ARTICLE

Efficacy of Noninvasive Tests in the Diagnosis of *Helicobacter pylori* Infection in Pediatric Patients

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Background: *Helicobacter pylori* infection is likely acquired in childhood. *Helicobacter pylori* is recognized as a cause of gastritis and peptic ulcer.

Objective: To investigate some noninvasive tests, particularly *H pylori* fecal antigen, for the diagnosis of *H pylori* infection in comparison with the gold-standard invasive test, esophagogastroduodenoscopy with biopsy.

Methods: We studied 250 patients (102 male; age range, 3-18 years) who underwent esophagogastroduodenoscopy with biopsy (histologic examination and rapid urease test) for a suspicious upper gastrointestinal disease; in all of them, fecal *H pylori* antigen, serum *H pylori* immunoglobulin G, and cytotoxin-associated gene product A immunoglobulin G were measured. Sensitivity and specificity of noninvasive tests were compared with those of the gold-standard esophagogastroduodenoscopy with biopsy.

Results: Ninety-three patients (37%) had positive histopathologic (Giemsa staining) and rapid urease test results. The *H pylori* fecal antigen revealed a sensitivity of 97%, a specificity of 98%, a positive predictive value of 97%, and a negative predictive value of 98%; serum *H pylori* immunoglobulin G had a sensitivity of 86%, a specificity of 80%, a positive predictive value of 72%, and a negative predictive value of 90%; and serum cytotoxin-associated gene product A immunoglobulin G had a sensitivity of 83%, a specificity of 80%, a positive predictive value of 71%, and a negative predictive value of 89%.

Conclusions: Our study demonstrates that among noninvasive and easily applicable tests, particularly in small children, *H pylori* fecal test is simple, suitable, and has high accuracy for the screening of *H pylori*–positive patients.

Arch Pediatr Adolesc Med. 2005;159:238-241

HELICOBACTER PYLORI INfection is common, even in pediatric patients. In European children, serum positivity of immunoglobulin G (IgG) antibodies is almost 5% to 15%. The infection is likely acquired early in childhood; in developing countries, the incidence of the infection in infancy may be up to 50%.[1,2]

Helicobacter pylori is now recognized as related to gastritis and peptic ulcer disease. Furthermore, *H pylori* infection is involved in the pathogenesis of gastric adenocarcinoma and lymphoma in adulthood.[3,4] However, the *H pylori* role in dyspepsia and extradigestive diseases (vascular, immunological, and skin pathologic features; sideropenic anemia; and delayed statural growth)[5-11] is quite controversial.

Children present an ideal population for studying the interaction between *H pylori* and gastric mucosa because pediatric age is free from common causes of secondary gastrointestinal diseases (drugs, tobacco, and alcohol).[12] Also, the natural history of diseases related to *H pylori* is conditioned by the early acquiring of the bacterium.[13]

The gold-standard diagnostic test remains endoscopy with biopsy analyses (histologic analysis and urease rapid test or culture of gastric biopsy specimens).[14,15] Invasive tests are not always suitable for the pediatric population; there is an increased interest in noninvasive tests for children. Serum (IgG and cytotoxin-associated gene product A [*CagA*] IgG) and salivary (IgG) antibodies are used for screening in epidemiological studies of large populations.[16-19] The detection of *H pylori* antigen in feces is considered useful for diagnosis and to confirm eradication.[20]

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Table 1. Sensitivity, Specificity, and Positive and Negative Predictive Values of Noninvasive Tests*

Noninvasive Tests	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Serum *Helicobacter pylori* immunoglobulin G	86	80	72	90
Serum cytotoxin-associated gene product A immunoglobulin G	83	80	71	89
Salivary *Helicobacter pylori* immunoglobulin G	66	91	82	79
Helicobacter pylori fecal antigen	97	98	97	98

*Data are presented as percentage.

The aim of this prospective study has been to validate the accuracy of some noninvasive diagnostic methods, particularly *H pylori* fecal antigen, in comparison with the invasive gold standard (endoscopy with biopsy analyses).

METHODS

The study involved 250 consecutive patients (102 male; mean age, 11 years; range, 3-18 years) with a suspicion of upper gastrointestinal disease (symptoms including recurrent abdominal pain, diurnal or nocturnal abdominal pain, nausea, vomiting, or iron deficiency) who were referred, in the last 2 years, to undergo esophagogastroduodenoscopy with biopsy. The recurrent abdominal pains were of such intensity that they prevented the normal activity of the child and were recurrent at least once a month during 3 months with asymptomatic intercritical periods.

In all the patients, noninvasive tests such as immunoassay for serological antibodies (IgG and *CagA*) against *H pylori* and detection of *H pylori* antigen in feces were measured. In 123 patients, salivary (IgG) antibody was also evaluated.

After signed informed consent was obtained, all patients received an upper gastrointestinal endoscopy with gastric biopsy, which is the gold standard for *H pylori* diagnosis. The biopsies were utilized for histologic examination and urease rapid test. The patients were considered *H pylori* positive if the histologic examination and urease rapid test indicated they had the bacterium.

Endoscopy was performed using Olympus videoscope XQ140 or GF100 (Olympus, Tokyo, Japan). Several biopsy specimens from 2 sites, the gastric body and the antrum, were obtained for histologic examination and urease rapid test. Section specimens were stained with hematoxylin-eosin and with Giemsa.

A rapid urease test result was obtained by adding a biopsy specimen to a urea broth (NaCl, KH_2PO_4, and NaOH); the result of the test was considered positive if there was a change of urea broth color from yellow-gold to pink-red due to an increase in pH induced by *H pylori*.[21]

An enzyme immunoassay (Premier Platinum HpSA; Meridian Diagnostics Inc, Cincinnati, Ohio) was used to detect *H pylori* in the frozen stool, utilizing polyclonal anti–*H pylori* antibody. A diluted feces sample and a peroxidase conjugated to polyclonal antibody were added to the wells and incubated for 1 hour at room temperature. A wash was performed to remove unbound material. The substrate was added and incubated for 10 minutes at room temperature. Color developed in the presence of bound enzyme. Stop solution was added and the results were interpreted visually or spectrophotometrically.

For the assay of salivary (IgG) and serum (IgG and *CagA*) antibodies, we used an enzyme immunoassay method still validated in children (EIA WELL-EUROSPITAL; Radim Spa, Pomezia, Italy), in which horseradish peroxidase was used as an enzyme tracer.[22,23]

Table 2. Clinical Features of 250 Patients Who Underwent Esophagogastroduodenoscopy for a Suspicion of Upper Gastrointestinal Tract Disease*

Symptoms and Signs	*Helicobacter pylori* Positive (n = 93)	*H pylori* Negative (n = 157)
Recurrent abdominal pain†	24	40
Nonrecurrent abdominal pain†	31	50
Nausea and vomiting	18	35
Iron deficiency	20	32
Site and Time of Abdominal Pain	**(n = 55)**	**(n = 90)**
Periumbilical diurnal	25	33
Epigastric diurnal	20	32
Epigastric nocturnal	10	25

**P* values were not significant.
†Recurrent abdominal pain was abdominal pain of such intensity that it prevented the normal activity of the child and was recurrent at least once a month during 3 months with asymptomatic intercritical periods.

For the serum antibodies, values between 0 and 22 (absorbance unit) UA/mL were considered normal; for the *CagA*, values greater than 7.5 UA/mL were considered positive. The cut off value for salivary antibodies was 20 UA/mL.

The accuracy of noninvasive tests was evaluated through sensitivity, specificity, and positive and negative predictive value (**Table 1**). Statistical analyses were performed with the χ^2 test. The result was considered significant with P value $<.05$.

The study was approved by the Scientific Secretariat, "Bambino Gesù" Pediatric Hospital, Rome, Italy.

RESULTS

In our case series, 93 (37%) of 250 patients tested *H pylori* positive by histologic examination and urease rapid test. Clinical features of the patients examined are reported in **Table 2**. There were no significant differences in symptoms between infected and noninfected patients ($P>.05$). Twenty-six infected children (28%) and 36 noninfected ones (23%) had parents affected by gastritis and duodenal ulcer. The domestic socioeconomic, hygiene, and living conditions were good.

The *H pylori* fecal antigen revealed a sensitivity of 97%, a specificity of 98%, a positive predictive value of 97%, and a negative predictive value of 98%; serum *H pylori* IgG had a sensitivity of 86%, a specificity of 80%, a positive predictive value of 72%, and a negative predictive value of 90%; and serum *CagA* IgG had a sensitivity of 83%, a specificity of 80%, a positive predictive value of 71%, and a negative predictive value of 89%.

The salivary antibodies (IgG) were evaluated in 123 patients. Fifty of them had *H pylori*–positive histologic and urease rapid test results; the urease rapid test results were positive in 33 (66%) of the infected children and in 7 (9%) of the noninfected ones.

The fecal antigen detection results were positive in 90 (97%) of the infected children and in 3 (2%) of the noninfected ones.

All the statistical differences between the noninvasive tests were significant ($P<.05$).

At endoscopic examination of the 93 affected children, hyperemia and friability of the gastric antrum were observed in 27 patients (29%), micronodular appearance in 48 patients (52%), and a normal picture in 18 patients (19%). The histologic examination results of all infected patients showed active microerosive gastritis (neutrophilic infiltration) and chronic gastritis (lymphoplasmacytic infiltration).

The *CagA*-positive children had an endoscopic finding of more intense hyperemia of the gastric antrum associated with an important lymphoplasmacellular infiltrate and degenerative and vacuolar lesions of the gastric epithelium.

All the 157 noninfected patients had a normal gastric finding except the 25 *H pylori*–negative patients (18%) with epigastric nocturnal pain who had cardial hyperemia and distal esophageal erosions. In noninfected children, there were no histologic signs of inflammation.

COMMENT

The high prevalence of *H pylori* disease (37%) could be explained by the selected population in a specialized center. Several invasive and noninvasive methods are available at present for detecting *H pylori* infection. Esophagogastroduodenoscopy with biopsy is the gold standard for diagnosing the pathologic features related to *H pylori* rather than the *H pylori* infection.

Among the noninvasive tests, the urea breath test (^{13}C urea breath test) is certainly the best, but it is more expensive, not always available (poor feasibility limit), and difficult to apply to the noncompliant child. In addition, its cut off value in pediatric patients remains unsettled. Regarding noninvasive tests, serum antibodies have the advantages of simplicity, low cost, and utility for epidemiological studies and screening programs.[14] Saliva testing has a role in epidemiological studies and in screening dyspeptic patients in general practice, especially in children in whom blood sampling is more difficult.

In 1998, enzyme-linked immunoassay (Premier Platinum *H pylori* stool antigen; Meridian Diagnostics Inc) in stool was approved by the Food and Drug Administration for both diagnosis in adult symptomatic patients and monitoring the response to treatment.[24,25] *Helicobacter pylori* detection from stool with polymerase chain reaction is limited by the presence of inhibitors of *H pylori* DNA amplification in feces.[14] Also, *H pylori* is difficult to culture from stool. The direct research of fecal antigen is simple, rapid, and inexpensive; in fact, only 1 stool specimen is required, the method does not need a technician or expensive equipment, and easy procedures are used for storing and transporting the stools, avoiding the need for freezing the stool sample.

Helicobacter pylori fecal antigen is a highly reliable diagnostic method for *H pylori* infection. Before extending its use in the general healthy population, many studies have compared the accuracy of this test with that of an invasive test in symptomatic patients.[21] It could be used in epidemiological studies to determine the prevalence of *H pylori* infections in asymptomatic subjects.[21] In general, *H pylori* prevalence is universally related to socioeconomic, hygiene, and living conditions.

Recently, a multicenter Italian study on the accuracy of the *H pylori* stool antigen test has reported the utility of fecal antigen in the diagnosis and follow-up of *H pylori* infection.[25,26] Our study confirms the high sensitivity and specificity of the *H pylori* stool antigen test (in relation to diagnoses based on invasive examinations) already found by other investigators either in adults or in children.

Fecal antigen must be considered useful for screening and monitoring the overall pediatric population. The position that fecal antigen testing is a highly reliable method for the diagnosis of *H pylori* infection in children is contentious and not substantiated by the currently available biomedical literature. The current study, however, helps to add support for this point of view.

In conclusion, our study demonstrates that among noninvasive and more applicable tests, especially in small children, *H pylori* fecal antigen has shown high sensitivity, specificity, and positive and negative predictive value and that this test is the most useful for the screening of *H pylori*–positive patients. There is no characteristic clinical feature that could help in the diagnosis of *H pylori* infection.

Accepted for Publication: October 13, 2004.
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