

### Short Communication

## Effect of *Helicobacter pylori* Eradication on Subsequent Development of Cancer after Endoscopic Resection of Early Gastric Cancer<sup>1</sup>

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#### Abstract

Although epidemiological studies strongly suggest an association between gastric cancer and *Helicobacter pylori* infection, there has been no clinical report indicating that cure of the infection prevents cancer. We conducted a nonrandomized *H. pylori* eradication trial in patients whose gastric cancer was removed by endoscopic resection (ER). We investigated the effect of treatment on the histopathology of the gastric mucosa, as well as on the incidence of metachronous gastric cancer during the long-term clinical and endoscopic follow-up. One hundred and thirty-two patients with early gastric cancer underwent ER and had *H. pylori* infection. Sixty-five (group A) were treated with omeprazole and antibiotics to eradicate the infection, and 67 (group B) were not. All patients were followed for 2 years post ER. After eradication treatment in group A, the disappearance of neutrophil infiltration in the antrum and body of the stomach was observed as was a decrease of the severity of intestinal metaplasia. Endoscopy after ER detected no new gastric cancers in these patients. After 3 years of follow-up, 6 (9%) of the 67 patients in group B had a new early-stage, intestinal-type gastric cancer endoscopically diagnosed. The above results suggest that *H. pylori* eradication may improve neutrophil infiltration and intestinal metaplasia in the gastric mucosa and inhibit the development of new carcinomas. This finding should be confirmed in a randomized, controlled trial.

#### Introduction

Based on epidemiologic evidence (1, 2), the WHO/International Agency for Research on Cancer (3) has assigned a causal role to the association between gastric cancer and *Helicobacter*

*pylori* infection. There has been, however, no clinical report indicating that cure of the infection prevents cancer.

In Japan where the incidence of gastric cancer is high, early-stage gastric cancer is often discovered by endoscopy. ER (4),<sup>3</sup> taking advantage of a strip biopsy technique developed by Tada *et al.* (5), has been conducted in many intestinal-type small gastric cancer cases. Because the background gastric mucosa in such cases is characterized by chronic atrophic gastritis accompanied by advanced intestinal metaplasia (6), the need to pay attention to synchronous multiple cancers (7) is well known. Also well established is an incidence of 5–10% of metachronous multiple cancer within 5 years (8). The residual gastric mucosa after ER is a high-risk microenvironment for the incidence and growth of the intestinal type of gastric cancer.

Given this background, we conducted *H. pylori* eradication treatment in the patients whose gastric cancer was resected by ER and investigated the effect of treatment on the histopathology of the gastric mucosa, as well as on the incidence of metachronous gastric cancer in other regions during the long-term clinical and endoscopic follow-up.

#### Patients and Methods

One hundred and sixty-five patients underwent ER of intestinal-type, early-stage gastric cancer lesion using the strip biopsy at Kure Kyosai Hospital between September 1991 and March 1995. After excluding those patients whose resection margins tested positive for cancer and those who received additional surgery due to submucosa infiltration, 132 (44–85 years of age; mean age, 69 years; 97 males and 35 females) had positive serology tests for *H. pylori* antibody and were followed up until September 1995.

Strip biopsy is an endoscopic mucosa resection technique developed by Tada *et al.* (5) in 1983. ER using this strip biopsy may be briefly explained as follows. Using an endoscope having a 2-channel clamp aperture (Olympus GIF 2T-200), physiological saline (3–10 ml) is first injected into the submucosal layer with a puncture needle (Olympus NM-1K) to raise the lesion; while the lesion is raised by a holding forceps (Olympus FG-42L) introduced from the other aperture, the lesion and the peripheral mucosa are cauterized by the snare (Olympus SD-5L) through the first aperture. Then high frequency electricity is passed to resect a part of the submucosal layer and the whole mucosa.

All patients received a thorough explanation of the study before ER, including a detailed description of the treatment intended to eradicate *H. pylori*, and all patients were given the option to undergo such treatment. Those who chose and gave consent were treated with omeprazole and antimicrobial medications. Sixty-five patients elected to receive antimicrobial treatment (group A). The remaining 67 were not given any treatment against *H. pylori* (group B).

After ER, the first *H. pylori* eradication treatment (20 mg

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<sup>3</sup> The abbreviation used is: ER, endoscopic resection.

	Group A (n = 65)	Group B (n = 67)	Statistical analysis
Mean age <sup>a</sup> (range)	69.4y (44–84y)	68.7y (47–85y)	NS <sup>b</sup>
Sex (female/male)	18/47	18/49	NS
Site (antrum/body/cardia)	48/24/3	42/31/2	NS
Mean size (cm)	11.6	11.2	NS
Intestinal type	65 (100%)	67 (100%)	NS
Synchronous cancers	5 (7.7%)	6 (8.9%)	NS

<sup>a</sup> In years.

<sup>b</sup> NS, not significant.

of omeprazole daily for 4 weeks and 400 mg of clarithromycin daily for 2 weeks) was given to the 65 subjects (group A). After 4 weeks, eradication had not been achieved in 35 of these patients. A second treatment (20 mg/day of omeprazole, 1500 mg/day of amoxicillin + 500 mg/day of Metronidazole for 2 weeks) was given to these patients, effectively eradicating *H. pylori* in all of them 4 weeks after the end of treatment. Thereafter, the patients were observed by endoscopy with biopsy conducted every 6 months. Biopsy specimens were taken at two points each from the greater curvature of the antrum and the body of stomach. These specimens were fixed in formalin and then stained (H&E and Giemsa staining). Eradication of *H. pylori* was assessed histologically by Giemsa staining. The severity of neutrophil infiltration and intestinal metaplasia was classified into four grades (none, 0; mild, 1; moderate, 2; and severe, 3) and expressed as a score by two pathologists according to the updated Sydney system (9). The pathologists were not aware of the clinical and endoscopic data, and the results were scored blindly with the use of patient codes. Consensus was reached through revision of all the slides by the combined panel of pathologists, leading to final conclusive scores. Endoscopy was also conducted to find any lesions in other sites every 6–12 months after ER in the 67 patients (group B) who did not receive any treatment against *H. pylori*. Whenever a suspicious lesion was detected in a subject from either group, a biopsy was done to histologically document the presence of any neoplastic lesion.

The scores of neutrophil and intestinal metaplasia are reported as mean (SE), and a two-tailed Wilcoxon Signed Rank test was used for paired comparisons before and after treatment (treatment-induced changes in a score of neutrophil and intestinal metaplasia). The incidence of cancer in other regions in the two groups was compared using the log rank test. The demographic and clinical characteristics of the study patients were compared statistically by the Wilcoxon rank sum test (age and size) or  $\chi^2$  test (sex, site, histological type, and synchronous multiple cases). Differences were considered significant at the 5% probability level. All statistical analyses were two tailed. Values were considered significant at the 5% probability level.

The present study was carried out in compliance with the ethical standards of Kure Kyosai Hospital including informed consent.

## Results

Table 1 shows the backgrounds of the 132 subjects in groups A and B. Comparison of age, sex, site, size, histological type, and synchronous multiple cancer cases did not indicate any difference between the two groups.

### Effect of *H. pylori* Eradication Treatment in the Background Gastric Mucosa of Early-Stage Gastric Cancer Pa-

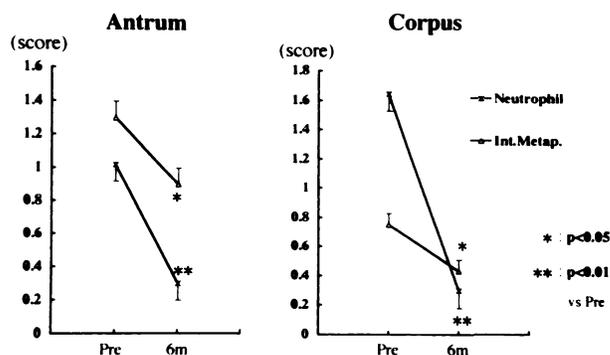


Fig. 1. The pretreatment status and the status 6 months after the completion of treatment in the 65 patients who underwent *H. pylori* eradication treatment. The fluctuations in the neutrophil infiltration score (x) and the extent of intestinal metaplasia ( $\Delta$ ) in the antrum (left) and the body of stomach (Corpus, right) are shown. Bars, SE.

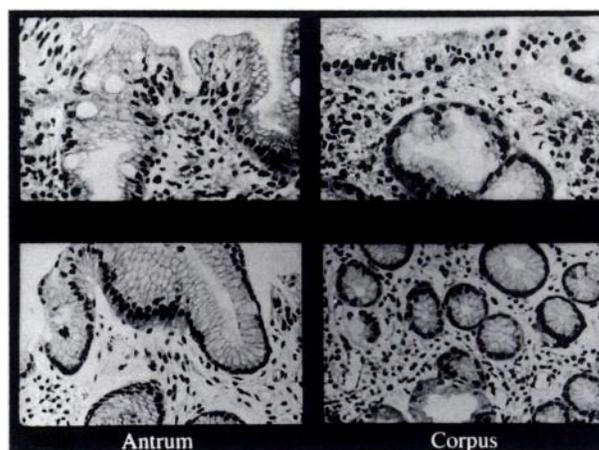


Fig. 2. The biopsy from the greater curvature of antrum (left) and body (Corpus, right; H&E staining) at the time of ER are shown at the top and those taken at 6 months after eradication as shown at the bottom. The neutrophil infiltration in the antrum and body of stomach before the treatment mostly disappeared after the treatment. The incomplete intestinal metaplasia, observed particularly in the antrum before treatment, considerably decreased after treatment.

tients. Fig. 1 shows the pretreatment status and the status 6 months after the completion of treatment in the 65 patients who underwent *H. pylori* eradication treatment. The fluctuations in the neutrophil infiltration score and the extent of intestinal metaplasia in the antrum (Fig. 1, left) and the body of stomach (Fig. 1, right) are shown. After *H. pylori* eradication, the neutrophil infiltration markedly decreased from the pretreatment level ( $1.02 \pm 0.1$  in the antrum and  $1.65 \pm 0.1$  in the body of stomach) to the posttreatment level ( $0.3 \pm 0.1$  and  $0.3 \pm 0.1$ , respectively) ( $P < 0.01$ ). The intestinal metaplasia demonstrated a significant regression ( $P < 0.05$ ) from  $1.30 \pm 0.1$  and  $0.7 \pm 0.1$ , respectively, before the treatment to  $0.90 \pm 0.1$  and  $0.46 \pm 0.1$ , respectively, after the treatment.

Biopsies from the greater curvature of antrum (Fig. 2, left) and body (Fig. 2, right) (H&E staining) at the time of ER are shown at the top of Fig. 2, and those taken 6 months after eradication are shown at the bottom for a single patient in the treated group. The neutrophil infiltration in the antrum and body of stomach before the treatment mostly disappeared after

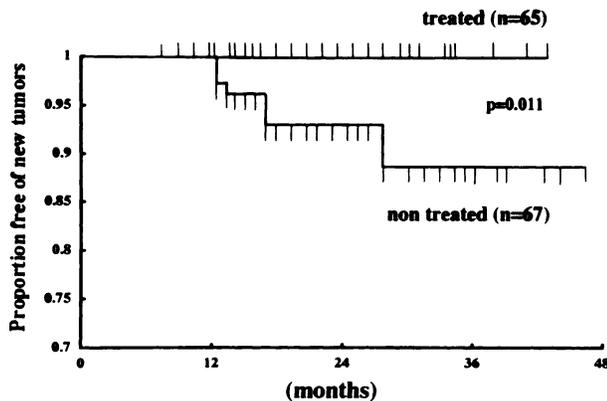


Fig. 3. Effect of *H. pylori* eradication after ER on the incidence of new cancers during follow-up. A Kaplan-Meier plot of the proportion free of new metachronous cancer by treatment for *H. pylori* infection ( $P = 0.011$  by the log-rank test) is shown.

the treatment. The incomplete intestinal metaplasia, observed particularly in the antrum before treatment, decreased markedly after treatment.

**Effect of *H. pylori* Eradication Treatment on the Incidence of Cancer in Other Regions.** Endoscopic observation was regularly conducted (every 6–12 months) both in group A and group B after ER. Fig. 3 shows the proportion of cases in which a new cancer was endoscopically detected in other regions. During the endoscopic observation period, no new cancer cases were detected for the 48 months in group A patients. On the other hand, the observation by the same method detected a new intestinal type of early gastric cancer in other regions in six (9%) patients in group B, demonstrating an incidence significantly higher than that in group A ( $P = 0.011$  by the log-rank test). These six patients included five males and 1 female aged 48 and 75 years (mean, 68 years). The type of adenocarcinoma initially detected and that of the early-stage gastric cancer subsequently diagnosed were all intestinal type.

New lesions were found in five of the six patients within 2 years of ER: three at 12–13 months; two at 18 months; and one at 28 months after ER. The size of early-stage gastric cancer discovered during the course of observation was 10 mm or less in diameter in all six patients, and each of them was completely extirpated by ER. Upon discovery of the secondary cancer in these six patients, the mucous membrane around the cancer lesions tested histologically positive to *H. pylori* infection, and the histological examination by H&E staining indicated moderate or severe neutrophil infiltration.

## Discussion

Epidemiological studies (10), as well as the results of long-term observation in Japanese monkeys after *H. pylori* infection (11), indicate that chronic atrophic gastritis, which is well known to be closely related to the incidence of an intestinal-type of gastric cancer (12), is caused by *H. pylori* infection. Epidemiological studies (1, 2) have also demonstrated the close relationship between *H. pylori* infection and incidence of gastric cancer. WHO/International Agency for Research on Cancer stated in 1994 that “there is sufficient evidence in humans for the carcinogenicity of infection with *H. pylori* (3).” However, there have not been any reports of a direct effect of *H. pylori* infection on the incidence and growth of gastric cancer.

Various reports have shown that eradication of *H. pylori* inhibits recurrence of peptic ulcer and markedly improves neutrophil infiltration, characteristics of *H. pylori*-related gastritis (13, 14). Recently, it has been reported that *H. pylori* infection is causally related to the incidence and growth of low-grade gastric lymphoma (MALT lymphoma (15)), and the eradication leads to the regression of MALT lymphoma (16). Although the mechanism of MALT lymphoma remission by *H. pylori* eradication has yet to be clarified, it has been suggested that the genetically abnormal cell clones are eliminated by the eradication of *H. pylori* (17).

Because of sophisticated technology in endoscopy, gastric cancer is detected at an early stage in many cases, and ER is well established in Japan. This treatment method circumvents the need for gastrectomy, and its clinical usefulness has been proven (5); however, a new gastric cancer in another region of the stomach is discovered by endoscopic observation in 5–10% of these patients within 5 years after ER (8).

It is well known that the intestinal type of adenocarcinoma of the stomach has chronic atrophic gastritis with marked intestinal metaplasia in the background (6). In the present study, we used two combinations of omeprazole and antibiotics to eradicate *H. pylori* in the 65 patients with the intestinal type of gastric cancer after ER. After eradication, neutrophil infiltration was significantly reduced, and a decrease of the severity of intestinal metaplasia was observed. Intestinal metaplasia has been considered as an advanced stage of chronic atrophic gastritis and, therefore, a nonreversible change. However, Tsutsumi *et al.* (18) reported that intestinal metaplasia may represent an immune response against chronic inflammation. According to his theory, the remission of inflammatory status after *H. pylori* eradication leads to the elimination of antigens and a possible regression of intestinal metaplasia. The multifocal distribution of metaplasia may lead to selection bias in endoscopic biopsy specimens, thus indicating a possibility that our evaluation of the extent of metaplasia may be inaccurate. In this regard, new randomized clinical trials with extensive mapping of the gastric mucosa are needed in the future to reach a reliable conclusion on the regression intestinal metaplasia.

Our results regarding the incidence of cancer in other regions suggest that eradication of *H. pylori* inhibited the growth of the intestinal type of adenocarcinoma in the initial stage. A study of 1159 gastrectomies for gastric cancer after detailed histopathological examination reported that minute synchronous multiple cancers, which could not be detected macroscopically, were present in more than 5% of the patients (7). This suggests that in our patients, *H. pylori* eradication achieved inhibition of the cancer growth rather than inhibition of cancer initiation. No report suggesting that *H. pylori* infection promoted the gastric cancer growth in the past is available. The results of present study strongly suggested that, just as in MALT lymphoma (16), gastric cancer is reversible in its initial stages and may regress after the eradication of *H. pylori*. The present results should strongly encourage new randomized clinical trials with proper design.

The results of this study are insufficient to clarify the mechanism by which *H. pylori* eradication may inhibit the growth of gastric cancer. Recent reports indirectly suggest a relation between *H. pylori* infection and the growth of gastric cancer. These reports have shown that long-term administration of ammonia enhances cell turnover in the gastric mucosa of rats (19), and that DNA damage (20) by free radicals, ammonia, and neutrophils may increase the incidence and progression of cancer. Still another report suggests decreased activity of ornithine decarboxylase (a rate-determining enzyme of polyamine

metabolism) in the antrum of stomach after *H. pylori* eradication (21). The present study is consistent with a hypothetical neutrophil infiltration and ammonia production by *H. pylori* infection in the growth of the intestinal type of gastric cancer in the initial stage.

Finally, the residual gastric mucosa after ER appears to be useful for elucidating the mechanisms of gastric carcinogenesis. Further investigation may contribute to our understanding of gastric carcinogenesis, and the results of this study warrant confirmation in a randomized trial.

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