Immunoglobulin G Antibody Against *Helicobacter pylori* Is an Accurate Test for Atrophic Gastritis

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Background: Enzyme-linked immunosorbent assay (ELISA) is the most commonly used serologic test for *Helicobacter pylori*. This study aimed to investigate the effects of age and atrophic gastritis on the diagnostic accuracy of an immunoglobulin G (IgG) antibody test against *H. pylori* in adults.

Methods: One hundred and seventy dyspeptic patients (age range, 20–70 years) were evaluated. *H. pylori* infection was diagnosed when culture or both urease and histological tests were positive. Serum pepsinogen-I (P-I) and pepsinogen-II (P-II) levels were measured. Atrophic gastritis was defined when P-I $\leq$ 70 μg/L and P-I/P-II $\leq$ 3. A quantitative ELISA test (HEL-pTEST II) was used for IgG antibodies against *H. pylori*.

Results: The *H. pylori* prevalence rate was 62.1%. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of ELISA were 93.5%, 94.4%, 95.6%, 91.9%, and 93.9% in patients aged < 45 years, and 100%, 81.3%, 94.3%, 100%, and 95.6% in patients aged $\geq$ 45 years, respectively. Twenty-six patients had atrophic gastritis. There was 100% sensitivity and 86.7% specificity in atrophic gastritis and 96.5% sensitivity and 91.9% specificity in non-atrophic gastritis.

Conclusion: The quantitative ELISA test is a good noninvasive test even in older age groups and is a suitable test in patients with atrophic gastritis due to its excellent sensitivity. [*J Chin Med Assoc* 2010;73(7):355–359]

Key Words: atrophic gastritis, *Helicobacter pylori*, serology test

Introduction

Helicobacter pylori is one of the most common worldwide infections, affecting approximately half of the world's population.[1] These bacteria play a causative role in the development of gastritis, peptic ulcers, gastric B cell lymphoma, and gastric cancer,[2] and their eradication reduces the recurrence of peptic ulcer disease cost-effectively.[3] *H. pylori* was also classified as a class I carcinogen by the World Health Organization in 1994.

The test-and-treat strategy for *H. pylori* infection is a proven management strategy for patients with uninvestigated dyspepsia who are under the age of 55 years and have no "alarm features".[4] Deciding which test to use in a particular situation depends heavily on whether a patient requires evaluation with upper endoscopy and an understanding of the strengths, weaknesses, and costs of individual tests.[4] Noninvasive test-and-treat strategies are widely recommended in the primary care setting.[5] A simple, rapid, accurate, and cost-effective diagnostic method is essential for *H. pylori* detection.

Serology is a widely available, discriminating (high negative predictive value), and cost-effective noninvasive test.[4] It requires no specialized equipment or technique and can be performed in most hospitals or clinic laboratories.[5] Enzyme-linked immunosorbent assay (ELISA) has been the most commonly used serologic test because it is suitable for screening large populations.[6,7]

There are several studies showing lower sensitivity and higher specificity in younger age groups (< 45 years) compared with older groups ($\geq$ 45 years) in the serologic diagnosis of *H. pylori* infection.[8–10] It has been reported that the specificity of the immunoglobulin G (IgG) antibody test for *H. pylori* declines in older age groups because of increased atrophic gastritis

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at older ages.[11] It has also been demonstrated that the performance of diagnostic assays may vary between different races and geographic regions, possibly due to the different antigenic properties of local bacterial strains and antibodies of commercial kits used for *H. pylori* detection.[12–14] Therefore, serologic assays for *H. pylori* should be evaluated in a local setting.

This study aimed to investigate the effects of increasing age and atrophic gastritis on the diagnostic accuracy of the *Helicobacter* IgG antibody test in adults. To the best of our knowledge, this is the first study on IgG antibodies against *H. pylori* in patients with atrophic gastritis in Taiwan.

Methods

Patient population

Dyspeptic patients scheduled for upper gastrointestinal endoscopy were recruited from July 1998 to August 1999. Patients with any of the following conditions were excluded: (1) ulcer complications such as bleeding, stenosis, or perforation; (2) previous gastric surgery; (3) intake of any substituted benzimidazoles or preparations containing bismuth within 1 month prior to the test; and (4) previous or current treatment with anti-*H. pylori* therapy.

H. pylori status

During endoscopy, 3 sets of gastric biopsy specimens from the greater curvature of the mid-body and the antral lesser curvature near the incisura were obtained for urease testing (CLO test; Delta West Ltd., Bentley, Australia), histology, and culture. The CLO test was considered positive for *H. pylori* if there was a color change from orange to pink within 24 hours.[15] For histology, *H. pylori* was considered present when curved rods were identified in hematoxylin and eosin, or modified Giemsa staining. In culture, the biopsy sample was homogenized with 0.3 mL of broth, plated on chocolate agar, and incubated at 37°C in a micro-aerobic (15% CO_2 and 5% O_2) incubator until the colony appeared, which was usually after 3 days. Negative plates were kept for 7 days. The growth of *H. pylori* was confirmed by the characteristic morphology (Gram-negative and curved) and if positive catalase, oxidase and urease reactions were observed.

A patient was classified as *H. pylori*-positive if the culture or both CLO and histologic tests were positive. A patient was classified as *H. pylori*-negative if all 3 methods (culture, CLO, and histology) were negative. Patients with only 1 positive test on CLO or histology were considered "indeterminate".

Serology

For serology studies, blood was drawn immediately after endoscopy and sera were collected and stored at –70°C until assay. Fasting serum pepsinogen-I was measured in all patients by radioimmunoassay (Pepsik; Sorin Biomedica, Saluggia, Italy) according to the manufacturer's instructions. Basal pepsinogen-II levels were determined using a specific enzyme immunoassay (BIOHIT Oyj, Helsinki, Finland).

Quantitative ELISA test

Serum specimens were tested for the presence of IgG antibodies against *H. pylori* using a quantitative ELISA test (HEL-pTEST II; AMRAD, Kew, Australia) according to the manufacturer's instructions.[16] Reference standards were used to produce a standard curve to quantify *H. pylori* antibody levels in patient samples. Results were expressed in arbitrary units per mL. A specimen was considered positive if it contained > 50 units/mL and negative if it contained < 30 units/mL. Samples with a reading between 30 and 50 units/mL were classified as undetermined.

Statistical analysis

Statistical analyses were performed using the χ^2 test. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined for ELISA and compared with the "gold standard", which is positive culture or both urease and histology test positive. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

Results

One hundred and seventy patients (88 males and 82 females; mean age, 44 years; age range, 20–70 years) were included in the study. The *H. pylori* prevalence rate was 54.9% (50/91) in patients aged < 45 years and 69.6% (55/79) in those ≥ 45 years ($p = 0.058$). Twenty-one patients were ELISA indeterminate, and 1 was *H. pylori* indeterminate.

In patients < 45 years old, 8 were excluded because of an indeterminate ELISA test. Of the 79 patients ≥ 45 years, 13 were ELISA indeterminate. The specificity was lower in those aged ≥ 45 years, but this was not statistically significant (Table 1).

Twenty-six patients were diagnosed with atrophic gastritis by the criteria (serum pepsinogen-I ≤ 70 μg/L and a pepsinogen-I/pepsinogen-II ratio ≤ 3).[11] One hundred and twenty-two patients were diagnosed with non-atrophic gastritis by these criteria (Table 2). ELISA

Table 1. Effect of age on the diagnostic value of ELISA in detecting *H. pylori* infection

Age	True positive	False positive	True negative	False negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
< 45 yr (n = 90)	43	2	34	3	93.5	94.4	95.6	91.9	93.9
≥ 45 yr (n = 79)	50	3	13	0	100	81.3	94.3	100	95.6

PPV = positive predictive value; NPV = negative predictive value; ELISA = HEL-pTEST II (quantitative ELISA test).

Table 2. Effect of atrophic gastritis on the diagnostic value of ELISA in detecting *H. pylori* infection

Age	True positive	False positive	True negative	False negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Atrophic gastritis* (n = 26)	11	2	13	0	100	86.7	84.6	100	92.3
Non-atrophic gastritis (n = 122)	82	3	34	3	96.5	91.9	96.5	91.9	95.1

**Atrophic gastritis was defined as serum pepsinogen-I ≤ 70 ng/mL and pepsinogen-I/pepsinogen-II ratio ≤ 3. PPV = positive predictive value; NPV = negative predictive value; ELISA = HEL-pTEST II (quantitative ELISA test).*

showed a high sensitivity (96.5%) and specificity (91.9%) in the non-atrophic gastritis group. Specificity (86.7%) and PPV (84.6%) were lower in the atrophic gastritis group, but sensitivity and NPV were 100%.

Discussion

The prevalence of *H. pylori* has epidemic variations, infecting more than 80% of adults in Japan and 40% in the UK.[17] The prevalence of infection increases with age, although this may be largely a cohort effect.[1] The choice of an initial test for detecting *H. pylori* depends on the prevalence of *H. pylori* infection and the diagnostic accuracy.[5]

A single test, except for culture, is not sufficient for diagnosis.[5] Therefore, the European Guidelines indicate that the gold standard should be generally represented by at least 2 different tests.[5,18] In this study, a patient was diagnosed as having *H. pylori* infection when the culture or both CLO and histology were positive. A serology test has the lowest cost per correct diagnosis, but its diagnostic accuracy is low (80–84%).[5] However, some serology kits with a high accuracy (>90%) have been reported in validated settings.[18,19] The sensitivity and specificity of ELISA depends on the antigen used, the clinical context, the gold standard used for comparison, and the prevalence of *H. pylori* in the community.[5] Although setting a gray zone for ELISA will decrease its effectiveness as a screening tool, it is still a very useful and suitable tool for epidemiological studies. Studies in the UK have also demonstrated that serology is the method of choice in screening before direct access upper gastrointestinal endoscopy in those < 45 years old because it shows a high sensitivity for peptic ulcer disease with a large reduction in unnecessary negative endoscopies.[15,20]

The sensitivity of ELISA-based testing ranges between 90% and 97%, while specificity is between 50% and 96%.[5,21–23] Serology has a similarly high sensitivity in different age groups but declining specificity in older groups.[11] In the current study, the quantitative ELISA test with IgG antibodies against *H. pylori* disclosed a high sensitivity in different age groups but lower specificity (86.7%) in the older age group, which is consistent with previous studies.[11]

Atrophic gastritis is an important risk factor for gastric cancer. Approximately one-third of *H. pylori*-infected patients have atrophic gastritis in Finland.[24] *H. pylori* infection is associated with 84% of atrophic corpus gastritis.[24] Although histology is currently the standard method for the detection of atrophy, it is not a "gold standard" because of interobserver variability, especially in mild atrophy gastritis and potential sampling error in patients with patchy distribution of the mucosal alterations.[25] The current study concluded that a pepsinogen-I/pepsinogen-II ratio ≤ 3 was a reliable marker for the diagnosis of atrophic gastritis.[25] In the present study, we recruited patients aged between 20 and 70 years (mean age, 44 years). Twenty-six of the 170 patients had atrophic gastritis by the serum pepsinogen criteria. The prevalence of atrophic gastritis was low (26/170, 15.3%), which may be due to a relatively younger age, geographic differences, and difference in race.

Serologic tests are recommended for assessing *H. pylori* in patients with a low bacterial density [extensive mucosal atrophy and MALT (mucosa-associated

lymphoid tissue) lymphoma].[18,24,26] In serology assays, prior use of antibiotics, anti-secretory treatment, location, and reduced number of *H. pylori* in the gastric mucosa have no effect on diagnostic accuracy.[12] In contrast, anti-secretory treatments before gastroscopy may lead to false-negative results for histology, culture, and urease tests.[27,28] Although histology, culture, and even the urea breath test remain negative, patients with atrophic corpus gastritis often have positive serology results.[11,24,29,30] Such patients may still be infected because antibody titers rapidly fall after eradication therapy.[31]

The sensitivity of both the urea breath test and histology are low in atrophic gastritis.[24] Low bacterial density results in a decreased *H. pylori* detection rate by the gold standard and increases the false-negative rate of the gold standard. When the gold standard is used for *H. pylori* detection in patients with a low bacterial load, the false-positive rate of serology increases, with concomitant decreased specificity. The exclusion of patients with atrophic gastritis improves the specificity for those with an older age.[11] In the present study, ELISA showed a higher specificity in the non-atrophic group compared with the atrophic group. Taken together, these findings indicated that the lower specificity in the older age group was due to the false-negative of the gold standard rather than the false-positive of serology.

In conclusion, serology is a good noninvasive test, even in older age groups, and is a suitable test in patients with gastric ulcer, gastric cancer, or pernicious anemia in which atrophic gastritis is more prevalent.[5,32–34]

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