

ORIGINAL ARTICLE

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

Chikara Iino¹⁾, Tadashi Shimoyama¹⁾, Takao Oyama¹⁾, Daisuke Chiba¹⁾,
Takashi Umeda²⁾, Ippei Takahashi²⁾, Masashi Matsuzaka²⁾, Kaori Iwane²⁾
and Shinsaku Fukuda¹⁾

Abstract Severe atrophic gastritis in aged *Helicobacter pylori*-positive patients leads to the decrease of the level of amount 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.

Hirosaki Med. J. 63 : 48—54, 2012

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

原 著

Helicobacter pylori 便中抗原との比較による *Helicobacter pylori* 血清抗体 (E-plate) のカットオフ値についての検討

飯 野 勢¹⁾ 下 山 克¹⁾ 小 山 隆 男¹⁾ 千 葉 大 輔¹⁾
梅 田 孝²⁾ 高 橋 一 平²⁾ 松 坂 方 士²⁾ 岩 根 かほり²⁾
福 田 眞 作¹⁾

抄録 *Helicobacter pylori* 感染高齢者の多くは萎縮性胃炎が高度となり、感染菌量が減少・消失するが、これまで *H. pylori* 血清抗体測定法のカットオフ値は年齢の影響が考慮されてこなかった。2005年の青森県岩木地区の健診受診者を対象とし、1950年以前の出生者594人を高齢群、以降の出生者400人を若年群について、便中抗原測定法を gold standard とした場合の両群での E-plate の至適カットオフ値を調べた。ROC 曲線より、E-plate の至適カットオフ値は高齢群で12.5 U/ml (感度94.2%, 特異度84.0%), 若年群では14.5 U/ml (感度93.4%, 特異度89.4%)となり、高齢者で低かった。高齢群では E-plate の偽陽性の頻度も高かった ($P < 0.001$)。E-plate のカットオフ値は対象者の年齢も考慮して設定すべきである。

弘前医学 63 : 48—54, 2012

キーワード: *Helicobacter pylori* ; E-plate ; cut-off value.

¹⁾ Department of Gastroenterology and Hematology, Hirosaki University Graduate School of Medicine

²⁾ Department of Social Medicine, Hirosaki University Graduate School of Medicine

Correspondence: T. Shimoyama

Received for publication, December 22, 2011

Accepted for publication, December 28, 2011

¹⁾ 弘前大学大学院医学研究科消化器・血液内科学講座

²⁾ 弘前大学大学院医学研究科社会医学講座

別刷請求先: 下山 克

平成23年12月22日受付

平成23年12月28日受理

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^{1, 2)}. Eradication of *H. pylori* prevents recurrence of peptic ulcer³⁾ and reduces metachronous gastric cancer after endoscopic resection of gastric cancer⁴⁾. Eradication of *H. pylori* is also the treatment of the first choice for gastric MALT lymphoma⁵⁾. Furthermore, *H. pylori* infection is associated with extragastric disorders such as immune thrombocytopenic purpura⁶⁾, iron deficiency anemia⁷⁾ and chronic urticaria⁸⁾. 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 patients⁹⁾. 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 method¹⁰⁾, 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 patients¹¹⁾. 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 age¹²⁾. Severe atrophic gastritis leads the decrease of *H. pylori* colonization

and the decrease of *H. pylori* antibodies occurs¹³⁾. 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)¹³⁾. Serum samples were stored at -20°C and tested for the prevalence of IgG antibody to *H. pylori* by E-plate (Eiken, Tokyo, Japan)¹¹⁾. The

Table 1 The characteristics of younger subjects and elderly subjects.

	Younger subjects	Elderly subjects
Male / Female	163 / 237	218 / 376
Age (mean \pm SD)	42.9 \pm 9.16	66.2 \pm 6.81
<i>H. pylori</i> 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 + ve	EIA - ve	sensitivity (%)	specificity (%)	accuracy (%)
All	+ 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.

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 $< 70 \mu\text{g/l}$ and a PG I/II ratio of < 3.0 were obtained and severe atrophic gastritis when both a PG I level of $< 30 \mu\text{g/l}$ 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 χ^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 in elderly 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

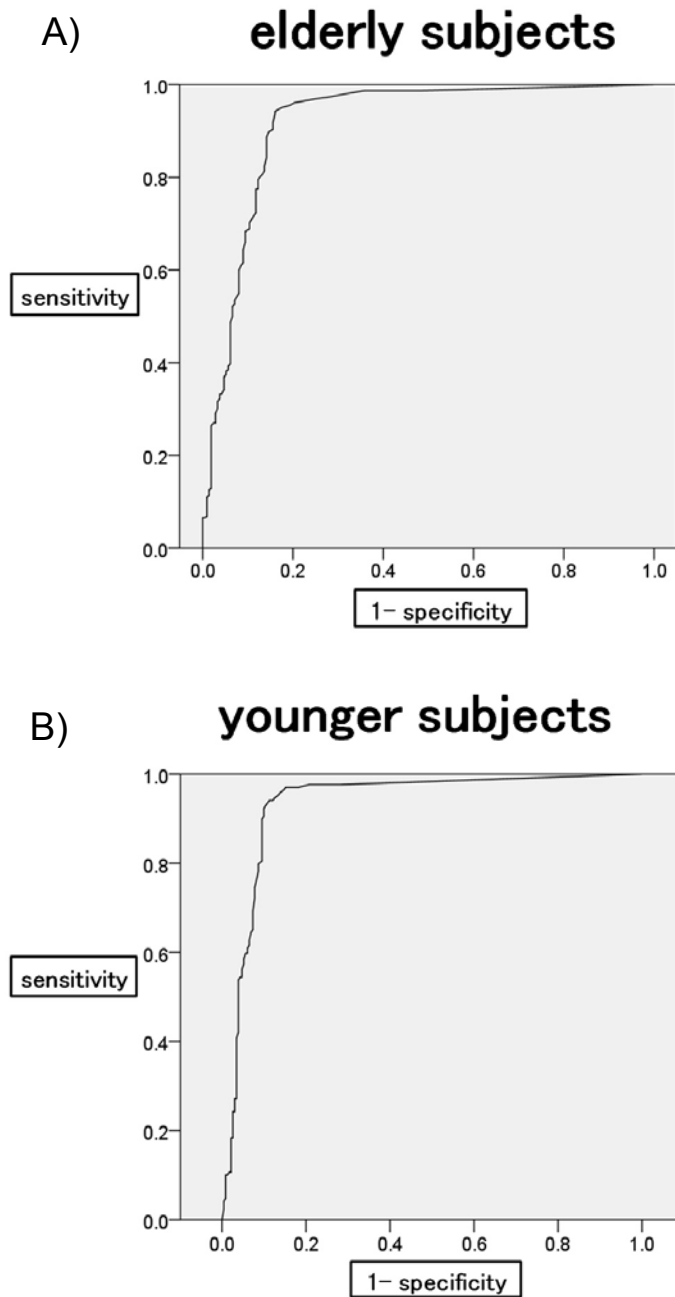


Figure 1 ROC curve of E-plate using TPAg EIA as the standard. A) for elderly subjects and B) for younger subjects, respectively.

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 age¹²⁾. 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 positive 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 test⁹⁾. TPAg EIA was developed in Japan and has been shown to have high sensitivity and specificity^{15, 16)}. 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 levels¹⁰⁾. 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^{17, 18)}. 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.

Acknowledgements

This study was partly supported by Grant-in-Aids from the Ministry of Education, Culture, Sports, Science and Technology, Japan (no. 18200044-00 and 23659342), and was based on the Iwaki Health Promotion Project as a project by Hirosaki University Graduate School of Medicine, in collaboration with Aomori Health Evaluation and Promotion Center and Hirosaki City Office, Department of Health Promotion.

References

- 1) Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001;345:784-9.
- 2) Ohkusa T, Takashimizu I, Fujiki K, Suzuki S, Shimoi K, Horiuchi T, Sakurazawa T, et al. Disappearance of hyperplastic polyps in the stomach after eradication of *Helicobacter pylori*. A randomized, clinical trial. *Ann Intern Med* 1998; 129:712-5.
- 3) Malfertheiner P, Leodolter A, Peitz U. Cure of *Helicobacter pylori* associated ulcer disease through eradication. *Baillieres Best Pract Res Clin Gastroenterol* 2000;14:119-32.
- 4) Fukase K, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, Terao S, et al. Effect of eradication of *Helicobacter pylori* on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. *Lancet* 2008;372:392-7.
- 5) Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, Isaacson PG. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. *Lancet* 1993;342:575-7.
- 6) Emilia G, Longo G, Luppi M, Gandini G, Morselli M, Ferrara L, Amarri S, et al. *Helicobacter pylori* eradication can induce platelet recovery in idiopathic thrombocytopenic purpura. *Blood* 2001; 97:812-4.
- 7) Marignani M, Angeletti S, Bordi C, Malagnino F, Mancino C, Delle Fave G, Annibale B. Reversal of long-standing iron deficiency anaemia after eradication of *Helicobacter pylori* infection. *Scand J Gastroenterol* 1997;32:617-22.
- 8) Fukuda S, Shimoyama T, Umegaki N, Mikami T, Nakano H, Munakata A. Effect of *Helicobacter pylori* eradication in the treatment of Japanese patients with chronic idiopathic urticaria. *J Gastroenterol* 2004;39:827-30.
- 9) Asaka M, Kato M, Takahashi S, Fukuda Y, Sugiyama T, Ota H, Uemura N, et al. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2009 revised edition. *Helicobacter* 2010; 15:1-20.
- 10) Inoue K, Fujisawa T, Haruma K. Assessment of degree of health of the stomach by concomitant measurement of serum pepsinogen and serum *Helicobacter pylori* antibodies. *Int J Biol Markers* 2010;25:207-12.
- 11) Kawai T, Kawakami K, Kudo T, Ogiyama S, Handa Y, Moriyasu F. A new serum antibody test kit (E plate) for evaluation of *Helicobacter pylori* eradication. *Intern Med* 2002;41:780-3.
- 12) Haruma K, Kamada T, Kawaguchi H, Okamoto S, Yoshihara M, Sumii K, Inoue M, et al. Effect of age and *Helicobacter pylori* infection on gastric acid secretion. *J Gastroenterol Hepatol* 2000;15:277-83.
- 13) Sato M, Shimoyama T, Takahashi R, Kajiyama H, Sano Y, Sakaedani N, Kato A, et al. Characterization and usefulness of stool antigen test using

- monoclonal antibody to *Helicobacter pylori* catalase. J Gastroenterol Hepatol (in press).
- 14) Miki K. Gastric cancer screening using the serum pepsinogen test method. Gastric Cancer 2006;9:245-53.
- 15) Shimoyama T, Oyama T, Matsuzaka M, Danjo K, Nakaji S, Fukuda S. Comparison of a stool antigen test and serology for the diagnosis of *Helicobacter pylori* infection in mass survey. Helicobacter 2009;14:87-90.
- 16) Shimoyama T, Kato C, Kodama M, Kobayashi I, Fukuda Y. Comparison of a monoclonal with a polyclonal antibody-based enzyme immunoassay stool test in diagnosing *Helicobacter pylori* infection before and after eradication therapy. Jpn J Infect Dis 2009;62:225-7.
- 17) Watabe H, Mitsushima T, Yamaji Y, Okamoto M, Wada R, Kokubo T, Doi H, et al. Predicting the development of gastric cancer from combining *Helicobacter pylori* antibodies and serum pepsinogen status: a prospective endoscopic cohort study. Gut 2005;54:764-8.
- 18) Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura H, Yoshikawa A et al. Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. Int J Cancer 2004;109:138-43.