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.

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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 μl 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]) ± 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 absorbances 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 evidence 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 92% (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. 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.](http://jcm.asm.org/DownloadedFrom)
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.

children’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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REFERENCES


