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Serological Detection of *Helicobacter pylori* Antibodies in Children and Their Parents

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The antibody response to *Helicobacter pylori* was examined in 56 children (ages 5 to 18) to determine whether serological tests can be used for diagnosis. Twenty-four children (43%) were *H. pylori* positive and 32 children (57%) were *H. pylori* negative by culture and histological examination of endoscopic biopsy specimens. The immune response was also examined in 39 nonendoscoped parents of the children. *H. pylori*-specific immunoglobulin G (IgG) and IgA antibodies were detected by the flow microsphere immunofluorescent assay (FMIA). IgG was also detected by using the Pyloristat enzyme-linked immunosorbent assay (ELISA). The sensitivity, specificity, and positive and negative predictive values for the FMIA for IgG were 100, 97, 96, and 100%, respectively. The respective values for the Pyloristat ELISA for IgG were 96, 94, 92, and 97%. The respective values for the FMIA for IgA were 50, 100, 100, and 73%. Both assays identified the same 19 parents as IgG positive, while FMIA identified 17 of the 19 parents as IgA positive.

Helicobacter pylori has been established as the cause of chronic active type B gastritis and as the most important etiological agent for relapsing duodenal ulcers in all age groups (10, 11). The humoral immune response to *H. pylori* infection is reflected in the immunoglobulin levels in serum (7). While the combination of culture and histological examination of gastric biopsy specimens has been considered the "gold standard" for the diagnosis of *H. pylori*, serodiagnosis has proven to be more sensitive (12, 13). Serological tests for *H. pylori* are advocated to replace endoscopy as a primary diagnostic procedure in patients under the age of 45 years (9, 10). We previously reported the development of a rapid and easily performed flow microsphere immunofluorescent assay (FMIA) (1). FMIA uses a sonicated, urease-enriched, whole-cell *H. pylori* antigen to detect specific antibodies which are collected and quantified by using a flow cytometer. The FMIA for immunoglobulin G (IgG) was 100% sensitive and 89% specific for samples from adults. The Pyloristat enzyme-linked immunosorbent assay (ELISA) for IgG was 96% sensitive and 89% specific with samples from the same patient population (1).

It has been stated that children differ from adults in their antibody responses to antigenic *H. pylori* proteins and that the cutoff values for positivity are different in children and adults (2, 8, 13). Serological tests for the detection of *H. pylori* in children must be validated in that population. The present study was carried out by using FMIA to measure the IgG and IgA responses to *H. pylori* in 56 endoscoped children whose *H. pylori* status was determined by the results of culture and histology and in 39 nonendoscoped parents of the study children (3). A previously established negative adult pool was assessed to determine whether or not the same cutoff value could be used with children (1). The results of the FMIA for IgG for the children and their parents were compared with the results obtained by the Pyloristat ELISA for IgG.

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MATERIALS AND METHODS

Patients. Two antral biopsy specimens were obtained from 56 children (ages, 5 to 18 years; mean, 12.6 years) referred to the Gastroenterology Department at the Hospital for Sick Children in Toronto with upper gastrointestinal symptoms (3, 4). Endoscopies were performed with Olympus GIF gastroscopes either under general anesthesia or after sedation. Informed parental consent was obtained for all procedures. Serum samples were collected when venipuncture was performed for preendoscopic blood work. Serum samples were also collected from 39 consenting parents (3, 4). The serum samples were coded and stored in cryovials at −70°C. The study was approved by the Human Subjects Review Committee of the Hospital for Sick Children.

Culture and histology. One antral biopsy specimen was fixed in formalin, sectioned, and stained with hematoxylin and eosin. Sections were subsequently stained with silver to identify *H. pylori* organisms. The second antral biopsy specimen was immediately taken to the microbiology laboratory. The tissue was ground with a pestle and was then inoculated onto Belo-Horizonte agar plates, which were incubated at 37°C under reduced oxygen for up to 7 days. Organisms were identified as *H. pylori* if they were gram negative with a characteristic spiral shape and had urease, oxidase, and catalase activities but no hippurase or nitrate reductase activity and were susceptible to cephalothin and resistant to nalidixic acid (4).

FMIA. The coded serum samples were assayed by FMIA as described previously (1). Briefly, the serum was diluted and reacted at 37°C with *H. pylori* antigen-coated microspheres. The microspheres were washed by centrifugation and were resuspended in either fluorescein isothiocyanate-conjugated Fab-specific goat anti-human IgG (Sigma Chemical Co., St. Louis, Mo.) or *r*-phycoerythrin-conjugated goat anti-human α-chain-specific IgA (Sigma). After incubation and washing,

TABLE 1. Comparison of serological methods for detection of antibodies to *H. pylori* in children

Method	Result	No. of patients who were C/H[a]:		Percent[b]			
		Positive	Negative	Sensitivity	Specificity	PPV	NPV
FMIA for IgG	Positive	24	1	100	97	96	100
	Negative	0	31				
FMIA for IgA	Positive	12	0	50	100	100	73
	Negative	12	32				
ELISA for IgG	Positive	23	2	96	94	92	97
	Negative	1	30				

[a] C/H, culture and/or histology.
[b] PPV, positive predictive value; NPV, negative predictive value.

the microspheres were kept at 4°C in the dark until analysis. Three aliquots of pooled sera from six biopsy-negative, asymptomatic adults were used to establish the cutoff value for the test (1). Negative, weakly positive, and strongly positive control sera were used to validate each set of assays. Collection of the microspheres and analysis of the data were carried out by using a flow cytometer, and values were determined relative to the values for the negative pool by using the Kolmogorov-Smirnof (KS) statistical method (1). For the data analyzed by using KS statistics, a difference of ≥ 8 was significant; therefore, relative to the negative pool, the cutoff value for a positive result was a KS value of 8.

Pyloristat ELISA. The Whittaker ELISA IgG kit was used according to the manufacturer's instructions. Each of the three 15-min incubations was carried out at room temperature with shaking. Coded serum samples were tested at a dilution of 1:20, and sera that gave equivocal results were retested. Plates were read at 550 nm with a Dynatech microplate reader. By using the standards and control sera included with the kit, a curve was derived and validated and was used to calculate predictive index (PI) values. PI values of 0.80 to 0.99 were equivocal, PI values of ≥ 1.00 were positive, and PI values of ≤ 0.79 were negative.

Statistical analysis. Sensitivity, specificity, and positive and negative predictive values were calculated, and correlation coefficients were determined (Systat 5.2; Systat Inc., Evanston, Ill.).

RESULTS

Determination of *H. pylori* status. Children were considered to be positive if *H. pylori* was demonstrated to be present in antral biopsy specimens by culture or histology, or both. Of the 56 children in the study, 24 (43%) were positive for *H. pylori* and 32 (57%) were negative for *H. pylori*. Of the 32 *H. pylori*-negative children, 23 had normal antral histologies, 6 had gastritis caused by Crohn's disease, and 3 had eosinophilic gastroenteritis (3). Duodenal ulcers were observed in 12 of the *H. pylori*-positive children. The *H. pylori* status of the nonendoscoped parents was determined by the FMIA for IgG and the ELISA for IgG. Both tests identified the same 19 *H. pylori*-positive and 20 *H. pylori*-negative parents. Thirteen parents of *H. pylori*-positive children and 6 parents of *H. pylori*-negative children were positive for *H. pylori*. Three parents of *H. pylori*-positive children and 17 parents of *H. pylori*-negative children were negative for *H. pylori*.

FMIA. Relative to the previously established negative pool, 19 parents were positive for IgG by FMIA and 20 were negative, and 17 were positive for IgA by FMIA and 22 were negative. The FMIA for IgG identified all of the 24 biopsy specimen-positive children and 1 of the 32 biopsy specimen-negative children (Table 1). No change in the negative pool was necessary to efficiently detect the presence of IgG antibodies to *H. pylori* in children. The FMIA for IgA identified 12 of the biopsy specimen-positive children but failed to identify 12 biopsy specimen-positive children, and all 32 biopsy specimen-negative children were negative by the FMIA for IgA (Table 1).

Pyloristat ELISA. By using the cutoff value stated by the manufacturer, the Pyloristat ELISA for IgG identified 23 of the 24 biopsy-positive children and 2 of the 32 biopsy-negative children (Table 1). Nineteen of the parents were IgG positive and 20 were IgG negative. There were no equivocal results.

Comparison of results. The FMIA for IgG detected 24 true-positive, 1 false-positive, no false-negative, and 31 true-negative children. The Pyloristat ELISA for IgG detected 23 true-positive, 2 false-positive, 1 false-negative, and 30 true-negative children. One biopsy-negative 18-year-old without antral gastritis was positive by both the FMIA for IgG and the ELISA for IgG. One biopsy-negative 17-year-old with Crohn's disease was negative by the FMIA for IgG and positive by the ELISA for IgG. One biopsy-positive 13-year-old with a duodenal ulcer was positive by the FMIA for IgG and negative by the ELISA for IgG. Both the FMIA for IgG and the ELISA for IgG identified the same 19 *H. pylori*-positive parents. The correlation coefficient for the two tests was highly significant (Fig. 1). In each test, the mean and range of values for children and their parents were similar (Fig. 2). The FMIA for IgA detected 12 true-positive, 12 false-negative, and 32 true-negative children and 17 of the 19 parents who were positive by the FMIA for IgG. The positive and negative mean values were substantially further removed from the cutoff value of a KS of 8 by the FMIA for IgG than by the FMIA for IgA (Fig. 2).

DISCUSSION

Serodiagnostic tests permit the rapid, noninvasive, sensitive, and specific detection of *H. pylori* (2, 7, 9). Measurement of *H. pylori* antibodies has been shown to be slightly more sensitive than the conventional gold standard combination of culture and histology because of the patchy nature of infection which may lead to endoscopic sampling error (5). Endoscopic examination of children presents some difficulties and often must be performed under general anesthesia. Serodiagnosis of *H. pylori* infection has been advocated as a replacement for endoscopy as a primary diagnostic test in patients under 45 years of age (6, 9).

It has been stated that the cutoff values for the detection of

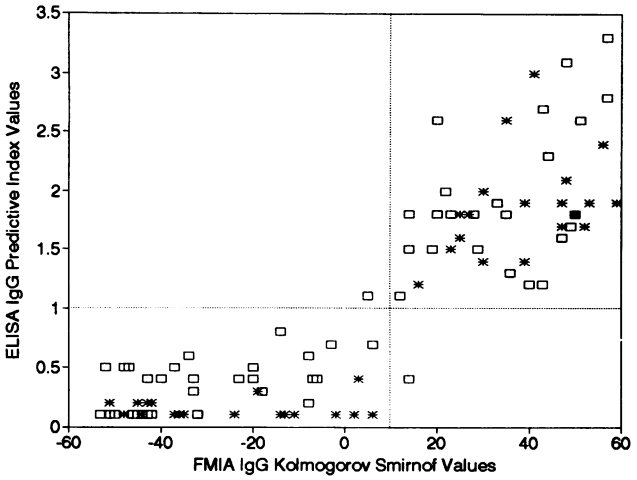

FIG. 1. Correlation of the KS statistical values of the FMIA for IgG and the PI values for the Pyloristat ELISA for IgG. The symbols represent children (□) and parents (*). The cutoff value for a positive FMIA result is 8.0, and that for a positive ELISA result is 1.0. There were no results in the equivocal range (between 0.80 and 0.99). The correlation coefficients for the two tests were 0.891 for children, 0.901 for their parents, and 0.887 ($P < 0.0001$) overall.

H. pylori antibodies by ELISA are different in children and adults (2, 8, 13). In our previously reported FMIA for IgG, the cutoff value was established relative to the value for pooled sera from biopsy specimen-negative, asymptomatic adults (1). We anticipated that it might be necessary to use pooled serum from biopsy specimen-negative, asymptomatic children to establish the cutoff value for positive results in children. However, the previously established cutoff value for the negative adult pool efficiently distinguished infected and noninfected children. The Pyloristat ELISA for IgG gave virtually identical results with the cutoff value stated for the kit. The FMIA for IgG and the ELISA for IgG gave identical results for the 39 parents.

None of the children or parents produced an IgA response in the absence of an IgG response. Many of the children failed to mount a significant systemic IgA response to *H. pylori*; therefore, detection of IgA antibodies is not sufficiently sensitive for the diagnosis of *H. pylori* infections in children.

We conclude that both the FMIA for IgG and the Pyloristat ELISA for IgG are sensitive and specific tests for the noninvasive diagnosis of *H. pylori* infection in children.

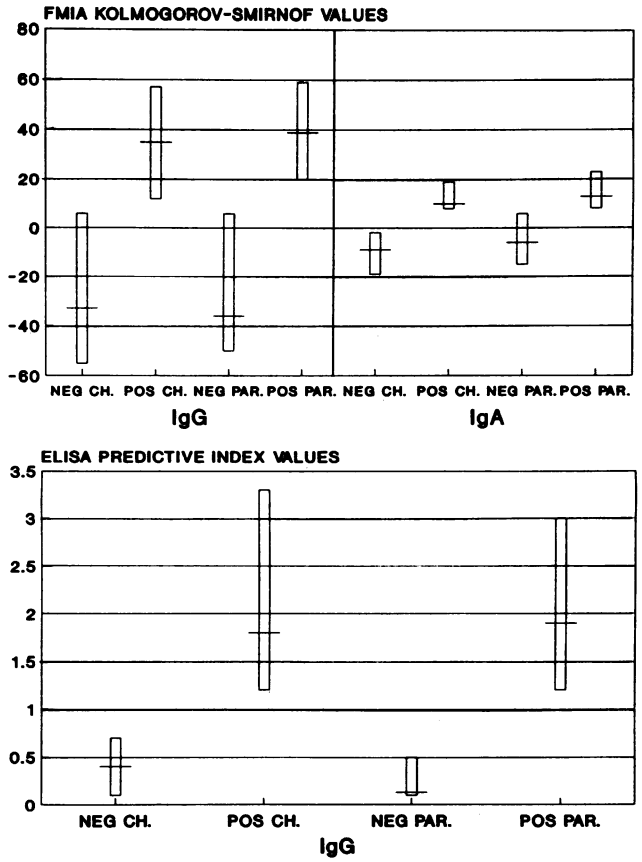

FIG. 2. Mean and range of the positive and negative KS values for the FMIA for IgG and IgA and the PI values for the ELISA for IgG for children and their parents. The mean values of the FMIA for IgG were −33 for negative children, 35 for positive children, −36 for negative parents, and 39 for positive parents. The mean values of the FMIA for IgA values were −10 for negative children, 11 for positive children, −6 for negative parents, and 14 for positive parents. The mean values of the ELISA for IgG values were 0.35 for negative children, 1.89 for positive children, 0.13 for negative parents, and 1.91 for positive parents. CH, children; PAR, parents.

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