**Helicobacter pylori** Eradication Suppresses Metachronous Gastric Cancer and Cyclooxygenase-2 Expression after Endoscopic Resection of Early Gastric Cancer

**Background/Aims:** The impact of *Helicobacter pylori* (*H. pylori*) eradication after endoscopic resection (ER) of early gastric cancer (EGC) has not been fully evaluated. We tried to find out the effect of *H. pylori* eradication therapy on the development of metachronous gastric cancers and changes in Cyclooxygenase-2 (COX-2) expression following attempts to eradicate *H. pylori* after ER of EGC. **Materials and Methods:** We eradicated *H. pylori* in the patients with EGC after ER. Biopsy samples were taken according to the follow-up schedules for surveillance after ER. **Results:** Fifty five patients were enrolled and finished the follow up schedules. Of the 55, 28 were successfully treated *H. pylori* infection, and the other 27 were failed eradication of *H. pylori*. The mean follow-up period was 60.8 months. Five in the *H. pylori* ongoing infection group developed metachronous gastric cancer, whereas no new gastric cancers were found in the 28 eradication group (*P*=0.023). COX-2 expression in the eradication group was significantly decreased (1.4±0.2, n=28), compared to that in *H. pylori* ongoing infection group (3.0±0.4, n=27, *P*=0.0001) after the follow-up. **Conclusions:** The eradication of *H. pylori* seems to have a preventative effect on the development of metachronous adenocarcinomas and a suppressive effect on COX-2 expression in the patients after ER for EGC. (Korean J Helicobacter and Upper Gastrointest Res 2011;11:117-123)

**Key Words:** *Helicobacter pylori*; Gastric cancer; Cyclooxygenase 2

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**INTRODUCTION**

Despite its decreasing incidence over recent decades, gastric cancer is still the second leading cause of cancer-related deaths worldwide.\(^1\) The development and progression of gastric cancer seem to result, in part, from the accumulation of multiple genetic alterations, which lead to oncogene overexpression and tumor suppressor loss.\(^2,3\) The most important single factor responsible for the development of gastric cancer is *Helicobacter pylori* (*H. pylori*) infection, which affects more than 50% of the world’s population.\(^4\) The risk for developing gastric cancer in patients with *H. pylori* infection is about two- to six-fold increases in non-infection individuals, according to epidemiological studies.\(^5,7\)

When diagnosed at an advanced stage, gastric cancer is usually an incurable disease. However, resection of the tumor at an early stage can confer a relatively favorable prognosis. Complete cures have been obtained in some early gastric cancers (EGC), especially with the use of endoscopic treatment modalities such as endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD).\(^8,9\) The incidence of lymph node metastasis is 0.36% in mucosal gastric cancer patients who have no lymphatic invasion, no histological ulceration, and a tumor diameter of less than 30 mm.\(^10\)

After endoscopic resection (ER), the disease can recur in the stomach in two patterns, local recurrence or metachronous gastric cancers. Many metachronous gastric cancers are thought to be de novo cancers and may arise from the same high-risk background mucosa.

The relationship between metachronous recurrence and *H. pylori* eradication therapy in gastric cancer patients treated by ER has not been evaluated.
pylori infection remains controversial. Arima et al. reported that H. pylori infection showed no significant relationship with metachronous recurrence. However, others have observed that H. pylori eradication inhibited the development of metachronous carcinoma in the remnant stomach. Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme in prostaglandin synthesis from arachidonic acid. COX-2 expression is thought to be a relatively early event during gastric carcinogenesis. Previous studies have demonstrated increased COX-2 expression in both H. pylori-related gastritis and gastric adenocarcinomas. COX-2 expression is increased in intestinal-type gastric cancers and in the crypts with intestinal metaplasia. Relatives of gastric cancer patients showed higher gastric COX-2 expression and higher incidence of precancerous lesions after H. pylori infection than did relatives of H. pylori-infected patients with duodenal ulcers. Furthermore, patients with intestinal-type adenocarcinomas had frequent atrophy and intestinal metaplasia in the surrounding gastric mucosa, compared to patients with diffuse-type adenocarcinoma, gastric lymphoma, or no neoplasia.

H. pylori infection resulted in overexpression of COX-2 that was reversed by H. pylori eradication. Therefore, eradication therapy may reduce the risk of gastric cancer development.

Given this information, we sought to assess the effects of H. pylori eradication on the development of new gastric cancers in patients who underwent endoscopic resection to treat early gastric cancer and on the expression of COX-2.

MATERIALS AND METHODS

1. Patients

The study was enrolled a total of 55 consecutive patients with H. pylori infection (36 males, 19 females; mean, age 60.7 years) who had received successful ER for EGC between March 2001 and November 2003 and follow-up for more than 24 months at Bucheon Hospital, Soonchunhyang University, Korea. Patients taking aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, bismuth preparations, or proton pump inhibitors during the month before the examination were excluded from the study. All patients provided informed consent about histological analysis and preserve of H. pylori status after primary eradication therapy for H. pylori.

2. Study design

Biopsy samples were taken from the cancer, the adjacent non-cancerous area, and two additional areas (two samples from the antrum, within 2 cm of the pyloric channel, and two from the corpus) before the EMR procedure. All gastric specimens were fixed in 10% buffered formalin for H&E staining or COX-2 immunostaining. The presence of H. pylori was determined by a CLO test (Delta West Ltd., Perth, Australia) and a 13C-urea breath test.

Patients with EGC were considered suitable candidates for endoscopic resection at our institution if they met all of the following criteria: clinical findings of intramucosal adenocarcinoma, differentiated-type adenocarcinoma, protruding-type or superficial elevated type no more than 3 cm in diameter, or superficial depressed type no more than 2 cm in diameter; no ulceration in the cancer; and no evidence of lymph node or distant metastases. Endoscopic resection was performed using conventional EMR or endoscopic submucosal dissection.

After the endoscopic resection procedure, all patients were attempted to treat for H. pylori infection using proton pump inhibitors based triple therapy. Endoscopic follow-up with biopsies was performed on each patient at 3, 12, 24, 36, 48, 60, and 72 months after ER. In addition, in patients with upper gastrointestinal system symptoms, we performed esophagogastroduodenoscopy at that time. We looked for any metachronous gastric cancer and cancer relapse, and collected gastric biopsy specimens for histological examination and COX-2 immunostaining. Each patient underwent a CLO test and a 13C-urea breath test to determine H. pylori status, with endoscopic surveillance.

3. Immunohistochemistry for COX-2

A paraffin-embedded mucosal biopsy specimen was mounted on a silane-coated glass slide. Immunohistochemical staining was performed using antibodies specific for COX-2; the COX2 antibody was a monoclonal antibody raised in a mouse against a synthetic peptide corresponding to human COX-2, amino acids 580-598 (DAKO, Carpinteria, CA, USA). For immunostaining, the COX-2 antibody (diluted 1:100) was incubated with the sections for 1 h at 37°C, using a microwave method. The primary antibody was omitted as a negative control.

The immunoreactivity was recorded using semi-quantitative and subjective grading, considering both the intensity of staining and the number of immunoreactive cells. The intensity was
recorded as 0 (no staining) to 3 (strong staining), using fundic glandular cells as controls. Positive reactions similar to those in the control cells were scored as 2 (moderate staining). Tissue specimens showing significantly more intense staining than the control cells were scored as 1 (weak staining), and specimens with undetectable or negligible expression were scored as 0 (no staining). The staining area, defined as the total amount of positively staining cells, was recorded as 0 (none or positive staining in <5% of the cells), 1 (positive staining in 5∼30%), 2 (positive staining in 31∼60%), or 3 (positive staining in >60%). The staining index, which ranged from 0 to 9, was obtained by multiplying the score for staining intensity times the staining area.

4. Statistical analysis

All results are expressed as means±SEM. For statistical analysis, ANOVA single-factor analysis was performed. Differences were deemed significant when \( P < 0.05 \). The incidence of cancer in other regions was compared between the two groups using the \( \chi^2 \) test and Student’s \( t \)-test. All statistical computations were performed using SPSS v.10 (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1 shows the characteristics of the 55 patients. The mean follow-up period of the patients was 60.8 months. A total of 55 patients with \( H. pylori \) infection (36 males, 19 females; mean age, 60.7 years) who underwent successful ER for EGC and were followed up. Of the 55, 28 were successfully treated with triple therapy for \( H. pylori \) infection, and the other 27 were failed eradication of \( H. pylori \) or couldn’t take those medications by poor compliance or side effects. There was no significant difference in any demographic characteristic between the groups. During the endoscopic follow up period, no newly developed cancers were observed in the eradication group. However, metachronous gastric cancers were detected in other regions in five patients (18.5%) belonging to the ongoing in-

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<tr>
<th>Table 1. Characteristics of the Patients</th>
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<tr>
<td>Number</td>
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<td>Age (^{a}) (years)</td>
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<td>Depth of EGC (m1/m2/m3)</td>
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<td>Mean size of EGC (mm)</td>
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NS, not significant. \(^{a}\)Mean (range).

| Table 2. Demographic Features of Patients with Metachronous Gastric Cancer |
|-----------------------------|-------------------------|-------------------|----------|---------|-----------|---------|
| Age (yr) | Gender | Time to 2\(^{nd}\) cancer (months) | Depth of 2\(^{nd}\) cancer | Size of 2\(^{nd}\) cancer | Location 1\(^{st}\) cancer | Location 2\(^{nd}\) cancer |
| 1       | 46 Male | 13 | m2 | 0.7×0.6 cm | Antrum | Antrum |
| 2       | 68 Male | 14 | m2 | 0.8×0.4 cm | Corpus | Antrum |
| 3       | 64 Male | 48 | m2 | 0.6×0.4 cm | Antrum | Antrum |
| 4       | 75 Female | 36 | m3 | 1.0×0.8 cm | Antrum | Angle |
| 5       | 54 Female | 36 | m2 | 1.2×1.0 cm | Antrum | Antrum |
COX-2 expression in the mucosa adjacent to the cancer. COX-2 expression in the adjacent mucosa was significantly lower in the eradication group than in the ongoing infection group at the last follow-up. In the eradication group, we analyzed the grade of gastritis in the pre-eradication period and that of last follow-up biopsies after *H. pylori* eradication. The neutrophil infiltration scores in the antrum and the corpus were markedly decreased (antrum: 1.1±0.1, corpus: 1.4±0.1), compared to the scores in the pre-treatment period (antrum: 1.7±0.2, corpus: 2.3±0.1, *P*<0.01). The atrophy score declined from 1.9±0.1 to 1.6±0.1 (*P*=0.030) in the corpus and from 2.0±0.2 to 1.6±0.1 in the antrum (*P*=0.048) after *H. pylori* eradication. Furthermore, the grade of intestinal metaplasia (IM) in the corpus significantly decreased, from 1.9±0.2 to 1.6±0.2 (*P*=0.043).

Slightly increased COX-2 expression was found in the cancer tissues (4.5±0.5), compared to their respective paired *H. pylori*-infected normal adjacent mucosa (3.2±0.3), but the difference was not statistically significant (*P*=0.07). There was no difference in COX-2 immunoreactivity in the mucosa adjacent to cancer tissues between the groups before EMR therapy (3.3±0.3 versus 3.0±0.4, *P*=0.914). However, COX-2 expression in adjacent mucosa in the eradication group was significantly decreased (1.4±0.2), compared to that in the ongoing infection group (3.0±0.4, *P*=0.001), at the last follow-up (Fig. 2, 3). COX-2 expression of the mucosa adjacent to cancer tissue was high in the patients with metachronous gastric cancer compared to that in the patients without metachronous cancer of ongoing infection group (5.6±0.9 versus 2.5±2.0, *P*=0.003).

**DISCUSSION**

The intestinal type of gastric adenocarcinoma forms glandular structures that somewhat resemble the glands of the gastrointestinal tract. This type has glandular atrophy with IM in the background mucosa. The second histological type, the diffuse carcinoma, invades the organ without forming well-defined...
structures such as glands. No clear precancerous lesions have been identified for the diffuse type. The diffuse type of gastric adenocarcinoma has different features and characteristics from the intestinal type.24,25 Furthermore, a diffuse histotype has been related to earlier presences of lymph node metastases. An absolute indication for ER of EGC can be considered the intestinal type of intramucosal cancer. Accordingly, patients with ER therapy for EGC retain potentially carcinogenic mucosa, which can develop into overt gastric adenocarcinoma at any time.

COX-2, an inducible isoinform of cyclooxygenase enzyme, converts arachidonic acid to prostanoids and is strongly expressed throughout the *H. pylori*-associated gastric carcinogenesis pathway, from chronic active gastritis, to gastric atrophy and IM, and finally to gastric adenocarcinoma.19,20 All our subjects had carcinogenic mucosa and *H. pylori* infection, and COX-2 expression was demonstrated in their epithelium. The expression of COX-2 in the eradication group was significantly decreased after *H. pylori* eradication. However, the ongoing infection group revealed continuous, strong expression of COX-2 in our study.

Mucosal COX-2 expression has been shown to continue in patients with IM, even after eradication of *H. pylori* infection.26 Other investigators have found that patients with IM exhibited reduced COX-2 expression at 1 year after successful *H. pylori* eradication, compared to expression before eradication therapy.27 Our results are consistent with the latter observation. Our cancer patients with high carcinogenic background mucosa, including IM, showed decreased carcinogenic potential of the mucosa at more than 3 years after *H. pylori* eradication. This is based on decreased COX2 expression and the regression of atrophy and corporeal IM in our patients after *H. pylori* eradication. Furthermore, newly developed metachronous early gastric cancers were detected only in the ongoing infection group.

Some studies have suggested that the suppression of COX-2 reduced tumor development.28-30 Methods of suppressing COX-2 in gastric mucosa include the ingestion of probiotics and *H. pylori* eradication therapy for *H. pylori*-infected mucosa, in addition to COX-2 inhibitors.29 However, the ability of these methods to actually suppress the incidence of gastric cancer development in high-risk patients remains controversial.

*Helicobacter pylori* infection plays an important role in gastric carcinogenesis, but the mechanism of COX-2 expression in *H. pylori*-related carcinogenesis is still unclear. Several studies have suggested that genetic polymorphisms in COX-2 are associated with risk for colorectal, lung, bladder, and gastric cancers.31-34 Subjects with the −1195 AA genotype had increased risk for gastric cancer, and an additionally elevated risk for gastric cancer was observed in subjects exhibiting the −1195 AA genotype and *H. pylori* infection or smoking.34 Subjects with the −1195 AA genotype also had high COX-2 expression, compared to subjects with the 1195 GG genotype. This genetic variation leads to differences in the metabolism of NSAIDs, in prostaglandin synthesis, and in individual cancer risk. Although we did not evaluate COX-2 genotypes, we believe that the elimination of *H. pylori* blocked over-expression of COX-2 and suppressed metachronous gastric cancer in our patients who were at high risk for gastric adenocarcinoma.

The eradication rate of this study was 50.9% by intention-to-treat analysis. Hwang, et al. showed that the eradication rate of *H. pylori* depends on the antibiotic resistance.35 In the study, the overall eradication rate of PPI triple therapy was 63.6% by intention-to-treat analysis. Our study also included the patients who did not complete the medicines for *H. pylori* eradication on account of poor compliance or side effects. We also assumed that a lot of the patients with antibiotic resistance were enrolled in the study.

We found 5 patients with metachronous gastric cancer in the ongoing infection group. The rate of the metachronous gastric cancer was relatively high compared to previous study in Japan.13 Most of previous studies were performed during only 3 years after ER.13,36 Three metachronous cancers of our study were observed at 36 months and 48 months after ER. Fukase’s study showed that 167 patients of control group could be followed up at 3 years. The cumulative incidence of metachronous gastric cancer was 14.4% among the 167 patients. The cumulative incidence rate of the study is very similar to the rate of our study at 3 years after ER.

Limitations of our study include the involvement a relatively small number of patients. A randomized multicenter trial assessing the preventive effect of *H. pylori* eradication on occurrence of new gastric carcinomas after ER was published in Japan.16 However, the results of this Japanese study showed a relatively short-term outcome during 3 years. In our study, we observed long-term suppression of the development of metachronous gastric adenocarcinomas and decreased COX-2 expression after *H. pylori* eradication therapy in the patients who underwent ER of EGC. Further studies on a larger scale may confirm the usefulness of *H. pylori* eradication therapy in these
patients.

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