

Helicobacter pylori Eradication Reduces the Metachronous Recurrence of Gastric Neoplasms by Attenuating the Precancerous Process

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Purpose: The importance of *Helicobacter pylori* eradication after endoscopic resection (ER) of gastric neoplasms remains controversial. In this study, we clarified the importance of *H. pylori* eradication for metachronous lesions after ER.

Materials and Methods: This study included 3,882 patients with gastric neoplasms who underwent ER. We included patients infected with *H. pylori* who received eradication therapy. Among them, 34 patients with metachronous lesions after ER and 102 age- and sex-matched patients (nonmetachronous group) were enrolled. Background mucosal pathologies such as atrophy and intestinal metaplasia (IM) were evaluated endoscopically. The expression levels of CDX1, CDX2, Sonic hedgehog (SHH), and SOX2 were evaluated based on *H. pylori* eradication and the development of metachronous lesions.

Results: The eradication failure rate was higher in the metachronous group than in the nonmetachronous group ($P=0.036$). Open-type atrophy ($P=0.003$) and moderate-to-severe IM ($P=0.001$) occurred more frequently in the metachronous group. In patients with an initial diagnosis of dysplasia, the eradication failure rate was higher in the metachronous group than in the nonmetachronous group ($P=0.002$). In addition, open-type atrophy was more frequent in the metachronous group ($P=0.047$). In patients with an initial diagnosis of carcinoma, moderate-to-severe IM occurred more frequently in the metachronous group ($P=0.003$); however, the eradication failure rate was not significantly different between the two groups. SHH and SOX2 expression was increased, and CDX2 expression was decreased in the nonmetachronous group after eradication ($P<0.05$).

Conclusions: Open-type atrophy, moderate-to-severe IM, and *H. pylori* eradication failure were significantly associated with metachronous lesions. However, eradication failure was significantly associated with dysplasia, but not carcinoma, in the metachronous group. Thus, *H. pylori* eradication may play an important role in preventing metachronous lesions after ER for precancerous lesions before carcinomatous transformation.

Key Words: *Helicobacter pylori*; Metachronous; Endoscopic resection; Stomach neoplasms

Introduction

Helicobacter pylori infection is closely related to gastric atrophy, intestinal metaplasia (IM), and progression to dysplasia or cancer.¹ However, the incidence of gastric cancer did not differ between Chinese patients receiving *H. pylori* eradication treatment and those receiving placebo during 7.5 years of follow-up.² Several reports exist on the effect of *H. pylori* eradication in patients at a high risk for gastric cancer. Previous studies illustrated

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that eradication of *H. pylori* after endoscopic resection (ER) of early gastric cancer (EGC) helped prevent the development of metachronous gastric cancer.^{3,4} However, Choi et al.⁵ revealed that *H. pylori* eradication after ER of EGC did not reduce the incidence of metachronous gastric cancer. Therefore, the importance of *H. pylori* eradication after ER for gastric neoplasms remains controversial. Defining the subgroup of patients for whom *H. pylori* eradication after ER for gastric neoplasms is beneficial is important because patients who undergo ER is increasing and the incidence of metachronous lesions is also consequently increasing.

Several gastric and intestinal epithelial markers are associated with the progression of IM, a precancerous lesion.^{6,7} Sonic hedgehog (SHH) is an essential regulator of developmental and physiological processes.⁸⁻¹⁰ In humans, Shh mRNA and SHH protein are expressed in the normal gastric fundus but not in the esophagus and intestine.^{11,12} Loss of SHH is an early change associated with the presence of IM.^{11,13} CDX proteins (intestine-specific caudal-related homeobox transcription factor) are intestine-specific transcription factors encoded by human *CDX1* and *CDX2* genes. Moreover, Mizoshita et al.¹⁴ reported that the expression of CDX1 and CDX2 was found in IM of the human stomach. Shiotani et al.⁷ demonstrated the reexpression of SHH and reduction of CDX2 levels after *H. pylori* eradication. Sox2, a gastric transcription factor, is expressed in the human stomach, and its expression gradually decreases as IM progresses.¹⁵ That is, Sox2 expression decreases and Cdx expression increases gradually during the progression of IM. Therefore, these markers might help identify patients for whom *H. pylori* eradication is advantageous. In this study, we evaluated the clinicopathological factors and gastric and intestinal markers according to the development of metachronous gastric neoplasms after ER, clarified the role of *H. pylori* eradication in the development of metachronous lesions, and investigated the patient subgroup for whom *H. pylori* eradication was beneficial, especially after ER.

Materials and Methods

1. Patients

Between June 2006 and June 2012, gastric neoplasms were diagnosed in 3,882 patients and treated with ER. Among the patients, we identified those who were infected with *H. pylori* and received *H. pylori* eradication treatment; that is, all patients included in this study received *H. pylori* eradication treat-

ment. After the *H. pylori* eradication treatment, 34 patients with metachronous gastric neoplasms after ER for gastric neoplasms (metachronous group) and 102 age- and sex-matched patients (nonmetachronous) who underwent ER for gastric neoplasms and who did not develop metachronous gastric neoplasms were enrolled on the basis of propensity score matching at a ratio of 1:3. The metachronous and nonmetachronous groups were matched according to age and sex by using propensity score matching. We defined metachronous lesions as those that developed at another site (not a previous ER site) at 6 months or later after the previous ER. The period between the development of metachronous gastric neoplasms and *H. pylori* eradication was at least 6 months, to accurately evaluate the effect of *H. pylori* eradication on the incidence of metachronous lesions after ER.⁴

In addition, there was a median interval of 3 months (range, 0~32 months) between ER and *H. pylori* eradication. To determine the *H. pylori* infection status, rapid urease and urea breath tests and histological assessment were done. Patients who had a positive result for at least one of these tests were defined as the group with a positive *H. pylori* infection. In contrast, those who showed negative results for all three examinations were defined as the group with negative *H. pylori* infection. The first eradication regimen was conventional triple therapy consisting of standard doses of a proton pump inhibitor (PPI) twice daily, 1,000 mg amoxicillin twice daily, and 500 mg clarithromycin twice daily for 7 to 14 days. In addition, in cases of eradication failure with the initial conventional triple therapy, quadruple therapy including two standard doses of a PPI, 500 mg metronidazole thrice daily, 120 mg bismuth four times daily, and 500 mg tetracycline four times daily for 7 to 14 days was used. The patients were evaluated in terms of their clinicopathological characteristics, such as age, sex, tumor location, gross appearance of the tumor, tumor histological type, tumor size, tumor invasion depth, tumor multiplicity, the presence of ulcers, background mucosa, and *H. pylori* eradication failure rate. Atrophy and IM were evaluated endoscopically at the time of ER. In particular, atrophic gastric mucosa was categorized by using the Kimura classification.^{16,17} The severity of atrophy was categorized as open or closed, and that of IM was classified as none, mild, moderate, or severe. Mild grade was characterized as metaplastic mucosa with fine or granular plaques. Moderate grade was defined as coarse plaques or patches. Severe grade was characterized as mucosa with coarser and larger plaques or patches.¹⁷ *H. pylori* infection was evaluated via histopathological examinations of the resected

specimen, the urea breath test, or the rapid urease test. The clinicopathological characteristics were analyzed retrospectively and compared between the metachronous and nonmetachronous groups. The median period between the initial resection and the subsequent identification of metachronous neoplasm was 30.1 months (range, 9.5~74.1 months), and that between the initial resection and the determination of no neoplasm (nonmetachronous group) was 49.1 months (range, 16.7~92.7 months). We excluded metachronous recurrence occurring within 6 months because the lesions detected during this time were most likely lesions that were missed before and that should be categorized as synchronous cancers or neoplasms.

The Institutional Review Board of Severance and Gangnam Severance Hospital approved this study (3-2014-0117).

2. Immunohistochemistry

Immunohistochemical staining was performed for SHH, CDX1, CDX2, and SOX2. Twenty patients were selected for immunohistochemistry on the basis of the development of metachronous neoplasms before and after *H. pylori* eradication, with 10 specimens obtained from each group both before and after *H. pylori* eradication. Immunohistochemical staining was performed after *H. pylori* eradication at the time of the subsequent identification of metachronous neoplasm (metachronous group, median 30.1 months) and the determination of no neoplasm (nonmetachronous group, median 49.1 months). Biopsy specimens were obtained from the antrum and corpus for evaluating the background mucosa by using immunohistochemical staining. We used paraffin-embedded tissue specimens and rabbit polyclonal anti-SHH (Santa Cruz Biotechnology, Santa Cruz, CA, USA), goat polyclonal anti-SOX2 (Santa Cruz Biotechnology), mouse monoclonal anti-CDX1 (Santa Cruz Biotechnology), and rabbit polyclonal anti-CDX2 (Santa Cruz Biotechnology) antibodies. The expression levels were determined by scoring both the positivity rate and intensity of SHH, CDX1, CDX2, and SOX2 staining. The positive expression rate was determined by counting the number of immunopositive cells in three areas randomly chosen in the tissue for a total of 300 cells and scored (0=0%, 1=1%~25%, 2=26%~35%, 3=36%~45%, 4=46%~65%, 5=66%~100%). The staining intensity was scored as follows: 0, no detectable cytoplasmic staining; 1, mild cytoplasmic staining; 2, moderate cytoplasmic staining; and 3, severe cytoplasmic staining. The two scores were then summed to obtain a final score between 0 and 8.

3. Statistical analysis

The chi-square test and Fisher exact test were used to compare the clinicopathological factors between the groups according to the development of metachronous gastric neoplasms. The t-test was used for noncategorical variables in the intergroup comparisons of clinicopathological characteristics. The Wilcoxon signed-rank test and Mann-Whitney U-test were used for evaluating immunohistochemical expression levels. The meta-

Table 1. Comparisons of the clinicopathologic findings between the metachronous and control groups

Variable	Metachronous group (n=34)	Control group (n=102)	P-value
Age (yr)	62.1±7.0	63.1±7.5	0.495
Sex (male)	26 (76.5)	78 (76.5)	1.000
Tumor size (mm)	11.0±9.3	11.7±7.5	0.659
Multiplicity	4 (11.8)	13 (12.7)	1.000
Location			0.351
Upper	3 (8.8)	7 (6.9)	
Middle	13 (38.2)	27 (26.5)	
Lower	18 (52.9)	68 (66.7)	
Macroscopic type			0.247
Elevated	21 (61.8)	76 (74.5)	
Flat	11 (32.4)	19 (18.6)	
Depressed	2 (5.9)	7 (6.9)	
Ulcer	18 (52.9)	52 (51.0)	1.000
Atrophy*			0.003
Open	23 (67.6)	36 (35.3)	
Close	8 (23.5)	39 (38.2)	
No	3 (8.8)	27 (26.5)	
Intestinal metaplasia*			0.001
No	1 (2.9)	20 (19.6)	
Mild	25 (73.5)	75 (73.5)	
Moderate	4 (11.8)	7 (6.9)	
Severe	4 (11.8)	0 (0.0)	
Histopathology			0.912
Low-grade dysplasia	10 (29.4)	29 (28.4)	
High-grade dysplasia	4 (11.8)	15 (14.7)	
Carcinoma	20 (58.8)	58 (56.9)	
<i>Helicobacter pylori</i> eradication			0.036
Success	26 (76.5)	93 (91.2)	
Failure	8 (23.5)	9 (8.8)	

Values are presented as mean±standard deviation or number (%). *Background mucosal pathologies, such as atrophy and intestinal metaplasia, were evaluated endoscopically.

Table 2. Comparisons of the clinicopathologic findings between the metachronous and control groups in patients with dysplasia

Variable	Metachronous group (n=14)	Control group (n=44)	P-value
Age (yr)	63.6±7.0	62.4±7.3	0.579
Sex (male)	12 (85.7)	31 (70.5)	0.317
Tumor size (mm)	11.6±10.4	11.1±7.3	0.841
Multiplicity	2 (14.3)	5 (11.4)	1.000
Location			0.326
Upper	1 (7.1)	3 (6.8)	
Middle	6 (42.9)	10 (22.7)	
Lower	7 (50.0)	31 (70.5)	
Macroscopic type			0.030
Elevated	7 (50.0)	36 (81.8)	
Flat	6 (42.9)	5 (11.4)	
Depressed	1 (7.1)	3 (6.8)	
Ulcer	7 (50.0)	9 (20.5)	0.043
Atrophy*			0.047
Open	10 (71.4)	15 (34.1)	
Close	3 (21.4)	19 (43.2)	
No	1 (7.1)	10 (22.7)	
Intestinal metaplasia*			0.194
No	1 (7.1)	10 (22.7)	
Mild	11 (78.6)	30 (68.2)	
Moderate	1 (7.1)	4 (9.1)	
Severe	1 (7.1)	0 (0.0)	
<i>Helicobacter pylori</i> eradication			0.002
Success	10 (71.4)	44 (100.0)	
Failure	4 (28.6)	0 (0.0)	

Values are presented as mean±standard deviation or number (%). *Background mucosal pathologies, such as atrophy and intestinal metaplasia, were evaluated endoscopically.

chronous and nonmetachronous groups were matched according to age and sex by using propensity score matching at a ratio of 1:3. Statistical significance was set at $P < 0.05$. All statistical analyses were performed by using SPSS ver. 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

1. Clinicopathological characteristics of the metachronous and nonmetachronous groups

The clinicopathological features of 34 patients with metachronous gastric neoplasms and 102 nonmetachronous patients

Table 3. Comparisons of the clinicopathologic findings between the metachronous and control groups in patients with carcinoma

Variable	Metachronous group (n=20)	Control group (n=58)	P-value
Age (yr)	61.0±7.0	63.6±7.7	0.184
Sex (male)	14 (70.0)	47 (81.0)	0.351
Tumor size (mm)	11.9±10.3	12.1±7.7	0.954
Multiplicity	2 (10.0)	8 (13.8)	1.000
Location			0.767
Upper	2 (10.0)	4 (6.9)	
Middle	7 (35.0)	17 (29.3)	
Lower	11 (55.0)	37 (63.8)	
Macroscopic type			0.956
Elevated	14 (70.0)	40 (69.0)	
Flat	5 (25.0)	14 (24.1)	
Depressed	1 (5.0)	4 (6.9)	
Ulcer	11 (55.0)	43 (74.1)	0.159
Atrophy*			0.063
Open	13 (65.0)	21 (36.2)	
Close	5 (25.0)	20 (34.5)	
No	2 (10.0)	17 (29.3)	
Intestinal metaplasia*			0.003
No	0 (0.0)	10 (17.2)	
Mild	14 (70.0)	45 (77.6)	
Moderate	3 (15.0)	3 (5.2)	
Severe	3 (15.0)	0 (0.0)	
<i>Helicobacter pylori</i> eradication			0.730
Success	16 (80.0)	49 (84.5)	
Failure	4 (20.0)	9 (15.5)	

Values are presented as mean±standard deviation or number (%). *Background mucosal pathologies, such as atrophy and intestinal metaplasia, were evaluated endoscopically.

are shown in Table 1. No differences were found in tumor size, multiplicity, location, macroscopic type, ulcer, and histology between the metachronous and nonmetachronous groups. Open-type atrophy ($P=0.003$), moderate and severe IM ($P=0.001$), and failure of *H. pylori* eradication ($P=0.036$) occurred more frequently in the metachronous group (Table 1).

2. Demographic and clinicopathological characteristics of the metachronous and nonmetachronous groups according to histopathology

Of the 136 patients who underwent ER for gastric neoplasms, 58 (42.6%) underwent ER for dysplasia and 78 (57.4%)

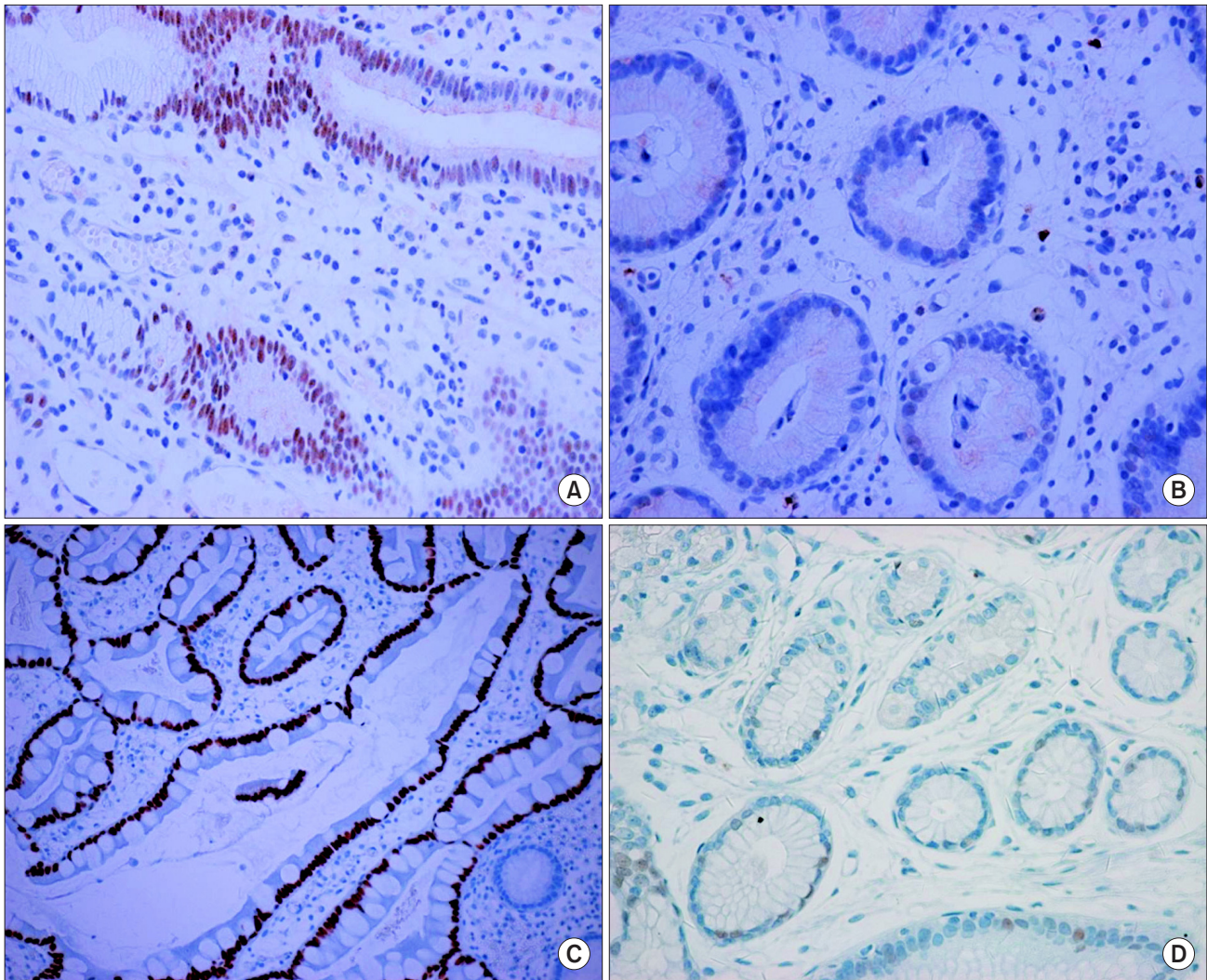


Fig. 1. Protein expression of SOX2, sonic hedgehog (SHH), CDX2, and CDX1 in the background gastric mucosa ($\times 400$). (A, B) Immunohistochemical staining for SOX2. Nuclear SOX2 expression increases after *Helicobacter pylori* eradication in the nonmetachronous group (A) in which metachronous gastric neoplasms do not arise (B). (C, D) Immunohistochemical staining for CDX2. The nuclear expression of CDX2 decreases after *H. pylori* eradication in the nonmetachronous group (D) in which metachronous gastric neoplasms do not arise (C). (E, F) Immunohistochemical staining for SHH. Cytoplasmic SHH expression increases after *H. pylori* eradication in the nonmetachronous group (E) in which metachronous gastric neoplasms do not arise (F). (G, H) Immunohistochemical staining for CDX1. The nuclear expression of CDX1 is not significantly different after *H. pylori* eradication between the nonmetachronous and metachronous groups. (G) Positive CDX1 expression; (H) negative CDX1 expression.

underwent ER for carcinoma. In patients with dysplasia, endoscopically flat-type lesions ($P=0.030$), ulcers ($P=0.043$), and open-type atrophy ($P=0.047$) were found predominantly in the metachronous group. The failure rate of *H. pylori* eradication was significantly higher in the metachronous group than in the nonmetachronous group ($P=0.002$) (Table 2). In patients with carcinoma, moderate-to-severe IM was observed more frequently in the metachronous group ($P=0.003$). The failure rate of *H. pylori* eradication was not different between the metachronous and nonmetachronous groups ($P=0.730$) (Table 3).

3. Immunohistochemistry

To investigate gastric and intestinal markers according to the development of metachronous gastric neoplasms after ER, immunohistochemical staining was performed for 10 metachronous and 10 nonmetachronous patients before and after *H. pylori* eradication (Fig. 1). The intensities of SOX2 staining in the antrum and SHH staining in the antrum and corpus were increased in the nonmetachronous group after *H. pylori* eradication ($P < 0.05$). In addition, the intensity of CDX2 staining in the antrum and corpus was decreased in the nonmetachronous group after *H. pylori* eradication ($P < 0.05$). However, such changes in the in-

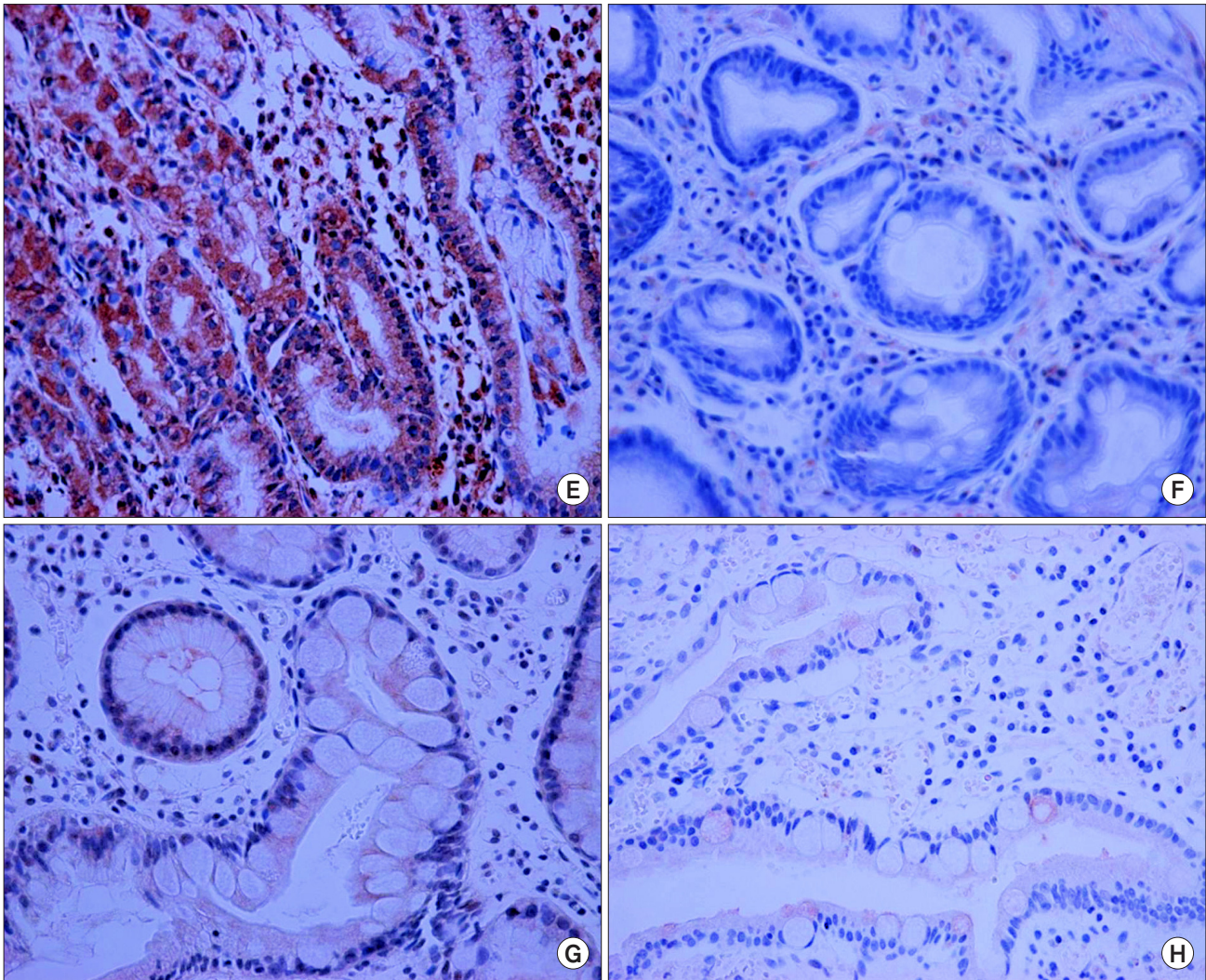


Fig. 1. Continued.

tensity of SOX2, SHH, and CDX2 staining were not observed in the metachronous group. The intensity of SOX2 and SHH staining in the antrum after *H. pylori* eradication was significantly lower in the metachronous group than in the nonmetachronous group ($P < 0.05$). Moreover, the intensity of CDX2 staining in the antrum before *H. pylori* eradication was significantly higher in the metachronous group than in the nonmetachronous group ($P < 0.05$) (Table 4).

Discussion

The 5-year survival rate of EGC was favorable ($>95\%$).¹⁸ ER is currently accepted as a local treatment for EGC, and the long-term outcome of ER for EGC in Korea is favorable.^{19,20} ER has the advantages of preserving the entire stomach and increasing

the postoperative quality of life of patients; however, the risk of synchronous or metachronous gastric cancer may be higher in the remnant stomach compared with that after gastrectomy. Metachronous gastric cancer developed in 2.7% to 14.0% of patients after ER within 3 to 5 years.^{21,22} Multiple gastric lesions are more frequent in EGC than in advanced gastric cancer. Therefore, the detection of metachronous lesions after ER is important during follow-up after ER. In addition, identifying patients at a high risk of developing metachronous lesions and investigating the clinicopathological factors associated with metachronous lesions are clinically important.

Chronic *H. pylori* infection is associated with gastric cancer; however, the effect of *H. pylori* eradication on the prevention of metachronous gastric cancers remains controversial. Choi et al.⁵ reported that *H. pylori* eradication after ER of gastric tumor did

Table 4. Expression of SOX2, SHH, CDX2, and CDX1 proteins in background gastric mucosa

Variable	Control group (n=10)		Metachronous group (n=10)	
	Antrum	Corpus	Antrum	Corpus
SOX2 [†]				
Before eradication	3.0 (2.0~3.0) [†]	3.0 (2.0~4.5)	3.5 (2.0~4.3)	3.0 (2.0~3.3)
After eradication	3.0 (2.0~5.3) ^{*†}	2.5 (2.0~5.3)	2.0 (2.0~2.0) [*]	2.0 (2.0~2.3)
SHH [‡]				
Before eradication	3.0 (2.8~5.3) [‡]	5.0 (3.8~5.5) [‡]	5.0 (4.0~5.3)	4.5 (3.0~6.0)
After eradication	4.5 (3.8~6.0) ^{*‡}	7.0 (4.0~7.0) ^{*‡}	3.0 (2.8~4.0) [*]	4.0 (2.0~6.0) [*]
CDX2 [§]				
Before eradication	4.0 (1.5~7.0) ^{*§}	6.0 (3.8~8.0) [§]	7.5 (4.8~8.0) [*]	5.0 (2.0~8.0)
After eradication	3.5 (0.0~6.3) [§]	3.0 (1.5~7.3) [§]	7.5 (4.5~8.0)	6.5 (5.8~8.0)
CDX1				
Before eradication	2.0 (2.0~4.5)	2.5 (2.0~5.3)	3.0 (2.0~3.3)	3.0 (2.0~5.3)
After eradication	2.5 (2.0~3.5)	2.0 (2.0~2.3)	2.0 (2.0~2.8)	2.0 (2.0~3.0)

Values are presented as median (interquartile range). The Wilcoxon signed-rank test and Mann-Whitney U-test were used for evaluating the immunohistochemical expression levels. *P<0.05, compared between the control and metachronous groups. The expression levels were determined by scoring both the positive rate and the intensity of Sonic hedgehog (SHH), CDX1, CDX2, and SOX2 staining. The positive expression rate was determined by counting the number of immunopositive cells in three areas randomly chosen in the tissue in a total of 300 cells and given scores (0=0%, 1=1%~25%, 2=26%~35%, 3=36%~45%, 4=46%~65%, 5=66%~100%). The staining intensity was scored as follows: 0, no detectable cytoplasmic staining; 1, mild cytoplasmic staining; 2, moderate cytoplasmic staining; and 3, severe cytoplasmic staining. Two scores were then summed to obtain a final score of 0 and 8. [†]The expression of SOX2 staining in the antrum increases in the control group after *Helicobacter pylori* eradication. [‡]The expression of SHH staining in the antrum and corpus increases in the control group after *H. pylori* eradication. [§]The expression of CDX2 staining in the antrum and corpus decreases in the control group after *H. pylori* eradication.

not reduce the incidence of metachronous gastric carcinoma. In this study, persistent *H. pylori* infection was found in 80 of 439 patients who underwent *H. pylori* eradication therapy. Moreover, metachronous gastric cancer developed in 10 patients in whom *H. pylori* had been eradicated (10 of 439, 2.3%). According to histology, metachronous gastric cancer developed in three patients with dysplasia (3 of 226, 1.3%) and seven patients with carcinoma (7 of 213, 3.3%) after *H. pylori* eradication. Kwon et al. reported that persistent *H. pylori* infection was significantly associated with metachronous gastric cancer. The incidence of metachronous gastric lesions was significantly higher in patients with persistent *H. pylori* infection than in those in whom the bacterium was eradicated (18.8% vs. 8.4%, P=0.016).²³

In the present study, all patients were infected with *H. pylori* and received *H. pylori* eradication treatment. We selected such a subgroup because we wanted to identify the patients for whom *H. pylori* eradication treatment would be helpful. Previous studies^{3,5} reported controversial results about the effects of *H. pylori* eradication treatment on metachronous gastric neoplasms. Therefore, we selected patients who received *H. pylori* eradica-

tion treatment, and then investigated the clinicopathological factors that affected the development of metachronous neoplasms. In this study, the number of patients with metachronous gastric neoplasms after ER was 34 (14 with dysplasia, 20 with carcinoma). However, the number of patients with metachronous gastric neoplasms was not small. Similar to previous studies, metachronous recurrence was observed in 33 patients during 3 years of follow-up³ and in 27 patients during 3 years of follow-up.⁵ Although the 3,882 patients were found to have gastric neoplasms and were scheduled for ER, the final group for the study consisted of 136 patients because we focused on those patients who received *H. pylori* eradication treatment. In addition, we investigated the role of *H. pylori* infection on the development of metachronous lesions by comparing the failure rate of *H. pylori* eradication; that is, we assessed the effect of persistent *H. pylori* infection on metachronous lesions after ER. Open-type atrophy, severe IM, and failure of *H. pylori* eradication were significantly associated with metachronous gastric neoplasms. Notably, failure of *H. pylori* eradication was significantly more frequent in metachronous patients with gastric dysplasia but not

those with carcinoma.

McDonald et al. reported that genetic/epigenetic changes in IM would lead to dysplasia by identifying identical genetic alterations in dysplastic lesions and the surrounding IM; indeed, gastric cancer can arise from dysplastic lesions through further genetic alterations, referred to as ‘cancer in adenoma’.^{24,25} The causes of genetic and epigenetic changes in IM or dysplasia are diverse, and several genetic and epigenetic changes were reversed by *H. pylori* eradication. In this study, persistent *H. pylori* infection was significantly more frequent in patients with gastric dysplasia but not in those with carcinoma, in the metachronous group than in the nonmetachronous group. Therefore, *H. pylori* infection may affect the development of metachronous gastric neoplasms in patients with dysplasia or precancerous lesions before carcinomatous transformation. Only moderate-to-severe IM was significantly associated with carcinoma in the metachronous group. The reason might be the ‘point of no return’ the point at which *H. pylori* eradication could no longer prevent metachronous lesions. Accurately defining the point of no return is difficult because the molecular process cannot be measured precisely. However, our data suggest that *H. pylori* eradication may be more effective before the status of the background mucosa progresses to severe IM.

Currently, *H. pylori* eradication for preventing metachronous gastric carcinoma after ER is permitted but is not covered by the Korean National Health Insurance. Meanwhile, *H. pylori* eradication is not covered for precancerous lesions such as atrophy, IM, or dysplasia because of the lack of sufficient data. However, in this study, the role of *H. pylori* eradication in the prevention of metachronous lesions was more important in dysplasia than in carcinoma. Therefore, *H. pylori* eradication would be more helpful in patients with dysplasia than in those with carcinoma after ER. Previous reports about the association between *H. pylori* eradication and metachronous lesions have focused on EGC. However, on the basis of our results, *H. pylori* eradication after ER should be performed in patients with dysplasia for the prevention of metachronous lesions.

Our study has some limitations. This study was retrospective, and *H. pylori* eradication treatment could not be randomized. In addition, the period between ER and *H. pylori* eradication varied. The background mucosa such as atrophy and IM were evaluated endoscopically, not histologically.

Bornschein et al.²⁶ reported that the expression levels of CDX1, CDX2, and SOX2 were similar in gastric cancer and in

the surrounding nontumorous mucosa. They compared the gene expression between the tumor and the tumor-distant mucosa. In addition, upregulation of CDX1 and CDX2 and downregulation of SOX2 in the nonneoplastic gastric mucosa of patients with gastric cancer were confirmed. Moreover, *H. pylori* infection had no significant effect on the regulation of CDX1, CDX2, and SOX2 at the gastric cancer stage. In this study, the expression of CDX2 decreased and that of SOX2 increased after *H. pylori* eradication in the nonmetachronous group in which metachronous gastric neoplasm did not arise. The transgenic expression of *cdx2* in the murine stomach can induce IM, which progresses to invasive gastric cancer.²⁷ Furthermore, SOX2 is downregulated in gastric carcinoma.^{15,28} Therefore, the negative correlation of CDX2 and inverse relation with SOX2 expression in this study might be associated with the effects of *H. pylori* eradication on premalignant lesions before carcinoma. SHH expression also increased after *H. pylori* eradication in the nonmetachronous group. Shiotani et al.⁷ reported that *H. pylori* eradication was associated with an increase in SHH expression in the corpus, especially in the absence of incomplete IM. Incomplete IM at the corpus is more common in patients with gastric cancer,¹³ which is in agreement with our finding of a positive correlation with SHH expression in the nonmetachronous group after *H. pylori* eradication. In summary, the expression of SHH and SOX2 increased and that of CDX2 decreased after *H. pylori* eradication in the nonmetachronous group in this study. Although the results were derived from a small sample size, these markers could be used to define the subgroup in which *H. pylori* eradication would be beneficial after ER.

In conclusion, *H. pylori* eradication can play an important role in the prevention of metachronous lesions after ER in patients with precancerous lesions before carcinomatous transformation. In addition, the changes in SHH, SOX2, and CDX2 expression after *H. pylori* eradication may be predictive markers for metachronous lesions after ER.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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