Eradication of *Helicobacter pylori* Induces Immediate Regressive Changes in Early Gastric Adenocarcinomas

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Keywords

Helicobacter pylori · Eradication · Regression · Proliferative zone

Abstract

Objective: *Helicobacter pylori* eradication is expected to prevent gastric cancer. However, morphological alterations after eradication often hinder accurate diagnosis. Therefore, we evaluated endoscopic and histological changes in gastric tumors after eradication of *H. pylori* in a time-dependent manner. **Methods:** We classified 144 cases of endoscopic submucosal dissection (ESD) of early gastric cancer into the following categories: (i) patients positive for *H. pylori* with no eradication history, (ii) patients positive for *H. pylori* who underwent ESD 2 months after eradication, (iii) patients negative for *H. pylori* with an eradication history of at least 6 months before ESD, and (iv) patients negative for *H. pylori* with an unknown history. We compared endoscopic and histological factors between the groups. **Results:** The characteristics of cancers positive for *H. pylori* were exploding shape, superficial high-grade atypical epithelium, and a surface proliferating zone. *H. pylori* eradication induced a series of endoscopic and histological changes, including shape

depression, appearance of surface regenerative and lower-grade atypical epithelium, and a downward shift of the proliferative zone within a period as short as 2 months. **Conclusion:** *H. pylori* eradication rapidly causes cancer regression and leads to tumor shrinkage, diminished atypism, and shortened proliferative zone, resulting in drastic morphological changes.

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Introduction

Gastric cancer remains a prevalent cause of disease-related deaths worldwide [1]. *Helicobacter pylori* has been identified as a causative factor for gastric disorders [2], and its infection has been widely accepted as a carcinogenic factor [3]. Epidemiological and experimental studies have strongly indicated that *H. pylori* eradication markedly reduces the risk of gastric disorders [4], and health insurance programs have confirmed the efficacy of this endeavor in preventing metachronous primary gastric cancers [5]. Although there have been negative reports, especially for patients with severe atrophic and metaplastic gastritis [6–8], a recent meta-analysis has

confirmed the effect of eradication [9]. Thus, it is important to ascertain the morphological changes in gastric cancers detected after successful eradication of *H. pylori* compared to those arising from an underlying active infection.

Several clinical and histopathological studies have indicated that the gastric cancers detected after eradication of *H. pylori* not only display poor demarcation and lack typical endoscopic cancerous characteristics [10–14], but they also show a pathologically lower-grade atypia of the neoplastic epithelium [12, 14, 15], making a precise diagnosis difficult. In the present study, we characterized the changes that occur in gastric cancers immediately and up to several years after *H. pylori* eradication, in order to understand the changes in tumor characteristics over time.

Materials and Methods

Patients

We included 144 patients who underwent endoscopic submucosal dissection (ESD) owing to early gastric cancer (pT1a and pT1b) at the Endoscopy Center of Fujita Health University Hospital between 2008 and 2015.

Eradication of *H. pylori*

H. pylori infection was detected through immunohistochemical analysis using anti-*H. pylori* polyclonal antibody (Dako, Glostrup, Denmark) from biopsy specimens, ¹³C-urea breath test with UBIT tablets (Otsuka Pharmaceutical, Tokyo, Japan), and/or serum IgG titer against *H. pylori*. When at least one of the tests was positive, we administered standardized eradication therapy (30 mg of lansoprazole, 200 mg of clarithromycin, and 750 mg of amoxicillin, twice a day for 1 week) to the patient [10]. Subsequently, the infection status was reevaluated, and the eradication was considered successful when all the tests yielded negative results.

Immunohistochemical Analysis

We performed immunohistochemistry for the following: MUC5AC (CLH2, Novocastra, Newcastle, UK), MUC6 (CLH5, Novocastra), and CDX2 (CDX2-88, BioGenex, San Ramon, CA, USA) to perform gastric and/or intestinal phenotyping; Ki-67 (MIB-1, Dako) to reveal proliferative activity; p53 (DO-7, Novocastra) to detect abnormal expression due to mutation; and *H. pylori* (anti-*H. pylori* polyclonal antibody, Dako). *HER2* gene amplification was evaluated using immunohistochemistry using 4B5 (Roche Diagnostics, Basel, Switzerland) or with fluorescent in situ hybridization employing the PathVysion® *HER2* DNA Probe Kit (Abbott Laboratories, Abbott Park, IL, USA). All immunohistochemical analyses were performed using the iView DAB Universal Kits run on Ventana Benchmark Ultra (Roche).

Patient Grouping Based on *H. pylori* Infection Status

Patients were classified based on their infection status and the times of *H. pylori* eradication (Table 1):

(i) Non-eradicated (non-E) group ($n = 76$): patients who did not undergo eradication therapy previously and were simultaneously diagnosed with early gastric cancer and *H. pylori* infection; these patients were treated with ESD without eradication.

(ii) Non-eradicated at diagnosis but eradicated before ESD (preoperatively eradicated, POE) group ($n = 10$): these patients shared a background similar to that of the non-E patients, but *H. pylori* was eradicated up to 2 months before ESD (Table 2).

(iii) Already eradicated at diagnosis (prediagnostically eradicated, PDE) group ($n = 43$): for these patients, the infection was eradicated when the cancer was diagnosed endoscopically. This group was further divided into two subgroups based on the timing of cancer detection: between 6 and 36 months after eradication ($n = 11$, PDE-early) and beyond 36 months ($n = 25$, PDE-late) (Table 2). Seven cases, for which the eradication treatment date was not precisely recorded, were excluded from the trend analysis.

(iv) Spontaneously eradicated (SE) group ($n = 15$): although *H. pylori* tests were negative for these patients with no history of *H. pylori* eradication, endoscopic findings of infection such as corpus atrophic or metaplastic gastritis, and gastric cancer, were identified. Spontaneous eradication due to past antibiotic therapy may have been the underlying cause.

Endoscopic and histological findings in the non-E and PDE groups were reflected by *H. pylori*-positive and -negative gastric backgrounds, respectively. In the POE group, these findings were compatible with ongoing histopathological changes following *H. pylori* eradication, because the cancer was dissected within 2 months after eradication.

Endoscopic and Histopathological Evaluation of Gastric Tumors

The location of the tumor in the stomach was anatomically divided into 3 sections: upper, middle, and lower.

According to the Paris endoscopic classification of superficial neoplastic lesions and the Japanese classification of gastric cancers, the morphology of the tumor was classified as “protruded/elevated” or “flat/depressed.” The former consisted of 0-I (protruded type), 0-IIa (superficially elevated type), and 0-IIa + IIc (superficially elevated, but partially depressed type). The latter was compatible with 0-IIb (flat type), 0-IIc (superficially depressed type), and 0-III (excavated type) (Table 1) [16, 17].

The microscopic shape of the tumor was sorted into 4 types: “polypoid,” “superiorly exploding,” “intermediate,” and “laterally infiltrating” (Fig. 1). Superiorly exploding tumor is a bowl-shaped tumor that expands more proliferously in the upper layer than in the lower layer. Conversely, laterally infiltrating tumor is a rounded tumor that spreads laterally in the middle layer. The intermediate type has an appearance between that of the superiorly exploding and laterally infiltrating types.

Histopathological grading of the tumor was performed based on the World Health Organization’s criteria for the classification of digestive system tumors [18].

Based on the microscopic findings, the surface epithelium over the tumor was categorized as follows: (i) regenerative columnar epithelium (RCE), (ii) epithelium with low-grade atypia (ELA), (iii) a combination of RCE and ELA, and (iv) epithelium with high-grade atypia (EHA) [12].

Table 1. The clinical and endoscopic characteristics of the patients

	Total	Non-E (non-eradicated)	POE (preoperatively eradicated)	PDE (prediagnostically eradicated)	SE (spontaneously eradicated)	<i>p</i> values ^a
Total, <i>n</i>	144	76	10	43	15	
Sex						0.293
Male	111	63	8	30	10	
Female	33	13	2		5	
Age (range, median), years	53–92 (72.0)	53–89 (70.5)	61–87 (72.5)	53–87 (74.0)	68–92 (75.0)	0.219
Location						0.691
Upper (fundus)	17	7	2	6	2	
Middle (corpus)	55	32	4	16	3	
Lower (antrum/pylorus)	72	37	4	21	10	
Endoscopic morphology						0.016
Protruded/elevated type	68	43*	4	12**	9	
Flat/depressed type	76	33*	6	31**	6	
Endoscopic maximum diameter (mean ± SD), mm	12.47±6.48	12.79±5.56	13.50±4.20	11.21±7.82 ^b	14.00±7.57	0.029

SD, standard deviation. * $p < 0.05$, ** $p < 0.01$ (Harberman's residual analysis). ^a χ^2 test (sex, location, and endoscopic morphology) and Kruskal-Wallis test (age and endoscopic maximum diameter). ^b Significantly smaller than the non-E group ($p < 0.05$, Dunn's multiple comparison test).

Table 2. The interval between endoscopic submucosal dissection and *Helicobacter pylori* eradication

	Non-E (<i>n</i> = 76)	POE (<i>n</i> = 10)	PDE-early (<i>n</i> = 11)	PDE-late (<i>n</i> = 25)
Interval between HP eradication and ESD	0	0–2 months	6–36 months	36–180 months
Mean ± SD	NA	33.6±12.3 days	18.4±8.7 months	90.6±48.1 months
Range	NA	19–56 days	6–34 months	36–180 months

HP, *Helicobacter pylori*; ESD, endoscopic submucosal dissection; non-E, non-eradication; POE, preoperatively eradicated; PDE, prediagnostically eradicated; SD, standard deviation; NA, not applicable.

The phenotype was identified based on the expression of MUC5AC, MUC6, and CDX2. MUC5AC- or MUC6-positive tumors were classified as “gastric,” while CDX2-positive tumors were classified as “intestinal.” Tumors with both markers were categorized as “gastrointestinal,” while those harboring neither of the markers were placed under the “null” phenotype [19]. Distinct staining of >5% of the tumor cells was considered positive.

The proliferative zone of the tumor was alternatively evaluated according to the Ki-67-positive tumor cell distribution and categorized into the “upper layer” when the positive cells were distributed in and beneath the surface epithelium, or into the “middle layer” when the positive cells were located in the middle of the neoplastic mucosa.

p53 mutation was speculated in the event of a positive cell ratio for its protein, determined using immunohistochemistry. We defined the “wild type pattern” as 1–33% positivity and the “aberrant pattern” as <1% or >33% positivity.

HER2 scoring was performed based on guidance provided in a previous report [20].

Statistical Analysis

Differences among the groups were analyzed using the Kruskal-Wallis and Dunn's multiple comparison tests. To discern the association between the categorical variables in the contingency tables, Pearson's χ^2 test and Harberman's residual analysis were applied when all expected values were >1.0 and at least 20% of the

Fig. 1. The 4 microscopic shapes of gastric adenocarcinoma. **a** Polypoid type is a tumor that forms a polypoid shape. **b** Superiorly exploding type is a bowl-shaped tumor that expands more proliferously in the upper layer than in the lower layer of the tumor. **c** Intermediate type is a tumor with an uneven border between the

tumor and background mucosa, showing an intermediate position between superior exploding and laterally infiltrating types. **d** Laterally infiltrating type is a round-edged tumor that laterally spreads through the middle layer of the tumor. Hematoxylin and eosin staining. Bar, 600 μ m for left panels and 200 μ m for right panels.

Fig. 2. Trend analyses for endoscopic morphology (a), microscopic shape (b), surface epithelium over the tumor (c), and proliferating zone (d) over time after eradication for the non-eradicated (non-E) group, the preoperatively eradicated (POE) group, and the prediagnostically eradicated (PDE) group. The protruded/elevated endoscopic morphology, superiorly exploding microscopic shape, epithelium with high-grade atypia (EHA) in the surface above the tumor, and a proliferating zone located in the

upper layer were all observed more frequently in non-E group cancer. Serial endoscopic and histopathological changes to the flat/depressed and laterally infiltrating type, regenerative columnar epithelium (RCE) and epithelium with low-grade atypia (ELA) in the surface, and a downward-proliferating zone were noted gradually over time after eradication with statistical significance. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ for the Cochran-Armitage trend test.

expected values were >5 . Otherwise, Yates' correction for continuity was adjusted to Pearson's χ^2 test (Yates' χ^2 test). Trend analysis was performed using the Cochran-Armitage test. Kruskal-Wallis and Dunn's multiple comparison tests and the Cochran-Armitage tests were evaluated using GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA). $p < 0.05$ was considered statistically significant.

Results

Differences in Endoscopic and Histopathological Appearances Based on the H. pylori Infection Status

The clinical and endoscopic characteristics of gastric cancers for the 4 patient groups are summarized in Table 1. No statistically significant differences were observed in sex, age, and location of the tumor. However, Harberman's residual analysis following Pearson's χ^2 test revealed that the protruded/elevated and flat/depressed

types were more common in the non-E (adjusted standardized residual, ASR = 2.338, $p = 0.018$) and PDE groups (ASR = 3.029, $p = 0.003$), respectively. The Cochran-Armitage trend test indicated that the endoscopic morphology shifted from the protruded/elevated type to the flat/depressed type over time after eradication ($p = 0.009$) (Fig. 2a). Dunn's multiple comparison test following the Kruskal-Wallis test revealed that the maximum diameter of the tumor was significantly smaller in the PDE group than in the non-E group ($p = 0.048$). These findings suggest that posteradication gastric cancer becomes smaller, flatter, and more depressed than *H. pylori*-associated cancer.

The histopathological findings are summarized in Table 3. Pearson's and Yates' χ^2 test ascertained that significant differences in the microscopic shape, surface epithelium over the tumor, and proliferating zone existed among the groups. Nonetheless, no significant differenc-

Fig. 3. Representative cases of cancer in the non-eradicated (non-E) and prediagnostically eradicated (PDE) groups. The tumor grows toward the superficial lumen in the non-E group, whereas the tumor spreads laterally in the middle layer in the PDE group, and the surface is covered with regenerative normal surface foveolar epithelium and pyloric glands. The Ki-67-labeled proliferative zone is located in the upper and middle layers in non-E and PDE group cancers, respectively. We evaluated one case each of non-E and PDE group cancers as the p53 aberrant pattern and the wild-type pattern, respectively. Both tumors show CDX2+, MUC5AC-, and MUC6-intestinal phenotypes. HE, hematoxylin and eosin staining. Bar, 200 μ m.

Table 3. Histological findings of gastric cancers classified based on the *Helicobacter pylori* infection status

At clinical diagnosis:	HP+		HP–		Total	<i>p</i> value ^a
At pathological sampling:	HP+	HP–				
	non-E (<i>n</i> = 76)	POE (<i>n</i> = 10)	PDE (<i>n</i> = 43)	SE (<i>n</i> = 15)	(<i>n</i> = 144)	
Microscopic shape						
Polypoid	6	0	0	1	7	<0.001
Superiorly exploding	50***	2	8***	9	69	
Intermediate	10	4*	6	3	23	
Laterally infiltrating	10***	4	29***	2	45	
Histological type						0.200
Tubular	76	10	42	13	141	
Papillary	0	0	0	2	2	
Poorly cohesive	0	0	1	0	1	
Depth of invasion						0.999
pT1a (mucosa)	69	9	38	14	130	
pT1b (submucosa)	7	1	5	1	14	
Surface epithelium over the tumor						<0.001
RCE	3**	0	10**	3	16	
ELA	11	2	14**	1	28	
RCE + ELA	8	6***	8	0	22	
EHA	54***	2*	11***	11	78	
Phenotype						0.546
Gastric	15	2	9	8	34	
Gastrointestinal	35	5	22	2	64	
Intestinal	23	3	11	5	42	
Null	3	0	1	0	4	
Proliferating zone (Ki-67)						0.021
Upper layer	49**	5	15**	8	77	
Middle layer	27**	5	28**	7	67	
p53 immunoreactivity						0.746
Normal	46	5	22	8	81	
Aberrant	30	5	21	7	63	
HER2 gene amplification						0.770
Yes	6	1	3	3	13	
No	70	9	40	12	131	

^a Assessed by χ^2 test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Harberman's residual analysis). HP, *Helicobacter pylori*; non-E, non-eradicated; POE, preoperatively eradicated; PDE, prediagnostically eradicated; SE, spontaneously eradicated; RCE, regenerative columnar epithelium; ELA, epithelium with low-grade atypia; EHA, epithelium with high-grade atypia.

es were noted in the histopathological type, depth of invasion, phenotype, p53 immunoreactivity, and *HER2* gene amplification (Fig. 3).

Serial Changes in the Microscopic Shape from Superiorly Exploding to Laterally Infiltrating Type

With respect to the microscopic shape, 65.8% (50/76) of the non-E group cancers were of the superiorly exploding

type, whereas 67.4% (29/43) of the PDE group cancers belonged to the laterally infiltrating type (Table 3). Based on statistical analysis, the most common tumor types were the superiorly exploding type in the non-E group (ASR = 4.539, $p < 0.001$), the intermediate type in the POE group (ASR = 2.150, $p = 0.032$), and the laterally infiltrating type in the PDE group (ASR = 6.114, $p < 0.001$). Laterally infiltrating tumors in the non-E group (ASR = -4.952, $p <$

0.001) and superiorly exploding tumors in the PDE group (ASR = -4.594, $p < 0.001$) manifested themselves to a lesser extent. The tumors were broadly divided into two categories: polypoid/superiorly exploding and intermediate/laterally infiltrating. This classification revealed a trend, wherein the latter type was most frequently manifested in the PDE (particularly the PDE-early) group, followed in order by the POE and non-E groups, with statistical significance ($p < 0.001$, Fig. 2b). These findings suggest that the microscopic shape of gastric cancer may change from the superiorly exploding to laterally infiltrating type over time after eradication treatment.

Gradual Decrease of Atypia in the Surface Epithelium over the Tumor

EHA was observed in the surface over the tumor as well as in the cancer itself in 71.0% (54/76) of the non-E group, while the surface epithelium displayed milder atypia, such as RCE, ELA, or both, in 74.4% (32/43) of the PDE group (Table 3). With statistical significance, EHA (ASR = 4.299, $p < 0.001$), RCE + ELA (ASR = 4.075, $p < 0.001$), and either RCE (ASR = 3.026, $p = 0.002$) or ELA (ASR = 2.594, $p = 0.009$) were more frequently observed above the tumor in the non-E, POE, and PDE groups, respectively. Trend analysis revealed that the atypia gradually became milder, while the regenerative change became more prominent in the surface epithelium over time after eradication ($p < 0.001$, Fig. 2c). Thus, the regenerative and low-grade epithelium may become proliferously covered with the tumor after the eradication of *H. pylori* infection.

Downward Shifting of the Ki-67-Labeled Proliferating Zone

The Ki-67-labeled proliferative zone was frequently observed in the upper layer of the neoplastic mucosa in the non-E group (ASR = 2.798, $p = 0.005$), whereas it was often distributed in the middle layer in the PDE group (ASR = 2.918, $p = 0.004$), with statistical significance. Trend analysis comparing the patient groups revealed that the proliferative zone moved downward in the neoplastic mucosa as the time from eradication increased ($p = 0.021$, Fig. 2d).

Discussion

This study demonstrates the differences between *H. pylori*-eradicated and non-eradicated gastric cancers in not only the endoscopic morphology and size, but also in

the histopathological characteristics. We also show the gradual endoscopic and histopathological changes over time after *H. pylori* eradication treatment.

Endoscopic observations revealed that the maximum diameter in the *H. pylori*-negative (PDE) group was smaller than that in the positive (non-E) group. Moreover, the morphology of gastric cancer shifted gradually from protruded/elevated to flat/depressed upon *H. pylori* eradication. These results are compatible with those of previous studies that reported a decrease in the height of superficially elevated tumors and the fading of the demarcation line 1 month after successful eradication [15]. Furthermore, the tumor size was significantly smaller in patients who had received eradication therapy before cancer dissection [21–23].

We also found that the superiorly exploding type in the microscopic shape, EHA in the surface above the tumor, and the proliferating zone located in the upper layer were more common in *H. pylori*-positive gastric cancer, while sequential changes to the laterally infiltrating type, RCE and ELA in the surface, and a downward-proliferating zone were noted after eradication. Gastric cancer positive for *H. pylori* was of the superiorly exploding type because the proliferating zone was distributed in the upper layer of the neoplastic mucosa and the tumor was more proliferous in the upper layer facing the lumen than in the lower layer. However, *H. pylori*-negative gastric cancer manifests itself as the laterally infiltrating type because the proliferating zone is in the middle layer of the neoplastic mucosa, while the regenerative epithelium instead grows in the upper and lower layers. Regenerative change and atypism in the surface epithelium above the tumor may depend on proliferative activity in the upper layer of the tumor [12, 15]. Thus, the shifting of the proliferative zone from the upper to the middle part of the neoplastic mucosa as a result of *H. pylori* eradication may influence the endoscopic and histopathological characteristics [24].

Herein, the gastric tumors were histopathologically differentiated tubular adenocarcinomas, except for 2 cases of differentiated papillary carcinoma in the SE group and 1 case of poorly cohesive carcinoma in the PDE group. No significant differences were noted in the histopathological types of the eradicated and non-eradicated cancers, and this result was consistent with previous reports [11, 14, 22, 25, 26]. Additionally, independently of the eradication status, a gastrointestinal phenotype was observed more often than the gastric, intestinal, and null phenotypes in our analysis. However, Yamamoto et al. [26] reported that the gastric phenotype was more com-

mon than the intestinal phenotype, particularly after eradication. Further studies are needed to address this discrepancy.

Intriguingly, in this study, trend analysis revealed that shrinking, flattening, and depression of the gastric cancer began immediately (within 2 months) after *H. pylori* eradication and reached a plateau after 36 months. The drastic endoscopic and histopathological changes observed after eradication are associated with an endoscopically indistinct alteration called “gastritis-like appearance” [14]. According to the present study, the gastritis-like appearance is witnessed even immediately after eradication. We therefore call the attention of endoscopists to the screening of *H. pylori*-eradicated gastric cancer because the early discovery of gastric cancer after eradication may contribute to a better prognosis for the patients [27].

Meanwhile, gastric cancers over 36 months after eradication tended to be slightly reversed in terms of their morphology and proliferative activity. Recent studies have revealed that DNA methylation was decreased in certain genes such as *LOX*, whereas it was unchanged in *APC*. Methylation status in the *MOS* gene could be associated with continuous atrophy and intestinal metaplasia after eradication [28, 29]. It has also been reported that DNA methylation and microsatellite abnormalities are regressed to normal levels in the long term after eradication in some cases [28, 30]. Regenerative mucosal proliferation could be due to mucosal status whether it had passed the “point of no return” as characterized as intestinal metaplasia or dysplasia [31]. In consequence, the

subsequent altered gene expression may lead to development of post-eradication gastric cancers, which could be more proliferative than those from unrecovered mucosa.

Our statistical analyses suggest that the pathological diagnosis of *H. pylori*-eradicated gastric cancer is sometimes challenging due to cancer regression, such as diminished atypism and shortened proliferative zones in the tumor area, particularly in biopsy specimens, although several pathologists have empirically noticed the difficulty. *H. pylori* infection status is extremely important in pathological diagnosis to prevent overlooking gastric cancer. Hence, pathologists and endoscopists should be careful during the evaluation of *H. pylori*-eradicated gastric cancer.

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Statement of Ethics

The study was conducted with approval from the Institutional Review Board of Fujita Health University.

Disclosure Statement

The authors have no conflicts of interest to declare.

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