A New Serum Antibody Test Kit (E Plate) for Evaluation of *Helicobacter Pylori* Eradication

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Abstract

Objective Serological antibody test have been widely performed to detect the presence of *H. pylori*, but they have not been used to evaluate the status of *H. pylori* after eradication. In this study we evaluated the diagnostic accuracy of a new serological test kit (E-plate) after eradication.

Method Eradication of *H. pylori* was performed in 100 patients by proton pump inhibitor (PPI)+amoxicillin (AMPC)+clarithromycin (CAM) or PPI+AMPC therapy. Evaluation of *H. pylori* was done by culture, histology and rapid urease test before, and 8 weeks after, the treatment. Serological tests were also performed before and after treatment using the E plate. Cure was defined as no evidence of *H. pylori* at 8 weeks after the treatment. Receiver operating characteristic (ROC) analysis was performed to determine the ideal cut-off value for percentage change in the serological test.

Result Success was obtained in 73 patients, failure in 20 patients and there were 7 dropouts. Serological test value was significantly decreased after treatment (44.3 ± 29.6 U/ml) compared to before treatment (94.8 ± 73.2 U/ml) in the successful cases. In contrast, those with no significant change after treatment (62.7 ± 31.3 U/ml) compared to before treatment (72.9 ± 47.7 U/ml) were considered as failure cases. ROC analysis revealed that cut-off values of a 20%, 30%, and 40% decrease on E plate result yielded a sensitivity of 95.5%, 92.4%, 71.2% and a specificity of 73.3%, 84.2%, 94.7%, respectively.

Conclusion The new E plate serological test kit for *H. pylori* was useful for distinguishing success from failure 8 weeks after completion of eradication therapy for *H. pylori*.

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Key words: *Helicobacter pylori*, serological test, pepsinogen

Introduction

Helicobacter pylori (*H. pylori*) has been shown to be related to peptic ulcers, chronic gastritis, MALT lymphoma and gastric cancer (1–4). Recurrence of gastric and duodenal ulcers can be significantly prevented by its eradication (2). In Japan, *H. pylori* eradication therapy in patients with gastric and duodenal ulcers has been included in health insurance coverage since November 2000. Diagnostic methods for detecting *H. pylori* infection can be divided into invasive and non-invasive methods. The former methods can be classified into the culture, rapid urease test, and histological examination using upper gastrointestinal endoscope, while the latter methods include the urea breath test and antibody determination method (using serum or urine samples). Although the non-invasive serum antibody determination method is the most common at present, it has not been used in the evaluation of eradication of *H. pylori* infection. In the present study we evaluated the usefulness of the E Plate, a new serum antibody kit developed in Japan, in the assessment of *H. pylori* eradication therapy.

Subjects and Methods

The subjects consisted of 100 consecutive patients who were found to be *H. pylori*-positive by upper GI endoscope between October 1999 and June 2000. There were 65 men and 35 women aged 53.2 ± 11.6 years on the average. The 100 subjects included 27 gastric ulcer cases (or ulcer scar), 20 duodenal ulcer cases (or ulcer scar), 9 gastro-duodenal ulcer cases (or ulcer scar), 40 chronic gastritis cases and 4 gastric polyp cases.

The study protocol was approved by the hospital clinical ethics committee on human experiments and with the Helsinki Declaration of 1975, as revised in 1983. After obtaining written informed consent, the patients were randomly assigned to one of the following 4 groups: 1) the RA Group, receiving a 2-week course of 20 mg/day rabeprazole (RPZ) once a day and 500 mg of amoxicillin (AMPC) 4 times a day, 2) the OAC Group, receiving a 1-week course of 20 mg/day omeprazole (OPZ) once a day, 500 mg AMPC 3 times a day, and 200 mg

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clarithromycin (CAM) 3 times day, 3) the LAC Group, receiving a 1-week course of 30 mg lansoprazole (LPZ) once a day together with AMPC and CAM in the same way as in the OAC Group, and 4) the RAC Group, receiving a 1-week course of 10 mg of RPZ once a day together with AMPC and CAM in the same way as in the OAC Group. A third party-controlled central management system was employed for medication regardless of the diagnosis or severity of the disease.

Evaluation of *H. pylori* infection

Three biopsy specimens each were obtained endoscopically from the greater curvature at the gastric antrum and body (total 6 specimens) in order to determine the presence or absence of *H. pylori* infection before starting eradication therapy and 8 weeks after its completion. Each specimen was examined by the rapid urease test (CLO test; Delta West Pty Ltd., Bentley, WA, Australia), culture and histological examination. For histological examinations, specimens were fixed in formalin and stained with hematoxylin and eosin and also Giemsa in order to determine the existence of *H. pylori* under an optical microscope. For culture studies, biopsy specimens of the gastric mucosa was homogenized, and the homogenate was inoculated to Skirrow's medium (Nissui Seiyaku Company Ltd., Tokyo) for culture for 5 to 7 days under microaerophilic conditions at 37°C (O₂ 5%, CO₂ 10%, N₂ 85%). Eradication therapy was regarded as successful if the results of culture, histological examination and RUT were all negative.

Evaluation of serum *H. pylori* antibody titer and serum pepsinogen levels

Blood samples were taken from patients at the time of endoscopy before eradication therapy and 8 weeks after its completion, and serum was separated to determine *H. pylori* antibody and pepsinogen I and II levels (PGI and PGII) by microplate EIA using an antibody determination kit (E Plate Eiken *H. pylori* Antibody, Eiken Chemical Co., Ltd., Tokyo, hereafter referred to as E Plate) and a microplate EIA pepsinogen kit (E Plate Eiken Pepsinogen I and II, Eiken Chemical Co., Ltd., Tokyo), respectively. Strains isolated from Japanese patients with gastric ulcers were used as antigens to prepare E plate. Furthermore, the total protein fraction, including high- and low-molecular weight (10,000–500,000 Da) fractions, obtained from the supernatant obtained by ultrasonic ultracentrifugation of bacteria was used as the antigen.

The plate solid phase 2-step sandwich EIA method was employed. *H. pylori*-antigen solid phase plates were incubated after adding buffer solution, followed by the standard *H. pylori* antibody or specimen. The reaction fluid was removed by washing and enzyme-labeled anti-human IgG antibody was introduced for further incubation. After reaction, non-reactive enzyme-labeled anti-human IgG antibody was removed by washing, and the enzymatic reaction was conducted after adding a substrate fluid. Coloration was determined using a spectrophotometer for microplates, and a standard curve was drawn based on the absorbance values obtained to determine *H. pylori* antibody concentrations. Pepsinogen was determined in

the same way, and the pepsinogen I/II ratio (PEG I/II) was calculated.

Percent changes before and after eradication were calculated using the following equation: (value obtained 8 weeks after eradication–pre-eradication baseline)/pre-eradication baseline × 100. In the E plate method, the cut-off value was set based on the receiver operating characteristic (ROC) curve to determine whether or not eradication was achieved. Percent changes in pepsinogen were used to determine whether or not eradication was achieved according to the method of Furuta et al (5). The results obtained by E plate and PEG I/II were compared using specimens obtained endoscopically.

Statistical analysis

Statistical analysis was conducted by student's t-test, and a P-value of less than 0.05 was regarded as indicating a statistically significant difference.

Results

Eradication therapy was successful in 73 patients and failure in 20, with 7 dropouts. The per-protocol eradication rate was 78.5% (73/93), while the eradication rate determined by intention-to-treat (ITT) analysis was 73% (73/100). The per-protocol and ITT eradication rates in each group were as follows: 79.2% and 76.0%, with success in 19 patients, failure in 5 and dropout in 1 in the RA Group; 82.6% and 76.0%, with success in 19 patients, failure in 4 and dropout in 2 in the OAC Group; 74.0% and 68.0%, with success in 17 patients, failure in 6 and dropout in 2 in the LAC Group; and 78.3% and 72.0%, with success in 18 patients, failure in 5 and dropout in 2 in the RAC Group (Table 1). No significant differences were observed among the four regimens.

H. pylori antibody titer was determined before and after eradication therapy in 66 and 19 patients in whom eradication was successful and failure, respectively. The reason for non-determination was failure to collect of serum samples before eradication therapy for patients' personal reasons. The *H. pylori* antibody titer decreased significantly from 94.8±73.2 U/

Table 1. Cure Rate of Each Regimen

	ITT	PP
RA	19/25 (76%)	19/24 (79.2%)
OAC	19/25 (76%)	19/23 (82.6%)
LAC	17/25 (68%)	17/23 (74.0%)
RAC	18/25 (72%)	18/23 (78.3%)
total	73/100 (73%)	73/93 (78.5%)

ITT: intention to treat analysis, PP: per protocol analysis, RA: rabeprazole (RPZ)+amoxicillin (AMPC), RAC: RPZ+AMPC+clarithromycin (CAM), LAC: lansoprazole+AMPC+CAM, OAC: omeprazole+AMPC+CAM.

Table 2. Change of Value of IgG *H. pylori* Antibody (E plate) before and 8 weeks after Completion of Eradication in Success and Failure

	Success (U/ml)	Failure (U/ml)
before	94.8±73.2*	72.9±47.7
after	44.3±29.6	62.7±31.3

*p<0.05

Table 3. Change of Value Pepsinogen I/II Ratio before and 8 wks after Completion of Eradication in Success and Failure

	Success	Failure
before	2.66±1.10*	3.01±1.16
after	5.19±1.86	3.35±1.40

*p<0.01.

Figure 1. Receiver operating characteristic (ROC) curve for evaluation of eradication using IgG *H. pylori* antibody (E plate).

ml before therapy to 44.3±29.6 U/ml after therapy in patients in whom the therapy was successful, while no significant differences were seen before (72.9±47.7 U/ml) and after (62.7±31.3 U/ml) therapy in patients in whom it was failure (Table 2). The PEG I/II ratio increased significantly from 2.66±1.10 before therapy to 5.19±1.86 after therapy in the successful group. A slighter increase was observed in the failure group from 3.01±1.16 to 3.35±1.40 (Table 3).

The ROC curve for percent changes in *H. pylori* antibody titer are illustrated in Fig. 1. The cut-off value was set at -20%, -30% and -40% based on the ROC curve to determine whether or not eradication was achieved. High agreement rates were

obtained between this evaluation and the evaluation using biopsy materials: 95.5%/73.7%, 92.4%/84.2% and 71.2%/94.7% (sensitivity/specificity), respectively, at the cut-off values of -20%, -30% and -40%. On the other hand, the agreement rate between evaluation based on percent changes in the PEG I/II ratio and the evaluation using biopsy materials was 98.4%/84.2% (sensitivity/specificity).

Discussions

Serological diagnostic methods such as the serum *H. pylori* antibody method and the serum pepsinogen method have a number of advantages over other methods, such as being less invasive than endoscopic methods (culture, histological examinations and RUT), cheaper than the urea breath test, not requiring dietary restrictions before tests, and the possibility of treatment of a large number of specimens at a time. At present, various serum antibody determination kits are commercially available, but test results using these kits do not necessarily agree because they use different antigens. E Plate uses an antigen extracted from *H. pylori* strains isolated in Japan and requires only simple procedures because it does not require dilution of specimens. According to Fujioka and Tokieda (6), the relative sensitivity, specificity and agreement rates between results obtained using this plate and those obtained by culture/RUT were 100%, 80.0% and 97.1%, respectively. However, the serum antibody titer is reported to decrease gradually as eradication therapy is conducted, and at least one year is needed until negative conversion is achieved after eradication. Cutler et al (7) who monitored changes in serum antibody titer after eradication, reported that antibody titer decreases by 26%, 43% and 55%, respectively, 3, 6 and 12 months after eradication. The relative sensitivity and specificity were 86% and 88%, respectively, in the evaluation conducted when serum antibody cutoff titer was taken as a 20% decrease at 6 months after eradication. In the present study, we investigated whether it is possible to determine the presence or absence of *H. pylori* at an earlier stage after eradication using the E plate.

In the present study, the *H. pylori* antibody titer decreased significantly 8 weeks after completion of eradication therapy, to about 50% of the pre-treatment baseline, in patients in whom *H. pylori* was eradicated. According to Kosunen et al (8), the antibody titer decreased by 50% 6 months after eradication in 97% of patients in whom *H. pylori* was eradicated, while Wang et al (9) using a GAP-IgG kit, reported that the antibody titer decreased by at least one grade 6 months after eradication in 95% of patients in whom *H. pylori* was eradicated. With many of the conventional antibody determination kits, decreases in antibody titer cannot be determined until a long time (6 months) after eradication of *H. pylori*. However, our findings showed that E Plate shows marked decreases in antibody titer even at 8 weeks after eradication is achieved.

According to the reports by Leung et al (10) and Miwa et al (11) the diagnostic accuracy of the serum antibody determination method developed in the West is not as good in Chinese and Japanese as it is in Caucasians. Decreases in the antibody

titer observed at an early stage after bacterial eradication from E plates are thought to be related to the fact that strains isolated from Japanese were used as antigens to prepare the E plates.

Furuta et al (5), who compared the PEG I/II ratio determined by RIA one month after eradication with the pre-eradication baseline, reported that there were significant differences between patients in whom *H. pylori* was eradicated and those in whom it was not. Our findings obtained using the E Plate also shows similar changes in patients in whom *H. pylori* was eradicated. However, the ratio was slightly increased 8 weeks after eradication compared to the pre-eradication baseline in patients in whom *H. pylori* was not eradicated. Because the PEG I/II ratio reflects gastric mucosal inflammation, this increase may be attributable to the fact that eradication therapy transiently improves inflammation even if it does not completely eradicate *H. pylori*. In three patients in whom the diagnosis based on the PEG I/II ratio was false positive, histological findings showed a decrease of one grade or two grades in lymphocyte and neutrophil infiltration at the greater curvature in the gastric body compared to pre-eradication, indicating a slight improvement in inflammatory findings.

In the evaluation based on percent changes after eradication therapy compared to the pre-treatment baseline, the sensitivity and specificity of the E Plate method were 92.4% and 84.2%, respectively, at a percent change of -30%. The sensitivity and specificity of the evaluation based on percent changes in the PEG I/II ratio were 98.4% and 84.2%, respectively. Evaluation of eradication using the E plate method is equivalent to that using the PEG I/II ratio, and the former is more accurate for detecting *H. pylori* infection.

Among various methods now available for the evaluation of eradication of *H. pylori*, histological examinations, culture, RUT, antibody determination and urea breath test (UBT) are covered by health insurance programs in Japan, but only UBT is effective for early determination (12). According to Nishikawa et al (13), the sensitivity of the RUT is very poor (60.5%) when conducted 1 month after eradication although its specificity is high (99.2%). Murata et al (14) also conducted a similar study by using by RUT and found that both the sensitivity and specificity of RUT increase to 100%, 4 months after eradication.

Use of the antibody titer in the evaluation of *H. pylori* infection after eradication therapy involves a number of problems, such as a long period requires for the titer to become negative

after successful eradication and relative judgment based on percent changes. However, the importance of this method will increase in the future to spread *H. pylori* eradication therapy widely because E plate method is convenient and can make reliable evaluation 8 weeks after eradication is achieved.

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