Original Article

Analysis of long-term serological and histological changes after eradication of *Helicobacter pylori*

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Stratification of gastric cancer risk by measuring serological biomarkers is useful for screening of gastric cancer. However, this method has problem such as overlooking past infected patients. We aimed to evaluate the association between *Helicobacter pylori* infection status and serological biomarkers. We divided 5,268 patients according to *Helicobacter pylori* infection status and past infected patients were divided into 12 groups according to time elapsed since eradication. We analyzed mean serum *H. pylori* immunoglobulin G antibody, pepsinogen titers, histological and endoscopic atrophy score of each group. Mean *H. pylori* immunoglobulin G antibody showed a decreasing tendency, there was no significant difference from the uninfected group at 11 years after eradication ($p = 0.19$). PGI, PGII decreased in short term after eradication. However, both PGI and PGII gradually increased as long-term changes after eradication, became comparable to those in the uninfected group ($p = 0.41$, $p = 0.37$, respectively). Histological atrophy improved gradually, became equivalent to uninfected group. Endoscopic atrophy score did not improve for long term after eradication. In conclusion, patients with long term after eradication reach the uninfected condition serologically, histologically. Endoscopic assessment of gastric mucosal atrophy may be useful for accurate assessment of gastric cancer risk.

***Key Words*: *Helicobacter pylori*, *Helicobacter pylori* IgG antibody, pepsinogen, eradication, gastric cancer**

Gastric cancer (GC) is the sixth most common cancer, the fourth most common cause of cancer-related mortality, and one of the most common cancers in the world.[1] Particularly in Japan, the number of patients with GC is large, and patients with GC tend to increase.[2] Previous study revealed a close relationship between infection with *Helicobacter pylori* and development of GC.[3] *H. pylori* is a major pathogen of GC.[4,5] Correa *et al.*[6] also revealed gastric mucosal atrophy and intestinal metaplasia as precancerous lesions.

Therefore, not only diagnosis of *H. pylori* infection, but also evaluations for the status of the gastric mucosa as a risk factor for GC, such as atrophic gastritis and intestinal metaplasia, have been considered important for early detection of GC. In Japan, the measurement of serum *H. pylori* immunoglobulin G antibody (HPIgG) or combined assay using HPIgG and serum pepsinogen (PG)I and PGII levels have been performed to stratify the risk of GC.[7] Measurement of serum HPIgG is non-invasive and inexpensive and has therefore been used for the diagnosis of *H. pylori* infection. PG has also been reported as a valuable biomarker for predicting the degree of gastric mucosal atrophy and for determining risk of GC in recent years,[8–10] and patients with both PGI <70 ng/dl and PGI/II <3 were considered to have severe gastric mucosal atrophy and a high risk of GC.[11,12] Diagnosis of *H. pylori* infection by measuring HPIgG or stratification of GC risk using combined HPIgG and PG assays are non-invasive and inexpensive for mass screening of GC.

However, some problems remain, such as patients diagnosed as uninfected according to the HPIgG test and patients categorized as showing a low risk of GC by combination assay can include GC patients or previously infected patients at high risk of GC.[13–15] Since the number of previously infected patients is expected to increase in Japan in the future, long-term changes in serum biomarkers after eradication need to be clarified to prevent patients with a high risk of GC from being missed in GC screening.

However, while some studies have evaluated changes in serum biomarkers over the short term after eradication, long-term changes have not been evaluated. In addition, no study have simultaneously evaluated long-term serological, histological, and endoscopic changes after eradication. We therefore aimed to evaluate long-term serological, histological, and endoscopic changes after eradication and to clarify the risk of GC development after eradication.

Methods

Study subjects. This was a retrospective observational study. The study protocol was approved by the institutional review boards of Oita University Hospital (no. 2216). We analyzed a total of 6,001 patients who underwent endoscopic examination and culture, histology, rapid urease test (RUT), and serological tests (HPIgG, PG) to diagnose *H. pylori* infection at the same time at Oita University Hospital between October 1987 and December 2017. Of the 6,001 registered patients, participants in this study were the 109 patients for whom endoscopy, serum, and histological data from before to five years after eradication were available. Endoscopic, serological, and histological characteristics were compared between groups with serum antibody titers of ≥3 U/ml and <3 U/ml after 5 years.

Endoscopic evaluation. We performed endoscopic examinations using endoscopes (Q-260, HQ-260, and HQ-290 and others, Olympus, Tokyo, Japan and EG-L590ZW, Fujifilm Co, Tokyo, Japan). Endoscopic atrophy was assessed according to the Kimura–Takemoto classification.[16] This classification system includes the following classifications: 1) close-type, when an

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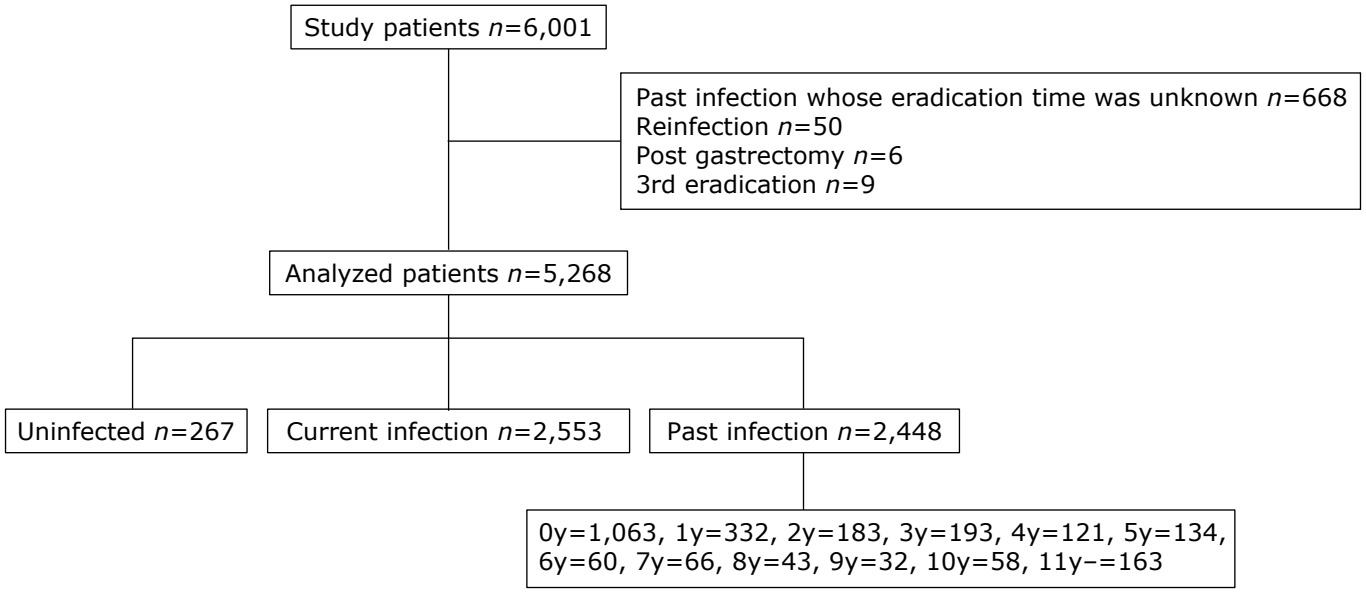

Fig. 1. Flow chart of enrolled patients.

atrophic border remains on the lesser curvature of the stomach; or 2) open-type, when the atrophic border extends along the anterior and posterior walls of the stomach and is not associated with the lesser curvature of the stomach. In this study, atrophy grade was scored as follows: absence of any atrophy, 0; C1, 1; C2, 2; C3, 3; O1, 4; O2, 5; and O3, 6, respectively.

Definition of infection status of *H. pylori*. The infection status of *H. pylori* was defined as follows: patients without atrophy apparent on endoscopic (C0/1) and for whom histological, culture, and RUT results were all negative were defined as uninfected. With or without endoscopic atrophy, if at least one of histological, culture, or RUT results were positive, the patient was defined as having current infection. If endoscopic atrophy (C2 or higher) was present but histological, culture and RUT results were all negative, the patient were defined as having past infection. Patients defined as having past infection were further divided into 12 groups according to time elapsed since *H. pylori* eradication: 0–11 months, 0 year (y); 12–23 months, 1y; 24–35 months, 2y; 36–47 months, 3y; 48–59 months, 4y; 60–71 months, 5y; 72–83 months, 6y; 84–95 months, 7y; 96–107 months, 8y; 108–119 months, 9y; 120–131 months, 10y; and 132 months–, 11y–. This study excluded previously infected cases for which eradication history and eradication time were unknown, reinfected cases, post-gastrectomy cases, and cases for which a third eradication was performed.

Serological examination. Serum samples were collected in a fasted state before endoscopy and stored at −80°C until laboratory assays were performed. Serum HPIgG was measured by enzyme-linked immunosorbent assay and an antibody determination kit (E plate "Eiken" *H. pylori* antibody II; Eiken Chemical, Tokyo, Japan). All samples were analyzed according to the instructions from the manufacturer, and the cutoff point was 10 U/ml. In addition, as a marker of atrophic gastritis, serum PGI were measured by latex agglutination (LZ test "Eiken" pepsinogen I, II; Eiken Chemical).

Histological evaluation. Biopsy specimens were obtained from the greater curvature of the antrum and the greater curvature of the corpus, as two of the five points recommended by the updated Sydney system.[17] Biopsy samples were subsequently evaluated according to the updated Sydney system with degree of inflammation and atrophy scored as: 0, 'normal'; 1, 'mild'; 2, 'moderate'; and 3, 'marked'. Histological evaluations were performed by MK who majored in pathology and diagnose the histological presence of *H. pylori* at Oita University Hospital. For patients with consistent results of histological atrophy of corpus and antrum, gastritis grade was also evaluated using operative link for gastritis assessment (OLGA) staging system.[18,19] These guidelines classify gastritis as grades 0–IV based on a combination of the degree of antrum and corpus atrophy. Moreover, grades III and IV are associated with the highest risks for gastric cancer.[19,20]

Statistical analysis. Statistical analyses were performed using SPSS software (IBM SPSS Statistics ver. 24.0; IBM, Armonk, NY), and data are expressed as the mean ± SD. Student's *t* test was performed to compare several serum biomarkers of several *H. pylori* infection status. Mann–Whitney *U* test was performed for comparison of histological scores according to the updated Sydney system in each *H. pylori* infection status. Mantel–Haenszel test was performed for trend test of ratio of OLGA stage 0 and stage I/II and stage III/IV in each year after eradication. Values of $p<0.05$ were considered significant.

Results

Participant flow. Of the 6,001 enrolled patients, 668 patients after spontaneous eradication or with unknown eradication time, 50 patients with suspected reinfection, six patients after gastrectomy, and nine patients with third eradication were excluded from this study, and the remaining 5,268 patients were included in this study (Fig. 1). These patients were divided into 3 groups according to *H. pylori* infection status (uninfected, $n = 267$; current infection, $n = 2,553$; past infection, $n = 2,448$). The past infection group was divided as follows according to the elapsed time since *H. pylori* eradication (0y, $n = 1,063$; 1y, $n = 332$; 2y, $n = 183$; 3y, $n = 193$; 4y, $n = 121$; 5y, $n = 134$; 6y, $n = 60$; 7y, $n = 66$; 8y, $n = 43$; 9y, $n = 32$; 10y, $n = 58$; and 11y–, $n = 163$) (Fig. 1).

Baseline characteristics of participants. Baseline characteristics of participants are shown in Table 1. Mean age was significantly higher in the current (55.9 ± 14.7 years old) and past infection (59 ± 13.6 years old) groups than in the uninfected

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Table 1. Baseline characteristics of study population in each *H. pylori* infection status

	Uninfected	Current infection	Past infection
n	267	2,553	2,448
Male	123 (46%)	1,545 (61%)	1,472 (60%)
Female	144 (54%)	1,008 (39%)	976 (40%)
Age (y), mean ± SD	51.2 ± 17.9	55.9 ± 14.7***	59 ± 13.6***
HPIgG (U/ml), mean ± SD	2.0 ± 4.4	43.7 ± 31.0***	10.3 ± 13.1***
PGI (ng/ml), mean ± SD	67.2 ± 66.7	78.8 ± 77.1	54.2 ± 70.3**
PGII (ng/ml), mean ± SD	11.6 ± 9.9	22.3 ± 14.8***	9.6 ± 7.2**
PGI/II, mean ± SD	5.9 ± 1.7	3.6 ± 1.8***	5.8 ± 1.9

Comparison between uninfected group, current infection group, and past infection group for each characteristic using Student's *t* test. Data are expressed as mean ± SD or *n* (%), as appropriate. **p<0.01, ***p<0.001. HPIgG, *Helicobacter pylori* IgG antibody; PGI, pepsinogen I; PGII, pepsinogen II; PGI/II, pepsinogen I/II ratio.

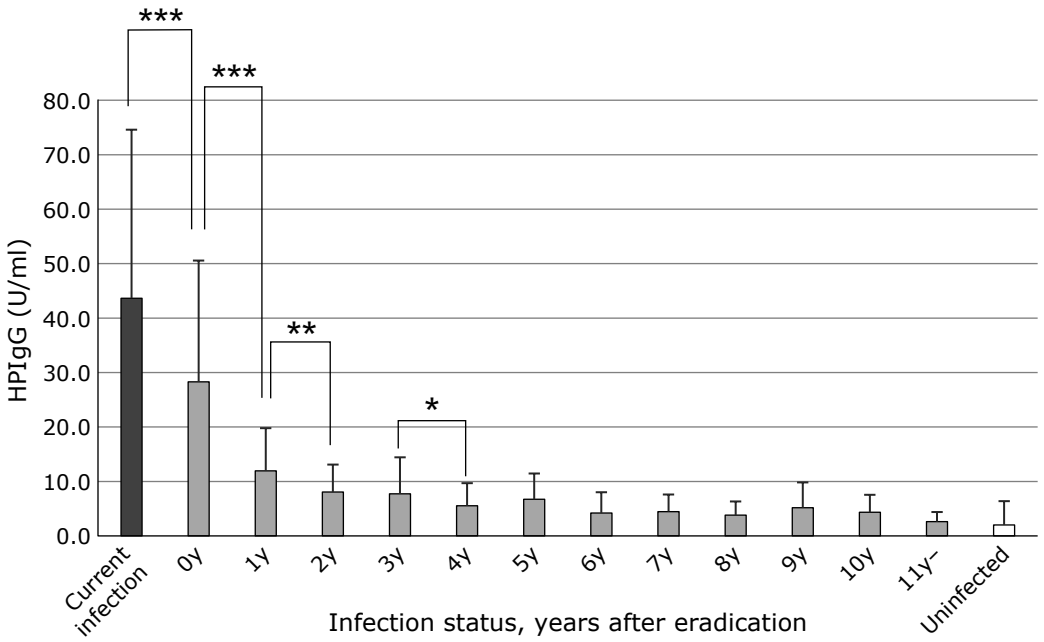

Fig. 2. Serum HPIgG titer in *H. pylori* current and past infection group divided by each year since eradication and uninfected group. HPIgG, *Helicobacter pylori* IgG antibody. Data are expressed as mean + SD. *p<0.05, **p<0.01, ***p<0.001.

(51.2 ± 17.9 years old) group (p<0.001 and p<0.001, respectively) (Table 1). Mean HPIgG was significantly higher in the current (43.7 ± 31.0 U/ml) and past infection (10.3 ± 13.1 U/ml) groups than in the uninfected (2.0 ± 4.4 U/ml) group (p<0.001 and p<0.001, respectively). Mean PGI in the uninfected group (67.2 ± 66.7 ng/ml) did not differ significantly from that in the current infection group (78.8 ± 77.1 ng/ml), (p = 0.203), but was significantly higher than that in the past infection group (54.2 ± 70.3 ng/ml), (p = 0.005). Mean PGII in the uninfected group (11.6 ± 9.9 ng/ml) was significantly lower than that in the current infection group (22.3 ± 14.8 ng/ml), (p<0.001), but significantly higher than that in the past infection group (9.6 ± 7.2 ng/ml), (p = 0.003). Mean PGI/II showed no significant difference between the uninfected group (5.9 ± 1.7) and past infection group (5.8 ± 1.9), (p = 0.381), but was significantly lower in the current infection group (3.6 ± 1.8) than in the uninfected group (p<0.001) (Table 1).

Comparison of HPIgG between current infection group, past infection group and uninfected group. The comparison of HPIgG titers between the uninfected group, current infection group and past infection groups is shown in Fig. 2. Mean HPIgG decreased significantly until the second year after eradication. A slow downward trend was subsequently seen, and no significant difference from the uninfected group was first seen at >11 years after eradication (p = 0.19).

Comparison between HPIgG <3 U/ml group and HPIgG ≥3 U/ml group at 5 years after eradication. In comparison between the HPIgG <3 U/ml group (n = 24) and ≥3 U/ml group (n = 85) five years after eradication, titers of HPIgG and PGII before eradication differed significantly between the two groups (Table 2). HPIgG and PGII before eradication were significantly higher in the ≥3 U/ml group (52.8 U/ml and 22.7 ng/ml, respectively) than in the <3 U/ml group (32.2 U/ml, p<0.001 and 18.2 ng/ml, p = 0.02, respectively). No significant differences between the two groups were seen in any other factors.

Comparing PGI, PGII, and PGI/II. In comparison between PGI, PGII, and PGI/II ratio between the uninfected group and current infection group and between each group according to elapsed years after eradication, mean PGI decreased significantly within one year after eradication, followed by a gradual increasing tendency, becoming equivalent to the uninfected group from six years after eradication (Fig. 3A). Mean PGII was

Table 2. Comparison between HPIgG ≥3 U/ml group and HPIgG <3 U/ml group at 5 years after eradication

	HPIgG<3 (n = 24)	HPIgG≥3 (n = 85)	p value
Age (y)	60.4 ± 9.4	57.2 ± 9.9	0.16
HPIgG (U/ml)	32.2 ± 20.0	52.8 ± 31.9	<0.001
PGI (ng/ml)	62.9 ± 27.6	70.6 ± 36.8	0.35
PGII (ng/ml)	18.2 ± 6.5	22.7 ± 11.8	0.02
PGI/II	3.7 ± 1.4	3.4 ± 1.4	0.34
Histological inflammation			
Antrum	2.4 ± 0.6	2.5 ± 0.5	0.84
Corpus	2.2 ± 0.6	2.1 ± 0.6	0.74
Histological atrophy			
Antrum	1.4 ± 0.7	1.4 ± 0.7	0.92
Corpus	0.5 ± 0.8	0.5 ± 0.8	0.76
Endoscopic atrophy	2.6 ± 0.5	2.4 ± 0.7	0.41

Comparison between HPIgG≥3 and HPIgG<3 groups at 5 years after eradication for each characteristic before eradication using Student's t test. Data are expressed as mean ± SD. HPIgG, *Helicobacter pylori* IgG antibody; PGI, pepsinogen I; PGII, pepsinogen II; PGI/II, pepsinogen I/II ratio.

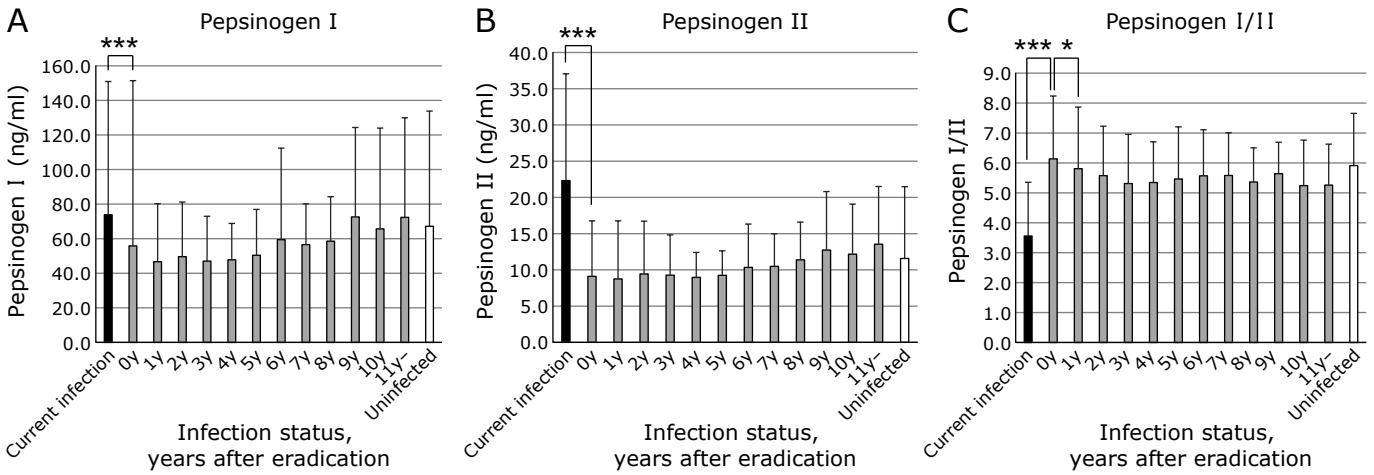

Fig. 3. Serum pepsinogen I and pepsinogen II and pepsinogen I/II ratio in *H. pylori* current and past infection group divided by each year since eradication and uninfected group. Data are expressed as mean + SD. *p<0.05, ***p<0.001.

significantly decreased within one year after eradication (p<0.001), showing a rising tendency as seen for PGI, and became comparable to the uninfected level from six years after eradication (Fig. 3B). Mean PGI/II ratio increased significantly within one year after eradication (p<0.001) to be comparable to that in the uninfected group. Mean PGI/II ratio subsequently fluctuated between 5.2 and 5.6 over the long term after eradication (Fig. 3C).

Endoscopic and histological atrophy. The gastritis grade evaluated by the OLGA staging system which indicated the grade of histological atrophy of both the antrum and the corpus using updated Sydney system atrophy score. It was found that the proportion of stage 0, gradually increased as the long term after eradication (p<0.001) (Fig. 4). For endoscopic atrophy, mean atrophy score based on the Kimura–Takemoto classification was 0.5 in the uninfected group and 3.3 in the current infection group, showing a significant difference during the observational period (p<0.001). Endoscopic atrophy remained for the long term after eradication (Fig. 5).

Discussion

Evaluations of serum HPIgG and pepsinogen titer are noninvasive, inexpensive tests. These tests are thus known to be useful for mass screening of *H. pylori* infection and risk of GC development. To date, various reports have investigated changes to serum HPIgG and PGs after *H. pylori* eradication, but each only reported follow-up for up to five years.[21–26] In our previous study, HPIgG were evaluated up to seven years with total 35 patients.[27] This is the first report to investigate serum HPIgG titer, PG titer, and histological changes simultaneously for more than a decade after eradication using a large number of eradicated patients.

HPIgG significantly decreased annually until two years after eradication, and still continued to decline slowly thereafter. No significant difference from the uninfected group was seen at 11 years after eradication. Previous reports have revealed short-term reductions in HPIgG after eradication,[21,22] but our study revealed that a slow downward trend may continue and finally clarify the decrease to levels indistinguishable from those of the uninfected group.

In the analysis of each biomarkers before and five years after

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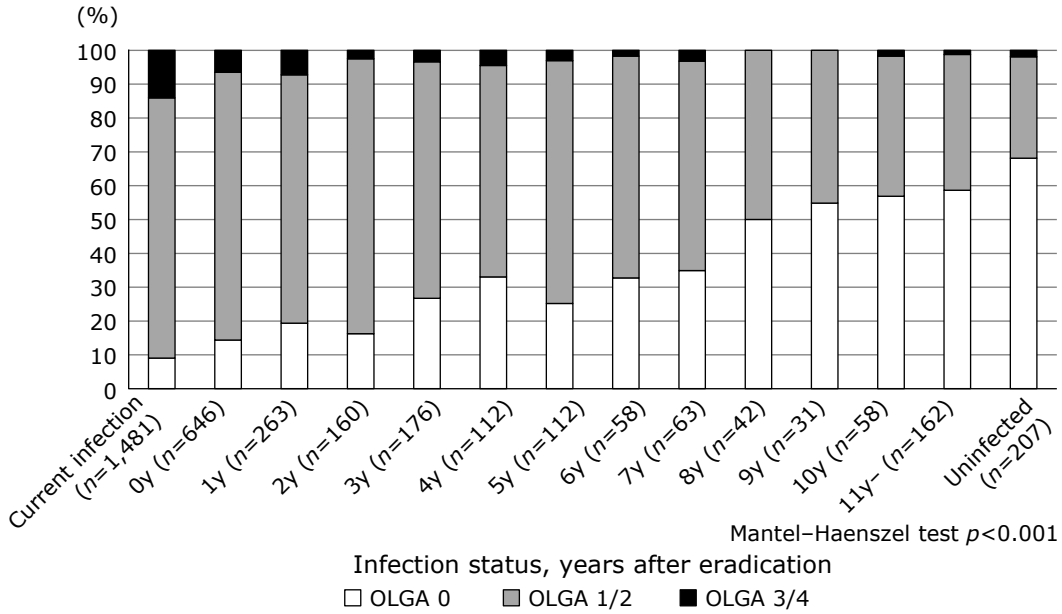

Fig. 4. Ratio of OLGA stage 0 and stage I/II and stage III/IV in *H. pylori* current and past infection group divided by each year since eradication and uninfected group. OLGA, operative link for gastritis assessment.

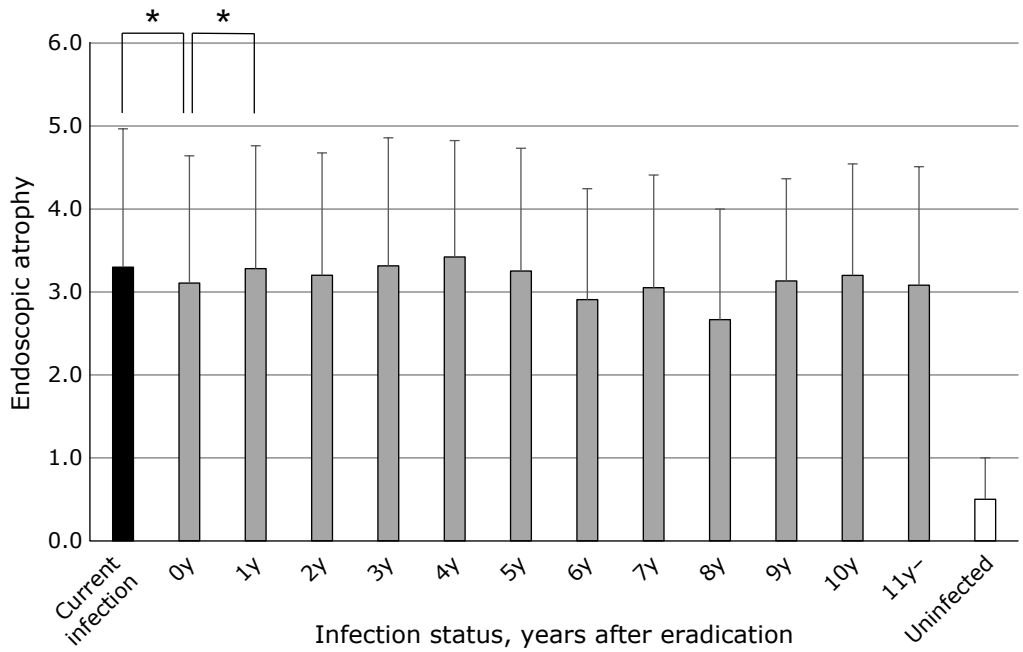

Fig. 5. Endoscopic atrophy in *H. pylori* current and past infection group divided by each year since eradication and uninfected group. Data are expressed as mean score + SD based on the Kimura–Takemoto classification, as follows: C0: 0, C1: 1, C2: 2, C3: 3, O1: 4, O2: 5, O3: 6. *p<0.05.

eradication of 109 cases, pre-eradication HPIgG tended to be significantly lower in the group with HPIgG <3.0 U/ml than in the group with HPIgG ≥3 U/ml at five years after eradication. It revealed that the patients with low HPIgG before eradication were more likely to show HPIgG titer lower than 3 U/ml five years after eradication. Previously, we reported that 75% of 137 patients with HPIgG 3.0–9.9 U/ml had endoscopic atrophy and 22% were current infection and 61% were past infection.[28] This study suggests that patients with HPIgG <3.0 U/ml after long term eradication may include past infected patients.

Regarding serum PG, both PGI and PGII decreased significantly compared to the current infection group and PGI/II increased to the same level as in the uninfected group within one year after eradication. These changes in PG levels were observed in the short term after eradication, as previously reported.[23–26] Serum PG levels were found to correlate positively with histological chronic inflammation and neutrophil activity due to *H. pylori* infection,[29–31] and both PGI and PGII levels were significantly elevated in the current infection group. Improvement of inflammation and neutrophil activity by eradication seems to cause PGI

and PGII levels to decrease rapidly in the short term after eradication. However, in this study, both PGI and PGII gradually increased as long-term changes after eradication, and became comparable to those in the uninfected group from around six years after eradication.

Long-term improvement of histological atrophy after eradication have been demonstrated in previous reports.[32–35] Likewise in this study, histological atrophy which was evaluated with OLGA staging system improved to a level equivalent to that in uninfected individuals. Previous study have revealed that there is significant correlation between serum PG and degree of histological atrophy.[36] In the Updated Sydney system,[17] degree of histological atrophy reflects the density of secretory glands in gastric mucosa. The gradual increase in serum PG levels may have relation with histological changes, such as atrophy, of the gastric mucosa after eradication. Therefore, improvement of histological atrophy also means recovery of secretory glands which causes serum PG level elevation. In the present study, serum PG and histological improvements after eradication indicated that the gastric mucosa improves not only histologically but also functionally after eradication. Previous reports have shown that eradication therapy affects not only serum PG levels but also various endocrine functions of the gastric mucosa in hemodialysis patients with mild and moderate gastric mucosal atrophy, improving nutritional status after eradication therapy.[37] This report also suggests the improvement of gastric mucosal function after eradication therapy and the efficacy of eradication therapy as in present study.

In this study, the proportion of stage 0 in the OLGA staging system showed significant gradual increase as the long term after eradication. Previous studies have shown that OLGA stage 0 is considered to be a low risk of gastric cancer, III, IV are at high risk for gastric cancer,[20] it has been found that the risk of developing gastric cancer is higher in groups III, IV than in stage 0–II even after eradication.[38] The gradual increase in OLGA stage 0 after eradication suggests that histological atrophy after eradication contributes to the prevention of gastric cancer development after eradication.

On endoscopic examination, atrophy after eradication persisted for a long time and did not reach the appearance in the uninfected group, even in long term after eradication. This tendency obviously differs from the serological or histological findings. These changes after eradication mean that a discrepancy exists between histological and endoscopic atrophy after eradication.

Changes in endoscopic atrophy after eradication remain controversial. The correlation between degree of histological atrophy according to the updated Sydney system and the degree of endoscopic atrophy using the Kimura–Takemoto classification has been investigated in several reports so far, both correlations have been observed in those reports.[39–41] However, few studies examined the correlations for both before and after eradication.

Kodama *et al.*[39] reported that the correlations between endoscopic and histological atrophy and intestinal metaplasia were recognized even after eradication, except atrophy in the antrum. Several studies showed that endoscopic atrophy and intestinal metaplasia hard to improve after eradication compared with histological atrophy.[42,43] These results of previous reports are consistent with the present study. The reason for the discrepancy between endoscopic and histological atrophy after eradication has not been clarified. Difficulty in improvement of intestinal metaplasia may be involved in this reason. To elucidate the underlying reasons, further study is needed to compare post-eradication gastric mucosa with uninfected gastric mucosa.

Some limitations to this study must be considered. First, we did not obtain data on the medications and underlying diseases of enrolled patients. Previous studies have revealed that patients taking proton pump inhibitors[44,45] and patients with renal dysfunction[46] showed significantly elevated serum PG. Such patients should have been excluded from the present study, but the present study did not have access to information on underlying diseases or medications and thus may well have included such patients.

Second, this was not a prospective study in which the same patients were followed each year from before eradication to many years after eradication.

Despite these limitations, there have been no study which showed such large numbers of patients in this study. Further, all cases were evaluated endoscopically, histologically, and serologically simultaneously, including patients in whom *H. pylori* had been eradicated for more than 10 years. No such studies have been conducted previously. This study may provide useful basic data to understanding trends in long-term endoscopic, histological, and serological changes after *H. pylori* eradication.

In conclusion, this study found that patients with long term after eradication reach the uninfected condition not only histologically, but also serologically. This knowledge suggests a functional improvement to the gastric mucosa after eradication, and such improvement may lead to the prevention of GC development following eradication. However, some risk of GC remains after eradication. Endoscopic assessment of gastric mucosal atrophy may thus be useful for accurate assessment of GC risk.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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