disappearance of polymorphonucleocytes is a histologically significant change shortly after *H. pylori* eradication, spotty redness of the fundic gland region is suggested to be related to histological activity.

Flat erosion of stomach and duodenum is related to recovery of acid output after successful eradication.[24] Basal gastric acidity increases after successful *H. pylori* eradication, but does not change for subjects with persistent infection.[25] Specifically, Japanese patients have a high likelihood of acid recovery after successful eradication because approximately 80% of *H. pylori*-infected patients end up with corpus-predominant gastritis. Although an increase in duodenal erosion is due to acid recovery, it occurs less frequently and only for a short duration after successful eradication.

The change in conventional endoscopic features with white light imaging has not been clarified. However, the change in magnifying endoscopic features with narrow band imaging during *H. pylori* eradication has been reported. Yagi *et al.* evaluated magnifying endoscopic change focusing on mucosal and microvascular patterns 1 year after successful eradication.[15] Changes in magnified findings after successful eradication included disappearance of erythema and swelling of areas between gastric pits, pinhole-like changing of white pits, and recovery of RAC. Okubo *et al.* also reported changes in gastric mucosal patterns observable by magnifying NBI.[16] The patterns of enlarged or elongated pits improved to small oval or pinhole-like round pits, and the density of fine irregular vessels decreased. However, a 5-year follow-up study using conventional endoscopy by Oda *et al.* reported that although histological atrophy improved, endoscopic examination revealed no consistent alteration in atrophic border.[26] Antral erosion became more conspicuous 5 years after successful eradication. Spotty redness in the corpus disappeared after 5 years.

The present study has limitations. Assessment of endoscopic findings depended on the endoscopist; however, a meeting was held to agree upon standards for endoscopic assessment. Because endoscopic change varies with the interval after successful eradication, short-term change is never relevant.

In conclusion, assessment of spotty redness after eradication treatment is useful in the diagnosis of *H. pylori* eradication.

CONFLICT OF INTERESTS

AUTHORS DECLARE NO conflict of interests for this article.

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Scientific Research

Coccoid *Helicobacter pylori* Can Directly Adhere and Invade in Agminated Formation to Human Gastric Epithelial Cells

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ABSTRACT

Helicobacter pylori (*H. pylori*) can infect into the epithelial cell to cause benign or malignant disorders. Under stressful environment, a spiral form of *H. pylori* is transformed into a coccoid form. The infectivity of the coccoid form is still controversial. Since spiral forms are transformed into two types of coccoid forms *via* different mechanisms, the infectivity of the two types of coccoid forms into human gastric epithelial cell was examined. A laboratory and a clinical strain of *H. pylori* were cultured in liquid medium under different conditions to produce the two types of coccoid forms. These coccoid *H. pyloris* were then co-cultured with human derived gastric epithelial cell, MKN-28. Adhesion and penetration of bacteria into MKN-28 cells were monitored by scanning-, standard transmission- and immunotransmission-electron microscopy (SEM, TEM and ITEM). We observed that both coccoid forms were able to adhere onto the surface of MKN-28 cells in agminated formation and also penetrated into the gastric epithelial cells besides the spiral form of *H. pylori*. Coccoid *H. pylori* is not a passive entity but can actively infect the human gastric epithelial cell.

Keywords: *H.pylori*; Electron Microscopy; Coccoid Form; Spiral Form; Bacterial Infection; *Cag A*

1. Introduction

Helicobacter pylori (*H. pylori*) is a microaerophilic bacterium that produces many benign or malignant disorders [1-3]. Eradication of *H. pylori* in patients after endoscopic resection during the early stage of gastric cancer has been shown to greatly reduce the development of metachronous gastric carcinoma [4]. Under stressful conditions, *H. pylori* changes from a spiral form to a coccoid one [5].

We reported that two types of coccoid forms could be produced in culture using different glucose-concentrations via different mechanisms [6-8]. One form (Type A) has irregular surface with few flagella and an indistinct cytoplasmic membrane cultured in Brucella-specific broth (Difco; USA) culture medium with 10% heat-activated horse serum (designated as CLM), and the other form (Type B) has smooth surface with tightly encircled flagella and comparatively clear membrane cultured in 300 mM glucose added CLM (designated as 300 mM-LM).

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Though the viability and characteristics of the coccoid forms have been a subject of controversy [9,10], there are many reports that non-spiral *H. pylori* is viable and there is a morphological manifestation for cell adaptation to severely non-optimal environment [11].

H. pylori can be seen adhering onto the surface of not only the biopsied gastric epithelial cell [12,13] but also cultured cells [14,15]. However, there had been several studies on adhesion and invasion of coccoid forms into human epithelial cells. One stated that coccoid forms could adhere and invade into human gastric epithelial cells [16]. Others suggested that coccoid forms could adhere only poorly onto such human cultured cells [17] or that the non-spiral bacteria produced as a result of antibiotic treatment would be passively adhered to such human cultured cells to be eventually destroyed [18].

Therefore, in this study, for the direct observation of the adhesion and invasion of the coccoid *H. pylori* into the epithelial cell, the infectivity of two different types of coccoid forms to human cultured epithelial cells was examined by SEM, TEM and ITEM comparing with that

of the spiral form as a control. A standard bacterial stock and a clinical strain of *H. pylori* were co-cultured with cells from a human gastric epithelial cell line and then observed by electron microscopy. Some biopsied specimens containing the bacteria and the gastric epithelial cells extracted from the patients with abdominal discomforts were also used as control.

2. Materials and Methods

2.1. Bacterial Culture

Both the ATCC 43504 strain and a clinical strain established from an *H. pylori*-positive patient were cultured in CLM for 24 hours and then were co-cultured under 8% CO_2 for 1 hour with MKN-28 cells raised in RPMI-1640 medium using a 12 wells plate (Transwell-COL, Corning Costar Co., USA) for 2 days. On the 1st day, approximately 95% of the bacteria showed spiral forms [6-8].

Both the ATCC 43504 strain and a clinical strain established from an *H. pylori*-positive patient were also cultured in CLM and in 300 mM-LM for 4 days. Organisms on the 4th day culture were used for co-culture with MKN-28 cell to examine the adhesion between bacteria and gastric epithelial cell. On the 4th day, approximately 99% of the bacteria in CLM was Type A coccoid form and more than 90% of those in 300 mM-LM was Type B one [7,8].

2.2. Morphological Preparation

After discarding the suspended *H. pylori* in the well, the bacteria that adhered to the MKN-23 cell were fixed in a solution containing 0.1% glutaraldehyde plus 2% paraformaldehyde. The membrane at the bottom of the well, onto which the cells were attached, was cut into three pieces, each for SEM, TEM and ITEM, respectively, as previously reported. Briefly, for SEM [6] or TEM [7,8], the specimens were fixed in 2% glutaraldehyde, for observing with an electron microscope. For ITEM [19,20], the fixed specimens were immediately treated with Lowicryl K4M (Polysciences; Tokyo, Japan) followed by ultra-sectioning. Ultrathin sections mounted on nickel grids were reacted with a rabbit IgG fraction specific for *H. pylori* (DAKO Japan) followed by reacting with gold particle-labeled goat antiserum specific for rabbit IgG. Biopsy materials from the gastric antrum of 24 patients examined by endoscopy for dyspepsia symptoms were also processed by the standard procedure for SEM, TEM and ITEM. No malignant lesion was diagnosed in all biopsy materials by standard light micros-copy.

3. Results

By ITEM, the gold particles indicating positive reaction for the presence of *H. pylori* antigen were detected on the flagella, on the bacterial surface and in the cytoplasm of the bacteria. Our electron microscopic study showed that *H. pylori* bacteria first aligned in agminated pattern to adhere onto the surface of epithelial cell to be incorporated into the cytoplasm.

In the SEM study using spiral bacteria, the gastric epithelial cell was adhered onto the surface by the bacteria in agminated pattern (**Figure 1(a)**). By TEM and

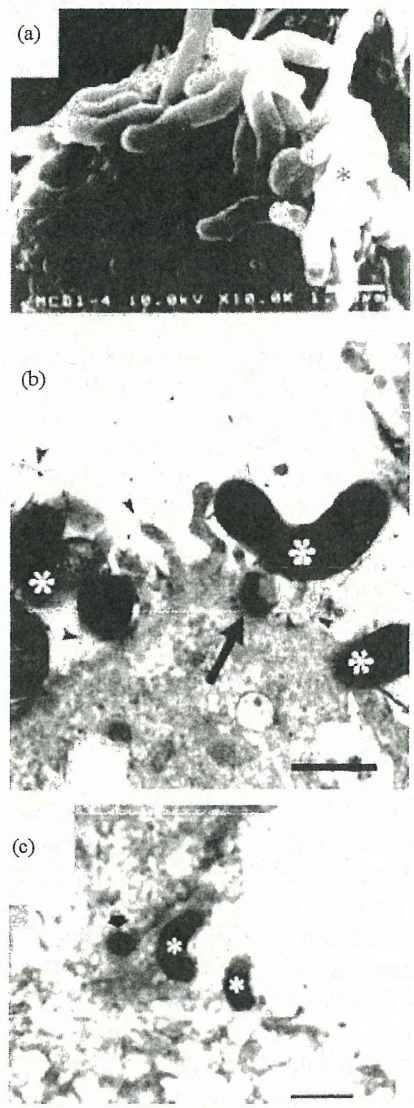

Figure 1. Spiral *H. pylori* as controls. (a) The bacterium (asterisk) agminates onto a certain spot of the surface of the epithelial cell (SEM: A clinical strain from the 1st day of culture. Bar indicates 1 μm); (b) The bacterial body (asterisks) directly attaches or is taken (black and white arrows) into the epithelial cell. Flagella (arrowheads) also adhere on the epithelial cell (TEM: ATCC43504 strain from the 1st day of culture, Bar indicates 0.5 μm); (c) The bacteria (asterisks) directly agminate to contact on the surface of the epithelial cell. A bacterial body (arrow) is observed in the cytoplasm (ITEM: A biopsy specimen, Bar indicates 1 μm).

ITEM, spiral bacteria were observed to be directly in close contact with the surface or microvilli of the epithelial cells (**Figures 1(b)** and **(c)**, asterisks) with flagella touching the microvilli (**Figure 1(b)**, arrowheads) and also their body being already in the cytoplasm of the epithelial cell (**Figures 1(b)** and **(c)**, arrow). We could not observe any agminated pattern of *H. pylori* on any of the biopsied specimens by SEM. Type A (**Figures 2(a)-(c)**) and Type B (**Figures 2(d)** and **(e)**) coccoid bacteria from the standard bacterial stock and a clinical strain of *H. pylori* adhered in similar manner to that of the spiral form. In Type A coccoid forms, the spherical bacteria were seen adhering in agminated pattern on the surface of the epithelial cells (**Figure 2(a)**). Remnants of the flagella and the bacterial body were seen to be directly in contact with the surface of the epithelial cells (**Figure 2(b)**, arrowheads). A vacuole as indicated by gold particles was considered to be the existence of the invaded bacteria in the cytoplasm (**Figure 2(c)**, arrow). Type B coccoid forms also adhered in agminated pattern on the surface of the epithelial cells (**Figure 2(d)**). Flagella (**Figure 2(e)**, arrowheads) and the bacterial body (**Figures 2(b)** and **(c)**, asterisks) were observed to be directly in contact with the surface of the epithelial cells. Amorphous structure with gold particles showing the presence of *H. pylori* antigen in the cytoplasm of the infected epithelial cells (**Figures 2(c)** and **(e)**, arrow) demonstrated that the adhered bacterium had been taken into the epithelial cell.

4. Discussion

In this study, we observed that not only the spiral *H. pylori* but also the coccoid ones assembled at a certain area on the surface of the epithelial cell before being taken into the cytoplasm. In general, a bacterium is thought to adhere to and invade into the epithelial cell only after the colony formation by its own cell-fission [21]. However, our study showed that many coccoid bacteria adhered simultaneously in a convergent fashion onto a narrow area on the surface of the epithelial cell and then invaded into the cytoplasm. In several biopsy specimens, we also observed the internalization of the agminated organisms by TEM and ITEM. In studies using cultured HEp-2 [14, 22] or AGS [23] cells, only spiral *H. pylori* has been reported to be taken into those cells. However, our study

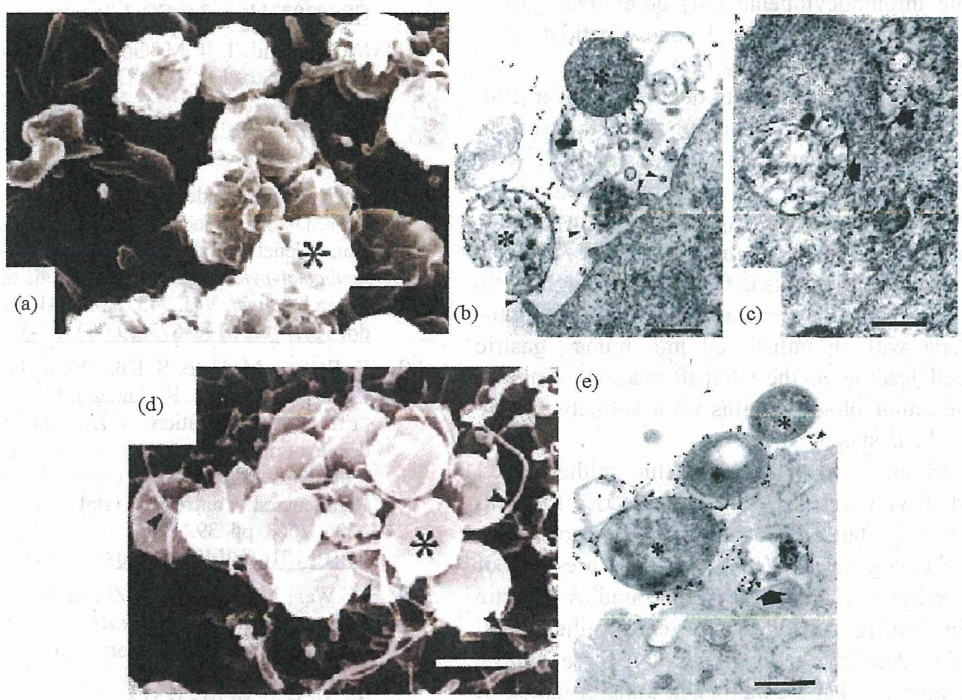

Figure 2. Coccoid *H. pylori* invasion into gastric epithelial cells (Bar indicates 0.5 μm). (a)-(c) show ATCC43504 strain from the 4th day in CLM. (a) Type A coccoid forms (asterisk) also agminates onto a certain area in similar way as shown by the spiral ones serving as control (SEM); (b) Type A coccoid forms (asterisks) are directly in contact with the surface of the epithelial cells using fragella and the bodies (arrowheads) (ITEM); (c) The anti-*H. pylori* antibody positive structures (arrows) are observed in the cytoplasm of the epithelial cells indicating successful penetration (ITEM); (d) and (e) show ATCC43504 strain from the 4th day in 300 mM-LM; (d) Type B coccoid forms (asterisks) also agminates onto a certain area as the spiral and coccoid A bacteria. (SEM); (e) Type B coccoid forms (asterisks) directly in contact with the epithelial cells in the same way as the type A coccoid ones (ITEM) Anti-*H. pylori* antibody positive flagella (arrowheads) are seen. Anti-*H. pylori* antibody positive structure (arrow) is seen in the cytoplasm of epithelial cell.

using Type A- and Type B-coccoid *H. pylori in vitro* [6-8,19] showed colony-like formation with attaching by themselves and invading into the epithelial cells, like that seen for the spiral form.

We have already reported that coccoid *H. pylori* is divided into four categories, namely, the dying one, the living one with culturability, the viable *but* non-culturable one and a special form prepared for species-preservation [19]. In this study, we could observe that the adhered coccoid forms showed an agminated pattern converging at a certain site and then penetrated into the gastric epithelial cells. From this observation, we propose the existence of a fifth category of coccoid *H. pylori*, one which can aggressively adhere in agminated manner and infuse their own genes into the cytoplasm of epithelial cells just like those of the spiral forms. Finally, the genes of the coccoid *H. pylori* could be involved in the production of human gastric ulcers, cancers and other diseases after the invasion into the epithelial cell.

From our observation, whole genes of coccoid *H. pylori* appeared to be finally incorporated into the epithelial cells. It is well-known that this bacterium also elicits many benign disorders such as coronary heart disease [2] or idiopathic thrombocytopenia [24] other than gastric carcinoma. Moreover, almost all Japanese patients with peptic ulcer, who are infected by *Cag A*-positive *H. pylori* [25], do not develop further to duodenal cancer [26]. From this clinical observation, it is suggested that several different genes other than *Cag A* are simultaneously taken into the epithelial cell and are transported together with *Cag A* to play their own respective roles in the mutation of the human cell. Since the infection of *H. pylori* into the gastric epithelial cells is facilitated through direct contact between the cells, several genes from the agminated bacteria will be transferred into human gastric epithelial cell leading to their transformation in accordance to the immunological status vis-a-vis between the bacteria and the host cell.

We actually observed that a transgenic epithelial cell being inserted with just *Cag A* gene gave rise to ultrastructural changes, but did not ultimately led to the direct onset of oncogenesis. We observed the presence of glycogen clusters and increased in the bundles of actin filaments, in the *Cag A* inserted transgenic epithelial cell (not shown in detail here. In preparation for a paper). Thus, it is suggested that the malignant transformation of gastric epithelial cell would need the interaction of several other genes, rather than just *Cag A*. This study supports the clinical phenomena that all other genes attached together with *Cag A* are simultaneously taken into epithelial cells resulting in several disorders. Furthermore, coccoid *H. pylori*, while trying to adapt to the harsh environment, would probably also elicit several *H. pylori*-related disorders.

Since several biopsied specimens showed agminated and invading bacteria, we raised an alarm that these patients should be given due attention for the treatment of sporadic *H. pylori*-related disorders because the bacterial genes might have been inserted into their gastric epithetlial cells. Thus, our study demonstrated that coccoid *H. pylori* is not a passive entity but can actively infect into the human gastric epithelial cell.

In conclusion, from this study, we proposed that coccoid *H. pylori* possessed a fifth characteristic; the ability to directly infect into gastric epithelial cells. This will be added to the already known 4 categories, viz, the dying one, the living one with culturability, the viable *but* non-culturable one and the active form for species-preservation.

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Frequency of *Helicobacter pylori*-Negative Gastric Cancer and Gastric Mucosal Atrophy in a Japanese Endoscopic Submucosal Dissection Series Including Histological, Endoscopic and Serological Atrophy

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Key Words
Helicobacter pylori · Gastric cancer · Endoscopic submucosal dissection

Abstract
Background: The definition of *Helicobacter pylori*-negative gastric cancer depends on the accuracy of diagnosis of *H. pylori* infection. The aim of this study was to determine the frequency of *H. pylori*-negative gastric cancer and to clarify relationships with histological atrophy, endoscopic atrophy, and serological atrophy. ***Methods:*** A total of 240 early gastric cancers were included in this study. The status of *H. pylori* infection was determined from the rapid urease test, ^{13}C-urea breath test, *H. pylori* culture, histopathological examination and examination of IgG antibodies. In *H. pylori*-negative gastric cancer, histological atrophy and intestinal metaplasia, endoscopic atrophy and serological atrophy were assessed by pepsinogen. ***Results:*** The rate of *H. pylori* infection was 77.9% and 19 patients (7.9%) had a history of eradication. 34 patients (14.2%) were diagnosed with *H. pylori*-negative gastric cancer using diagnostic tools of *H. pylori*. However, most of the patients with *H. pylori*-negative gastric cancer had histological atrophy and intestinal metaplasia. Only 1 gastric cancer (0.42%) occurred in the mucosa without histological atrophy, endoscopic atrophy or serological atrophy. ***Conclusion:*** Early gastric cancers in the Japanese endoscopic submucosal dissection series were strongly related to current or past infection with *H. pylori* and to gastric mucosal atrophy.

Introduction

The technique of endoscopic surgery has undergone various developments, particularly in Japan, because of the high frequency of early gastric cancer [1]. The advantage of endoscopic submucosal dissection (ESD), which is a current standard method of endoscopic resection, is curative treatment and accurate pathological diagnosis based on en-bloc resection [2, 3]. On the other hand, the high frequency of metachronous gastric cancer is problematic [4, 5]. Fukase et al. [6] showed in a prospective study that eradication of *Helicobacter pylori (H. pylori)* reduced the incidence of metachronous gastric cancer in one-third of the patients. Maehata et al. [7] disagreed with this opinion and they showed that eradication of *H. pylori*

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did not reduce metachronous gastric cancer from a retrospective follow-up of more than 5 years. Since 2010, the Japanese insurance system has allowed patients who have undergone endoscopic resection to receive eradication of *H. pylori* [8]. However, no evidence of *H. pylori* infection was found in some patients with gastric cancer, and there have been few reports on *H. pylori* infection status of early gastric cancer resected by endoscopic surgery [9, 10].

Determination of *H. pylori* infection status for gastric cancer is problematic because of false negatives for *H. pylori* tests and because of spontaneous disappearance in severely atrophic mucosa [11, 12]. Some studies on *H. pylori*-negative gastric cancer have focused on serological atrophy, histological atrophy and intestinal metaplasia [13–15]. However, there have been few studies on *H. pylori*-negative gastric cancer that have focused on endoscopic atrophy. In this study, we determined the ratio of *H. pylori*-negative early gastric cancer resected by ESD according to diagnostic tools of *H. pylori* and estimated relations with histological atrophy, endoscopic atrophy, and serological atrophy.

Materials and Methods

Patients

A total of 294 gastric epithelial neoplasms that were consecutively treated by ESD between January 2004 and December 2010 were retrospectively investigated. Patients who met the following criteria were excluded from this study: patients who were using an immunosuppressant, patients who had a history of gastric surgery and radiation therapy, and patients in whom *H. pylori* infection status was not determined.

Determination of H. pylori *Infection Status*

H. pylori-Positive

The status of *H. pylori* was determined to be positive when the results of at least one of the following were positive before ESD: rapid urease test (RUT), ^{13}C-urea breath test (^{13}C-UBT), *H. pylori* culture, and histopathologic examination.

In the RUT, by using a Helicocheck kit (Otsuka Pharmaceutical Co., Tokyo, Japan), the presence of *H. pylori* was determined by color changes after 20 min in specimens of normal mucosa biopsy obtained from the gastric antrum and body [16].

H. pylori was cultured by using modified Skirrow agar in a certified central laboratory. Biopsy specimens were obtained from the antrum and the body of the stomach. Static culturing was performed with the use of sheep blood agar M58 (Eiken, Tokyo, Japan) and *H. pylori* isolation medium (Eiken) as culture media with the addition of 10% carbon dioxide (CO_2) at 35°C for 7 days.

For the ^{13}C-UBT, breath samples were obtained before and at 20 min after administration of 100 mg of ^{13}C-urea. $^{13}CO_2$ excretion was measured in a UBiT-IR200 (Photal Otsuka Electronics, Tokyo, Japan), and a level >2.5‰ indicated *H. pylori* infection [17].

In the histopathologic examination, all biopsy specimens from the antrum and the body were stained with Giemsa stain and classified by pathologists according to the updated Sydney System [18].

When results of the above four tests and examinations were all negative, serum *H. pylori* antibody was examined by using the E plate test (Eiken Kagaku, Tokyo, Japan) [19]. The cut-off value was 10 U/ml.

Post-Eradication of *H. pylori*

If the patient had a history of *H. pylori* eradication and no bacteria were found by the RUT, ^{13}C-UBT, culture and histopathology, the patient was diagnosed with post-*H. pylori* eradication without current infection.

H. pylori-Negative

Status of *H. pylori*-negative was determined when results of all *H. pylori* tests (RUT, ^{13}C-UBT, culture, histopathology, and IgG antibody) were negative without a history of eradication.

Definitions of Endoscopic Atrophy and Serological Atrophy

Endoscopic atrophy was defined according to the Kimura-Takemoto classification system before ESD. Kimura and Takemoto [20] divided gastric mucosal atrophy into six groups (closed type: C1, C2, C3, open type: O1, O2, and O3) according to endoscopically recognized differences in the color and height of the gastric mucosa. This classification correlated well with the histological features and showed progress from C1 to O3.

In addition, fasting pepsinogen (PG) I and II levels were assayed using the chemiluminescence enzyme immunoassay for determination of serum atrophy in patients who did not have a history of use of antacids within the previous 2 weeks [21]. Serum samples were collected before ESD. Serological mucosal atrophy was evaluated from these PG levels according to previous reports, and no severe atrophy was defined as PG I level >70 ng/ml and PG I/PG II (PG I/II) ratio >3.0 [22]. PG I and PGI/PG II ratio were compared according to histological atrophy and endoscopically defined atrophy using Student's t test.

Histological Score of Background Mucosa

Biopsy specimens were taken from five sites of the stomach, as recommended by the updated Sydney System [18]: the greater curvature of the antrum, the lesser curvature of the antrum, the lesser curvature of the angulus, the lesser curvature of the corpus, and the greater curvature of the corpus. The state of the gastric mucosa was evaluated according to the updated Sydney System by pathologists. The degrees of atrophy and intestinal metaplasia (IM) were classified into four grades: 0, 'normal'; 1, 'mild'; 2, 'moderate', and 3, 'marked'. Histological non-atrophy was defined as score 0 in both atrophy and IM. Mild atrophy was defined as score 1 of atrophy and/or IM in one site or more. The remainder was defined as severe atrophy. The scores of histological atrophy and IM were compared according to endoscopic atrophy using the Mann-Whitney U test.

Ethics

This study was carried out in accordance with the principles embodied in the Declaration of Helsinki 1975 and was approved by our institutional review board. Informed consent was obtained from all subjects.

Results

Frequency of H. pylori*-Negative Gastric Cancer Determined by Using Diagnostic Tools*

H. pylori infection status in our 294 consecutive patients with gastric cancer resected by ESD is shown in figure 1. 51 patients were excluded according to our exclusion criteria and 3 patients who had a history eradication of *H. pylori* after a finding of gastric lesions were excluded. The *H. pylori*-positive rate in our ESD series was 77.9% (187/240), and 19 patients (7.9%) had a history of eradication. Finally, 34 patients (14.2%) who were negative for all *H. pylori* tests and had not received eradication were diagnosed with *H. pylori*-negative gastric cancer using diagnostic tools.

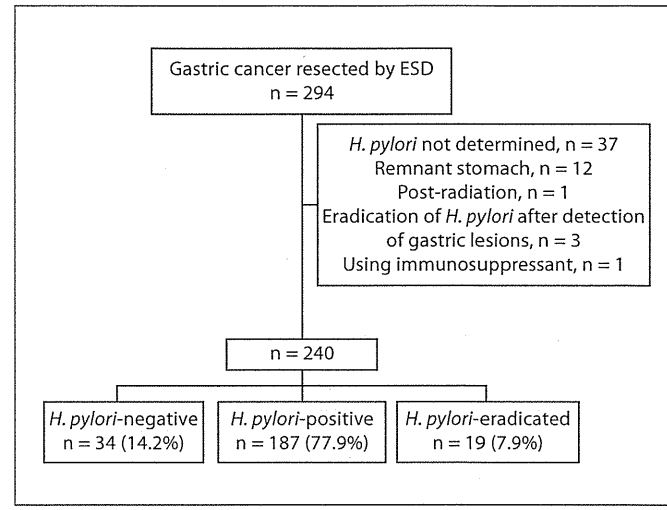

Fig. 1. Flow diagram of *H. pylori* infection status in the present ESD series. *H. pylori* infection was defined as positive from a RUT, histology, culture, UBT and IgG antibody.

Histological Atrophy and IM of H. pylori*-Negative Gastric Cancer*

Most of the *H. pylori*-negative gastric cancers occurred in histologically mild or severe atrophic mucosa, and only 3 patients had neither histological atrophy nor IM in background mucosa (fig. 2).

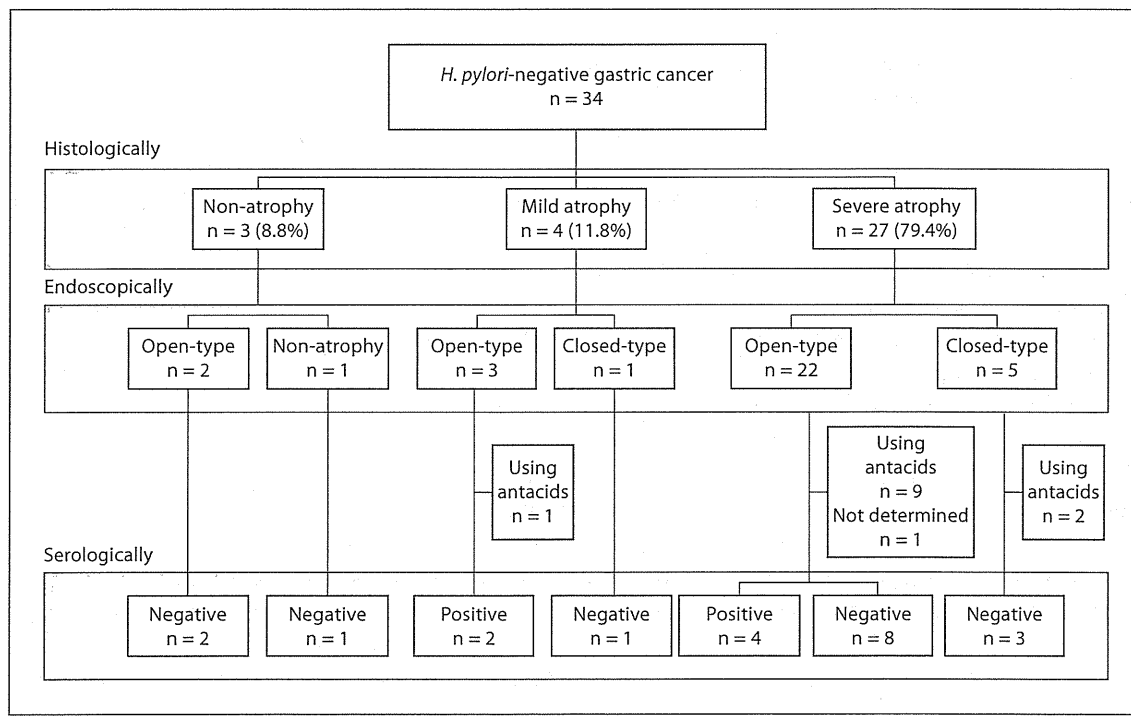

Fig. 2. Flow diagram of histological atrophy, endoscopic atrophy and serological atrophy of *H. pylori*-negative gastric cancer. Histological atrophy was classified into three groups according to updated Sydney System. Endoscopic atrophy was defined according to the Kimura-Takemoto classification system. Serological atrophy was determined when the PG I level was <70 ng/ml and/or the PG I/PG II ratio was <3.0.

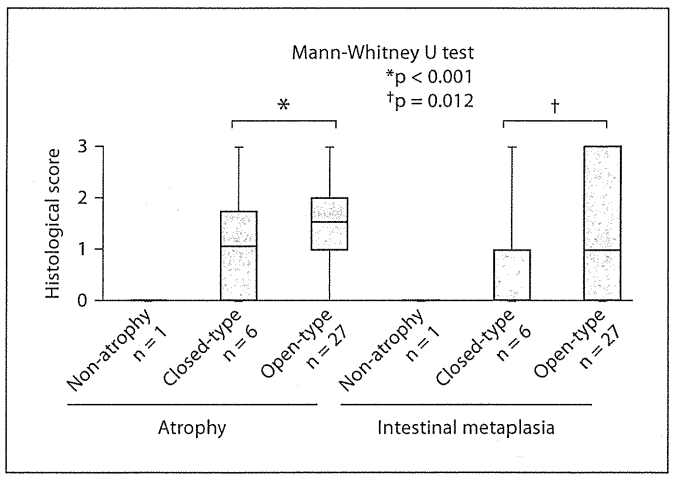

Fig. 3. Histological scores of atrophy and IM in patients who had *H. pylori*-negative gastric cancer. Mean scores of atrophy and IM in 5 sites of background gastric mucosa are shown. Box plots according to endoscopic atrophy show the 25th and 75th percentile with the lowest and highest values, respectively. Scores of atrophy and IM were significantly higher in the endoscopic open-type atrophy group.

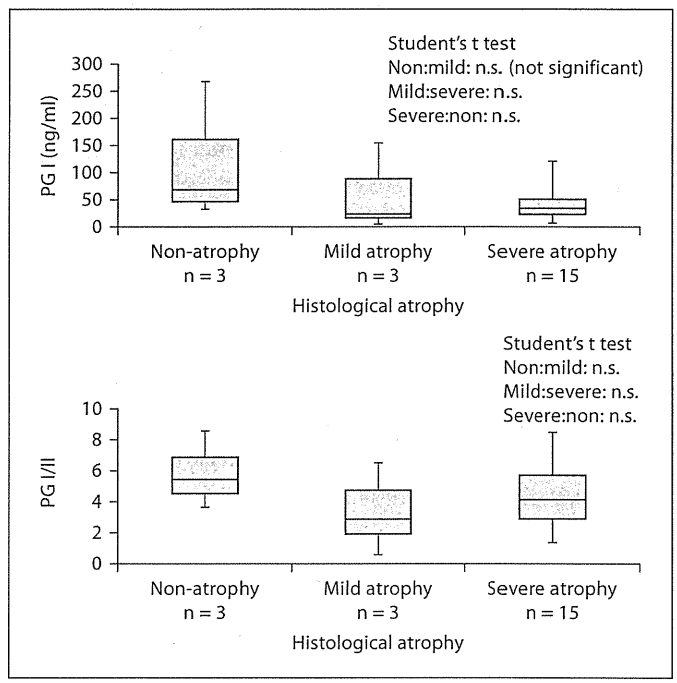

Fig. 4. Correlation between serum PG and histological atrophy in patients who had *H. pylori*-negative gastric cancer. PG I and PG I/II ratio are shown according to degrees of histological atrophy and IM. There were no significant differences in the values of PG I and PG I/II ratio. Horizontal bar: median; box: 25th–75th interquartile range; vertical lines: range of values.

Endoscopic Atrophy and Serological Atrophy of H. pylori*-Negative Gastric Cancer*

Although mild histological atrophy and severe histological atrophy were correlated with endoscopic atrophy, two-thirds of the patients with histological atrophy were negative for serological atrophy. Only 1 cancer in the 34 *H. pylori*-negative gastric cancers occurred in the gastric mucosa without histological atrophy, endoscopic atrophy or serological atrophy (fig. 2).

Mean histological scores of atrophy and IM according to endoscopic atrophy are shown in figure 3. The degrees of endoscopic atrophy were correlated with severity of histological atrophy and IM.

Excluding 13 patients who were using antacids, PG levels were measured in 21 patients of 34 *H. pylori*-negative patients. PG I and PG I/II levels according to histological atrophy and endoscopic atrophy are shown in figures 4 and 5, respectively. There were no significant differences in the values of PG I and PG I/II according to degrees of histological atrophy and endoscopic atrophy.

A Case of H. pylori*-Negative Gastric Cancer without Atrophy*

Figure 6 shows *H. pylori*-negative gastric cancer in a patient without histological, endoscopic or serological atrophy. The tumor was located in the posterior wall of the antrum and was depressed type (a), and the gastric mucosa was smooth with no endoscopically atrophic change (b, c, d). Pathological diagnosis of the tumor, which was completely resected by ESD, was intestinal-type cancer. PG I and II levels were 55.7 and 10.8 ng/ml, respectively, and there was no histological atrophy or IM in the background mucosa.

Discussion

Results of retrospective studies conducted in Japan have indicated that the incidence rate of *H. pylori*-negative gastric cancer is about 2–10% [13–15]. Recently, Yoon et al. [23] reported that the incidence rate was at least 5.4% among South Korean patients. However, advanced gastric cancers, in which the rate of *H. pylori* positivity was lower than that of early gastric cancer, were included into those studies [24, 25]. Therefore, to evaluate the frequency of *H. pylori*-negative gastric cancer, samples obtained from patients with early stage cancer would be more suitable.

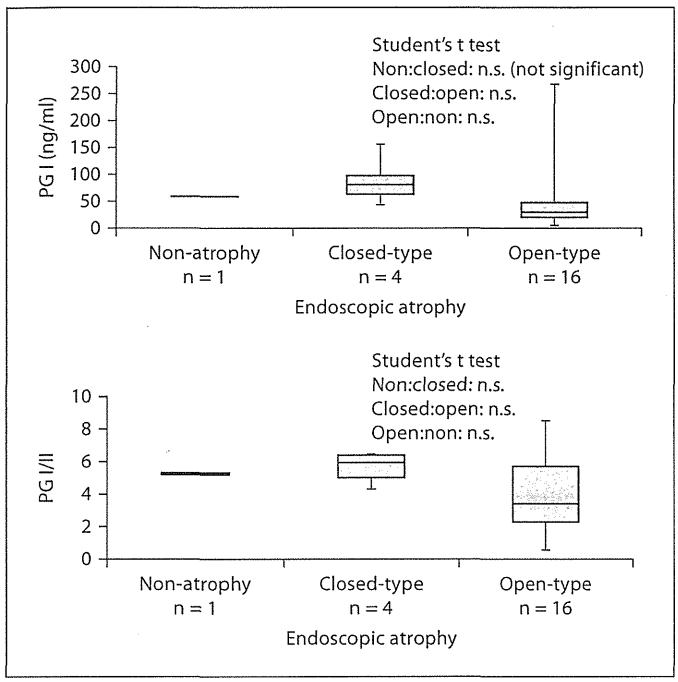

Fig. 5. Correlation between serum PG and endoscopic atrophy in patients who had *H. pylori*-negative gastric cancer. PG I and PG I/II ratio are shown according to endoscopic atrophy. There were no significant differences in the values of PG I and PG I/II ratio between closed-type atrophy and open-type atrophy. Horizontal bar: median; box: 25th–75th interquartile range; vertical lines: range of values.

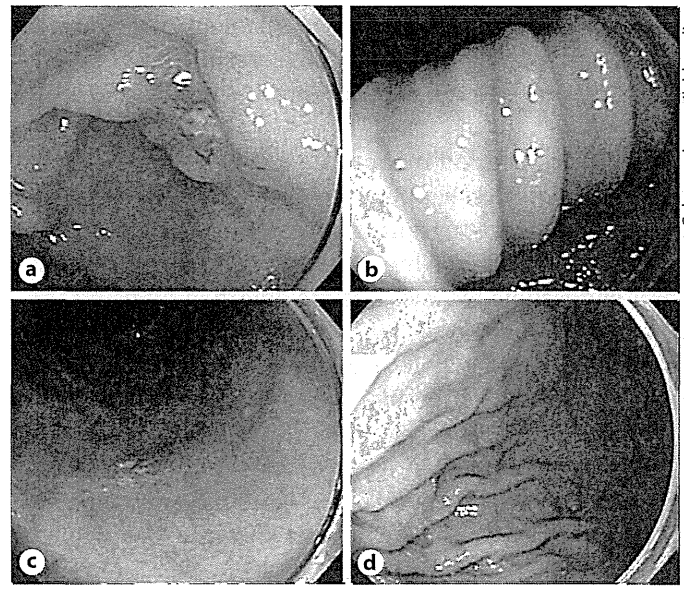

Fig. 6. A case of *H. pylori*-negative gastric cancer without atrophy. **a** A depressed lesion of 15 mm in size is observed in the posterior wall of the antrum. **b** A regular arrangement of collecting venules is seen in the lesser curvature of the angle. **c** Endoscopic atrophy is not seen in the lesser curvature of the corpus. **d** Gastric mucosa in the greater curvature of the corpus is smooth without rugal hypertrophy.

In this study, current or past infection with *H. pylori* was determined by five tests. Histological atrophy and IM, serological atrophy and endoscopic atrophy were analyzed to exclude patients with spontaneous elimination patients as much as possible. Finally, there was only 1 case (0.42%) of early gastric cancer that occurred in patients without current or past *H. pylori* infection and without atrophic mucosa, and the frequency was lower than that in previous studies [13–15, 23].

Recently, Matsuo et al. [26] reported that the frequency of *H. pylori*-negative gastric cancer in many samples of gastric cancer was 0.66%. In that study, *H. pylori*-negative gastric cancer was defined by (1) *H. pylori* antibody, (2) microscopic observation, (3) endoscopic atrophy, and (4) UBT or RUT. Although serological atrophy was not used for their analysis, their data and our data were similar in the low frequency of gastric cancer in patients without *H. pylori* infection and gastric atrophy.

Endoscopic atrophy is commonly used in Japan, but there is no worldwide consensus. The frequency of *H. pylori* infection in patients with atrophic gastritis according to the Kimura-Takemoto classification was 92.7% in Japan, and the severity of endoscopic gastric atrophy according to this classification could help to predict histological atrophy and IM in Vietnam [27, 28]. Recently, image-enhanced endoscopy technology has been developed, and narrow-band imaging with magnifying endoscopy and autofluorescence imaging endoscopy are used for staging atrophic changes in the gastric mucosa [29, 30]. In particular, autofluorescence imaging endoscopy may be useful for objective endoscopic evaluation of atrophic gastritis. Unfortunately, we used conventional endoscopy for evaluating endoscopic atrophy in this series, though our endoscopic atrophy was correlated with histological atrophy and IM.

Uemura et al. [31] reported that gastric cancer in the Japanese population developed only in persons infected with *H. pylori* and intestinal-type gastric cancer did not develop in persons with no or mild endoscopic atrophy. Autoimmune gastritis is very rare in Japan, a *H. pylori* infection might therefore be the most important factor of atrophic gastritis. Although 2 of our cases had diffused

endoscopic atrophy, there was no histological atrophy or serological atrophy. False-negative results of *H. pylori* tests or spontaneous disappearance is suggested in these cases.

Recently, the combination of a serum PG test and a *H. pylori* antibody test has been incorporated into the gastric cancer screening program in Japan [32]. Although the serum PG test might be more accurate and is less invasive than endoscopic biopsies, results obtained by using this test can be affected by age, sex, antacids and infection status of *H. pylori* [22]. *H. pylori* eradication decreases the values of serum PG I and II and increases PG I/II ratio [33]. If the patients in our ESD series had received this screening examination, at least 15 (6.3%) of the patients without a history of eradication would have been included in the lowest risk group of gastric cancer that includes patients who were negative for both *H. pylori* antibodies and PG tests. For reduction of false-negative results of this check-up program, further analysis of PG levels of *H. pylori*-negative gastric cancer is necessary.

Finally, our results must be interpreted in consideration of the following limitations: the sample size was very small and most of our subjects had intestinal-type gastric cancer resected by ESD. The present study should be considered as a preliminary study for countries in which *H. pylori* infection is common and the incidence of gastric cancer is high.

In summary, the prevalence of gastric cancer without current or past *H. pylori* infection and without gastric mucosal atrophy is very low in the Japanese ESD series.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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GASTROENTEROLOGY

Identification of a high risk gastric cancer group using serum pepsinogen after successful eradication of *Helicobacter pylori*

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Key words
gastric cancer screening, gastric carcinoma, *Helicobacter pylori* eradication, pepsinogen I/II.

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Abstract

Background and Aim: Pepsinogen (PG) method is widely used to identify high risk groups of gastric cancer. It is very useful before *Helicobacter pylori* eradication, but after eradication the method becomes useless because the PGI, PGII, PGI/II ratios change. Therefore, we aimed to identify a high risk group for gastric cancer using serum pepsinogen after successful eradication of *H. pylori*.

Methods: A total of 261 participants were enrolled after successful eradication of *H. pylori* in Hokkaido University Hospital from 1995 to 2010. Participants with renal failure, taking proton pump inhibitors, and those with advanced gastric cancer were excluded. Serum levels of PGI and II were measured using chemiluminescent immunoassay method.

Results: Receiver operating characteristic curves using cancerous and non-cancerous data in post-eradication determined the optimal cut-off value of PGI/II as 4.5. The sensitivity and the specificity were 65.9% and 79.3%, respectively. The usual PG method includes 48.9% of cancer cases, and the PGI/II $\leq$ 4.5 in post-eradication includes 65.9% of them, and it includes approximately half of the high risk group of diffuse type cancer. PGI/II $\leq$ 4.5 in post-eradication included many gastric cancer cases detected after eradication (12/16 = 75%).

Conclusion: In the identification of a high risk group for gastric cancer, we suggest that the optimal cut-off value of PGI/II after successful eradication of *H. pylori* is 4.5. PGI/II $\leq$ 4.5 in post-eradication includes more gastric cancer cases compared with the traditional PG method, and 75% of gastric cancer cases detected after eradication.

Introduction

Gastric mucosal atrophy resulting from chronic inflammation caused by persistent infection with *Helicobacter pylori* is well known to be strongly associated with the development of differentiated gastric cancer.[1,2] Moreover, gastric cancer risk is known to be increased as atrophic gastritis, based on endoscopic findings, increases.[3,4] Histological studies have also found that gastric cancer risk is incrementally increased by progression of mucosal atrophy, separately, of the gastric corpus and antrum.[5,6] Atrophy involving the entire stomach, referred to as multifocal atrophic gastritis, has been revealed to have the highest risk for development of gastric cancer among background gastric mucosal factors.[7]

In Japan, barium radiography of the stomach is performed as a part of gastric cancer screening for secondary prevention. However, the morbidity and mortality of gastric cancer have not been reduced, for various reasons such as a decreased screening rate and low detection rate of early gastric cancer, showing the limits of the present gastric cancer screening system.[8] Thus, the pepsinogen (PG) method, which is superior for detecting gastric mucosal atrophy, was devised as a more efficient and less invasive gastric cancer screening method and applied to the opportunistic and organized population-based forms of screening.[9–13]

Pepsinogen is the inactive precursor of pepsin specifically produced in the stomach, of which 99% is secreted into the gastric lumen and 1% into the blood stream. PG is comprised mainly of two biochemically and immunologically different isozymes (PGI and PGII). PGI is secreted only from the oxyntic mucosa,[14] PGII from the fundic, pyloric and proximal duodenal glands.[15] Serum PGI levels and PGI/II ratios are known to correlate with the extent of mucosal atrophy in the gastric corpus and gastric acid secretion ability. The PG method is considered to provide useful indices reflecting morphological and functional states of the gastric mucosa and is also referred to as a serological gastric

biopsy.[16,17] In Japan, in which the frequency of mucosal atrophy in the gastric corpus is high, the PG method is extremely suitable. Recent prospective cohort studies also confirmed that measurement of serum PG levels before eradication of *H. pylori* is useful for assessing gastric cancer risk[18–20] and Yanaoka *et al.* reported that atrophy-negative subjects with pepsinogen I of > 70 ng/mL and pepsinogen I/II ratio of < or = 3.0 (reflecting putative inflammation-based high pepsinogen II level) are at high risk for cancer, particularly diffuse-type cancer, with a cancer incidence rate comparable with atrophy-positive subjects.[20] In 2007, a case-control study on the effect of gastric cancer screening using the PG method on mortality reduction showed for the first time that this method is effective for reducing mortality from gastric cancer.[21] Furthermore, the ABC method[18] using both anti-*H. pylori* antibody and serum PG levels allows classification of gastric cancer risk into the following groups based on these levels: Group A is negative for both PG method results and the antibody, Group B is negative for PG method results and positive for the antibody, Group C is positive for both PG method results and the antibody, and Group D is positive for the PG method results and negative for the antibody. According to reports on cohort studies[22] undergoing comprehensive medical examinations[23] etc., based on ABC method results, Groups A, B, C and D represent low, moderate, high, and very high risk, respectively. In cases with classification changing from Group B or C before eradication of *H. pylori* to Group A after eradication, odds ratios are reduced. However, it is known that gastric cancer risk is still higher compared to that in Group A cases before eradication.[22] If no pre-eradication PG level is available, the ABC method is not applicable to the conventional risk classification.

Regarding serological assessment of gastric cancer risk, many studies used serum PG levels before eradication of *H. pylori* as described above, whereas there have been no reports on risk classification of gastric cancer using serum PG levels after eradication of *H. pylori*. The *H. pylori* infection rate in middle-aged and older people in Japan is still higher than in Europe and the United States. Because eradication of *H. pylori* does not result in complete disappearance of gastric cancer risk, using post-eradication serum PG levels for risk classification of gastric cancer may be extremely important.

In this study, we aimed to examine the usefulness of serum PG levels after eradication of *H. pylori* by classifying gastric cancer risk based on these levels.

Methods

Patients. Among patients visiting the outpatient unit of Hokkaido University Hospital between January 1995 and December 2010, we enrolled 261 patients who underwent successful eradication after diagnosis of *H. pylori* infection.

Patients taking proton pump inhibitors,[23] those with a history of upper gastrointestinal tract surgery,[24,25] those with chronic renal failure defined by serum creatinine of 2.0 mg/dL or higher,[26] and those with advanced gastric cancer were excluded because serum PG levels might be affected by their conditions.[27]

Participants included 47 cases of early gastric cancer (gastric cancer group) and 213 non-cancer cases (control group). The gastric cancer group was composed of 34 men and 13 women with a median age of 71 years (64–76). In all 47, early-stage cancer had been confirmed by histopathologic examination of resected samples and endoscopically treated. Cases with advanced gastric cancer were excluded because this disease extensively destroys the normal gastric mucosal structure and may thus affect serum PG levels. The histological type in the gastric cancer group was differentiated cancer in 44 participants and undifferentiated cancer in three.

Table 1 Background of subjects in the study of a high risk gastric cancer group using serum pepsinogen after successful eradication of *Helicobacter pylori*

Total number	261
Age median (range)	57 (45–67)
M : F	142:119
Disease	
GU/DU (including scar)	112 (44%)
Atrophic gastritis	43 (16%)
Hyperplastic polyp	19 (7%)
Nodular gastritis (NG)	35 (13%)
Early gastric cancer	47 (18%)
MALT lymphoma	5 (2%)

DU, duodenal ulcer; GU, gastric ulcer; MALT, mucosa-associated lymphoid tissue.

The control group was composed of 142 men and 119 women with a median age of 54 years (44–62). Endoscopic diagnoses in the control group revealed gastroduodenal ulcer (including scarring) in 112 participants, atrophic gastritis in 43, nodular gastritis in 35, gastric hyperplastic polyp in 19, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma in five (Table 1).

In 16 participants who underwent measurement of serum PG levels after eradication of *H. pylori* at our hospital, gastric cancer was detected after successful eradication. The median time from eradication to detection of cancer was 39.1 months (12.3–69.5). The diseases at the time of eradication included gastric ulcer in six participants, gastric polyp in two, MALT lymphoma in two, and gastric cancer in four.

This study was approved by the ethics committee of Hokkaido University School of Medicine. We adequately explained the study to each participant and obtained his or her written consent.

Measurement of serum samples. Fasting blood samples were collected immediately before endoscopy from all participants. The samples were immediately centrifuged at 4°C, and the serum was frozen and stored at –20°C. Serum PGI and PGII levels were measured by chemiluminescent immunoassay (CLIA) using ARCHITECT analyzer, an automatic CLIA analyzer manufactured by Dainabot (Abbott Japan, Matsudo, Japan). The positive levels were set at PGI ≤ 70 and PGI/II ≤ 3.0 for the conventional PG method. Serum PG was checked in 226 subjects at 3 months after eradication, in four subjects at 4–6 months, 31 subjects at 7–12 months.

Endoscopic examination. We checked the endoscopic examination at 1, 6 or 12 months and then once every year after eradication therapy.

Methods of* H. pylori *infection diagnosis and eradication. *Helicobacter pylori* infection was assessed by microscopy using Giemsa staining, measurement of serum *H. pylori*-immunoglobulin G antibody levels, rapid urease test, urea breath test, and culture test. Participants were judged to be positive for *H. pylori* based on a positive result on any of these tests. After eradication therapy with a combination of a proton pump inhibitors and several antibiotics, eradication was judged to be successful based on negative results for all of the above tests.

Statistical analysis. The data of continuous variables were expressed as medians (interquartile range).

Friedman test was done to compare the long term value of serum pepsinogen after eradication. The Wilcoxon signed rank test was used to determine a statistically significant difference in continuous variables between two related groups. The Mann–Whitney *U*-test was used to determine a statistically significant difference in continuous variables between two unrelated groups. Receiver operating characteristic (ROC) analysis was used to set cut-off values for the PGI/II ratio in the gastric cancer and non-cancer cases. The detectability of gastric cancer with new cut-off values was analyzed through sensitivity and specificity.

Results

***Changes in serum PG levels after eradication of* H. pylori.** In all participants, changes in mean serum PG levels before *versus* after eradication were examined. Before eradication, the mean serum PGI level was 59.7 (40.5–79.5) ng/mL, the mean serum PGII level was 19.3 (13.1–27.6) ng/mL, and the mean PGI/II ratio was 3.1 (2.1–3.9). After eradication, these values were 38.2 (25.2–49.8) ng/mL, 6.9 (5.0–8.9) ng/mL and 5.6 (4.1–7.2) ng/mL, respectively. The serum PGI and PGII levels were significantly reduced, whereas the PGI/II ratio was increased (Table 2) (Wilcoxon signed rank test, $P < 0.001$). These results are similar to those of previous reports.[28,29] Compared to before eradication, serum PG levels change significantly after eradication.

Table 2 Successful eradication of *Helicobacter pylori* decreased both serum pepsinogen (PG)I and PGII levels and increased PG I/II ratio

n = 261	PGI Median (range)	PGII Median (range)	PGI/II Median (range)
Pre-eradication (ng/mL)	59.7 (40.5–79.5)	19.3 (13.1–27.6)	3.1 (2.1–3.9)
Post-eradication (ng/mL)	38.2* (25.2–49.8)	6.9* (5.0–8.9)	5.6* (4.1–7.2)

*$P < 0.001$, Wilcoxon signed rank test.
PG, pepsinogen.

Time-trend of serum PG values for long-term after* H. pylori *eradication. We investigated time-trend of serum pepsinogen value using samples at 1, 3, 6 or 12 months and every year after eradication treatment. In following up 40 patients, the values of Serum PGI, PGII, PGI/II ratio were not significantly changed from 3 months to 36 months after successful eradication in our study (Fig. 1) (Friedman test, *N.S.*).

ROC analysis using PGI/II ratio post-eradication. The ROC curves of post-eradication PGI/II ratios in the gastric cancer and non-cancer cases are shown in Figure 2. Based on the ROC curve, the optimal cut-off value for the PGI/II ratio appeared to be 4.5. Screening with the PGI/II ratio ≤ 4.5 had sensitivity of 65.9% and specificity of 79.3% for gastric cancer.

Examination of gastric cancer risk using the PGI/II ratio of post-eradication. The gastric cancer and non-cancer cases were examined with a cut-off value of 4.5 for the serum PGI/II ratio of post-eradication. In the non-cancer cases, the conventional PG method detected 29.1% as positive, whereas with the new cut-off value used post-eradication 20.7% were detected as positive. The proportion of positive non-cancer cases was reduced by the new cut-off value. Especially, only 6.1% of the low-risk for gastric cancer group (serum PG > 70 and PGI/II > 3) were included at risk of gastric cancer after eradication (Fig. 3). The conventional PG method in pre-eradication identified 47.9% of gastric cancer cases, whereas the PGI/II ratio ≤ 4.5 in post-eradication identified 65.9% of these cases (Fig. 4). Furthermore, it was suggested that use of the ratio in post-eradication cases may

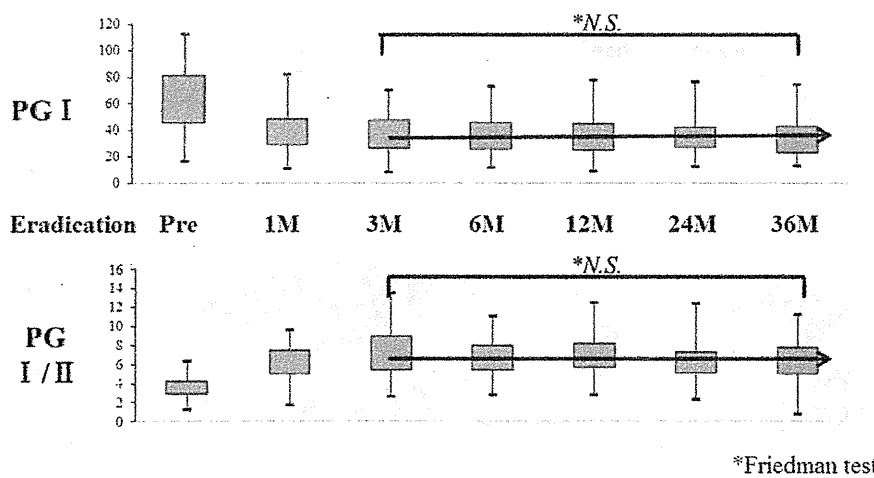

Figure 1 Time-trend of serum pepsinogen (PG) values for long-term after *Helicobacter pylori* eradication. In following up 40 patients, the values of Serum PGI, PGII, PGI/II ratio were not significantly changed from 3 months to 36 months after successful eradication in our study.

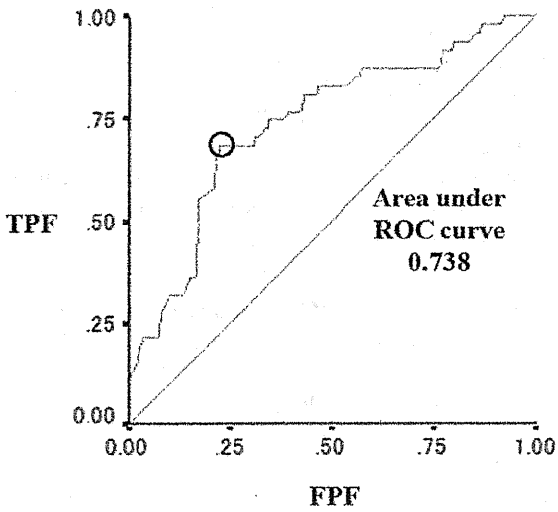

Figure 2 Receiver operating characteristic (ROC) curve and cut-off value using cancerous and non-cancerous data in post-eradication. Cut-off value of PGI/II was determined as 4.5. The sensitivity and the specificity were 65.9% and 79.3%.

identify approximately half of cancers difficult to identify by the conventional PG method, such as those in Group γ (PGI/II ratio ≤ 3 and PGI > 70 ng/mL pre-eradication) who are considered to be at risk for undifferentiated cancer[20] and those with serum PGII levels ≥ 30 ng/mL in pre-eradication[30] (Figs 4 and 5).

Out of 16 participants who were diagnosed as having gastric cancer after eradication at our hospital, 12 (75%) were identified as being at risk by examination with a cut-off value of PGI/II ratio ≤ 4.5 (Fig. 6).

The odds ratio for gastric cancer was 7.44 (95% confidence interval: 3.74–14.8) in participants with serum PGI/II ratios ≤ 4.5 in post-eradication. The gastric cancer risk was significantly higher in this subset than in those with PGI/II ratios > 4.5.

Discussion

Measurement of serum PG levels has often been reported to be useful not only for gastric cancer screening, but also for assessing its risk and identifying patients at high risk for gastric cancer. However, because serum PG levels change after eradication of *H. pylori*, the conventional PG method cannot be applied after successful eradication. We investigated time-trend of serum pepsinogen value after eradication treatment. The values of serum

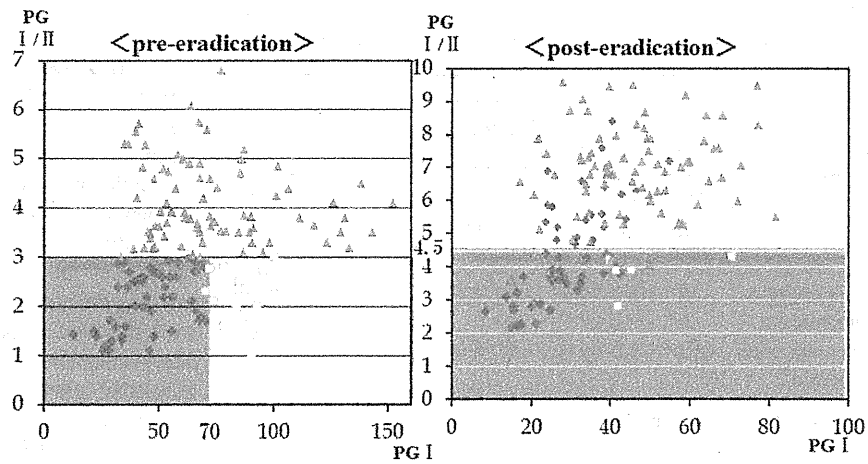

Figure 3 Distribution of non-cancerous cases in pre-eradication and post-eradication. PGI/II ≤ 4.5 includes 20.7% of non-cancer group, whereas traditional PG method includes 29.1%. Especially, only 6.1% of low risk group of cancer (PGI/II ≥ 3 in pre-eradication) was included. ■, PGI/II ≤ 3.0 and PGI ≤ 70 ng/mL (positive PG method group); ▲, PGI/II > 3.0 and PGI > 70 ng/mL (low risk group); ▩, PGI/II ≤ 3.0 and PGI > 70 ng/mL (γ group).

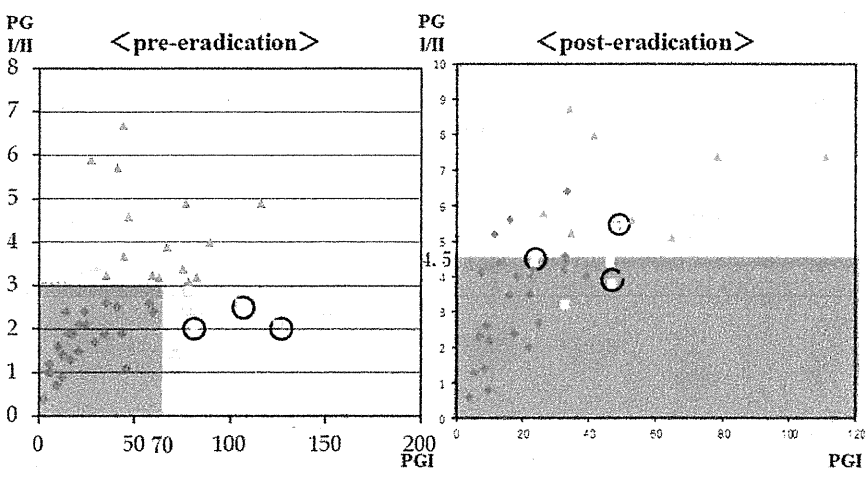

Figure 4 Distribution of gastric cancer cases in pre-eradication and post-eradication. PGI/II ≤ 4.5 included the high risk group of diffuse type cancer (4/8 = 50%) comparing with 0% in traditional PG method. And the traditional PG method in pre-eradication identified 47.9% of gastric cancer cases, whereas the PGI/II ≤ 4.5 in post-eradication identified 65.9% of these cases. ■, PGI/II ≤ 3.0 and PGI ≤ 70 ng/mL (positive PG method group); ▲, PGI/II > 3.0 and PGI > 70 ng/mL (low risk group); ▩, PGI/II ≤ 3.0 and PGI > 70 ng/mL (γ group); ○, actual diffuse type gastric cancer.

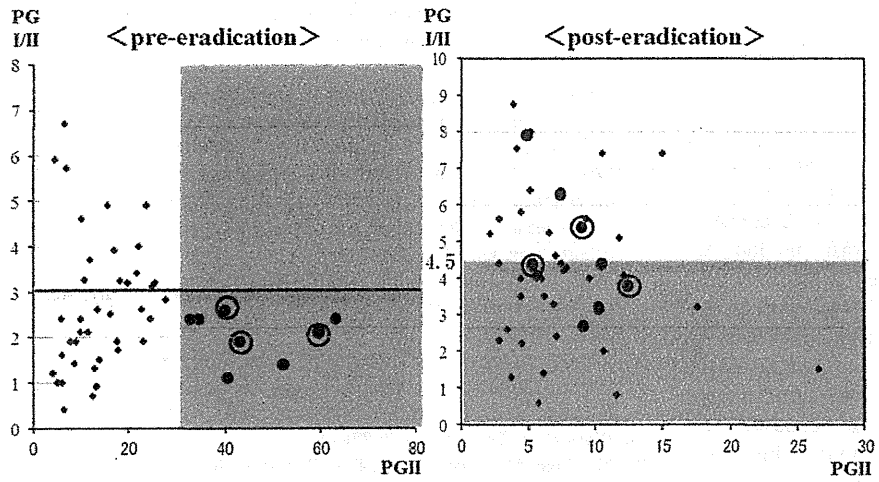

Figure 5 Distribution of gastric cancer cases in pre-eradication and post-eradication. PGI/II ≤ 4.5 included the high risk group of diffuse type cancer(5/8 = 62.5%). ◇, PGII < 30 ng/mL; ●, PGII ≥ ng/mL; ○, actual diffuse type gastric cancer.

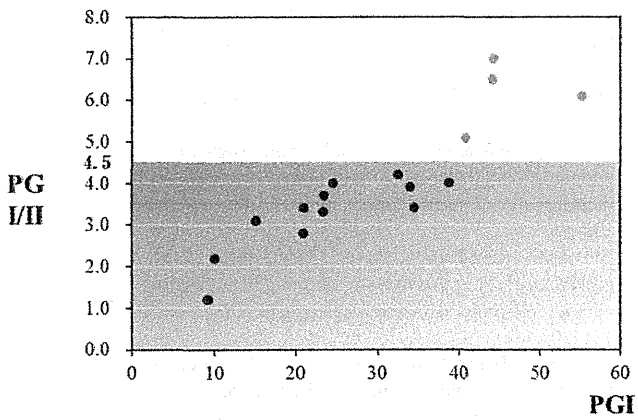

Figure 6 Distribution of gastric cancer cases detected after eradication. PGI/II ≤ 4.5 included gastric cancer cases detected after eradication (12/16 = 75%). ●, PGI/II ≤ 4.5; ○, PGI/II > 4.5.

PGI, PGII, and PGI/II ratio were not significantly changed from 3 months to 36 months after successful eradication in our study.

According to the report by Ito *et al.* the *H. pylori* infection rate was 42.6% in subjects undergoing their initial comprehensive medical examinations, and the rate of eradicated subjects was 8.3%. However, in those who had undergone several examinations, the infection rate was reduced to 16.9%, and the rate of eradicated subjects was increased to 36.7%. These results show that approximately two-thirds of subjects positive for *H. pylori* had undergone eradication therapy.[31] Based on this report, it is anticipated that patients who have achieved eradication (classified into Group E) will increase among those undergoing medical examinations. There has been no report on identification of a subgroup at risk for gastric cancer based on serum PG levels after eradication of *H. pylori* in Group E. The risk classification of gastric cancer based on a PGI/II ratio ≤ 4.5 in post-eradication, as reported in this study, is intended for patients who have achieved eradication (Group E) for whom the conventional method is not indicated in primary screening of the general population for gastric cancer. When the ABC method, which uses both anti-*H. pylori* antibody and serum PG levels in pre-eradication,[22] is applied, the classification using a PGI/II ratio ≤ 4.5 may be useful for setting a cut-off value for high-risk gastric cancer cases who received successful eradication treatment that is confirmed by interview.

If subjects in whom eradication of *H. pylori* has been successful are identified by interview during medical examination, we propose separately treating them as Group E and classifying them into high-risk and low-risk for gastric cancer subgroups based on the PGI/II ratio cut-off value of 4.5, which was determined in this study, for follow-up.

Helicobacter pylori is considered to act as not only an initiator, but also a promoter of gastric cancer. It has been reported that the incidence and growth rate of gastric cancer may be reduced after eradication.[32] Thus, the PGI/II ratio of 4.5 may contribute to determining the optimal surveillance interval. For example, follow-up endoscopy may be performed once every 2 years for those with PGI/II ratios ≤ 4.5 post-eradication or once every 3 years for those with PGI/II ratios > 4.5. Because there has been no report on setting appropriate intervals for endoscopic surveillance after eradication of *H. pylori*, it seems that follow-up endoscopy is often performed annually. We consider the new cut-off value determined in this study to be a useful guide for setting appropriate intervals for endoscopic surveillance in post-eradication.

However, it should be noted that, even if a high-risk group for gastric cancer is identified by serum PG levels among subjects in whom eradication of *H. pylori* has been successful, a low-risk group post-eradication will continue undergoing endoscopic surveillance. In those in whom eradication of *H. pylori* has been successful, endoscopic surveillance should be performed regularly, given that neoplastic cells arising during persistent infection may persist after eradication.

In this study, selection of the control group was biased, and the general population might not have been represented. This is a potential limitation of case-control studies. In order to confirm our results, that is, whether a high-risk group for gastric cancer is identified by a PGI/II ratio ≤ 4.5 in post-eradication, prospective cohort studies on the incidence of gastric cancer are needed. Moreover, the gastric cancer group in the present study was composed mainly of patients with differentiated cancer, with only a few patients with undifferentiated cancer. The association between

gastric mucosal atrophy and undifferentiated cancer is known to be weaker than that of differentiated cancer.[33,34] Thus, further studies on undifferentiated cancer may also be needed.

When serum PG levels after eradication of *H. pylori* were used to identify a high-risk group for gastric cancer, the optimal cut-off value of the PGI/II ratio was considered to be 4.5. The classification using a PGI/II ratio $\leq$ 4.5 had better sensitivity and specificity for identification of patients with gastric cancer than the conventional PG method. It was suggested that the former may include more than half of patients with undifferentiated gastric cancers, which had been difficult to identify. Moreover, 75% of participants who were diagnosed as having gastric cancer after eradication at our hospital were included in the high-risk group. In the future, it may be necessary to accumulate further cases and conduct a prospective multicenter study. The present study may contribute to effective gastric cancer surveillance by identifying a high-risk group for gastric cancer after eradication of *H. pylori*.

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