Comparison of the HM-CAP and E-Plate Serum Antibody Kit for the Assessment of Helicobacter pylori Eradication in Japan

Kohei Kawakami¹, Takashi Kawai²*, Mikinori Kataoka², Kazuo Takei¹, Takao Ito³, Fuminori Moriyasu¹, Yuu Takagi³, Tatsuya Aoki³, Jun Matsubayasiu¹, Kiyoshi Mukai¹, Emiko Rimbara³, Norihisa Noguchi⁵, and Masanori Sasatsu⁵

¹Fourth Department of Internal Medicine, Tokyo Medical University, Tokyo 160-0023, Japan
²Endoscopy Center, Tokyo Medical University Hospital, Tokyo 160-0023, Japan
³Third Department of Surgery, Tokyo Medical University, Tokyo 160-0023, Japan
⁴Division of Pathology, Tokyo Medical University Hospital, Tokyo 160-0023, Japan
⁵Department of Pathogenic Microbiology, Tokyo University of Pharmacology and Pharmacy, Tokyo 192-0392, Japan

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Summary   Helicobacter pylori (H. pylori) is known to be associated with peptic ulcer, chronic gastritis, MALT lymphoma, and gastric cancer. In this study, we investigated the usefulness of a new E-plate serum antibody kit, developed in Japan using H. pylori strains isolated from Japanese patients, in the assessment of eradication, with an HM-CAP kit for comparison. The subjects were 100 H. pylori positive patients who underwent upper gastrointestinal endoscopy between October 1999 and June 2000. Eradication assessment performed using E-plate kit with the cut-off values for the delta value of the antibody titre set at −20%, −30%, and −40%, yielded sensitivity/specificity of 93.9%/85.7%, 92.4%/85.7%, and 72.7%/92.9%, respectively, for agreement rates with eradication assessments using endoscopic biopsy specimens at 2 months post-eradication therapy. Use of the HM-CAP kit with cut-off values of −5%, −10%, and −15% yielded sensitivity/specificity of 97.0%/28.6%, 83.3%/85.7%, and 53.0%/100%, respectively. These results indicated that the E-plate kit is more useful for eradication assessment in Japan.

Key Words: Helicobacter pylori, antibody

Introduction

Helicobacter pylori (H. pylori) is known to be associated with peptic ulcer, chronic gastritis, MALT lymphoma, and gastric cancer [1–6]. Eradication of H. pylori has been shown to significantly reduce the risk of recurrence of gastric and duodenal ulcers [2]. H. pylori eradication regimens were approved for Japanese national insurance in 2000. Methods for diagnosing H. pylori infection can be classified into invasive methods, using upper gastrointestinal endoscopy (UGITE), and non-invasive methods, that do not require endoscopy. The former include culture, the rapid urease test (RUT), and histological examination, whereas the latter includes the urea breath test (¹³C-UBT), antibody testing (serum and urine) and faecal antigen testing [7]. Of these, serology is non-invasive and the easiest to perform. Until now, serology has been used for the diagnosis of infection, but rarely for assessment of eradication. In this study, we investigated the usefulness of a new E-plate serum antibody kit, recently developed in Japan using H. pylori strains isolated from Japanese patients, in the assessment of
eradication, with an HM-CAP kit using strains derived from Western patients for comparison.

Subjects and Methods

Subjects

This study is prospective study. The subjects were 100 consecutive H. pylori patients who attended this hospital for UGITE between October 1999 and June 2000. There were 65 males and 35 females, average age 53.2 ± 11.6 years. The diagnosis was gastric ulcer in 27 subjects, duodenal ulcer in 20, gastroduodenal ulcer in 9, chronic gastritis in 40, and gastric polyps in 4.

Subjects with a past history of oesophageal or gastric surgery, those who had taken a proton pump inhibitor (PPI) or antibiotic in the preceding 4 weeks were excluded, as were those who had previously undergone H. pylori eradication therapy.

With the approval of the hospital ethics committee, subjects from whom informed consent had been obtained were randomly allocated to one of four eradication regimen groups: I, rabeprazole (RPZ) group 20 mg daily + amoxicillin (AMPC) 500 mg qid for 2 weeks (RA); II, omeprazole (OPZ) 20 mg daily + AMPC 500 mg tid + clarithromycin (CAM) 200 mg tid for 1 week (OAC group); III, lansoprazole (LPZ) 30 mg daily + AMPC + CAM as for II (LAC group); and IV, RPZ 10 mg daily + AMPC + CAM as for II (RAC group).

Assessment of H. pylori infection

Patients underwent UGITE prior to, and 8 weeks after, eradication therapy, at which time 3 mucosal biopsies each were taken from the greater curvature of the antrum and corpus. The biopsy samples were respectively used for culture, RUT (Helicocheck test kit; Institute of Immunology, Tokyo, Japan), and histological examination. For the histological examination, specimens were fixed in formalin, stained with either Haematoxylin and Eosin or Giemsa, and examined under light microscopy for the presence of H. pylori. Gastric mucosal specimens for culture were homogenised, placed in Skirrow’s medium (Nissui Seiyaku Co. Ltd, Tokyo, Japan), and cultured for 5–7 days at 37°C in microaerophilic conditions (O₂: 5%, CO₂: 10%, N₂: 85%). Eradication was deemed successful only if culture, histological examination, and the RUT were all negative.

Eradication assessment was repeated at 6 months after eradication therapy using UBT. On an empty stomach, each subject was instructed to drink 100 mg of¹³C-urea dissolved in 100 ml of water. They then immediately rinsed their mouth out with tapwater. Subjects breathed into the collection bag, while seated, before swallowing the urea and 20 minutes later. Pre- and post-ingestion ¹³CO₂ concentrations in the breath were measured using UbiT-IR 200 (Otsuka Pharmaceutical, Tokushima, Japan), with a difference over baseline of at least 2.5% after 20 minutes considered positive.

Measurement of serum anti-H. pylori antibody levels

Blood was taken from subjects at the time of UGITE before and 2 months after eradication therapy, and at the time of the UBT 6 months post-eradication. Following the separation of the serum, samples were tested by E-plate (E-Plate Eiken H. pylori Antibody, Eiken Chemical Co., Ltd., Tokyo, Japan) and HM-CAP (Enteric Products, Westbury, NY, USA). Results at or below the cut-off values for serum antibody levels of 10 U/mL for the E-plate and 1.7 U/mL for the HM-CAP were considered negative. For the HM-CAP, levels at or above 2.1 U/mL were considered positive, and 1.7–2.1 U/mL indeterminate. The delta value between pre- and post-eradication was calculated as follows. Using delta value (%) = value at 2 mth – pre-eradication value/pre-eradication value × 100, the cutoff values were determined from the receiver operator characteristics (ROC) curves. The success or failure of the eradication therapy was assessed, and the results compared with the endoscopic eradication assessments. Delta values at 6 months post-eradication therapy were calculated only for eradication successes.

Statistical analyses

These were performed using the t-test, with p<0.05 considered significant.

Results

Overall, there were 73 eradication successes, 20 failures, and 7 withdrawals, giving a per protocol (PP) eradication rate of 79.6% (73/93), and an intention-to-treat (ITT) rate of 73% (73/100). Looking at each regimen, for the RA group there were 19 successes, 5 failures, and 1 withdrawal, giving eradication rates of 79.2% (PP) and 76.0% (ITT). For the OAC group, there were 19 successes, 4 failures, and 2 withdrawals, and eradication rates of 82.6% (PP) and 76.0% (ITT). For the LAC group, there were 17 successes, 6 failures, and 2 withdrawals, and eradication rates of 73.9% (PP) and 68.0% (ITT). For the RAC group, there were 18 successes, 5 failures, and 2 withdrawals, giving eradication rates of 78.3% (PP) and 72.0% (ITT). No significant differences were seen between groups.

Serum anti-H. pylori antibody levels could be measured pre- and post-eradication therapy in 81 subjects at 2 months post-treatment, and 41 subjects in the successful eradication group at 6 months. Using the E-plate kit, antibody levels fell significantly in the successful eradication group from 92.2 ± 71.2 U/mL (mean ± SD) pre-eradication to 41.0 ± 26.9 U/mL at 2 months post-eradication. In the eradication
failure group, no difference was seen, with levels at 72.9 ± 49.7 U/mL pre-eradication and 69.3 ± 43.6 U/mL post-eradication. Using the HM-CAP kit, antibody levels fell significantly in the successful eradication group from 4.86 ± 1.20 U/mL pre-eradication to 3.96 ± 1.30 U/mL at 2 months post-eradication. In the eradication failure group, no difference was seen, with levels at 4.82 ± 1.50 U/mL pre-eradication and 4.23 ± 1.35 U/mL post-eradication. At 6 months post-successful eradication, antibody levels fell significantly in the successful eradication group, to 21.8 ± 14.1 U/mL (E-plate) and 2.89 ± 1.09 U/mL (HM-CAP). In cases of successful eradication, the delta value for antibody levels at 2 months were −50.0 ± 19.7% (mean ± SD) for the E-plate kit and −20.1 ± 14.4% for the HM-CAP kit, and at 6 months they were −81.3 ± 14.8% and −36.0 ± 18.3%, respectively. Antibody levels as determined using the E-plate kit were lower at 2 months post-eradication than pre-eradication in all subjects, whereas with the HM-CAP kit they had risen in 2 subjects. This finding also suggests that the former is more useful. At 6 months following successful eradication, antibody levels had become negative in 6 subjects each using E-plate and HM-CAP.

Eradication assessment was performed using the E-plate kit with cut-off values for the delta value of the antibody titre derived from the ROC curve set at −20%, −30% and −40%. These yielded high sensitivity/specificity values of 93.9%/85.7%, 92.4%/85.7%, and 72.7%/92.9%, respectively, for agreement rates with eradication assessments using endoscopic biopsy specimens at 2 months post-eradication therapy (Fig. 1). Use of the HM-CAP kit with cut-off values of −5%, −10% and −15% yielded inconsistent sensitivity/specificity values of 97.0%/28.6%, 83.3%/85.7%, and 53.0%/100%, respectively (Fig. 2).

Discussion

The greatest merits of serological methods of diagnosis of *H. pylori* infection, such as estimation of serum anti-*H. pylori* antibody levels, are that they are less invasive than endoscopic methods (culture, histology, RUT), they are cheaper than the UBT, fasting is not required, and many specimens can be processed at the same time. A number of serum antibody kits are presently available, with each kit using a different antigen, so results are not necessarily consistent. The E-plate kit used in this study uses antigens extracted from Japanese *H. pylori* strains, does not require specimen dilution, and is easy to use. Fujioka *et al.* reported that the E-plate kit is useful in the diagnosis of *H. pylori* infection, with sensitivity of 100%, specificity of 80.0%, and agreement rate of 97.1% with culture and RUT [8]. A common perception of serological methods is that antibody titres decline slowly after eradication therapy, and may take one year or more to become negative. In this study, we investigated whether it is possible to assess eradication early time after treatment using the new E-plate serum antibody kit.

In cases of successful eradication, we found that serum anti-*H. pylori* antibody levels were significantly reduced at 2 months post-eradication in comparison to pre-eradication levels, with mean values some 55% lower at 2 months and 78% lower at 6 months using the E-plate kit. The reductions were smaller using the HM-CAP kit, 18% at 2 months and 40% at 6 months. In earlier studies, Cutler *et al.* reported reductions in serum antibody levels of 26% at 3 months, 43% at 6 months, and 55% at 12 months post-eradication using the PyloriStat Test Kit [9]. Kosunen *et al.* reported that IgG levels declined by 50% at 6 months post-eradication in 97% of cases of successful eradication [10]. Wang *et al.* reported that at 6 months post-eradication antibody levels had declined by at least 1 step in 95% of cases of successful eradication [11]. In previous reports, with many serum antibody kits, antibody levels began to decline after considerable time has passed, often 6 months, post-eradication. In this study, however, a marked decrease was seen soon after successful eradication therapy (2 months).

Leung *et al.* [12] and Miwa *et al.* [13] have pointed out
that eradication assessment using Western serum antibody kits is appropriate for Caucasian patients, but not for Asian patients, such as the Japanese and Chinese. Obata et al. [14] compared the diagnostic ability of HM-CAP with that of J-HM-CAP, that uses H. pylori strains isolated from Japanese patients. They demonstrated a sensitivity of 95.5%, specificity of 81.9%, and diagnostic accuracy of 92.3% with J-HM-CAP, making it more useful than HM-CAP with a sensitivity of 87.5%, specificity of 84.8%, and diagnostic accuracy of 92.3%.

Ekstrum et al. [15] in Sweden investigated the correlation between H. pylori infection and the development of gastric cancer in a case-control study, using a traditional ELISA serum antibody kit and a CagA antibody immunoblot method. They found that using the traditional serum antibody kit, the H. pylori-positive rates in 279 gastric cancer patients and 238 controls were 72% and 55%, respectively, with an odds ratio of 2.2. Using the immunoblot method on the same serum samples, the CagA antibody positive rates were 56% in the controls, and much higher at 91% in the gastric cancer patients, the odds ratio also rising to 21.0. Maeda et al. [16] conducted a similar study, reporting a high incidence of false negative results only in gastric cancer patients. They noted that in past studies using serum antibody kits, the influence of H. pylori infection in the aetiology of gastric cancer has been underestimated statistically, and emphasised the importance of the antibodies used. Problems associated with the use of serum antibody testing for the assessment of the success or failure of H. pylori eradication include the time taken for serum antibody levels to become negative following successful eradication, and the use of delta values in the assessment. It is, however, a simple method, and our results show that the size of the changes in serum antibody levels seen using the E-plate kit, that uses H. pylori strains isolated from Japanese patients, made eradication assessment feasible at 2 months post-eradication therapy, in contrast to the HM-CAP using strains isolated from Western patients. This new test kit will be important in the future in further popularising H. pylori eradication therapy in Japan.

References


