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Evaluation of Enzyme Immunoassay for Detection of Salivary Antibody to *Helicobacter pylori*

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The Helisal test is a quantitative enzyme immunoassay for the measurement of *Helicobacter pylori*-specific immunoglobulin G antibodies in saliva. This test was evaluated in comparison with culture and histopathologic examination of gastric biopsy specimens obtained from 195 patients who underwent 200 endoscopic procedures for the investigation of gastrointestinal symptoms. Forty-one (21%) patients were found to have peptic ulcer disease, and one other patient had a gastric carcinoma. *H. pylori* was detected in gastric biopsy specimens obtained from 98 (49%) of the procedures. The sensitivity, specificity, and positive and negative predictive values of the Helisal test were 81, 75, 76, and 80%, respectively. The test was negative for 16 (38%) of the 42 patients with peptic ulcer disease or a gastric malignancy diagnosed at endoscopy. These results suggest that the Helisal assay is only moderately accurate for the detection of *H. pylori* infection in symptomatic patients.

Helicobacter pylori is now known to be the major cause of chronic superficial gastritis and peptic ulcer disease (1, 2). Many studies have confirmed that antimicrobial therapy that eradicates *H. pylori* from the gastric antrum is associated with a greatly reduced risk of ulcer recurrence compared with treatment that does not eradicate the organism (6, 9, 10, 18). A recent consensus statement by the National Institutes of Health has therefore recommended antimicrobial therapy for the eradication of *H. pylori* in all patients with peptic ulcer disease who are infected with the organism (18).

The definitive diagnosis of *H. pylori* infection has been based primarily on the isolation of the bacterium in culture or detection of the organism in histological sections of gastric biopsy specimens obtained at endoscopy. Several serological methods have also become available, and the presence of *H. pylori* immunoglobulin G (IgG) and IgA antibodies in serum has been found to correlate with infection (14, 22–24). More recently, a quantitative immunoassay for the measurement of salivary *H. pylori* IgG antibodies (Helisal; Cortecs Diagnostics, Deeside, United Kingdom) has been developed. For this test, saliva is collected and then assayed by a standard microplate enzyme immunoassay. It has been recommended that salivary antibody test results can be used to screen patients prior to gastroscopy or to determine the effect of antimicrobial therapy on the eradication of *H. pylori* (4, 21). For such an assay to be clinically useful, it must accurately detect the presence or absence of *H. pylori* infection. In the study described here, we compared the diagnostic accuracy of the Helisal salivary immunoassay with that of culture and histologic examination of gastric biopsy specimens from patients referred for endoscopy for the investigation of gastrointestinal symptoms.

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MATERIALS AND METHODS

All subjects over the age of 18 years undergoing gastroscopy for investigation of gastrointestinal symptoms, peptic ulcer, or gastrointestinal bleeding were asked to participate in the study. Exclusion criteria included a previous diagnosis of gastric carcinoma or treatment with antibiotics, bismuth-containing compounds, and/or omeprazole at the time of endoscopy or within the previous 2 weeks. Baseline demographic and clinical data were obtained by questionnaire from all study participants. Gastroscopy was performed by one of three qualified gastroenterologists. Multiple biopsy specimens of the gastric antrum and of any obvious abnormalities were obtained and sent for culture and histopathologic examination. Informed consent was obtained from all study participants in accordance with institutional review board requirements.

The Omni-SAL saliva collection device (Saliva Diagnostic Systems, U.K., Ltd.), and separator tubes were used as described by the manufacturer's instructions in order to collect salivary samples from patients. The specimens were separated by using the separator tubes and were then stored for up to 2 weeks at room temperature prior to testing. The Helisal quantitative enzyme immunoassay for measurement of salivary IgG antibodies to *H. pylori* was done according to the manufacturer's instructions. Following sample processing, A_{450} values were read with the LP400 microplate reader (Sanofi Diagnostics Pasteur, Montreal, Quebec, Canada). The recommended cutoff value for a positive test result was 0.90 enzyme-linked immunosorbent assay (ELISA) unit.

Gastric biopsy specimens were transported to the microbiology laboratory in sterile containers for processing within 2 h of procurement. One biopsy sample was inoculated directly onto a urea slant (Bacto Urea Agar Base Concentrate; Difco Laboratories), and the slant was incubated at 35°C for up to 4 h for rapid urease testing. Minced biopsy samples were used to prepare a Gram stain (with carbol fuchsin counterstain) and for inoculation onto selective *Campylobacter* agar and chocolate plates, and the plates were incubated microaerophilically at 35°C for up to 7 days. Standard methods were used for the isolation and identification of *H. pylori*. Biopsy specimens for histopathology were examined by using hemotoxylin-eosin and modified Giemsa stains by pathologists blinded to the endoscopy and culture results. The sensitivity, specificity, and positive and negative predictive values of the Helisal test were calculated by using detection of *H. pylori* by culture and/or histologic examination as the "gold standard."

RESULTS

A total of 195 eligible patients, who underwent 200 endoscopic procedures, participated in the study. There were 108 men and 87 women, with a mean age of 54.9 years (age range, 18 to 91 years). A total of 77 patients had been taking an H2-receptor antagonist, 8 had been taking antacids, and 3 had been taking sucralfate within the 2 weeks prior to endoscopy. Gastritis and/or duodenitis was detected at endoscopy in 85 (43%) procedures, duodenal ulcer was detected in 26 (13%) procedures, gastric ulcer was detected in 19 (10%) procedures, esophagitis was detected in 11 (6%) procedures, and other

TABLE 1. Comparison of the Helisal assay with culture and/or histopathology for detection of *H. pylori* infection

H. pylori detection by culture and/or histopathology (no. of procedures)	Helisal assay results	
	No. of procedures positive (% sensitivity)	No. of procedures negative (% specificity)
Positive (98)	79 (81)	19
Negative (102)	25	77 (75)

abnormalities were detected in 11 (6%) procedures. One patient was found to have a gastric carcinoma. The gastroscopy findings were reported to be normal in 68 (34%) procedures.

H. pylori was detected in gastric biopsy specimens obtained from 98 (49%) of the 200 procedures. The organism was grown in culture from 80 specimens and was visualized histopathologically in biopsy specimens from 85 procedures. The Helisal assay was positive for salivary samples obtained from 104 procedures. Compared with the detection of *H. pylori* by culture and/or histopathology, the sensitivity and specificity of the Helisal assay were 81% (95% confidence interval, 71 to 88%), and 75% (95% confidence interval, 66 to 83%), respectively (Table 1). The positive and negative predictive values in this patient population were 76 and 80%, respectively. Test results for procedures in which the Helisal and the culture and histopathology results were discrepant are summarized in Table 2.

The Helisal assay was negative for 16 (38%) of the 42 patients with peptic ulcer disease or a gastric malignancy diagnosed at endoscopy. Twelve of the patients with gastric or duodenal ulcers were under 45 years of age, and the Helisal assay was negative for 5 (42%) of these patients. *H. pylori* was

TABLE 2. Test results for 44 procedures in which the Helisal assay and the culture and histopathology results were discrepant

Helisal assay result (EU[a])	Prior history of peptic ulcer or *H. pylori* infection	Endoscopy result	Results		
			Culture	Gram stain	Histopathology (Giemsa stain)
− (0.528)	Yes	Duodenal ulcer	+	+	+
− (0.432)	Yes	Gastric ulcer	+	−	+
− (0.744)	Yes	Gastritis	+	+	+
− (0.078)	Yes	Normal	+	+	+
− (0.444)	Yes	Duodenal ulcer	+	+	+
− (0.834)	Yes	Gastric ulcer	+	+	+
− (0.073)	Yes	Gastritis	+	−	+
− (0.073)	Yes	Gastric ulcer	+	+	+
− (0.785)	Yes	Gastritis	+	+	+
− (0.828)	No	Normal	+	−	+
− (0.004)	No	Gastritis	−	−	+
− (0.077)	No	Normal	+	+	+
− (0.887)	No	Duodenal ulcer	+	+	+
− (0.856)	No	Normal	+	+	+
− (0.270)	No	Gastritis	+	+	+
− (0.797)	No	Normal	+	+	+
− (0.213)	No	Duodenal ulcer	+	+	+
− (0.812)	No	Duodenal ulcer	+	+	+
− (0.596)	No	Gastric ulcer	−	−	+
+ (1.248)	Yes	Normal	−	−	−
+ (2.934)	Yes	Partial gastrectomy	−	−	−
+ (0.984)	Yes	Gastritis	−	−	−
+ (5.772)	Yes	Normal	−	−	−
+ (2.06)	Yes	Normal	−	−	−
+ (>8.00)	Yes	Gastritis	−	−	−
+ (>8.00)	Yes	Gastritis	−	−	−
+ (1.265)	Yes	Pyloric stenosis	−	−	−
+ (1.058)	Yes	Gastritis	−	−	−
+ (1.835)	Yes	Gastritis	−	−	−
+ (0.902)	Yes	Normal	−	−	−
+ (14.65)	Yes	Gastritis	−	−	−
+ (6.822)	No	Normal	−	−	−
+ (1.47)	No	Gastritis	−	−	−
+ (2.424)	No	Normal	−	−	−
+ (7.992)	No	Gastric ulcer	−	−	−
+ (7.872)	No	Gastritis	−	−	−
+ (2.748)	No	Esophageal varices	−	−	−
+ (5.25)	No	Gastritis	−	−	−
+ (3.884)	No	Normal	−	−	−
+ (>8.00)	No	Esophagitis	−	−	−
+ (1.319)	No	Gastritis	−	−	−
+ (1.823)	No	Gastritis	−	−	−
+ (1.559)	No	Normal	−	−	−
+ (1.624)	No	Normal	−	−	−

[a] EU, ELISA units.

not detected in biopsy specimens obtained from one patient under age 45 who had been taking a nonsteroidal anti-inflammatory agent. Therefore, screening by the Helisal assay prior to endoscopy would have missed 4 (36%) of the 11 patients under age 45 with peptic ulcer disease not associated with nonsteroidal anti-inflammatory agent use.

DISCUSSION

The administration of antimicrobial agents along with standard antiulcer medications for patients with peptic ulcer disease and *H. pylori* infection has been recommended in order to eradicate the organism and thereby prevent ulcer relapses (9, 10, 18). Therefore, the ability to reliably detect infection caused by *H. pylori* has become an important determinant in the management of these patients. Endoscopy with gastric biopsy has been the standard diagnostic procedure. This allows visualization of the gastroduodenal mucosa, confirmation of the presence of ulcers, and provision of tissue for microbiologic and histologic examinations. However, it is a costly and invasive procedure with potential risks and discomfort for the patient. Radiolabelled urea breath tests have been found to be an accurate noninvasive means of detecting *H. pylori* infection (8), but these tests are not widely available.

In the past few years, commercial enzyme immunoassays have been developed for the detection of serum *H. pylori* antibodies. Although several studies have found an excellent correlation between *H. pylori* serology and the presence of infection (22, 24), other studies have failed to reproduce these results (12). The reported sensitivities and specificities of commercially available serologic test kits have ranged from 68 to 97% and 53 to 83%, respectively (3, 11–13, 24). Preliminary results suggest that serology may play a useful role in screening patients prior to endoscopy (5) or in monitoring the effect of antimicrobial therapy directed at the eradication of *H. pylori* (11), although these findings need to be confirmed in larger clinical trials.

Detection of antibodies in saliva has been used increasingly in the past few years for the diagnosis of a variety of infectious diseases (16, 19, 20). The use of this type of specimen offers definite advantages including ease of sample collection, lack of patient discomfort, and no risk of needlestick injury. Although studies have examined the use of saliva antibody tests in epidemiologic screening or surveillance (7, 16), there have been fewer evaluations of these tests for the diagnosis of disease in individual patients. An accurate assay for the detection of *H. pylori* antibodies in saliva would be a useful and noninvasive way to identify infection, permit selective use of endoscopy, and monitor the response to antimicrobial therapy.

Compared with culture and/or histopathologic detection of the organism, the overall accuracy of the Helisal assay was 78%; the reproducibility of the assay results was not specifically evaluated. The sensitivity (81%) and specificity (75%) found in the present study are comparable to those reported previously for salivary antibody tests used to diagnose *H. pylori* infection. Patel et al. (21) assessed a modification of a serum ELISA for the measurement of salivary *H. pylori* IgG and IgA antibodies in 119 patients referred for endoscopy. The sensitivity and specificity of the test were both 85%, and a good correlation between levels of salivary and serum IgG antibodies was found. The salivary IgA assay appeared to be less sensitive and less specific. Previous evaluations of the Helisal assay in relatively small numbers of patients referred for endoscopy have found sensitivities of the test ranging from 65 to 82% and specificities of between 72 and 96% (15, 17). Clancy and coworkers (4) compared the Helisal assay with serology and found a good correlation of salivary antibody results with serum antibody titers. There appeared to be a more rapid decline in salivary antibody levels than in serum antibody levels following antimicrobial treatment.

Several factors may have contributed to the discrepant results obtained in the present evaluation (Table 2). Saliva samples were stored at room temperature prior to testing, as recommended by the manufacturer. It is possible that immunoglobulin degradation by salivary protease may have contributed to false-negative Helisal assay results. In 5 of the 19 procedures with false-negative results, the Gram stain and/or culture results were negative, suggesting that infection in these patients may have been associated with a low inoculum of organisms. False-negative results may also possibly occur in patients recently infected, before an antibody response has developed. False-positive test results may be due to the presence of cross-reacting bacterial antigens. Apparently false-positive results may also occur because of sampling error in obtaining gastric biopsy specimens: infection of the gastric mucosa may be patchy, so that examination of biopsy specimens may occasionally fail to identify truly infected patients. This is unlikely to have been a major problem in the current study because a minimum of four biopsy specimens were obtained from each patient. Twelve of the 25 false-positive Helisal assay results occurred in patients who were either known to have had prior *H. pylori* infection or who were likely to have been previously infected because they had documented peptic ulcer disease in the past. The duration of detectable salivary secretion of antibodies following infection and after treatment is uncertain, but it may take 6 months for serum antibody levels to decline to less than half of pretreatment values (11, 14). Because the Helisal test is a quantitative assay, it may be able to measure a decline in antibody levels after treatment, although this was not evaluated in the current study. However, it is for this reason that the value of a single test result in patients who may have had previous *H. pylori* infection remains uncertain. Although the present study was not designed to determine the utility of this assay as a screening test prior to endoscopy, the results suggest that a significant number of patients with peptic ulcer disease would have been missed if patients with negative Helisal assay results were presumed to be uninfected and therefore not candidates for endoscopy.

In summary, the Helisal assay was moderately accurate for the detection of *H. pylori* infection in symptomatic individuals referred for endoscopy. Further studies are required in order to determine whether enzyme immunoassays for measurement of salivary antibodies can be used as a screening test prior to endoscopy or would be useful for monitoring the response to therapy.

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