ORIGINAL ARTICLE

EVALUATION OF THE APPROPRIATE CUT-OFF VALUE FOR SEROLOGICAL DIAGNOSIS OF HELICOBACTER PYLORI INFECTION BY COMPARISON WITH A STOOL ANTIGEN TEST

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Abstract Severe atrophic gastritis in aged Helicobacter pylori-positive patients leads to the decrease of the level of H. pylori in gastric mucosa. Cut-off value for E-plate (a serum H. pylori antibody kit) has been set regardless the age of the subjects. The aim of this study was to estimate appropriate cut-off values for E-plate in younger and elderly subjects. A total of 994 healthy adults who received a health survey in Iwaki area of Hirosaki City in 2005 were tested. We divided the subjects into two groups; the elderly group included 594 subjects who were born before 1950 and the younger group included 400 subjects who were born after 1950. We evaluated appropriate cut-off values of E-plate by stool antigen test as gold standard. The ROC curves showed the best cut-off level to be 12.5 U/ml (sensitivity: 94.2%, specificity: 84.0%) in elderly group and 14.5 U/ml (sensitivity: 93.4%, specificity: 89.4%) in younger group. False positive results of E-plate were more frequent in elderly group (P < 0.001). Cut-off values of E-plate should be decided according to the age of the subjects.

Key words: Helicobacter pylori; E-plate; cut-off value.


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Introduction

Infection of Helicobacter pylori induces chronic active inflammation of the gastric mucosa and associated with the development of several upper gastrointestinal diseases such as atrophic gastritis, peptic ulcer, gastric cancer, MALT lymphoma and hyperplastic polyp. Eradication of H. pylori prevents recurrence of peptic ulcer and reduces metachronous gastric cancer after endoscopic resection of gastric cancer. Eradication of H. pylori is also the treatment of the first choice for gastric MALT lymphoma. Furthermore, H. pylori infection is associated with extragastric disorders such as immune thrombocytopenic purpura, iron deficiency anemia and chronic urticaria. In 2009, the Japanese Society for Helicobacter Research revised the guidelines for the management of Helicobacter pylori infection and recommended H. pylori eradication for all the infected patients. As the indication for the treatment of H. pylori infection has been expanded, diagnosis of infection and evaluation of the results of eradication therapy have become more significant.

Measurement of serum antibody is one of the most widely-used methods for the management of H. pylori infection. More recently, serum antibody is used in the screening for gastric cancer known as ABC method, which is the combination of the detection of serum H. pylori antibody and determination of atrophic gastritis by serum pepsinogen (PG) levels. Among serum antibody tests, E-plate is the most commonly used test in Japan because this test was developed using Japanese H. pylori strains and highly accurate for Japanese patients. To date, cut-off value of E-plate test has been the same in any ages. In patients with H. pylori infection, the prevalence of atrophic gastritis increases with age. Severe atrophic gastritis leads the decrease of H. pylori colonization and the decrease of H. pylori antibodies occurs. Therefore, the appropriate cut-off value of E-plate would be different between younger and elderly populations. Particularly, low level of serum antibody can cause false-negative results of ABC screening for gastric cancer, and some people may miss the chance of having detailed examination without being aware of the risk of gastric cancer.

The aim of this study was to examine the difference of appropriate cut-off value for E-plate in younger and elderly patients by the comparison of a monoclonal antibody-based stool antigen test.

Materials and Methods

Among 1070 healthy adults who received health survey (Iwaki Health Promote Project) in Iwaki area of Hirosaki City in April 2005, 994 subjects agreed to participate in this study. Subjects took their stool samples in the morning of the day of health survey. The subjects were 381 men and 613 women; the mean age was 56.9 ± 13.9 years old. Subjects who had previous history of gastric surgery, successful H. pylori eradication were excluded. We divided the subjects into elderly and younger groups. The elderly group included 594 subjects, who were born before 1950 and the younger group included 400 subjects, who were born after 1950. We evaluated appropriate cut-off value of E-plate by using the stool antigen test as a gold standard. Stool samples were stored at -80°C and tested for the prevalence of H. pylori antigen with enzyme immunoassay (EIA) by using a monoclonal antibody kit (Testmate pylori antigen enzyme immunoassay; TPAg EIA, Wakamoto Pharmaceutical Co. Ltd. Kanagawa, Japan & Kyowa Medex, Tokyo, Japan). Serum samples were stored at -20°C and tested for the prevalence of IgG antibody to H. pylori by E-plate (Eiken, Tokyo, Japan). The
levels of PG I and PG II were also measured by radioimmunoassay. The result was considered indicative of atrophic gastritis when both a PG I level of $< 70 \mu g/l$ and a PG I/II ratio of $< 3.0$ were obtained and severe atrophic gastritis when both a PG I level of $< 30 \mu g/l$ and a PG I/II ratio of $< 2.0$ 14). Differences of the results of E-plate between younger and elderly groups were examined by $\chi^2$ analysis. A $p$-value of $< 0.05$ was considered statistically significant. Receiver operating characteristic (ROC) curves were used to determine the appropriate cut-off value.

All the subjects provided their written informed consent, and this study was approved by the ethics committee of Hirosaki University.

### Results

In younger group and elderly group, 29% and 53% of the subjects had atrophic gastritis and 4% and 21% of the subjects had severe atrophic gastritis by serum PGs (Table 1). The prevalence of subjects who had severe atrophic gastritis was significantly higher inelderly group than younger group ($P < 0.001$). When diagnosis of *H. pylori* infection was determined by TPAg EIA, 42% of subjects in younger group and 64% of subjects in elderly group were tested positive (Table 1). In all the 994 subjects, when present cut-off value of E-plate was used, 551 subjects were positive for TPAg EIA including 22 subjects negative for E-plate and 443 subjects were negative for TPAg EIA including 75 subjects positive for E-plate (Table 2). Sensitivity, specificity and accuracy of E-plate were 96.0%, 83.1% and 90.2%, respectively, in all the 994 subjects. Although the sensitivity and accuracy were not different, the specificity was 79.1% in elderly group and 86.1% in younger group, respectively. The specificity of E-plate was significantly lower in

| Table 1 The characteristics of younger subjects and elderly subjects. |
|-----------------|-----------------|-----------------|
| Male / Female   | 163 / 237       | 218 / 376       |
| Age (mean ± SD) | 42.9 ± 9.16     | 66.2 ± 6.81     |
| *H. pylori* infection (stool antigen positive) | 42 % | 64 % *|
| Atrophic gastritis | 29 % | 53 % *|
| Severe atrophic gastritis | 4 % | 21 % *|
| *: $P < 0.001$ |

| Table 2 Comparison of serology and stool antigen test for detection of *H. pylori* infection. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| E-plate         | TPAg            | EIA            | sensitivity (%) | specificity (%) | accuracy (%) |
| + ve            | 529             | 75             | 96.0           | 83.1           | 90.2         |
| - ve            | 22              | 368            |                |                |              |
| younger subjects | + ve            | 162            | 32             | 95.8           | 86.1         | 90.3         |
|                 | - ve            | 7              | 199            |                |              |
| elderly subjects | + ve            | 367            | 43             | 96.1           | 79.7         | 90.2         |
|                 | - ve            | 15             | 169            |                |              |

*: $P < 0.001$. Cut-off value is set as 10 U/ml according to the manufacturer’s instructions.
Appropriate Cut-Off Value for E-plate

elderly group comparing with younger group (P < 0.001).

Appropriate cut-off value determined by ROC curve analysis was 12.5 U/ml (sensitivity: 94.2%, specificity: 84.0%, accuracy: 90.6%) in elderly group (Figure 1) and 14.5 U/ml (sensitivity: 93.4%, specificity: 89.4%, accuracy: 91.0%) in younger group (Figure 1).

Discussion

The present study demonstrated that appropriate cut-off value of E-plate determined by ROC curve was higher in younger subjects.
than elderly subjects. The difference would be caused by the prevalence and extent of atrophic gastritis in *H. pylori* infected patients increased with age. In this study, the proportion of the subjects who had severe atrophic gastritis increased in elderly group. In patients with severe atrophic gastritis, *H. pylori* colonization is decreased or spontaneously disappeared resulting in the decrease of serum antibody. Therefore, in some patients with severe atrophic gastritis, the value of E-plate became under the cut-off value. There were also other patients with severe atrophic gastritis, the value of E-plate remains above the cut-off level while *H. pylori* colonization had been already disappeared. False positive results of serology in such patients would cause low specificity of E-plate in elderly subjects. Actually, although sensitivity and accuracy were not different between elderly and younger groups, specificity of E-plate was significantly lower in elderly group by both present cut-off and appropriate cut-off determined by ROC curves. Severe atrophic gastritis presented in aged subjects may cause false negative and negative serology. Thus, serological diagnosis of *H. pylori* infection, including E-plate, may be more reliable in younger populations.

In this study, a monoclonal antibody-based stool antigen test was used as a gold standard. According to the guidelines for the management of *Helicobacter pylori* infection in Japan, stool antigen tests based on monoclonal antibody appear to be an accurate test for evaluating the results of *H. pylori* eradication therapy as well as urea breath test. TPAg EIA was developed in Japan and has been shown to have high sensitivity and specificity. Since this study was taken place as a part of a health survey, the methods of estimation of *H. pylori* infection had to be easy and costless. Thus, we used TPAg EIA as a gold standard to estimate appropriate cut-off value of E-plate.

ABC method is a new gastric cancer screening developed in Japan by combination of the detection of serum anti-*H. pylori* antibody and the measurement of serum PG levels. In ABC method, subjects are classified into one of 4 risk groups (group A, B, C, and D) according to the presence of atrophic gastritis identified by serum PGs and *H. pylori* seropositivity. Group A comprised the subjects whose results are negative for both anti-*H. pylori* antibody and atrophic gastritis. Group B comprised the subjects whose results are seropositive for *H. pylori* but negative for atrophic gastritis. Group C comprised the subjects whose results are positive for both anti-*H. pylori* antibody and atrophic gastritis. Group D comprised the subjects with atrophic gastritis and anti-*H. pylori* antibody levels lower than the cut-off value. Several studies have shown the usefulness of this method for assessment of the risk of gastric cancer. However, one of the problems of ABC method is false negative result of *H. pylori* serology. Some elderly subjects who show false negative results by serology would be classified into group A which is recognized to have very low risk of gastric cancer. Such subjects would lose the chance of receiving endoscopy. Actually, in our department, the level of E-plate was estimated slightly below the recommended cut-off level in some elderly patients with early gastric cancer. Since the purpose of cancer screening is to reduce the mortality rate, it is important to distinguish the patients with early gastric cancer as the subjects who need endoscopy. Gastric cancer is more frequently occur in elderly patients who have severe atrophic gastritis. However, ABC method was performed without consideration of the possibility of the decrease of serum antibody level in elderly patients. Therefore, more consideration is necessary for the cut-off level of *H. pylori* serology particularly in elderly subjects.
### Conclusion

Appropriate cut-off value of E-plate test was lower in elderly people than in younger people by using the stool antigen test as a gold standard. The specificity of E-plate was significantly lower in elderly group. Appropriate cut-off value of E-plate should be examined according to age of the subjects and the purpose of serodiagnosis such as ABC screening particularly in elderly populations.

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