Diagnosis of *Helicobacter pylori* Infection by Using Pyloriset EIA-G and EIA-A for Detection of Serum Immunoglobulin G (IgG) and IgA Antibodies

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We evaluated the performance of new enzyme immunoassay (EIA) kits (Pyloriset; Orion Corporation, Orion Diagnostica, Espoo, Finland) for the detection of immunoglobulin G (IgG) and IgA antibodies to *Helicobacter pylori* in serum. Serum samples from 195 patients with upper abdominal complaints were collected. Biopsy specimens of the gastric mucosae were taken for histological analysis and bacterial culture. The sensitivity, specificity, positive and negative predictive values, and efficacy of the Pyloriset EIA-G in detecting IgG antibodies to *H. pylori* were 92, 84, 88, 90, and 89%, respectively, when compared with those of the reference methods used. The corresponding data for detection of IgA antibodies were 80, 89, 89, 79, and 84%, respectively. The overall prevalence of defined *H. pylori* positivity was 54%. Moreover, the antibody tests showed a very good correlation with the biopsy findings. IgG antibodies were found in 93% of sera from patients with documented gastritis and *H. pylori* positivity, whereas only 4% of the sera from patients with documented gastritis and *H. pylori*-negative patients was positive. The results obtained for IgA antibodies were 81 and 6%, respectively. We conclude that the Pyloriset EIA-G, the test for IgG antibodies, is a good and reliable test for the detection of antibodies to *H. pylori* and as an indication of *H. pylori* infection. The determination of IgA antibodies may be used as a test that complements the IgG antibody assay.

Following the successful isolation of *Helicobacter pylori* (*Campylobacter pylori*) in 1983 by Warren and Marshall (21), *H. pylori* has been a topic of much research. At present, an increasing amount of evidence indicates an association between *H. pylori* infection and active chronic gastritis, duodenal and gastric ulcers (5, 10, 17), and even *H. pylori* infection and gastric cancer (2, 3, 15, 16).

A variety of methods are available for detecting *H. pylori* infections (11). However, most of these are invasive and require upper gastrointestinal endoscopy in order to obtain biopsy specimens for culture, rapid urease test, or histological analysis. These methods are widely used, but the sampling method is costly and uncomfortable to the patient and restricts these studies to hospitals or outpatient settings. The noninvasive methods used include the urea breath test and serological detection of antibodies to *H. pylori* by enzyme immunoassay (EIA) or latex agglutination assay.

In the present study, we evaluated the performance of Pyloriset EIAs, a set of two new commercially available enzyme immunoassays for the detection of immunoglobulin G (IgG) and IgA antibodies to *H. pylori*.

MATERIALS AND METHODS

Patients. Biopsy specimens and serum samples were collected from 195 patients (84 males and 111 females) with upper abdominal complaints. The mean age of the males was 54 years, and the mean age of the females was 56 years (ranges, 7 to 89 and 4 to 87 years, respectively). The samples

were obtained at Turku University Central Hospital (Departments of Medicine and Pediatrics), Turku City Hospital, and the Hospital of Turunmaa (a district hospital).

Patients included in an *H. pylori* eradication program or patients who had undergone antimicrobial treatment for other reasons less than 1 month before endoscopy were excluded from the study.

Upper gastrointestinal endoscopy and retrieval of biopsy specimens. The endoscopies and the retrieval of biopsy specimens were performed by four gastroenterologists. The severity, localization, and special features of the endoscopic findings were graded according to the Sydney system (12). Three biopsy specimens for histological examination were taken from both the antrum and the corpus. Three additional specimens, for the culture of *H. pylori*, were taken from antral and corpus mucosae.

Histology. The biopsy specimens were fixed in 10% phosphate-buffered neutral formalin, embedded in paraffin, and cut into sections of 4 to 5 μm. The sections were stained by routine histological methods, including stains for mucins, to better evaluate possible atrophy and intestinal metaplasia of the mucosae. In addition, Giemsa staining was performed on antral and corpus mucosal specimens taken for the detection of *H. pylori*.

The histological findings were classified according to the Sydney system (12). Briefly, the following components were quantified: inflammation (amount of mononuclear inflammatory cells), activity (amount of polymorphonuclear leukocytes), intestinal metaplasia, and atrophy. The possible presence of a special type of gastritis was accounted for. If no bacteria meeting the morphological criteria were detected

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at the objective magnification of ×40, an oil immersion objective (×100) was also used.

Microbiology. The biopsy specimens for microbiological analysis were collected into tubes containing 0.9% saline. One piece of the biopsy specimen was cut and used for acridine orange staining. The remaining samples were homogenized and cultured (within 5 h after sampling) on a selective medium (DENT; Oxoid Ltd., Basingstoke, United Kingdom) with laked horse blood and chocolate agar both with 5% horse blood (Amersham, United Kingdom). The cultures were incubated in a microaerophilic atmosphere (Anaerocult C mini; Merck, Darmstadt, Germany) for 5 days. The cultures were examined for growth at days 3 and 5; identification of cultures was based on Gram staining, oxidase and catalase activities, and urease positivity. The acridine orange-stained smears were analyzed for typical curved fluorescent rod-shaped bacteria under fluorescence microscopy (Ernst Leitz Wetzlar, GmbH, Germany).

Serology. Blood (5 to 10 ml) was taken from the patients. The serum was separated and stored at 4°C until the EIAs were done (3 to 7 days), after which time the samples were stored at −20°C.

The Pyloriset EIA-G and EIA-A (Orion Corporation, Orion Diagnostica, Espoo, Finland) were performed according to the manufacturer's instructions. Briefly, the serum was diluted 1:200 for the EIA-G and 1:100 for the EIA-A. The diluted serum samples (100 μl) and four ready-to-use reference serum samples were pipetted into duplicate wells of microtitration strips, the wells were covered with plastic tape, and the plates were incubated for 60 min at room temperature. After this first incubation, the wells were washed three times with 200 μl of washing buffer. The enzyme conjugate, alkaline phosphatase (100 μl of swine anti-human IgG for EIA-G and swine anti-human IgA for EIA-A), was pipetted into each well, the wells were covered with plastic tape, and the plates were incubated for 60 min at room temperature. The wells were washed twice with 200 μl of washing buffer and twice with distilled water. In order to visualize a positive reaction, 100 μl of fresh substrate solution, *p*-nitrophenyl phosphate, was pipetted into each well and the plates were incubated for 30 min at room temperature. After stopping the enzyme reaction with stopping solution, A_{405} readings were determined in a spectrophotometer. The titer of the patient's serum was read from a graph based on the standard curve obtained on a semilogarithmic paper by using the mean A_{405} readings of the ready-to-use reference sera with known titers.

A serum sample was considered positive if the titer was ≥500 and negative if the titer was <500. The cutoff titer was the same for the determination of both IgG and IgA antibodies.

RESULTS

Of 195 samples, 98 were positive both for IgG antibodies to *H. pylori* and for verified *H. pylori* infection. Seventy-five samples were negative for both aspects. The corresponding figures for IgA antibodies to *H. pylori* were 85 positive and 79 negative specimens (Tables 1 and 2).

Eight of the 10 samples positive for IgA antibodies to *H. pylori* were also positive for IgG antibodies. Fifteen of the 21 samples negative for IgA antibodies were found to be positive for IgG antibodies (data not shown).

The prevalence of both IgG and IgA antibodies and *H. pylori* infection was age dependent, increasing with age. No major differences were observed among samples collected at the different clinics. However, a reduction in the specificity of the IgG assay was observed for samples collected at the Hospital of Turunmaa.

The relationship between biopsy data (gastritis and *H. pylori* infection, either positive or negative) and the frequency of antibodies to *H. pylori* is presented in Table 3.

When compared with the 20 diagnosed cases of duodenal ulcer and 19 cases of gastric ulcer, the results show that 100% of patients with duodenal ulcer were positive for *H. pylori*, 100% were positive for IgG antibodies, and 95% were positive for IgA antibodies. For patients with gastric ulcer, the results were 90% were positive for *H. pylori*, 79% were positive for IgG antibodies, and 74% were positive for IgA antibodies. The mean age of the patients with duodenal ulcer was 53 years while that of patients with gastric ulcer was 68 years.

DISCUSSION

The results of the present study show that the new EIAs for detection of IgG and IgA antibodies to *H. pylori* performed well in comparison with a set of reference tests. When examining the performances of the tests, it should be kept in mind that the material used in the present study was obtained only from patients with upper abdominal complaints.

Of the 14 patients positive for IgG antibodies to *H. pylori* and negative in the reference tests, 10 had either mild or moderate atrophy. These data are consistent with reports indicating that patients with certain histological findings, such as atrophy and/or intestinal metaplasia, may have an elevated antibody titer to *H. pylori* even when no bacteria can be detected in gastric biopsy specimens (4, 13). Moreover, the finding that the test result depends on the patient's age (6, 7) was also confirmed in the present study.

A positive IgA antibody finding without any evidence of IgG antibodies to *H. pylori* meant that there were two

TABLE 1. Results showing the correlation between Pyloriset EIA-G, EIA-A, and the reference tests

Interpretation of the reference test[a] and performance of Pyloriset	Pyloriset result (No. of specimens)			
	EIA-G		EIA-A	
	Positive	Negative	Positive	Negative
Reference				
Positive	98	8	85	21
Negative	14	75	10	79

[a] The reference tests, including culture, acridine orange staining, and histological Giemsa staining, were considered positive if *H. pylori* was detected by at least one of the tests.

TABLE 2. Results of Pyloriset EIA-G and EIA-A when compared with reference tests[a]

Pyloriset test	Percent (95% confidence interval)				
	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Efficacy
EIA-G	92 (85–96)	84 (75–91)	88 (79–92)	90 (81–95)	89 (83–92)
EIA-A	80 (71–87)	89 (80–94)	89 (81–94)	79 (69–86)	84 (78–88)

[a] The reference tests, including culture, acridine orange staining, and histological Giemsa staining, were considered positive if *H. pylori* was detected by at least one of the tests.

TABLE 3. Correlation between biopsy data and IgG and IgA antibodies to *H. pylori*

Biopsy data[a]	Total no. (%) obtained	No. (%) of specimens			
		IgG		IgA	
		Positive	Negative	Positive	Negative
Gastritis +, *H. pylori* +	104 (53)	97 (93)	7 (7)	84 (81)	20 (19)
Gastritis −, *H. pylori* −	46 (24)	2 (4)	44 (96)	3 (6)	43 (94)
Gastritis +, *H. pylori* −	43 (22)	12 (28)	31 (72)	7 (16)	36 (84)
Gastritis −, *H. pylori* +	2 (1)	1 (50)	1 (50)	1 (50)	1 (50)

[a] The definition of gastritis is based on the histological finding. The definition of *H. pylori* positive or negative is based on culture, acridine orange staining, and/or Giemsa staining positivity or negativity in all parameters, respectively.

additional true positive samples. A similar finding has been reported by Kosunen et al. (8), who showed that 2% of the patients produce an IgA response but not an IgG response. Therefore, the Pyloriset EIA-A can be recommended for use as a complementary test to the Pyloriset EIA-G, but the test is also valuable for measuring the fall in IgA antibody titers during an eradication program in the case of patients who have shown increased IgA titers only.

In agreement with earlier reports (14, 22), the results of the present study show that there is a close association between *H. pylori* and active chronic gastritis. When the biopsy findings are completely negative, an antibody response is found in only some of the sera. Of interest is also that there were only two patients with no verified gastritis but for whom the findings were positive for *H. pylori*.

As in earlier studies (1, 5), we found that *H. pylori* infection is present in all patients with duodenal ulcers and in most patients with gastric ulcers. Furthermore, the ulcer findings correlated well with the elevated IgG and IgA antibody titers also seen in the present study.

We conclude that the Pyloriset EIA-G is a sensitive and specific noninvasive test for the detection of IgG antibodies in patients with *H. pylori* infections. The findings of the present study support the importance of testing the patient's antibody response, especially the IgG antibody response, to *H. pylori* when the patient suffers from upper abdominal complaints or when chronic gastritis or peptic ulcer is suspected. Therefore, the endoscopic and biopsy findings play a central role in diagnosing chronic gastritis. However, studies by Sobala et al. (19) showed that the outcome of symptom-based screening is poor, which further strengthens the importance of using serological tests as diagnostic tools. According to Sobala et al. (19), in patients under 45 years of age, serology may reduce the number of endoscopic studies needed to diagnose *H. pylori*. It has been shown that eradication of *H. pylori* infection leads to the gradual disappearance of the gastritic inflammation (20) and permanent healing of peptic ulcers (9, 18). Therefore, it is very likely that serology also will become an established procedure for monitoring the therapeutic effect of antimicrobial treatment for the eradication of *H. pylori* (8) as well as for screening sera in epidemiological studies.

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