

Salivary Immunoglobulin G Assay To Diagnose *Helicobacter pylori* Infection in Children

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An in-house enzyme-linked immunosorbent assay (ELISA) for measurement of *Helicobacter pylori*-specific immunoglobulin G (IgG) and IgA in saliva was evaluated by comparison with histopathologic (Giemsa staining) and biochemical (urease quick test) examination of gastric biopsy specimens obtained from 112 children referred for diagnostic gastroscopy. Serum *H. pylori* IgG was also measured in a subgroup of 50 children by the same ELISA. Salivary *H. pylori* IgG levels were significantly higher in *H. pylori*-positive ($n = 57$) than in *H. pylori*-negative ($n = 55$) children ($P < 0.001$). The sensitivity and specificity of the salivary IgG test were 93 and 82%, respectively; the positive and negative predictive values were 84 and 92%, respectively; and the accuracy was 87.5%. Salivary *H. pylori* IgA did not distinguish *H. pylori*-positive from *H. pylori*-negative children. The performance of serum *H. pylori* IgG was slightly (3 to 6%) better than that of salivary *H. pylori* IgG. The salivary IgG test can be considered a useful tool for the screening of *H. pylori* infection in children.

Helicobacter pylori infection is the commonest chronic bacterial infection in humans and the major cause of chronic gastritis, peptic ulcer, and possibly gastric carcinoma and lymphoma (5, 12, 14, 18). The prevalence of the infection ranges between 25 and 90% and increases with age (12). Recent evidence indicates that in most populations *H. pylori* infection is commonly acquired in childhood (1, 7, 11, 15, 22). Thus, there is growing appreciation that studies with children are important in determining the epidemiology of *H. pylori* infection, providing valuable information on the natural history of the disease. Furthermore, the finding that *H. pylori* influences growth emphasizes the clinical relevance of early detection of *H. pylori* infection in childhood (17, 19). Studies targeted at the pediatric population have been indicated as a very high priority for future research on *H. pylori* infection (20). Salivary immunoglobulin G (IgG) antibodies specific against *H. pylori* have already been used for adults and in an important epidemiological study of children even without the test being validated, and this demonstrates the need for a noninvasive and inexpensive test (16, 17, 21). We have recently confirmed that in an adult population, salivary IgG antibodies to *H. pylori* may offer a noninvasive method for detecting *H. pylori* infection (8) and that their levels paralleled those of specific circulating IgGs (9). In the present study, we attempted to validate the salivary test with children. In particular, we assessed the accuracy of salivary *H. pylori* antibodies of the IgG and IgA classes in detecting biopsy-proven *H. pylori* infection, and we compared the performance of the salivary assay with that of serology.

(This work was presented in part at Digestive Disease Week, Washington, D.C., 11 to 14 May 1997 [10]).

A total of 112 children (55 males and 57 females; age range, 2 to 18 years; median age, 11 years) consecutively referred for diagnostic upper-gastrointestinal endoscopy in four gastroenterology and pediatric gastroenterology centers, two in Catan-

zaro (southern Italy) and two in Turin and Novara (northern Italy), were enrolled. Seven children who had taken proton pump inhibitors and/or antibiotics within the preceding 8 weeks and five children who did not have sufficient saliva for measurement were excluded from the study. The indications for endoscopy were as follows: recurrent abdominal pain ($n = 82$), failure to thrive or suspected celiac disease ($n = 21$), hematemesis ($n = 3$), vomiting ($n = 2$), melena ($n = 1$), dysphagia ($n = 1$), unexplained iron deficiency anemia ($n = 1$), and purpura ($n = 1$). Upper-gastrointestinal endoscopy was performed with Olympus GIF-XP20 and Fujinon UGI-PE7 gastroscopes. During endoscopy, at least three biopsy specimens were taken: one from the antrum for a urease quick test (Yamanouchi Pharma, Milan, Italy), and one from the antrum and one from the gastric body for histological examination. One to two milliliters of unstimulated saliva was collected in 5 to 10 min just by having the patient spit into a clean glass. Venous blood was also drawn from a subgroup of 50 children (25 males and 25 females; age range, 2 to 18 years; median age, 11 years). All samples were taken from each patient before endoscopy was performed. Samples were spun, and supernatants were coded and stored at -20°C until tested. Informed consent was obtained from the parents and the children. The study was approved by the Department Ethical Committee (University of R. Calabria, Catanzaro, Italy) and approved by and conducted within the Working Group of Gastric Diseases of the Italian Society for Pediatric Gastroenterology and Hepatology (SIGEP). Children were classified as *H. pylori* positive when the urease quick test was positive and/or the organism was identified in the Giemsa-stained section of either the antral or the body gastric sample, or both.

ELISA. All saliva samples were assayed for *H. pylori* IgG and IgA as previously described for an adult population (8). Saliva samples and conjugate dilutions were optimized, and a linear standard curve ($r = 0.97$) was obtained by using a reference pool of samples from children. In order to achieve optimal accuracy, we modified the working dilution of saliva samples from 1:4, previously utilized for samples from adults, to 1:2. In brief, a whole *H. pylori* strain isolated from samples of an

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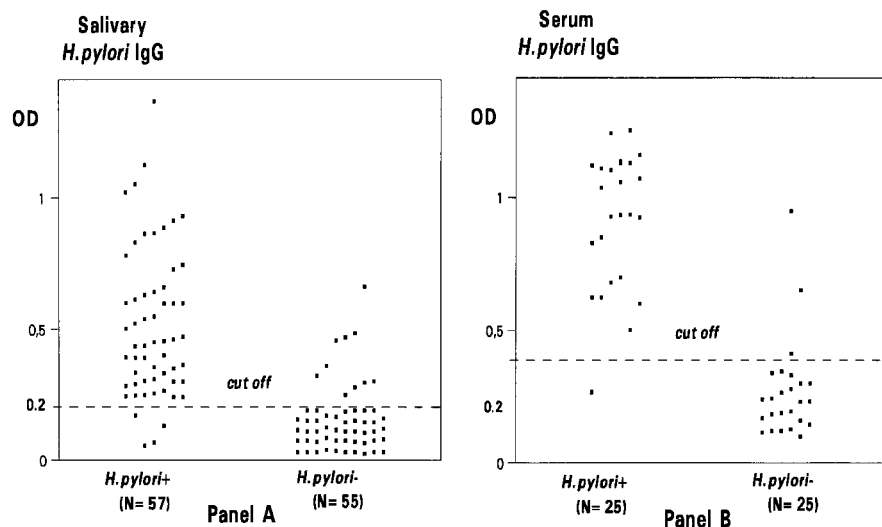


FIG. 1. Absorbancies of salivary ($n = 112$) and serum ($n = 50$) *H. pylori* IgG in children according to *H. pylori* status. Cutoff ODs of 0.200 and 0.400 were chosen for the saliva and serum assays, respectively.

Italian population (13) was used as a source of antigen, diluted with coating buffer, added to each well, and incubated for 2 h at 37°C. The plates were washed with washing buffer, and binding sites were blocked by addition of 2% serum albumin in washing buffer and incubation for 18 h at 4°C. Diluted saliva samples were added separately to each well and incubated for 90 min at 37°C. Anti-human IgG- or anti-human IgA-peroxidase conjugates (Sigma, St. Louis, Mo.) were added and incubated for 1 h at 37°C. Substrate was left for 45 and 60 min (for IgG and IgA, respectively), and finally a stopping solution (10 μ l of demineralized water plus 9.6 mg of sodium fluoride) was added and the absorbance of the wells at 405 nm was read with a microELISA plate reader (Labsystem Multiskan MCC/340; Labsystem Oy, Helsinki, Finland). All samples were tested in duplicate at the same time, in different plates (96 wells per plate). Results were expressed as mean absorbance (optical density [OD]) \pm 1 standard deviation (SD). The intra-assay coefficient of variation was calculated for each saliva sample. In addition, 10 samples were tested five times in the same assay, and 20 samples were collected on two different occasions from a subset of 10 children and tested in the same assay. In order to calculate the interassay coefficient of variation, 20 samples were tested in duplicate in four different assays. The mean values of the intra-assay and interassay coefficients of variation were below 10%. Furthermore, there was no variation with repeated tests in terms of *H. pylori* positivity and negativity. The OD cutoff of 0.200 was calculated by adding 2 SDs to the mean OD obtained from 20 saliva samples of biopsy-proven *H. pylori*-negative children. Serum *H. pylori* IgG was also measured by means of the same enzyme-linked immunosorbent assay (ELISA) (8).

Sensitivity, specificity, positive and negative predictive values, and accuracy of the salivary and serum assays were calculated together with 95% confidence intervals. Student's *t* test was used to compare levels of salivary or serum absorbance between *H. pylori*-positive and *H. pylori*-negative children. The relationship between salivary and serum absorbancies was measured by the correlation coefficient (Pearson's test). Probability values (two-sided tests) of less than 0.05 were considered significant.

Fifty-seven (51%) children (median age, 11 years) had evi-

dence of *H. pylori* infection. Salivary *H. pylori* IgG levels were significantly higher in *H. pylori*-positive children than in *H. pylori*-negative children (mean ODs, 0.495 ± 0.292 versus 0.150 ± 0.131 ; $P < 0.001$). Based on an OD cutoff of 0.200, we found that 4 *H. pylori*-positive children were saliva negative and 10 *H. pylori*-negative children were saliva positive (Fig. 1A). The sensitivity and specificity of salivary *H. pylori* IgG were 93% [95% confidence interval, 83 to 98%] and 82% [95% confidence interval, 70 to 91%], respectively; the positive and negative predictive values were 84% [95% confidence interval, 73 to 92%] and 92% [95% confidence interval, 80 to 98%], respectively; and the accuracy was 87.5% [95% confidence interval, 80 to 93%]. Serum *H. pylori* IgG levels were significantly higher in *H. pylori*-positive than in *H. pylori*-negative children (mean ODs, 0.953 ± 0.242 versus 0.269 ± 0.194 ; $P < 0.001$), giving one false-negative result and three false-positive results [96% [95% confidence interval, 80 to 100%] sensitivity, 88% [95% confidence interval, 69 to 97%] specificity, 89% [95% confidence interval, 71 to 98%] positive predictive value, 96% [95% confidence interval, 78 to 100%] negative predictive value, and 92% [95% confidence interval, 81 to 98%] accuracy] (Fig. 1B). Salivary *H. pylori* IgA levels were similar in *H. pylori*-positive and *H. pylori*-negative children (mean ODs, 0.317 ± 0.213 versus 0.343 ± 0.245 ; not significant). There was a good correlation between salivary and serum *H. pylori* IgG levels ($r = 0.762$; $P < 0.001$) (Fig. 2).

It is increasingly agreed that studies with children are crucial in investigating the epidemiology and natural history of *H. pylori*-related diseases. Nevertheless, a simple, inexpensive, and painless method for *H. pylori* testing in children is not currently available. Recent observations from our laboratory (Università di R. Calabria, Catanzaro, Italy) have shown that for an adult population, salivary *H. pylori* IgG was a fairly good diagnostic test. In contrast to adults, *H. pylori*-infected children may fail to uniformly mount a systemic humoral immune response by specific immunoglobulins (3, 23). Since our salivary test measures specific antibodies, the possibility that differences between the humoral immune responses of adults and children could affect the accuracy of *H. pylori* testing in children has led us to validate the test for the pediatric population. As suggested for serum antibody determination, we used chil-

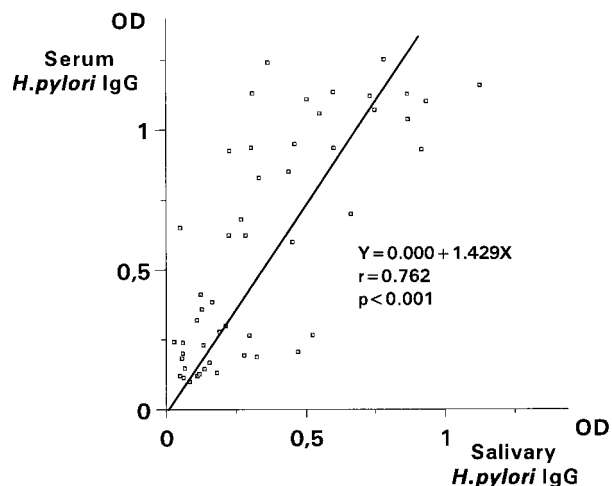


FIG. 2. Correlation between salivary and serum *H. pylori* IgG levels ($n = 50$ children). Salivary and serum ELISA determinations for each subject are given as ODs.

dren's serum samples to standardize the assay (2, 4). The positive and negative predictive values of any diagnostic test depend on the prevalence of infection in the referred population. Our test worked well with a prevalence of approximately 50%. Nevertheless, with a lower prevalence of *H. pylori* infection, the higher negative predictive value will enable us to be more confident that the child is free from the infection. Such a noninvasive test could accurately identify subjects at high risk of acquiring the infection at an early age in order to select them for a vaccination program, which hopefully will soon be available (6).

Using a widely accepted "gold standard" and a large number of patients, we demonstrated for the first time that the salivary IgG test is an accurate method with a high sensitivity and a high negative predictive value for detecting *H. pylori* infection in children. Furthermore, saliva collection is easy, saliva does not need any particular handling or storage, and use of this test could reduce the risk of blood-borne infection. The salivary IgG test fulfills requirements that make it more suitable than other available tests, and it can be regarded as a useful tool for the screening of *H. pylori* infection in children.

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