic Utility of Enzyme-Linked Immunosorbent Assay

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To evaluate the accuracy of IgG and IgA serological tests in establishing a diagnosis of Helicobacter (Campylobacter) pylori *gastric infection, 60 children presenting with chronic abdominal pain were prospectively studied. Endoscopic antral biopsies were obtained and analyzed for the presence of* H. pylori *using three standard methods: culture and identification of bacterial isolates, microscopic examination for morphologically characteristic bacteria, and urease production by the biopsy specimen. Concomitantly obtained serum samples were analyzed for the presence of IgG and IgA antibodies against* H. pylori *surface antigens using enzyme-linked immunosorbent assay (ELISA). Thirty-four of 60 (56.6%) had histological evidence of chronic active gastritis, eight of whom (13.3%) also had evidence of* H. pylori *infection by at least one criteria. Six of the eight infected patients had* H. pylori *demonstrated by all three methods. Of the eight infected patients, seven had IgG antibodies against* H. pylori *(sensitivity of 87%) and six had IgA antibodies (sensitivity of 75%). Among the six patients who had* H. pylori *infection confirmed by all three methods, all had IgG antibodies (sensitivity of 100%). In the patients without evidence of* H. pylori *infection, the IgG ELISA had a specificity of 96% (50/52), and the IgA ELISA had a specificity of 100% (52/52). Our data suggest that serological testing for the presence of antibodies against* H. pylori *may be a useful diagnostic tool in screening children with chronic abdominal pain for the presence of gastric infection with* H. pylori.

KEY WORDS: *Helicobacter pylori;* gastritis; ELISA; pediatrics.

Helicobacter pylori (formerly named *Campylobacter pylori*) infection is associated with inflammatory gastroduodenal disease in adults (1, 2). Recently in this journal, we reported our experience with *H. pylori*-related gastritis in children (3). This study confirmed the findings of others (4, 5) concerning the relative importance of *H. pylori* in pediatric gastroduodenal disease. In most centers, the diagnosis of *H. pylori* infection has required histopathological and microbiological evaluation of biopsy samples obtained during upper gastrointestinal endoscopy. Studies by Perez-Perez et al (6) and Evans et al (7) have demonstrated the clinical usefulness of serological assays for antibodies against *H. pylori* in infected adult patients. Czinn et al (8) identified

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serum IgG antibodies against *H. pylori* in infected children with gastritis. Additionally, Oderda et al (9) reported that the titer of anti-*H. pylori* antibodies decreased following antimicrobial therapy in children with *H. pylori*-associated gastritis. However, neither of these studies have determined prospectively the diagnostic utility of serum anti-*H. pylori* IgG levels in predicting the presence of *H. pylori* infection. In the only prospective study in children, Drumm et al (10) confirmed the utility of serologic testing in predicting the presence of gastric *Helicobacter*. The present study prospectively correlates the results of an ELISA *H. pylori* assay with both histological evidence of gastritis and microbiological evidence of *H. pylori* infection in children with chronic abdominal pain to determine the validity of serologic evaluation in identifying those with *Helicobacter*-associated gastroduodenal disease.

MATERIALS AND METHODS

Study Population. Over a six-month period (January 1–June 30, 1989), we prospectively studied 60 patients referred to the Division of Pediatric Gastroenterology and Nutrition at New York Medical College for evaluation of chronic abdominal pain. Evaluation included endoscopic examination of the upper gastrointestinal tract. None of these patients had received nonsteroidal antiinflammatory agents, antibiotics, H_2 receptor antagonists, bismuth subsalicylate, or antacids for one month prior to endoscopy.

Endoscopy and Biopsy. After informed consent was obtained, patients were sedated with intravenous meperidine and diazepam. Esophagogastroduodenoscopy was performed using an Olympus G1F-P1OL endoscope (Olympus Corporation, Lake Success, New York). Biopsy specimens from the esophagus, duodenum, and antrum were fixed in 10% neutral-buffered formalin, sectioned, stained with hematoxylin and eosin, and examined under light microscopy. Mucosal inflammation was identified by the finding of increased numbers of acute/chronic mucosal inflammatory cells and graded according to the criteria of Whitehead (11). Chronic active gastritis was characterized by infiltration of the lamina propria by lymphocytes and destruction of the gastric glands by polymorphonuclear cells. A methenamine silver stain (GMS) was performed on each sample to identify *H. pylori*.

Microbiological Assay. Antral biopsies were immediately placed in 0.5 cc of 0.9% saline solution. A portion of the specimen was inoculated into a urea agar slant (BBC Microbiology System, Cookelsville, Maryland) and urease activity was indicated by a color change from tan to pink after incubation at 37° C for 18 hr. A second section was inoculated onto blood agar and Skirrow's media and incubated at 37° C in a microaerobic environment for seven days. Growth on the media was evaluated for *H. pylori* colonies. A third section was stained with silver methenamine stain (GMS) using standard techniques. The criteria for *H. pylori* infection was defined as histopathological evidence of gastritis in the presence of *H. pylori* on culture or silver methenamine stain or the presence of urease activity.

ELISA Assay for *H. pylori*. During the endoscopy, 5 cc of whole blood was obtained and serum was frozen at −4° C. Sera were coded and were examined blindly within two to four weeks. Sera were examined by *H. pylori*-specific ELISA tests as described previously (6). The *H. pylori* antigen was diluted in 0.5 M bicarbonate buffer (pH 9.6) to yield the optimal protein concentration of 10 μg/ml. A 0.1-ml aliquot of this solution was added to each well of a flat-bottom Immulon 2 plate (Dynatech Laboratories, Alexandria, Virginia). The screening serum dilutions were 1:800 for IgG and 1:50 for IgA determinations. Peroxidase conjugates of goat anti-human IgG and IgA were diluted 1:2000 and 1:500, respectively. Optical density values of the patients' sera were compared with a panel of reference sera, a ratio of >1.0 was considered indicative of the presence of anti-*Helicobacter* antibodies in the serum (6). All assays were done in triplicate. The intraassay and interassay variations were less than 5%, as estimated with positive and negative control sera.

Statistical Analysis. Statistical significance was determined utilizing chi-squared analysis with Yates' correction, Fisher's exact test and the Student's *t* test. Sensitivity was defined as true positive results/total positive results, and specificity as true negative results/total negative results.

RESULTS

The mean age of our patient population was 12.5 years (range 3–18) and the male–female ratio was 27:33. All 60 patients had epigastric abdominal pain and 27 (45%) had vomiting associated with their pain. The duration of symptoms was 10.3 ± 0.6 months (mean ± SEM). There were no differences in the age or sex of the patients or the duration of symptoms between *H. pylori*-positive and -negative patients; 24 patients (40.0%) had histologic gastritis (14 with chronic active gastritis, 10 with chronic gastritis); 11 (18.3%) had chronic duodenitis; 10 (16.6%) had both chronic acute gastritis and chronic duodenitis; 1 (1.6%) had esophagitis, and in 14 (23.3%), no pathological abnormalities were found.

Eight patients (13.3%), all with chronic active gastritis, had evidence of *H. pylori* infection by at least one criteria. Six of these eight patients had *H. pylori* demonstrated by all three methods (Table 1). Of the eight infected patients, seven had IgG antibodies against *H. pylori* (sensitivity of 87%) and six had IgA antibodies (sensitivity of 75%) (Table 2). The remaining patient had gastritis and gastric ure-

TABLE 1. ELISA RESULTS ASSOCIATED WITH POSITIVE CULTURE AND/OR SPECIAL STAINING FOR *H. pylori* (N = 8)

Patient	*Age (years)*	*Sex*	*Sx**	*Duration (months)*	*Gastritis*	*Culture*	*Stain*	*Optical Density ratio†*	
								IgA	*IgG*
1	14	M	p/v	2m	+	+	+	0.776	1.949
2	9	F	p	24m	+	−	+	0.775	2.466
3	11	M	p/v	1m	+	+	+	1.453	1.754
4	12	F	p/v	5m	+	+	+	2.017	1.284
5	15	M	p	4m	+	+	+	2.547	2.332
6	12	M	p/v	18m	+	+	+	0.660	1.171
7	14	M	p	6m	+	+	+	1.945	2.008
8	14	F	p	1m	+	−	+	0.266	0.383

*Symptoms: p = epigastric pain, v = vomiting.
†Optical density of precipitated patient sera/optical density of blank control sera.

ase activity but a negative culture and silver methenamine stain. Among the six patients who had *H. pylori* infection confirmed by all three methods, all had IgG antibodies (sensitivity of 100%) and five had IgA antibodies (sensitivity of 83%). In those patients without evidence of *H. pylori* infection, the IgG ELISA had a specificity of 96% (50/52) and the IgA ELISA had a specificity of 100%. Two patients without *H. pylori* infection had a positive IgG (false positive), but a negative IgA.

The IgA and IgG optical density ratio (mean ± SEM) of the *H. pylori*-negative patients was 0.237 ± 0.02 and 0.276 ± 0.02, respectively, and the IgA and IgG optical density ratio of the *H. pylori*-positive patients was 1.304 ± 0.30 and 1.668 ± 0.26, respectively. These optical density ratios were significantly different ($P < 0.001$).

DISCUSSION

Using standard methodologies, we found gastric infection with *H. pylori* in 13.3% of the children evaluated for chronic abdominal pain. Although our patient numbers are small, the clinical characteristics of our population, the incidence of *H. pylori* and its association with gastritis in confirm our previous experience in children (3) and are comparable with other reports (4, 5, 10). However, the techniques used to identify gastric *Helicobacter* require direct analysis of biopsies obtained during upper endoscopy, with its associated expense and discomfort. Furthermore, prior administration of antibiotics, H_2-receptor antagonists, bismuth subsalicylate, and antacids prior to the procedure (12) and oropharyngeal anesthesia during the procedure (13) may interfere with isolation of *H. pylori* from the biopsy specimen. The patchy nature of the infection can also result in sampling errors, further diminishing the reliability of biopsy analysis.

Recently, Perez-Perez et al (6) and Evans et al (7) have reported that serological assays for anti-*Helicobacter pylori* antibodies in adult patients are both specific and sensitive for the diagnosis of gastric *H. pylori* infection. In a retrospective study involving 30 children, Czinn et al (8) reported that anti-Helicobacter IgG titers were higher in culture-positive than control patients and confirmed that these antibodies were against three outer membrane proteins of *H. pylori*. However, the authors did not determine the efficacy of prospective serologic testing in identifying those patients with gastric *H. pylori* infection. Oderda et al (9) followed serum

TABLE 2. SUMMARY OF RELIABILITY OF IgG AND IgA-SPECIFIC ELISA IN DETECTING CHILDREN WITH GASTRIC INFECTION WITH *H. pylori* (N = 60)

Criteria for diagnosis of H. pylori *infection*	*Positive IgA ELISA*	*Positive IgG ELISA*	*Positive IgA and IgG*
Gastritis + culture only			
Sensitivity	5/6 (83%)	6/6 (100%)	5/6 (83%)
Specificity	54/54 (100%)	52/54 (98%)	52/54 (98%)
Gastritis + culture, urease or stain			
Sensitivity	6/8 (75%)	7/8 (87%)	6/8 (75%)
Specificity	52/52 (100%)	50/52 (96%)	52/52 (100%)

IgG antibody concentrations in 32 children with *H. pylori*-associated gastritis treated with amoxycillin and tinidazole. After six weeks of therapy, 94% of their patients cleared the organism and had a significant decrease in serum IgG concentrations coincident with this response. In a recent prospective study in children undergoing upper endoscopy for evaluation of abdominal pain, Drumm et al (10) reported that 25% had histologic or culture evidence of *H. pylori*-associated gastritis. In their population, IgG ELISA was both sensitive (97%) and specific (99%) for the diagnosis of gastric *Helicobacter*.

We found that in our patients, IgG-specific ELISA for antibodies to *H. pylori* had a positive predictive value of 87% and a negative predictive value of 96%, confirming the experience of Drumm et al (10). The one patient in whom the both IgG and IgA ELISA failed to identify infection with *H. pylori* had a positive urease test, but negative culture and silver stain. Recently, *Gastrospirillum hominis*, a spiral organism that is morphologically distinct from *H. pylori*, has been identified as a source of gastric urease activity (14, 15). It is possible that the gastric urease activity originated from such a non-*Heliobacter* organism and, therefore, represents a false positive urease (16) rather than a false negative ELISA. If this patient was removed from analysis, the positive predictive value of the ELISA would be 100%. In addition, requiring both IgA- and IgG-specific ELISA to be positive would increase the negative predictive value to 100%. However, the small number of *H. pylori*-positive children in our study may have affected our results. Furthermore, the recent interest in using serological testing to confirm the presence of gastric *Helicobacter* highlights the need for standardization of the ELISA assay among testing centers.

The relative usefulness of culture, histology, serology, and urease testing in diagnosing *H. pylori* infection in adult patients was investigated by Schnell and Schubert (16) and Vaira and Holton (17). They found that except for the reduced sensitivity of biopsy culture (70% and 76%, respectively), the sensitivity and specificity of the other methods approximated 90%. Although similar comparative studies in children are lacking, and Carr (18) reported that rapid diagnosis of *H. pylori* using urease testing was both 100% sensitive and specific. Other than serologic evaluation, the [^{13}C] or [^{14}C]urea breath test is the only noninvasive method for detecting gastric urease activity (19, 20), presumably due to *H. pylori*. However, the inconvenience, cost, and radiation exposure associated with the administration of the carbon isotopes limit the clinical utility of the breath test.

Future confirmation of our results may support the use of serologic testing as a screening procedure, permitting the presumptive treatment of *H. pylori* infection in children with abdominal pain prior to endoscopic biopsy.

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