

***Host factors contributing to the discovery of gastric cancer after successful eradication therapy of Helicobacter pylori***

S. TAKATA<sup>1</sup>, M. ITO<sup>1</sup>, M. YOSHIHARA<sup>2</sup>, S. TANAKA<sup>3</sup>, S. IMAGAWA<sup>1</sup>,  
K. HARUMA<sup>4</sup>, K. CHAYAMA<sup>1</sup>

<sup>1</sup> *Department of Medicine and Molecular Science, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan.*

<sup>2</sup> *Health Service Center, Hiroshima University, Higashi-Hiroshima, Japan.*

<sup>3</sup> *Department of Endoscopy, Hiroshima University Hospital, Hiroshima, Japan.*

<sup>4</sup> *Gastroenterology Unit, Department of Internal Medicine, Kawasaki Medical School, Kurashiki, Japan.*

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*Abbreviation used in this paper: H. pylori, Helicobacter pylori; UBT, <sup>13</sup>C-urea breath test; PG, pepsinogen*

Correspondence:

Masanori Ito, M.D.

Department of Medicine and Molecular Science

Hiroshima University, Hiroshima 734-8551 JAPAN

TEL: +81-82-257-5191

FAX: +81-82-257-5194

E-mail: [maito@hiroshima-u.ac.jp](mailto:maito@hiroshima-u.ac.jp)

## ABSTRACT

*Background and Aim:* Clinical features of patients who develop gastric cancer after successful eradication of *Helicobacter pylori* are still unclear. We attempted to identify host factors associated with discovery of gastric cancer including change in the background gastric mucosa in patients with atrophic gastritis.

*Methods:* We enrolled 101 patients (59 men, 42 women; mean age, 56.0 years) who were underwent successful eradication therapy. All patients had no neoplastic lesion in the stomach and diagnosed as corpus atrophic gastritis histologically before the eradication therapy. After successful eradication, these patients were followed up by the annual endoscopic examination (mean follow-up time, 63.2 months; range, 12-157 months). Fasting sera were obtained before and after eradication therapy and the serum levels of gastrin/pepsinogens were evaluated.

*Results:* Gastric cancer occurred during follow-up in 8 of the 101 patients (7.9%). We compared the host features between cancer- discovered group (n=8) and non-discovered group (n=93). We found no difference in gender, history of previous treatment of gastric cancer, and serum pepsinogen/gastrin levels at entry between them. Moreover, the trends of alterations of serum markers did not differ between the two groups. However, gastric cancer was more frequently found in elder patients (over 54 years old at eradication) than in others ( $P<0.05$ ).

*Conclusion:* Improvement of gastric inflammation did not correlate with the discovery of gastric cancer after eradication, however, age at the time of eradication seems to be important. Strict follow-up after eradication is needed in elder patients with atrophic gastritis.

Keywords: *H. pylori*, eradication, gastric cancer, gastrin, pepsinogen

## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) plays an important role in the development of atrophic gastritis.<sup>1</sup> Strong association is recognized between *H. pylori*-associated gastritis and gastric cancer not only of the intestinal type but also of the diffuse type.<sup>2,3</sup> Therefore, chronic gastritis induced by *H. pylori* is thought to be an important risk factor for the development of gastric carcinoma, and *H. pylori* is classified as a definite carcinogen by WHO/IARC.<sup>4</sup> Gastric cancer was proved to be induced by *H. pylori* infection in an animal model.<sup>5,6</sup> In addition, Uemura *et al.* showed by prospective study that gastric cancer is seldom found in patients without *H. pylori* infection.<sup>7</sup>

There have reports that the incidence of gastric cancer decreased among patients who underwent successful eradication of *H. pylori*.<sup>8</sup> With successful eradication, gastric inflammation including atrophy and intestinal metaplasia improves, and eradication may be beneficial in preventing new development of gastric cancer.<sup>9,10</sup> However, gastric cancer is a common disease among Japanese especially in patients with atrophic gastritis, and we sometimes encounter patients with atrophic gastritis who develop gastric cancer even after having undergone successful eradication therapy. It is controversial whether eradication therapy decreases the incidence of gastric cancer in patients with atrophic gastritis. Concerning the development of gastric cancer after eradication, previous report indicated that gastric cancer seldom occurred in patients with duodenal ulcer,<sup>7</sup> however, the detail host factors, including the alteration of gastric inflammation, in patients with corpus atrophic gastritis are still unclear.

In the present study, we enrolled the patients with atrophic gastritis who received successful eradication therapy and examined the incidence of newly discovered gastric cancer by long-term follow-up of patients after eradication of *H. pylori*. We also attempted to clarify the clinical features, including changes in the background gastric mucosa, of patients with newly discovered gastric cancer after eradication therapy.

## PATIENTS AND METHODS

### *Patients*

Between September 1991 and September 2001, totally 1902 patients were diagnosed as *H. pylori*-positive in Hiroshima University Hospital by serum antibody against *H. pylori* (Eiken Eplate, Tokyo), <sup>13</sup>C-urea breath test (UBT) (Otsuka, Tokushima, Japan), rapid urease test (RUT) (Serim Research Corp., Indiana) or histological examination. Among the patients, we enrolled 101 patients with corpus atrophic gastritis (59 men, 42 women; mean age, 56.0 years; range, 18-77 years; 8 with gastric ulcer and 93 with gastritis) who were underwent successful eradication therapy in order to prevent the peptic ulcer recurrence or to improve gastric inflammation. Successful eradication was confirmed by UBT, RUT, and histological examination at 8 weeks. All of them were confirmed there were no neoplastic lesions in the stomach and diagnosed as corpus atrophic gastritis by histological examination. A biopsy specimen was taken from the gastric corpus, and we diagnosed the degree of atrophic change by HE section with the use of updated Sydney system. When we find the atrophy (mild to marked), we considered the patient has atrophic gastritis. Patients who underwent gastrectomy or still treated with proton pump inhibitors were not included in this study. We followed-up enrolled patients for 12 to 157 months (mean, 63.2 months). Twenty-eight of these patients had undergone endoscopic resection of gastric cancer prior to the entry. Endoscopic examination and histological examination were performed annually (sometimes more often) after successful eradication therapy. We confirmed no re-infection of *H. pylori*. The ethics committee of Hiroshima University approved the study protocol, and subjects gave written informed consent.

### *Serum gastrin and pepsinogens*

Fasting sera were collected from all the patients. The samples were centrifuged immediately at 4°C and stored at -20°C until use. Serum gastrin and pepsinogen (PG) concentrations were determined by means of a modified radioimmunoassay.<sup>11</sup>

### *Statistical analysis*

Values are reported as mean ± SE. Analysis was performed with the paired *t*-test or Fisher's exact test and a Kaplan-Meier estimator JMP software (SAS Institute Inc., Cary, NC) was used for all statistical analyses. A *P* value of <0.05 was considered

significant.

## RESULTS

### *Clinical features of patients*

Gastric cancer was discovered in 8 of the 101 study patients (7.9%) during the follow-up period. The incidence of newly discovered gastric cancer was 1.5%/year after eradication. Detailed data are shown per group (patients with gastric cancer (n=8) versus patients without gastric cancer (n=93)) in Table 1. The mean age of patients with cancer was higher than that of those without cancer, whereas it was not the statistical difference ( $P=0.07$ ). There were no differences in sex, disease, or the history of prior gastric cancer between two groups. Clinical features of patients with newly diagnosed gastric cancer are summarized in Table 2. In most cases, gastric cancer was discovered as mucosal limited cancer.

### *Relation between newly discovered gastric cancer and clinical features*

We classified the 101 patients into two groups according to the age at entry into the study. Concerning the age, we set the cut-off line at 54 years, which was the age of youngest patient with newly discovered gastric cancer. There was a statistical difference in cancer discovery between the younger and older patients ( $\geq 54$  years vs.  $< 54$  years at eradication;  $P=0.033$ ) (Figure 1-A). We then classified patients by sex, history of endoscopic resection for gastric cancer, and serum PG levels (high-PG; PG-I  $> 70$ ng/ml or PG-I/II ratio  $> 3$ ). However, statistical differences were not found between two groups in the discovery of gastric cancer (Figures 1-B, C, D).

### *Relation between discovery of gastric cancer and serum PG and gastrin levels at entry*

We chose 37 patients who were followed up for at least 58 months, that is the longest period for the diagnosis of newly discovered gastric cancer, and classified these patients into two groups; those with newly discovered gastric cancer (Group C; n=8) and those without gastric cancer (Group N; n=29). We compared serum PG and gastrin levels at entry between the two groups. As shown in Table 3, serum PG and gastrin levels were slightly lower in the Group C patients, but statistical significance was not reached. When we subclassified patients by high and low PG levels, there were 3 with gastric cancer among the high-PG patients and 5 among the low-PG patients. The difference was not statistically significant ( $P=0.60$ ) (Table 3).

*Changes in the serum PG-I/II ratio after eradication therapy*

We then analyzed changes in the serum PG-I/II ratio in the two groups after eradication therapy. Second sera (after eradication) were collected around 12-24 month after eradication. We showed changes and its amount in the serum PG-I/II ratio after eradication therapy in Figure 2-A. The PG-I/II ratio significantly increased after eradication in both groups ( $P<0.05$ , Figure 2-A), although no statistical difference was found between the trends of two groups (Figure 2-B). A low PG-I/II value before eradication therapy was characteristic of the group that discovered cancer. In addition, the serum PG-I level decreased with eradication therapy in both groups, and there was no statistical difference in the amount of decrease between two groups (data not shown).

*Changes in serum gastrin levels after eradication therapy*

Further, we examined the serum gastrin level at pre- and post-eradication therapy and examined the alteration in each group. The serum gastrin level decreased with eradication therapy in both groups and statistical difference was found in group N ( $P<0.01$ ; Figure 2-C). However, as demonstrated in PG I/II level, we could not detect statistical difference in the trends between two groups (Figure 2-D).

## DISCUSSION

Atrophic gastritis as well as gastric cancer is a very common disease in Japan. It is important to clarify whether gastric carcinogenesis is influenced by eradication therapy. In a recent randomized controlled trial in China, eradication of *H. pylori* prevented the development of gastric cancer in patients with no precancerous lesion upon presentation.<sup>12</sup> There was a concurrent 37% relative decrease in the incidence of cancer incidence in the overall population, but this difference did not reach a level of statistical significance. On the other hand, Take *et al* demonstrated that gastric cancer incidence decreased after eradication therapy in gastric ulcer patients.<sup>13</sup> These results seem to be contradictory in part, and it is still controversial whether or not eradication therapy truly diminishes gastric cancer incidence. Our data are not sufficient to answer this question, but we did show that gastric cancer occurred in a certain subset of patients in whom *H. pylori* was eradicated, sometimes just after therapy and sometimes much later (more than 4 years later). We recently showed that the morphology of gastric cancer changes after eradication.<sup>14</sup> Moreover, Kamada *et al* reported that the major part of gastric cancer after eradication showed depressive appearance.<sup>15</sup> It is possible that eradication therapy modifies the appearance of gastric cancer and thus our ability to diagnose it by endoscopic examination.

It is clinically important to speculate in whom gastric cancer will occur after eradication therapy. In the present study, we found one of the most important factors to be the age of the patients which may correspond to an infectious period of *H. pylori*. It is widely known that *H. pylori* infects the human gastric mucosa in the cradle; thus, the more important factor in the development of gastric cancer is the infectious period rather than the level of atrophic gastritis or a prior occurrence of gastric cancer. For example, the incidence of colitic cancer increases gradually among patients with ulcerative colitis who are followed up for a long time.<sup>16</sup> The incidence of hepatocellular carcinoma also increases gradually with long-term follow-up among patients with hepatitis C virus infection.<sup>17</sup> We have reported a close correlation between inducible nitric oxide synthase (iNOS)-producing gastritis and gastric carcinogenesis.<sup>18,19</sup> The longer the period of inflammation, the higher the risk of damaging DNA, the leading to increased carcinogenicity.<sup>20</sup> In an animal model, early eradication was shown to be more effective than late eradication for preventing the development of gastric cancer.<sup>21</sup> Thus, we believe eradication therapy should be

performed in young people.

As well known, low PG-I and PG-I/II level is an important risk factor for gastric carcinogenesis.<sup>11</sup> However, the degree of atrophic gastritis at entry and the degree of improvement in inflammation after eradication did not differ between patients in the two groups. In addition, we examined the histological change of gastritis and found that atrophy and metaplastic levels at entry were improved significantly in both groups. However, no statistical difference was found between the trends of two groups (data not shown). These results suggest that we can not predict the occurrence of gastric cancer only by evaluating the gastric inflammation after eradication therapy. However, theologically, eradication therapy should decrease the newly development of gastric cancer by diminishing the inflammatory change in its long-term effect. Focus must be placed on the observation period in the study. It is likely that a cancer cell requires a long time to reach a visible size. We should distinguish the “discovery” of the gastric cancer from the “development” of cancer. It is likely that one cancer cell requires a long time to reach a visible size. Therefore, we will need to perform a more longer-term study to determine the relationship between the gastric inflammation and carcinogenesis.

In addition to the observation period, we should point out some problems in our study design. First, the number of patients in the present cohort study may not be adequate for discuss relations between host factors and gastric cancer development after eradication. Secondary, it may be controversial that we set the cut-off age at 54 years. Because 54 were the youngest age in the Group C, we divided our subjects by this age. Indeed, when we set the cut-off age at 60 years, we could not find statistical difference between two groups. However the number of our subjects was so small and the follow-up period was too short, that we could not demonstrate the clear results in the present study. Therefore we have to set the study design with larger scale in the next step. Thirdly, the incidence of gastric cancer in our study was relatively high in comparison with other reports. We think that the difference is caused by baseline status of corpus atrophy. The patients in our study should be considered very high risky group in gastric cancer incident. Another reason is the diagnostic ability of gastric cancer in the endoscopic examination. Our protocol was carried out in the uni-center design and we confirmed the size of newly discovered cancer was very small ( $\leq 10$ mm in diameter as demonstrated in Table 2), that should be emphasized.

In conclusion, we showed that gastric cancer is often found by endoscopic examination in a subset of patients, i.e., in patients age 54 years and above, after successful eradication therapy. The improvement of gastric inflammation may not link to the diminished gastric carcinogenesis unless we follow up the patients in a longer period. Endoscopic examination should be performed annually in the elder patients with atrophic gastritis even they received successful eradication of *H. pylori*.

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Table 1. Clinical features of patients per study group

	Patients with cancer* (n=8)	Patients without cancer* (n=93)
Mean age (range)	63.8 (54-77)	55.4 (18-76)
Sex (M/F)	6 / 2	53 / 40
Disease (G/GU)	8 / 0	85 / 8
Prior EMR**	4	24

\* Eradication of *H. pylori* was confirmed in all patients.

\*\* Patients who had received endoscopic mucosal resection prior the entry.

G/GU, gastritis / gastric ulcer

EMR, endoscopic mucosal resection of gastric cancer

Table 2. Eight patients with newly diagnosed gastric cancer

Patient	Age (yr)	Sex	Endoscopic type of gastric cancer	diameter (mm)	Histology	Therapy	Period (months)
1	56	M	SD	5	intestinal	EMR	5
2	75	M	SD	5	intestinal	EMR	11
3	55	M	SD	10	intestinal	EMR	26
4	69	M	polypoid	5	intestinal	EMR	29
5	67	F	SD	8	intestinal	EMR	36
6	57	M	SD	5	intestinal	EMR	38
7	77	F	SD	15	intestinal	EMR	57
8	54	M	AD	22	intestinal	Surgery	58

SD, superficial depressive type

EMR, endoscopic mucosal resection

AD, advanced type

Period, until discovering gastric cancer after eradication

Table 3. Serum PG-I, PG-I/II, and gastrin levels in patients followed up for  $\geq 58$  months

	Group C (n=8)	Group N (n=29)	<i>P</i> value*
PG-I (ng/ml)	42.6	55.7	0.53
PG-I/II	2.0	2.7	0.26
Gastrin (pg/ml)	216.5	289.8	0.57
Low-PG	5 / 8 (63%)	19 / 29 (66%)	0.60

\* by Fisher's exact probability test

Group C, patients with newly discovered gastric cancer

Group N, patients without gastric cancer

Low-PG, PG-I  $\leq 70$ ng/ml and PG-I/II ratio  $\leq 3$

## Figure Legends

Figure 1. Kaplan-Meier analysis subclassified by host factors. (A) Subclassification by age at eradication. During follow-up, gastric cancer discovered in 8 of the 65 patients over 54 years of age (12.3%) but 0 of the 36 patients under 54 years of age ( $P=0.033$  by log rank test). (B) Subclassification by sex. During follow-up, gastric cancer was discovered in 6 male patients and 2 female patients. (C) Subclassification by history of endoscopic resection (EMR) for gastric cancer. Gastric cancer was discovered in 4 patients in each group. (D) Subclassification by PG levels. Gastric cancer was discovered in 5 low-PG (PG-I  $\leq 70$ ng/ml and PG-I/II ratio  $\leq 3$ ) patients and 3 high-PG (PG-I  $> 70$ ng/ml or PG-I/II ratio  $> 3$ ) patients.

N.S.= not significant

Figure 2. Changes in serum PG-I/II and gastrin levels after eradication therapy in patients followed up for  $\geq 58$  months. (A) The serum PG-I/II ratio increased with eradication therapy in both groups ( $*P<0.01$ ). (B) The trend was not statistically different in both groups. (C) The serum gastrin level decreased with eradication therapy in both groups and statistical difference was found in group N, but these trends were not statistically different. (D) The trend was not statistically different in both groups.

N.S.= not significant

Group C, patients with newly discovered gastric cancer

Group N, patients without gastric cancer

Fig. 1

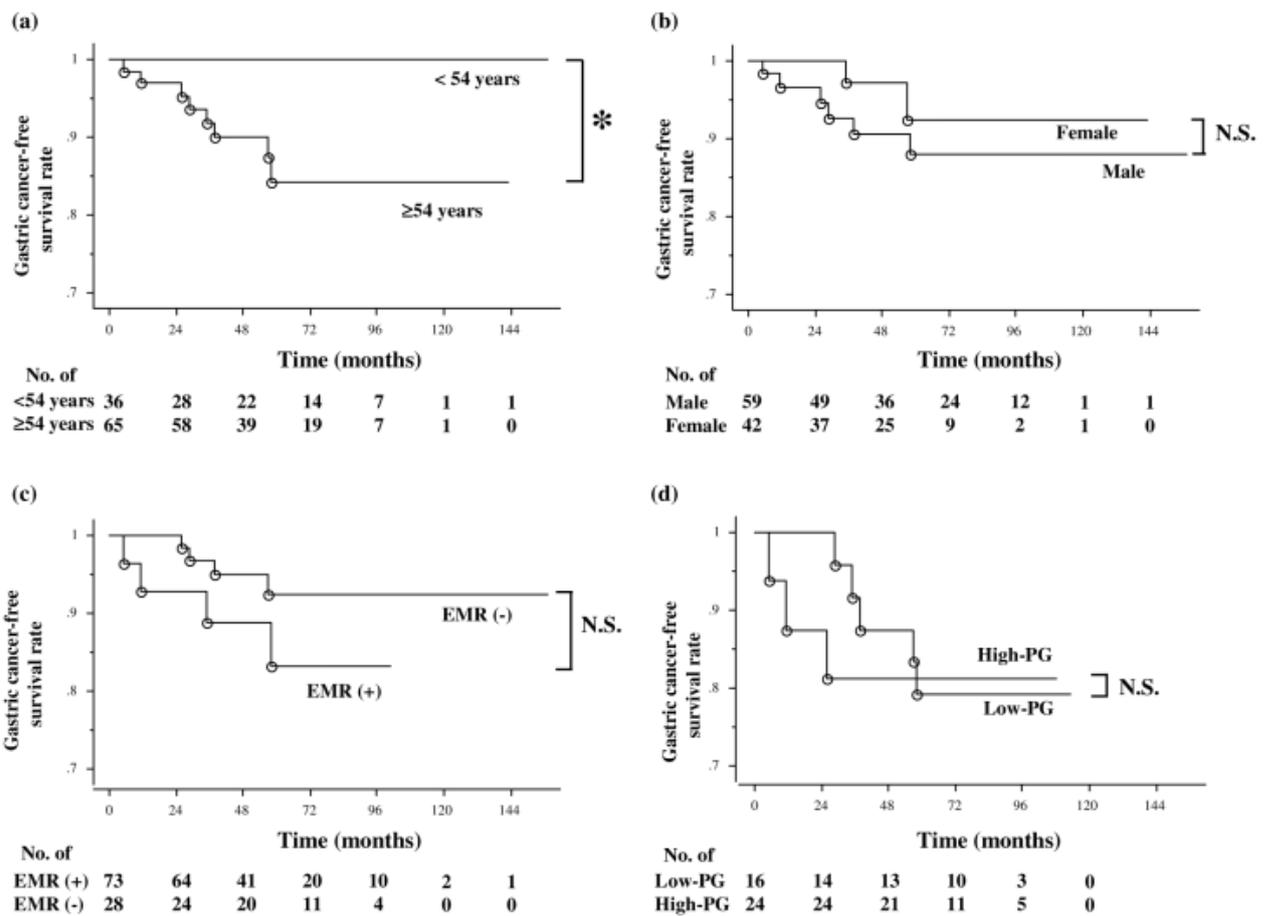


Fig. 2

