Table 2 LOH at specific chromosome loci in sporadic gastric cancers

Chromosome	Frequency of LOH *n* (%)
10p	17/57 (30)
18q	17/57 (30)
5q	6/29 (21)
17p	9/63 (14)
17q	6/51 (12)

LOH, loss of heterozygosity. Markers used were D5S505 (5q), D10S501, D10S602 (10p), TP53 (17p), D17S855 (17q), D18S58 and D18S61 (18q).

Table 3 Patterns of LOH and MSI-H for sporadic gastric cancers

	MSI-H	
	(–) *n* (%)	(+) *n* (%)
LOH (–)	47 (48)	8 (8)
LOH (+)	39 (40)	4 (4)

LOH, loss of heterozygosity; MSI-H, high-frequency microsatellite instability. Markers used were D5S505 (5q), D10S501, D10S602 (10p), TP53 (17p), D17S855 (17q), D18S58 and D18S61 (18q).

We next investigated the degree of overlap between tumors with LOH and those with MSI-H (Table 3). Of the 43 tumors with LOH, four (9%) were MSI-H. Of 86 tumors without MSI-H, 39 (45%) also contained an LOH event at one or more of the loci examined, as did four (33%) of the 12 MSI-H tumors. We found that 4% of all tumors were both LOH(+) and MSI-H(+), 8% were LOH(–) and MSI-H(+), whereas 40% of the tumors were LOH(+) and MSI-H(–). Interestingly, we identified a group of 48% of all gastric cancers examined that were LOH(–) and MSI-H(–), and therefore did not demonstrate signs for either of the two pathways of genomic instability.

DISCUSSION

This study indicates that CIN and MSI pathways are not always independent and that some tumors show overlap between these two mechanisms in sporadic gastric cancers. Our current understanding of gastric carcinogenesis suggests that at least two mechanisms are capable of producing the mutations that are required for a cell to demonstrate a malignant phenotype. These mechanisms include CIN, characterized in tumor DNA by the presence of multiple LOH events, and loss of MMR function, which is defined by MSI-H.

In the present study MSI-H was defined by the microsatellite assay with BAT26. BAT26 is a polyadenine tract localized in the fifth intron of the *hMSH2* gene.[19] The locus does not show important size variation between both alleles, nor between individuals in DNA from normal tissues and microsatellite stable tumors and cell lines, and was thus quasi-monomorphic. It has been indicated that a single test of BAT26 could identify the MSI-H cases defined by the National Cancer Institute (Bethesda consensus panel).[19–21]

To detect LOH, we used seven polymorphic markers mapped closely to key tumor suppressor genes that may be lost during gastric carcinogenesis. Detection of one or more of these loci was taken as evidence of loss of tumor suppressor activity by CIN. It is highly unlikely that a tumor could exhibit the widespread LOH that is characteristic of tumors arising in the setting of CIN without a single LOH event at the seven markers examined.

With obtained results, we classified the cancers into four subtypes; MSI-H(+)/LOH(–), MSI-H(+)/LOH(+), MSI-H(–)/LOH(–), and MSI-H(–)/LOH(+). The MSI-H(+)/LOH(–) tumors were found in 8% of the cancers analyzed. The MSI-H in the sporadic gastric cancers is caused by a defect in DNA MMR capability, which most commonly is achieved by hypermethylation of the *hMLH1* promoter.[10] Cancers associated with MMR defects may demonstrate amplifications and deletions of single alleles or chromosomes. In agreement with this, MSI-H and LOH events coincided in 4% of the cancers in the present study. The LOH events in MSI-H tumors may be caused by a general genomic instability that is typical for neoplasms.

Loss of heterozygosity was observed in 44% of the cancers examined. Tumors that contained LOH without MSI-H comprised 40% of the cancers. The presumed course of tumor progression in this subset involves accumulated allelic losses at tumor suppressor loci. In the literature, frequencies in gastric cancer range from 9 to 30% for LOH of the *APC* gene,[8,22] from 30 to 50% for LOH of the *DCC* gene,[23] and from 30 to 65% for LOH of the *p53* gene.[11,24] In the present study, LOH at the *APC*, *DCC* and *p53* gene loci was identified in 22%, 12%, and 14% of gastric cancers, respectively. Our data on the frequencies of LOH at the *APC* gene loci are consistent with those of previous studies. In contrast, LOH at the *DCC* and *p53* gene loci was relatively lower than those of previous studies. It has been reported that alterations of the *DCC* and *p53* genes were significantly more frequent in advanced-stage gastric cancers than in early stage cancers.[24,25] The present study included a relatively high frequency of early stage cases. This may be the reason why our data on the frequencies of LOH at the loci were relatively lower.

The most interesting subgroup identified was that containing approximately half of the sporadic gastric cancers, in which evidence of both MSI and LOH was lacking. These cancers may be associated with the transcriptional silencing of growth and differentiation genes by epigenetic alterations. Hypermethylation of the promoter region of tumor suppressor genes, which leads to loss of tumor suppressor function, has been observed in a variety of cancers. Even in normal tissues, methylation

was observed in an age-dependent manner.[10] Some tumors show the methylation phenotype, indicating simultaneous methylation of multiple loci. Genes associated with carcinogenesis, such as *APC*, *p16*, and *IGFII*, are silenced by their promoter methylation. Toyota *et al.* reported that approximately 40% of gastric cancers may be of the methylation phenotype, and suggest that methylation phenotype may be one of the major pathways that contribute to tumorigenesis in gastric cancers.[10] We did not examine the methylation status in the present cases. This will be addressed in a future examination.

The MSI-H tumors exhibited distinct clinicopathological features, including predominant antral location and a lower frequency of lymph node metastasis, compared with tumors without MSI-H.[12] These results strengthen the hypothesis that gastric cancers with MSI-H are clinicopathologically distinguishable from those without MSI-H. In the present study, we classified the cancers into four subtypes by LOH and MSI status. We did not find any significant differences in clinicopathological parameters, including patient age, sex, tumor location and histological subtype between these subtypes (data not shown). However, only a limited number of patients was examined in the present study, and to clarify the clinicopathological differences further examinations are necessary.

In conclusion, our data suggest that molecular mechanisms of genomic instability are not necessarily independent and may not be fully defined by either the MSI or CIN pathways in sporadic gastric cancers. Future studies that stratify gastric cancers on the basis of genetic and epigenetic changes may identify factors that contribute to one pathway or others.

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Digestive Endoscopy

Magnifying gastroendoscopy for diagnosis of histologic gastritis in the gastric antrum

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See related commentary on pages 251–252

Abstract

Background. We investigated the potential of magnifying endoscopy for diagnosis of histologic gastritis in the gastric antrum. In addition, we investigated whether magnifying endoscopy can be applied for evaluation of *Helicobacter pylori* eradication therapy.

Methods. We examined 176 Japanese patients including 53 with *H. pylori* eradication. We evaluated the antrum by magnifying observation and ordinary endoscopic findings, and compared these results. Biopsy specimens were taken from the sites observed.

Results. The magnified views were classified into four types. Histology of the biopsy specimens allowed us to match the four magnified views with normal mucosa with fundic glands, normal mucosa with pyloric glands, mucosa with gastritis and intestinal metaplasia/epithelial hyperplasia. The types of magnifying appearances were specific enough for the diagnosis of histologic gastritis (148 out of the 176 (82.4%) cases; sensitivity, 96.3%; specificity, 73.7%). We could accurately diagnose the histologic gastritis by magnifying endoscopy in 49 out of the 53 (92%) cases with *H. pylori* eradication, while only in 38% by ordinary endoscopy. The accuracy of diagnosis was statistically higher with the use of magnifying endoscopy than with ordinary endoscope ($P < 0.001$).

Conclusion. Magnifying gastroendoscopy is useful to judge the histologic gastritis, especially, in cases with *H. pylori* eradication.
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Keywords: Eradication therapy; Gastritis; *Helicobacter pylori*; Magnifying endoscopy

1. Introduction

Gastric diseases including peptic ulcer and gastric cancer are firmly linked with chronic gastritis induced by *Helicobacter pylori* infection [1–4]. The correct diagnosis of histologic gastritis is difficult by endoscopic observation, making gastric biopsy necessary. Histologic gastritis is recognised endoscopically by the atrophic border [5]. Changes in the areae gastricae are also used diagnostically [6]. Chromoendoscopy has been useful for identifying intestinal metaplasia [7,8]. Although endoscopic attempts have been made to classify gastritis, no markers of inflammation activity have been defined. Sakaki et al. established a classification system for magnifying gastroendoscopy, but it has not been applied clinically because of difficulties in observation and the complexity of classification [9]. Recently, it has been reported that the regular arrangement of collecting venules (RAC) finding is useful for judging gastritis in the gastric corpus [10]. However, no endoscopic criteria have been established for determination of gastritis till date, especially in the antrum.

We reported previously that feature of the gastric folds in the corpus showed good correlation with the degrees of histologic gastritis, because pan-gastritis is common in Japanese patients [11]. Changes in these gastric folds indicate the presence of histologic gastritis also in the antrum. In patients less than 49 years of age, however, it is difficult to diagnose gastritis correctly, because atrophic change is not as obvious in these patients as it is in patients over 50 years of age [11]. In addition, eradication therapy for *H. pylori* makes correct diagnosis even more complicated. It is likely that active histologic gastritis improves for a short

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period after eradication of *H. pylori*, though it takes a relatively long time for atrophic change [12,13]. And it is still unknown which part is proper to detect the histological modification of eradication therapy.

Progress in magnifying endoscopy has made it possible to observe microstructures of the gastrointestinal mucosae. Magnifying observation has become essential for the clinical diagnosis of colorectal diseases [14,15]. In the present study, we tried observing the features of the gastric antrum directly using a new magnifying gastroscope. We analysed our endoscopic findings of the antrum in comparison to histologic findings. We established a new classification system for endoscopic findings to be used in the diagnosis of histologic gastritis. In addition, we examined the possibility of evaluating *H. pylori* eradication therapy under magnifying gastroendoscopy.

2. Patients and methods

2.1. Patients

We examined 176 Japanese patients (84 men and 92 women, mean age 57.0 years, range 21–84 years) with dyspepsia including 53 patients who underwent *H. pylori* eradication therapy more than 12 months prior to our study at Hiroshima University Hospital. *H. pylori* infection was evaluated on the basis of anti-*H. pylori* antibody titres (Pylori Stat Kit, Whittaker Bioproducts Inc., Walkersville, MD), histologic examination and ^{13}C urea breath test. Patients in whom any of these assays were positive were classified as *H. pylori*-positive. Those in whom all three were negative were considered *H. pylori*-negative. All patients were informed of the purpose of the study and agreed to magnifying examination and biopsy.

2.2. Gastroendoscopy

We used a magnifying videoendoscope (EG-450ZH and EG-450ZW5, Fujinon Co. Ltd., Saitama, Japan) with a zoom (80×) and TV monitor (38 cm). We evaluated the presence of ordinary endoscopic gastritis by the criteria of Sydney system [16] and with the findings of gastric fold [11]. After routine endoscopic examination without using the cap, indigocarmine dye was applied to the gastric antrum. The gastric antrum was widely observed and it was recorded in image filing system (nexus sif SD, Nexus Co. Ltd., Tokyo, Japan). At the time of endoscopy, magnifying observation was carried out in the greater curvature of the gastric antrum (approximately 5 cm from the pyloric ring). Two endoscopy specialists independently classified the ordinary and magnifying endoscopic findings without the patient information including the result of the eradication therapy. When we found the heterogeneity in the magnifying observation, we judged by the major appearance of magnifying endoscopy. A biopsy specimen with the use of standard forceps was obtained from the lesions that were observed, and histologic gastritis in haematoxylin and eosin (HE)-stained sections were estimated with the use of updated Sydney system [17]. The pathologist judged the histological finding without having information of clinical and endoscopic findings.

2.3. Statistical analysis

Statistical analysis was performed by χ^2-test with StatView software (SAS Institute Inc., Cary, NC). A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Classification of magnifying view in the gastric antrum

Total magnifying endoscopy findings of the 176 patients were classified into four types: pinpoint pits on the flat field (type 1, Fig. 1A); a regular, trabecular ridge pattern or a regular, flat granular pattern (type 2, Fig. 1B); mucosa consisting of irregular and coarse granular structure (type 3, Fig. 1C); and a prominent clubbing (villous) or papillary pattern (type 4, Fig. 1D). Kappa value of the classification by two endoscopists was calculated as 0.906 (95% confidence interval (95% CI): 0.846–0.966).

Representative histologic features corresponding to each of these four patterns are shown in Fig. 2. Normal mucosa with fundic and pyloric glands with no or mild inflammation were found to correspond to types 1 and 2 of magnifying endoscopy findings, respectively (Fig. 2A and B). A small part of the patient shows the histological feature of fundic glands even if we have collected the biopsy specimen from the gastric antrum. Active inflammation was detected in the lesion showing type 3 (Fig. 2C). Intestinal metaplasia corresponded with type 4 (Fig. 2D). In type 4 cases, without intestinal metaplasia, severe inflammation with epithelial hyperplasia was frequently observed.

3.2. Relation between endoscopic findings and histologic gastritis

The histological findings characterising each magnifying endoscopy type are summarised in Table 1. All type 1 sections consisted of normal mucosa with fundic glands.

Table 1
Relation between magnifying endoscopic and histologic findings

	Type 1 ($n = 11$)	Type 2 ($n = 84$)	Type 3 ($n = 64$)	Type 4 ($n = 17$)
Atrophy[a]	0 (0)	12 (14)	44 (69)	11 (65)
Mononuclear cell infiltration[a]	1 (9)	14 (17)	44 (69)	14 (82)
Neutrophil infiltration[b]	0 (0)	15 (18)	44 (69)	13 (76)
Intestinal metaplasia[b]	0 (0)	9 (11)	28 (44)	7 (41)

Data is presented as number of cases (%).
[a] Moderate or severe.
[b] Mild to severe.

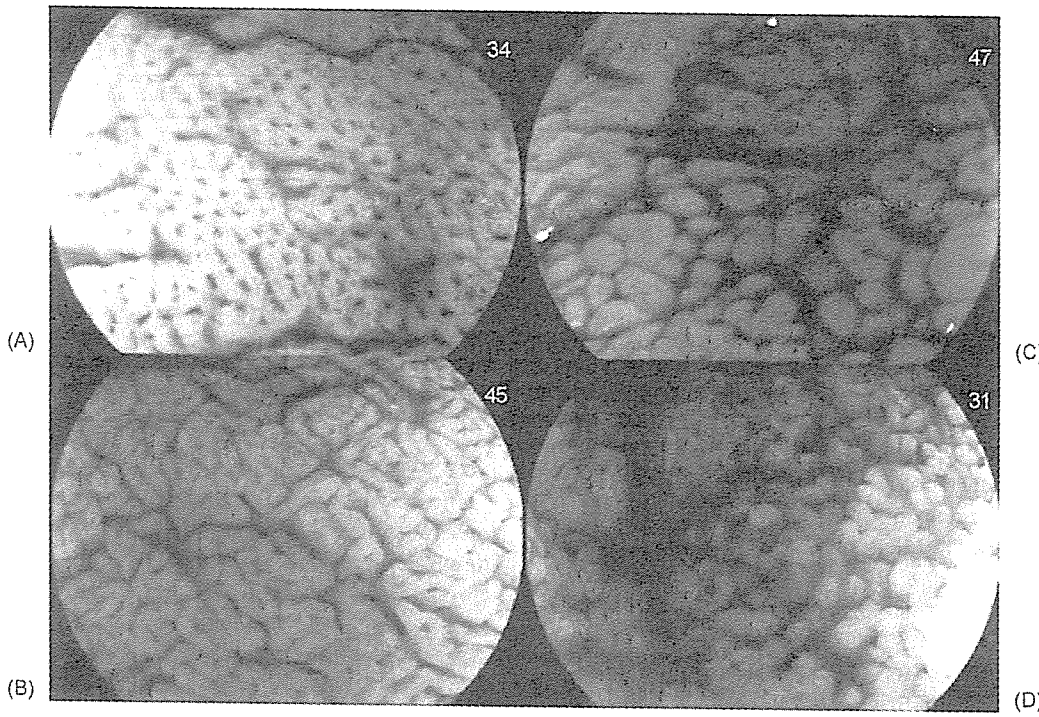

Fig. 1. The four types of magnifying endoscopy appearances. (A) Type 1 is pinpoint pits on the flat field. (B) Type 2 is a regular, trabecular ridge pattern or a regular, flat granular pattern. (C) Type 3 is mucosa consisting of irregular and coarse granular structure. (D) Type 4 is a prominent clubbing (villous) or papillary pattern (magnification 80×).

Type 2 sections included normal mucosa with pyloric glands. The histologic features of gastritis (mononuclear cell infiltration, neutrophil infiltration, atrophy and intestinal metaplasia) were more frequently observed in the type 3 and 4 sections than in the type 1 and 2 sections. Sixty-two out of the 64 (96.9%) type 3 sections showed histologic gastritis. In type 4 sections, no normal features were observed and intestinal metaplasia or severe inflammation was clearly seen.

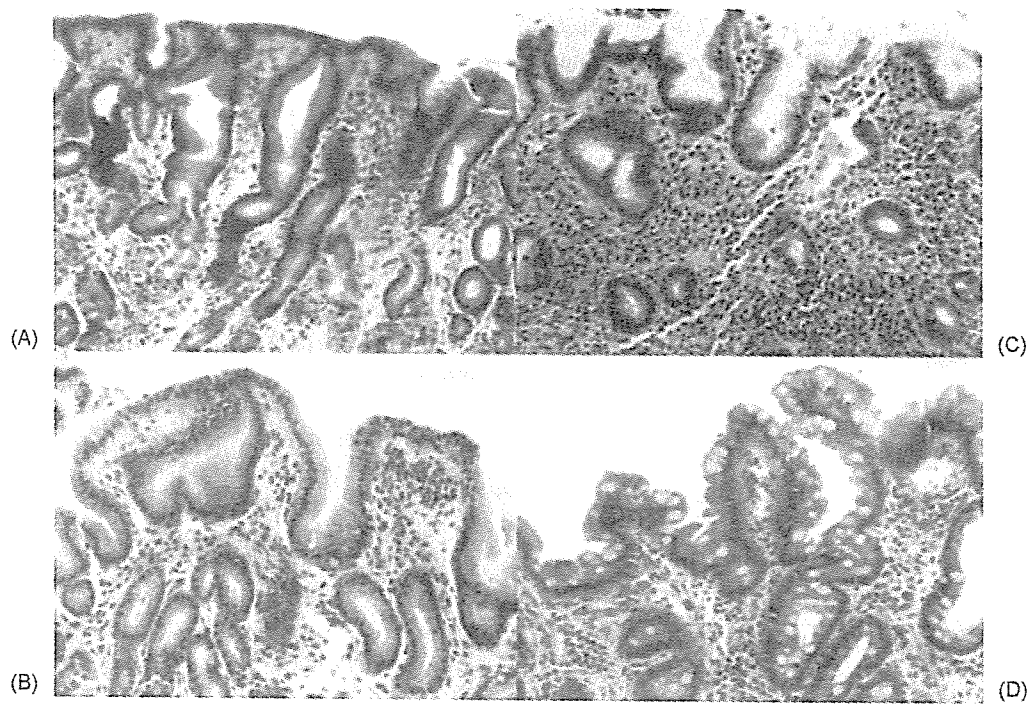

Fig. 2. Histologic features corresponding to the four magnifying appearances. (A) Type 1 represents normal fundic mucosa. (B) Type 2 represents normal pyloric mucosa. (C) Type 3 represents mucosa with active inflammation. (D) Type 4 represents intestinal metaplasia or severe inflammation with epithelial hyperplasia. Sections were stained with HE.

Table 2
Relationship between magnifying endoscopic findings and histologic gastritis

	Magnifying findings		Total
	Types 1 and 2	Types 3 and 4	
Histologic gastritis (−)	70	3	70
Histologic gastritis (+)	25	78	106
Total	95	81	176

Data is presented as number of cases.

3.3. Diagnosis of histologic gastritis

We regarded magnifying endoscopy type 1 and 2 images are gastritis-negative. Histologically, corresponding sections were normal, showing no neutrophil infiltration, no intestinal metaplasia and none or mild lymphocyte infiltration [18]. As shown in Table 2, the types of magnifying endoscopy were specific enough for diagnosis of histologic gastritis and its status in 148 out of the 176 (84.1%) cases. Sensitivity and specificity of diagnosis of histological gastritis by magnifying endoscopic examination were 96.3 and 73.7%, respectively.

Further, we evaluated the ordinary endoscopic findings in the same patients and compared the results with that of the magnifying endoscopy. One hundred and six cases were judged as "gastritis" and most of these were regarded as atrophic gastritis by Sydney system and by enlarged and swollen folds. With the use of ordinary endoscopy, we could diagnose the histologic gastritis correctly only in 107 out of the 176 (60.8%) cases (Table 3). Sensitivity and specificity of diagnosis of histologic gastritis by ordinary endoscopic examination were 66 and 52.9%, respectively. The accuracy was inferior to that of the magnifying endoscopy ($P = 0.006$).

3.4. Evaluation of gastritis in patients with H. pylori eradication therapy

Of the 53 patients who underwent *H. pylori* eradication therapy, 40 were considered to be treated successfully. In patients with successful eradication therapy, we confirmed the absence of active inflammation, whereas persistent active inflammation was detected in the patients with failed eradication therapy. Out of the 40 successful eradication therapies, 39 (98%) had mainly type 1 and 2 endoscopy findings (Table 4), whereas 10 out of the 13 patients, in whom *H. pylori* eradication treatment failed, had type 3 and 4 magnifying observation findings. In 49 out of the 53 (92%) patients, we could completely diagnose the status of histologic gastritis by magnifying endoscopy, while we could only diagnose only in 20 (38%) by ordinary endoscopy. The accuracy of diagnosis was statistically higher with the use of magnifying endoscopy than of ordinary endoscope ($P < 0.001$; Table 4). We have four patients in whom we could perform magnifying endoscope before and after eradication therapy. We confirmed that findings of magnifying endoscope improved from abnormal pattern (three of type 3 and one of type 4) to normal pattern of type 2 (data not shown), while the atrophic finding still remained by ordinary endoscopic observation.

Table 3
Relationship between ordinary endoscopic findings and histologic gastritis

	Endoscopic gastritis		Total
	−	+	
Histologic gastritis (−)	37	36	73
Histologic gastritis (+)	33	70	103
Total	70	106	176

Data is presented as number of cases.

Table 4
Diagnostic ability of gastritis in patients after *H. pylori* eradication

	H. pylori eradication		Accuracy (%)
	Success ($n = 40$)	Fail ($n = 13$)	
By magnifying endoscopy			
Types 1 and 2	39	1	49/53 (92.5)*
Types 3 and 4	3	10	
By ordinary endoscopy			
Gastritis (−)	14	5	20/53 (37.7)*
Gastritis (+)	28	6	

* $P < 0.001$, Yates' corrected χ^2-test.

4. Discussion

It is clinically important to diagnose the presence of histologic gastritis. Many gastric diseases are closely associated with the presence of histologic gastritis induced by *H. pylori* infection [1–3]. Notably, the presence of histologic gastritis with *H. pylori* infection is considered as a risk factor for gastric cancer [4,19,20]. Till date, diagnosis of gastritis has been dependent upon histologic examination by multiple biopsies and does not provide information for the entire stomach. Endoscopic examination for gastritis is considered a non-invasive examination and provides total observation of the stomach not as a "point" but as a "field". However, no objective clinical criteria have been established.

We were able to establish a four-grade classification system for magnifying endoscopy determination of histologic gastritis by matching magnifying examination appearances with histologic features. We focussed on the gastric antrum because the effect of heartbeats is less and few reports have mentioned the feature of magnifying endoscopy in the gastric antrum. We first clarified the normal appearance of the gastric mucosa. Type 1 features represent normal mucosa with fundic glands. The type 1 features represent the narrow and small orifices of the fundic glands, as also reported

previously [21]. Type 2 features represent normal mucosa with pyloric gland. The type 3 appearance indicates the presence of histologic gastritis. For type 1 or 2 appearance, in which flat and regular features are observed, cellular turnover in the gastric mucosa is probably strictly controlled. For a type 3 appearance, the regulation system might be broken down by mucosal inflammation caused by *H. pylori* infection. Indeed, it has been reported that the cell-proliferating zone of the gastric mucosa is altered by *H. pylori* infection [22,23]. A change in microstructure might reflect the disrupted cellular regeneration just as is seen in the pseudolobular formation of liver cirrhosis. The type 4 findings were indicative of intestinal metaplasia, most of these were complete types of intestinal metaplasia, or severe gastritis with epithelial hyperplasia. The papillary or clubbing (villous) structures represent the round tops seen in histologic sections. The mucosa with a type 4 appearance will not yield normal histologic findings.

By defining four magnifying endoscopy patterns, accuracy of diagnosis reached 84.1%. However, there were some false-negative type 2, because the type 2 appearance included mucosa with and without gastritis as shown in Table 1. First reason of false-negative diagnosis (type 2 appearance with presence of histological gastritis) was heterogeneous status of gastric mucosae. While we tried to obtain the biopsy specimen from the lesion of magnifying observation, mistaken sampling might exist. Moreover, heterogeneous appearance could be observed even in a single specimen. Secondly, some sections with false-negative diagnosis revealed normal columnar epithelium and massive infiltration of lymphocytes in mucosal layer. In such patients, pathogenic factors except for *H. pylori* might play a crucial role in the gastritis. It is also possible that some drugs modify the endoscopic features of surface gastric mucosa.

It is of particular interest that our classification system is useful for evaluation of *H. pylori* eradication therapy. We were able by magnifying endoscopy to identify all patients in whom eradication was achieved, and diagnosis with magnifying endoscopy was statistically more useful to judge the histologic gastritis in patients with eradication therapy than ordinary endoscopy. In ordinary endoscopy, the most remarkable finding of gastritis is the atrophic change in the gastric corpus [11]. Since in Japanese patients autoimmune gastritis is rare [24], we could estimate the findings in the corpus and could presume the status of antral gastritis indirectly. However, improvement of atrophy could not be demonstrated in all patients after eradication therapy [13]. In addition, it is hard to estimate the presence of inflammatory cells by ordinary endoscope, and its diagnosis is not reliable in patients with eradication therapy. An accurate diagnosis is possible when we use the magnifying endoscope to observe the antral mucosa directly.

Although our group of *H. pylori* infection patients was not so large, our results show promise that magnifying endoscopy will be useful in judging the result of *H. pylori* eradication therapy. The stomach lining has a heterogeneous appearance in some patients, so it may be difficult to establish a uniform classification system. However, such heterogeneity was not obvious in our patients in whom eradication was achieved. In the present study, the patients have undergone therapy more than 12 months prior to our study, perhaps long enough to show normal turnover of epithelial cells. It would be helpful to examine the changes over time after eradication of *H. pylori*. Since the number of eradication cases will increase in future, the magnifying endoscope will be more worthwhile to evaluate the histologic gastritis.

Taken together, magnifying gastroendoscopy is useful to judge the histologic gastritis, especially, in cases with *H. pylori* eradication.

Conflict of interest statement

None declared.

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Characteristics and Trends of Clarithromycin-Resistant *Helicobacter pylori* Isolates in Japan over a Decade

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Key Words
Helicobacter pylori · Clarithromycin resistance · 23S rRNA

Abstract
Clarithromycin has been administered to patients in Japan since 1991. Clarithromycin-resistant *Helicobacter pylori* strains have been on the rise in Japan. We obtained *H. pylori* isolates between 1989 and 2000 and examined mutations of the 23S rRNA gene, which are closely associated with clarithromycin resistance. Isolates were obtained from 356 patients with *H. pylori* infection treated at the Hiroshima University. Sixty-one of the patients received clarithromycin-based *H. pylori* eradication therapy. Mutations of the 23S rRNA gene were examined by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) followed by sequencing analysis. Mutant strains were found in 42 of the 356 patients (11.8%). The prevalence of mutant strains increased from 0 to 20.4% during the 12-year study period. The prevalence increased to more than 10% by 1995 and then to more 20% after 1999. The *H. pylori* eradication rate was significantly higher in patients with wild-type strains than in patients with mutant strains (72.0 vs. 36.4%, p = 0.024). Our data indicate that clarithromycin-resistant *H. pylori* strains have increased rapidly since 1995 and that the effectiveness of clarithromycin-based *H. pylori* eradication therapies may soon be compromised. Other new therapies may be necessary as first-line treatments in Japan.

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Introduction

Helicobacter pylori is a gram-negative bacterium that infects the human gastric mucosa [1]. This bacterium plays an important role in the pathogenesis of chronic gastritis, peptic ulcer disease, gastric carcinoma, and gastric mucosa-associated lymphoid tissue lymphoma [2–5]. At a National Institutes of Health Consensus Development Conference, a recommendation was made to administer antimicrobial agents with antisecretory drugs for the treatment of patients with *H. pylori*-associated peptic ulcer disease [6]. The antimicrobial agents most often

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Table 1. Diagnoses in study patients

Diagnosis	Number of patients (n = 356)
Chronic gastritis	159
Gastric ulcer	34
Duodenal ulcer	31
Gastric hyperplastic polyp	8
Gastric adenoma	17
Gastric cancer	107

used are amoxicillin, clarithromycin, and metronidazole. Therapy with two of these three antibiotics and an antisecretory drug usually achieves an eradication rate above 70% [7, 8]. Most *H. pylori* strains are susceptible to amoxicillin, whereas 5–20% of strains are resistant to clarithromycin, and 5–50% of strains are resistant to metronidazole [9–15].

Resistance of *H. pylori* to clarithromycin is closely associated with one of three known point mutations in domain V of the 23S rRNA gene: adenine to guanine at position 2143 (A2143G), adenine to guanine at position 2144 (A2144G), and adenine to cytosine at position 2143 (A2143C) [10–15]. It has been reported that these positions are the targets of ribosomal peptidyl transferase as well as the binding sites for clarithromycin [15]. Researchers have reported frequencies of clarithromycin resistance in *H. pylori* strains by detecting mutations of the 23S rRNA gene [10–15]. Clarithromycin has been administered to patients in Japan since 1991, and the prevalence of clarithromycin resistance in *H. pylori* strains is high at present [16]. So far, there have been no reports indicating when clarithromycin-resistant strains began to increase in Japan. Therefore, we examined mutations of the 23S rRNA gene of *H. pylori* strains isolated from 1989 to 2000, i.e. over more than a decade.

Patients and Methods

Patients

Patients with *H. pylori* infection treated at the Hiroshima University Hospital between 1989 and 2000 (n = 356) were enrolled in the study. The male/female ratio was 224/132, and the mean age was 56.0 years (range 13–90 years). Diagnoses are shown in table 1. Gastric corporeal biopsy specimens were obtained endoscopically from each patient. *H. pylori* infection was examined histologically with Giemsa staining and by rapid urease test. Sixty-one patients underwent *H. pylori* eradication therapy for 1 or 2 weeks with 40 mg omeprazole, 2,000 mg amoxicillin, and 800 mg clarithromycin (29 with chronic gastritis, 10 with gastric ulcer, 6 with duodenal ulcer, 3 with gastric hyperplastic polyp, 5 with gastric adenoma and 8 with gastric cancer). *H. pylori* eradication was confirmed histologically and by a rapid urease test. Three months to 1 year after antibacterial treatment, eradication was evaluated by biopsy specimens and ^{13}C-urea breath test.

DNA Extraction

Tissue sections 10 μm in thickness were placed on glass slides and stained with hematoxylin and eosin. The tissue sections were then dehydrated in graded ethanol solutions and dried without a cover glass. Tissues were scraped from the slides with sterile needles. DNA was extracted from the tissues with 20 μl of extraction buffer (100 m*M* Tris-HCl; 2 m*M* EDTA, pH 8.0; 400 μl/ml proteinase K) at 55°C overnight. The tubes were boiled for 7 min to inactivate the proteinase K, and then 2 μl of the extracts was used for each polymerase chain reaction (PCR) amplification.

PCR-Single Strand Conformation Polymorphism Analysis of the 23S rRNA Gene

PCR primers targeting the 23S rRNA gene were 5′-TGT AGT GGA GGT GAA AAT TCC TCC-3′ (positions 2101–2125) and 5′-GAT ATT CCC ATT AGC AGT GCT-3′ (positions 2172–2192). To detect mutations at positions 2143 and 2144, PCR-single strand conformation polymorphism (PCR-SSCP) analysis was used as described elsewhere [17, 18]. Briefly, each 25 μl of reaction mixture contained 1 × AmpliTaq Gold Buffer [8.0 m*M* Tris-HCl (pH 8.3), 40 m*M* KCl] (Perkin-Elmer, Branchburg, N.J., USA), 4 m*M* of $MgCl_2$, 0.3 m*M* of each deoxynucleotide triphosphate, 100 pmol of each primer, 10–20 ng of genomic DNA, 2.5 mCi of (alpha-^{32}P)dCTP (3,000 Ci/m*M*, 10 mCi/ml), and 1.25 units of AmpliTaq Gold DNA polymerase (Perkin-Elmer). Heating of reaction mixtures to 95°C for 10 min was followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and strand elongation at 72°C for 30 s. After PCR, the samples were electrophoresed on 6% polyacrylamide gels (acrylamide:bisacrylamide, 19:1) with 10% glycerol at 4°C. The gels were subjected to autoradiography overnight at −80°C.

DNA Sequencing

To confirm the mutations detected by PCR-SSCP, a direct sequencing analysis was performed as described by Yokozaki et al. [19]. The aberrant migration band on the SSCP gel was removed, amplified again, and directly sequenced on both strands with an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer ABI, Foster City, Calif., USA). For the sequencing reaction, a PRISM AmpliTaq DNA polymerase FS Ready Reaction Dye Terminator Sequencing Kit (Perkin-Elmer ABI) was used.

PCR-Restriction Fragment Length Polymorphism Analysis of the 23S rRNA Gene

For detection of the A to G mutation at position 2143 of the 23S rRNA gene, PCR-restriction fragment length polymorphism (RFLP) was performed according to the method described by Maeda et al. [11]. The PCR products were digested with *Mbo*II (Takara, Otsu, Japan) at 37°C for 1 h.

Statistical Analysis

Statistical differences were evaluated by means of the χ^2 test. A value of $p < 0.05$ was regarded as significant.

Table 2. 23S rRNA mutant *H. pylori* strains for 1989–2000

Period	Number of 23S rRNA	Mutant strains
1989–1990	32	0
1991–1992	32	1 (3.1%)
1993–1994	38	2 (5.3%)
1995–1996	82	9 (11.0%)
1997–1998	118	19 (16.1%)
1999–2000	54	11 (20.4%)

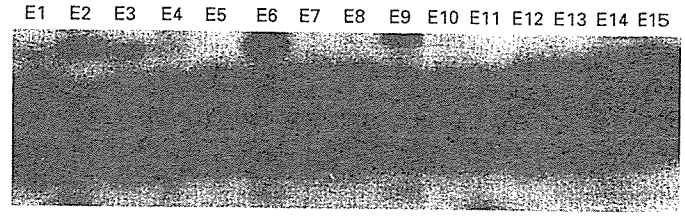

Fig. 1. PCR-SSCP analysis of the 23S rRNA gene of *H. pylori* strains. Patient numbers are shown above the lanes. Mobility shifts were detected in patients E8, E12, and E15 which were A to G point mutations at position 2144 by sequencing analysis.

Table 3. Characteristics of patients and the respective 23S rRNA mutant *H. pylori* strains isolated between 1995 and 2000

	Number of 23S rRNA	Mutant strains
Total	254	41 (16.1%)
Sex		
Male	170	27 (14.1%)
Female	84	14 (16.7%)
Age, years		
≤39	30	6 (20.0%)
40–59	106	18 (17.9%)
≥60	118	16 (13.6%)
Diagnosis		
Chronic gastritis	101	19 (18.8%)
Gastric ulcer	29	5 (17.2%)
Duodenal ulcer	21	3 (14.3%)
Gastric hyperplastic polyp	4	0
Gastric adenoma	12	0
Gastric cancer	87	11 (12.6%)

Table 4. 23S rRNA mutant *H. pylori* strains and outcome of clarithromycin-based *H. pylori* eradication therapy

	H. pylori eradication therapy		Eradication rate
	failure	success	
Wild-type strains	14	36	36/50 (72%)[a]
Mutant strains	7	4	4/11 (36.4%)

[a] p = 0.024 vs. mutant strains.

Results

The 23S rRNA mutant *H. pylori* strains were found in 43 of the 356 patients (11.8%) (table 2, fig. 1). The prevalence of mutant strains increased from 0 to 20.4% over the 12 years. The prevalence was above 10% after 1995, and it increased to more than 20% between 1999 and 2000. Mutant strains were found in only 3 of 102 patients (2.9%) during the initial 6 years (1989–1994) but were found in 39 of 254 patients (15.4%) during the latter 6 years (1995–2000). We examined the association between the clinical features of the patients and 23S rRNA mutant *H. pylori* strains isolated between 1995 and 2000. No significant associations were observed between sex, age, patient diagnosis and frequencies of the mutant strains (table 3). Sixty-one patients underwent clarithromycin-based *H. pylori* eradication therapy. Eradication was achieved in 40 of the total 61 patients (65.6%), specifically in 36 of 50 patients (72.0%) with wild-type strains, and in 4 of 11 patients (36.4%) with mutant strains (table 4). The eradication rate was significantly higher in patients with wild-type strains than in patients with mutant strains (p = 0.024). Mutations detected by PCR-SSCP followed by sequencing analysis were all A2144G mutations and no A2143G and A2143C mutations were observed. When PCR-RFLP analysis was performed with *Mbo*II digestion, no A2143G mutations were found.

Discussion

Clarithromycin is an often used and important antibiotic in eradication treatment for *H. pylori* [2, 8]. Because the presence of clarithromycin-resistant *H. pylori* strains may result in eradication failure, it is important to be able to predict bacterial resistance [11]. Clarithromycin inhib-

its protein synthesis by binding to the peptidyl transferase loop of 23S rRNA, which has been shown at residues A2058 and A2059 in the 23S rRNA gene of *Escherichia coli* [20]. When these positions mutate, the affinity of clarithromycin binding to ribosomes is reduced, resulting in the drug resistance. Versalovic et al. [14] reported that the transitional mutations A2143G and A2144G were associated with clarithromycin resistance. Van Doorn et al. [21] reported that 97.7% of clarithromycin-resistant strains contained 23S rRNA gene mutations, whereas 98.8% of susceptible strains contained wild-type sequences. Several other reports have confirmed that the mutations are responsible for the resistance [10–13, 15, 22, 23]. Antimicrobial resistance of *H. pylori* is thought to be a consequence of the overuse antibiotics in the community. In Japan, the government (i.e. the Ministry of Health and Welfare) did not allowe the use of antibiotics against *H. pylori* until November 2000. Resistance to clarithromycin observed before the year 2000 in this study is probably due to the use of the antimicrobial agent for other infections. The reported prevalence of *H. pylori* resistance to clarithromycin in Japan is reported at 6–21% [11, 13, 16, 24, 25]. The reported prevalence in western countries is from 1 to 17% [9, 10, 15, 19, 26]. Few researchers, however, have examined the trends in clarithromycin resistance among *H. pylori* strains over time. Boyanova et al. [27] examined the frequencies of resistant strains isolated in Bulgaria over 4 years. Ferrero et al. [28] examined resistant strains isolated in Spain over 3 years. Both studies showed an increase in resistant strains over time. However, the observation periods were relatively short. Indeed, ours is the first reported study of trends in resistant strains isolated in one country over a fairly long period of time (more than a decade). Our data shows clearly that the prevalence of clarithromycin resistance in *H. pylori* strains is high in Japan and that it is increasing. Clarithromycin was developed in Japan and is commonly used for the treatment of respiratory tract infections. This may be a reason for the high prevalence of resistant strains here. If this trend continues, the recommended first-line *H. pylori* eradication therapy, i.e. the combination of amoxicillin, clarithromycin, and proton pomp inhibitor, should be reconsidered. Other therapies, such as metronidazole-based therapy, have been reported to be effective [9]. Other new therapies may be necessary as first-line treatments. Alternatively, reducing the use of clarithromycin for other infections might reduce the prevalence of clarithromycin resistance. A 50% decrease in macrolide consumption in Finland between 1988 and 1992 led to a decrease in resistance of group A streptococci from 19 to 9% [29]. In the present study, the prevalence of resistant strains was shown to be higher in younger patients. In contrast, in Spain resistant strains have been common in older patients [4] and resistance rates have been lower in patients with peptic ulcers than in patients without peptic ulcers. The discrepancies between populations may be due to differences in the administration in clarithromycin. Younger Japanese individuals may have been given more clarithromycin than was given to the Spanish.

Several studies in western countries have reported similar prevalences of the A2143G and A2144G mutants [10, 12, 14, 26]. Versalovic et al. [14], for example, reported 53% of resistant strains to be A2143G mutants and 39% to be A2144G mutants. Most mutants detected in Japan, however, were A2144G mutants [11, 13, 25]. Maeda et al. [11] reported 70 of 75 (93%) mutant strains to be A2144G mutants. The A2144G mutants we detected may be representative of clarithromycin resistance in *H. pylori* strains in Japan. Another mutation, A2143C, is also known to be associated with clarithromycin resistance and has been reported to account for 7% of the resistant strains in western countries [12]. Maeda et al. [11] reported an absence of the A2143C mutation in Japan and we also observed this absence. This may also be a characteristic of clarithromycin-resistant strains in Japan. This geographical difference would be difficult to explain if random mutations occurred at these sites. It is well known that *H. pylori* strains differ between western and East Asian countries including Japan. The manifestations of *H. pylori* infection also differ between western countries and East Asia. Duodenal ulcers are often seen in *H. pylori*-infected populations in western countries, whereas atrophic gastritis and gastric ulcers are often seen in East Asia [3]. The differences may be associated with differences in the mutation spectrum, and further examinations are necessary to clarify this issue.

In summary, our data indicate that clarithromycin-resistant *H. pylori* strains have increased rapidly since 1995 and that the effectiveness of clarithromycin-based *H. pylori* eradication therapies may soon be compromised. Thus, other new therapies may be necessary as first-line treatments in Japan.

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Morphological changes in human gastric tumours after eradication therapy of Helicobacter pylori *in a short-term follow-up*

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SUMMARY

Background: It is controversial as to whether the development of gastric cancer is influenced by *Helicobacter pylori* eradication. If eradication itself influences the tumour morphology, this may affect the tumour discovery rate.

Aim: To investigate the morphological changes in the gastric neoplasm after *H. pylori* eradication.

Methods: We studied 37 patients with eradication therapy. After a 1-month follow-up, endoscopic re-evaluation was performed and the appearance was compared with first image. All lesions were resected endoscopically, and were subjected to histological assessment and to immunohistochemistry. Serum gastrin levels were determined before and after eradication.

Results: Twenty-nine of 37 patients underwent successful eradication. The appearance of 11 lesions (33% of 33 lesions) became indistinct after successful eradication. All lesions were of the superficial-elevated type and the height of the lesions decreased. We detected normal columnar epithelium over the neoplasm in eight of the lesions. Higher expression of single-stranded deoxyribonucleic acid in the deep area was characteristic in tumours with an indistinct appearance. These changes did not correlate with the serum gastrin levels.

Conclusions: The morphology of the gastric neoplasm change after eradication in the short-term. This may contribute to the decreased tumour discovery rate.

INTRODUCTION

Helicobacter pylori plays an important role in the promotion of atrophic gastritis.[1] Long-term infection of *H. pylori* results in glandular atrophy and intestinal metaplasia. It has been accepted that there is a strong association between *H. pylori*-associated gastritis and gastric cancer.[2–4] Uemura *et al.* clearly demonstrated that gastric cancer developed only in patients with *H. pylori* infection by prospective study.[5] *Helicobacter pylori* eradication therapy is widely accepted as a prevention of peptic ulcer. We have previously demonstrated that the extent of gastric atrophy and intestinal metaplasia improved in some cases after successful eradication therapy.[6] Severe gastric atrophy induced by *H. pylori* is thought to be an important risk factor in the development of gastric carcinoma; therefore, it is speculated that control of histological gastritis is linked to the control of gastric cancer developments. Indeed, Uemura *et al.* had reported that eradication therapy of *H. pylori* decreased the occurrence of second gastric cancer in patients with pre-treated gastric cancer by

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endoscopic mucosal resection.[7] We also found a low Ki-67 labelling index in gastric cancer cells in *H. pylori*-negative gastric cancer tissue compared with *H. pylori*-positive tissue, suggesting that *H. pylori* has a growth promoting role on gastric cancer cells.[8] However, it is still controversial as to whether eradication therapy of *H. pylori* diminishes the incidence of gastric cancer.

One of the difficulties of this field seems to be based on the methodology used to evaluate the gastric carcinogenesis. Researchers can evaluate the degree of carcinogenesis only by the discovery rate of gastric cancer by endoscopic examination. Due emphasis must be placed on the differences in diagnostic ability of each examination. Moreover, endoscopic morphology might be influenced directly by eradication therapy, this affecting the discovery rate of gastric cancer.

In the present study, we focused on the morphological changes in gastric neoplasms after the eradication therapy with a short-term follow-up study. We then examined the histological and molecular biological changes induced by eradication therapy, and discussed the clinical implication.

METHODS

Patients

Thirty-eight patients with gastric neoplasm (27 men, mean age: 69.1 year old) were included in this study, and 45 lesions (28 gastric carcinomas and 17 gastric adenoma) were studied. All patients received an endoscopic examination and the endoscopic features were recorded in a database. No patients who had undergone gastrectomy were included in the study. All patients had histological gastritis in both corpus and antrum and were confirmed as being *H. pylori*-positive by rapid urease test (PyloriTek, Serim Research, Elkhart, IN, USA), Giemsa staining, ^{13}C-urea breath test (UBT; Otsuka UBiT-IR200, Tokushima, Japan) or the presence of serum IgG antibodies against *H. pylori* (E-plate, Eiken, Tokyo, Japan). Patients were considered as *H. pylori*-positive if at least two of them were positive. After diagnosis of the *H. pylori* infection, all patients received eradication therapy by the use of a proton-pump inhibitor (lansoprazole 60 mg, twice daily), amoxicillin (1500 mg, twice daily) and clarithromycin (400 mg, twice daily) for 1 week. The successful clearance of *H. pylori* was judged more than 4 weeks later by UBT or the *H. pylori* stool antigen test (Meridian Diagnostics, Cincinnati, OH, USA). A second endoscopic observation was performed prior to endoscopic mucosal resection (average 33.9 days) of the gastric tumour. From the patients we received written informed consent and the Ethical Committee of Hiroshima University approved our protocol.

Evaluation of endoscopic findings

First, endoscopic pictures were saved in the database. Secondly, endoscopic observations were performed using the same endoscopic system and saved in the same manner. Later, the pictures were printed out and three specialists judged the alterations of endoscopic appearance independently, unaware of the clinical information including the evaluation of the eradication therapy. They evaluated the endoscopic changes concerning: (i) difficulties to point out the tumour itself or its margin (whether tumour became indistinct or not), (ii) tumour height or depth, (iii) tumour surface and (iv) the degree of redness in background mucosa. If more than two specialists recognized the finding, we regarded it as being significant.

Determination of serum pepsinogen and gastrin levels

Fasting serum was collected from all patients. The samples were centrifuged immediately at 4 °C and stored at −20 °C until use. Serum concentrations of pepsinogens (PGs) and gastrin were determined by enzyme-linked immunosorbent assay and modified radioimmunoassay.[9]

Immunohistochemistry

About 4-μm sections of formalin-fixed paraffin-embedded tissues were used for immunohistochemical staining. After deparaffinization and hydration, internal peroxidase was blocked by incubating with 0.3% H_2O_2 in methanol for 15 min. After incubation with 5% skim milk/phosphate-buffered saline (PBS) for 20 min, the sections were reacted with the primary antibody (diluted with PBS) for 2 h at room temperature. The primary antibodies used were anti-single-stranded DNA (ssDNA) polyclonal antibody (dilution of 1:300; Dako, Kyoto, Japan),[10] and antihuman Ki-67 antigen (MIB-1, dilution of 1:100; Dako).[8] We performed the immunostaining using an LSAB2 kit (Dako). Antigen retrieval was carried out with microwave

treatment before reacting with anti-Ki-67. Strong signals in the nuclei of the epithelial cells were taken to be positive result.

Statistics

Results are reported as mean ± s.d. Statistical analysis was performed by chi-square test with STATVIEW software (SAS Institute Inc., Cary, NC, USA). A *P*-value of <0.05 was considered statistically significant.

RESULTS

Clinical features of patients and changes in endoscopic findings

Following initial enrolment of 38 patients, one patient dropped out of this protocol because of a suspicious tumour invasion into the submucosal layer, which was followed by an operation. Therefore, 37 patients with 44 lesions (27 carcinomas and 17 adenomas) were finally enrolled. *Helicobacter pylori* eradication therapy succeeded in 29 patients (78%) with 33 lesions. The clinical features are summarized in Table 1. In 11 of the lesions, we found that the presence of the lesion came to be indistinct compared with the primary image (Table 1). All of these lesions were found in patients who underwent successful eradication therapy and no lesions in the cases of failed eradication showed this alteration.

Table 1. Clinical features of patients and alterations of tumour findings

	Eradicated (n = 29)	Non-eradicated (n = 8)	*P*-value
Clinical features			
Mean age (range)	69.8 (48–84)	69.3 (54–78)	N.S.
Gender (male/female)	19/10	8/0	N.S.
Period (days)	33.3	36.0	N.S.
Lesions			
Number	33	11	
Elevated/depressed	20/13	3/8	N.S.
Tumour diameter (mm, mean ± s.d.)	15.9 ± 5.6	13.1 ± 10.7	N.S.
Carcinoma/adenoma	21/12	6/5	N.S.
Endoscopic change			
Indistinct	11 (33%)	0 (0%)	**0.03**[1]

[1] Chi-square test.

Table 2. Clinicopathological features of 33 gastric tumours with successful eradication; comparison between adenoma and carcinoma

	Adenoma (n = 12)	Carcinoma (n = 21)	*P*-value
Tumour features			
Elevated/depressed	11/0	9/13	
Mucosal/submucosal	12/0	21/0	
Diameter (average; mm)	11.5	18.5	
Endoscopic alterations			
Indistinct	6 (50%)	5 (24%)	0.12[1]

[1] Chi-square test.

Comparison between adenoma and carcinoma

We compared the endoscopic alteration in patients with gastric adenoma and in those with adenocarcinoma. The clinicopathological features of patients with the adenomas and carcinomas were summarized in Table 2. All adenocarcinoma tissues were confirmed histologically to be limited in the mucosal layer. We could find the endoscopic alteration not only in six adenomas but also in five carcinomas (Table 2). The representative endoscopic features were demonstrated in Figures 1 and 2. After eradication, the tumours became flattened and indistinct, and it was difficult to point out the tumour itself or to set the clear horizontal margin of the tumours. Although this alteration was frequently detected in adenoma tissue, we could not find the statistical difference in the endoscopic change of tumours between two groups.

Characteristics of the lesions that became unclear after eradication

We tried to clarify the characteristics of the lesions that became indistinct after successful eradication therapy. As shown in Table 3, this phenomenon was characteristically found in elevated lesions. Moreover, a flattened appearance had a close association with the incidence of unclear change. Although it is well-known that the redness of the background mucosa often diminishes after eradication therapy, it was not associated with the indistinct appearance.

Changes in histological findings by eradication

We then examined the histological features using sections taken from the endoscopic resection stained

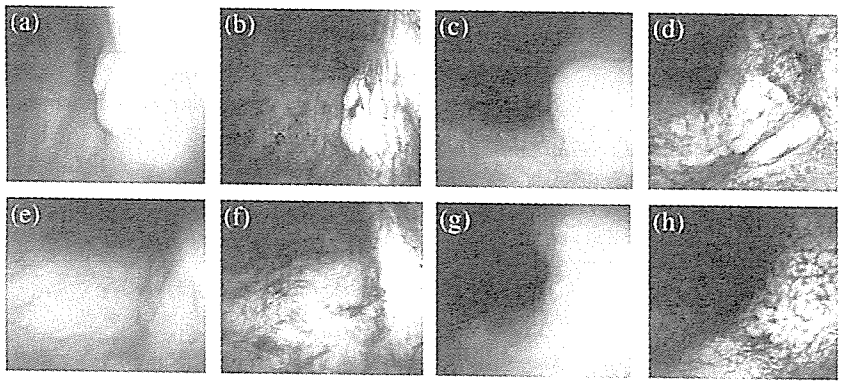

Figure 1. Endoscopic features of the gastric adenoma at pre- (a–d) and post-eradication therapy (e–h). Patients were 71 years female (a, b, e, f) and 67 years male (c, d, g, h). Ordinary (a, c, e, g) and dye-endoscopic (b, d, f, h) observation. Tumours became flattened and indistinct after eradication therapy.

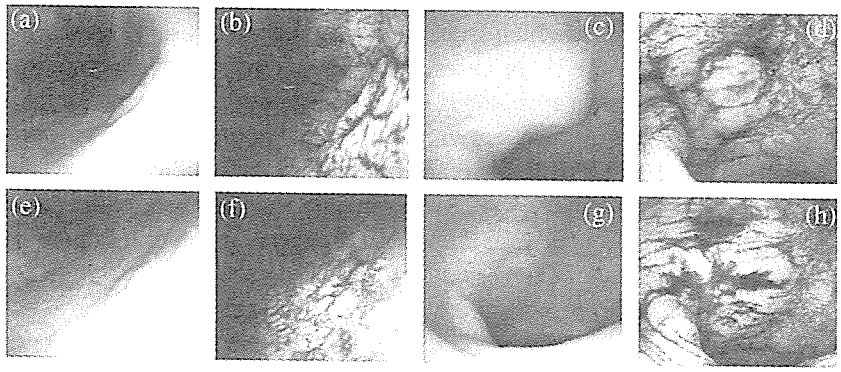

Figure 2. Endoscopic features of the gastric adenocarcinoma at pre- (a–d) and post-eradication therapy (e–h). Patients were 64 years male (a, b, e, f) and 75 years male (c, d, g, h). Ordinary (a, c, e, g) and dye-endoscopic (b, d, f, h) observation. Tumours became flattened and indistinct after eradication therapy as seen in cases with adenoma.

Table 3. Characteristics of the lesions, which became indistinct after successful eradication therapy

	Indistinct (n = 11)	No change (n = 22)	P-value
Tumour features			
Elevated/depressed	11/0	9/13	<0.01[1]
Carcinoma/adenoma	5/6	16/6	0.12[1]
Endoscopic alterations			
Flattened	11 (100%)	2 (9%)	<0.01[1]
Diminished redness	6 (55%)	6 (27%)	0.12[1]
Histological alterations			
Normal columnar			
Epithelium over the tumour	8 (73%)	3 (14%)	<0.01[1]
Serum pepsinogens (pre-eradication)			
PG I (ng/mL, mean ± s.d.)	33.0 ± 23.9	30.0 ± 25.5	N.S.
PG II (ng/mL, mean ± s.d.)	18.2 ± 7.9	19.2 ± 11.9	N.S.
PG I/II (mean ± s.d.)	1.73 ± 0.97	1.49 ± 0.87	N.S.

[1] Chi-square test.

with haematoxylin and eosin. We could detect the appearance of normal columnar epithelium to various degrees over the tumour tissue (Figure 3) in 12 lesions. Of 12, 11 were found in patients who underwent successful eradication therapy. Especially, in three cases, the atypical epithelium covers the bulk of the tumour tissue. This change was found not only in adenoma (nine lesions) but also in carcinoma tissue (three lesions) and showed a close association with the endoscopic finding of unclear margin (Table 3).

Serum pepsinogen levels and endoscopic alteration

Sera from patients were collected before eradication therapy and the serum level of PGs was estimated. We examined the relationship between serum levels of PGs

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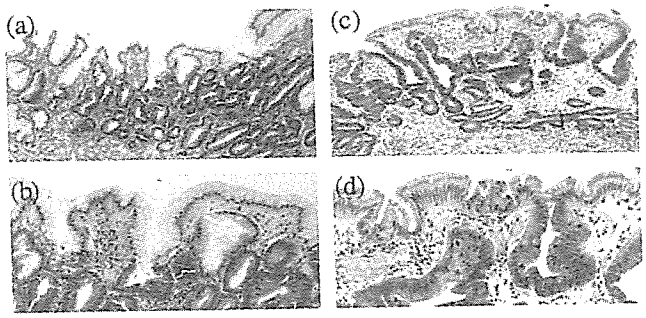

Figure 3. Histological features of gastric neoplasm at posteradication. Cases of gastric adenoma (a, b) and adenocarcinoma (c, d) with successful eradication therapy. (a, c) Low magnification of gastric tumour specimen, (b, d) high magnification of surface epithelium. Patients were 71 years female (a, b) and 82 years male (c, d).

and the endoscopic findings. As shown in Table 3, relative high levels of PG I and a high I/II ratio were found in patients with endoscopic changes compared to those in the patients with no-changes; however, this is not statistically significant.

Relationship between serum gastrin level and endoscopic changes

Further to this, we studied the alteration of serum gastrin levels. Fasting sera were collected before and after eradication therapy. As shown in Table 4, a decrease of the serum gastrin level was not so obvious after 1 month of eradication therapy. We could not find a difference in the level of gastrin between patients with indistinct tumour appearance and those with no change.

Expressions of Ki-67 and ssDNA in tumour cells

We examined the cell kinetics in these lesions using immunohistochemical staining with the use of tumour specimens at posteradication. We could not detect any difference in the Ki-67 labelling index, which is a marker for cell proliferation, or the ssDNA labelling index, which is a marker for cell apoptosis (Table 5).[11] However, in cases where there were indistinct tumour appearance after eradication, ssDNA expression was more frequently detected from deeper within the tumour at posteradication (Figure 4, Table 5). In other specimens, ssDNA expression was uniformly detected, and no lesions showed luminal side-dominant pattern in ssDNA expression.

Table 4. Changes in serum gastrin levels after eradication therapy

		Gastrin level (pg/mL, mean ± s.d.)	
	Number	Before eradication	After eradication
Successful eradication			
Indistinct	10	322.1 ± 356.0	296.1 ± 314.3
No change	16	309.9 ± 271.1	211.0 ± 173.1
Failed eradication	7	176.8 ± 162.5	245.6 ± 279.5

Table 5. Expressions of Ki-67 and ssDNA in gastric tumour cells

	Number	Ki-67 LI (%)	ssDNA LI (%) (deep area dominant)
Successful eradication†			
Indistinct	6	17.5 ± 16.3	46.2 ± 13.2 **(3/6)***
No change	14	15.4 ± 12.9	45.9 ± 20.8 **(0/14)***
Failed eradication†	7	9.0 ± 5.5	40.3 ± 27.6 (0/7)

LI, labelling index; ssDNA, single-stranded deoxyribonucleic acid.
* $P < 0.05$, chi-square test.
†Mean ± s.d.

DISCUSSION

In the present study, we demonstrated the direct effect of *H. pylori* eradication therapy on the morphological appearance in gastric adenomas and carcinomas. The

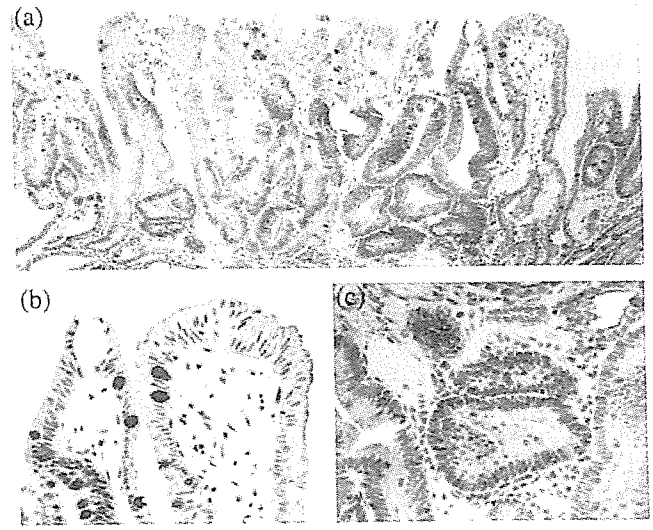

Figure 4. Expression of single-stranded DNA (ssDNA) in adenoma cells. Immunohistochemical analysis was performed as described in Methods. (a) Low magnification of gastric tumour specimen, (b) high magnification of the surface area, (c) high magnification of the deep area. Patient was 67 years male.

typical changes after eradication were (i) a flattened aspect to the elevated lesion and indistinct border of tumour lesion when viewed at endoscopy and (ii) the appearance of a normal columnar epithelium over the neoplastic lesion. Uemura *et al.* previously demonstrated the low incidence of a second cancer development by the eradication therapy in patients who underwent endoscopic mucosal resection of the gastric cancer.[7] In addition, we have published data demonstrating a low Ki-67 labelling index in those gastric cancer cells without *H. pylori* infection.[8] These results are indicative of the promoting effect of *H. pylori* on the growth of gastric cancer cells.

We found that the gastric tumour had flattened and showed indistinct feature after a short period and this result completely agrees with the previous findings. It is of interest that the main morphological change revealed by endoscopic observation was that it had flattened and this was only in the elevated lesions, regardless of the tumour's histology. No morphological change was found in the cases that had depressed features. This indicates that *H. pylori* eradication may inhibit the upward (expansive) growth of the gastric tumour. We have also found that most gastric cancers discovered after successful eradication therapy were of the flat, depressed type (under submission). This phenomenon also agrees with our hypothesis.

The mechanism of the tumour promoting effect of *H. pylori* is still unknown. In the *in vitro* studies, *H. pylori* itself was found to have the effect of modifying the expressions of several genes in gastric carcinoma cells.[12] And in the *in vivo* studies, *H. pylori* has been proved to modify directly the state of apoptosis or the cell cycle regulatory system including p27 expression.[13, 14] Recent evidence has clarified the direct mechanism of the translocation of harmful proteins (Cag A) from *H. pylori* to the host cell followed by specific intracellular signalling.[15, 16] Semino-Mora *et al.* recently demonstrated the presence of *H. pylori*-derived toxic proteins and mRNAs in gastric tumour cells *in vivo*.[17] However, their theory is still controversial, and, until now, it has been believed that *H. pylori* cannot exist on the surface of gastric carcinoma cells. Indeed, no evidence has demonstrated *H. pylori*-induced signalling, including CagA phosphorylation, in the human gastric carcinoma cells *in vivo*.

Thus, it is likely that *H. pylori* indirectly influences tumour cell growth by regulating the inflammatory reaction around the tumour tissue. Several cytokines have been reported to be induced by *H. pylori* infection[18] and some of them, such as interleukin-1 and hepatocyte growth factor, may act as growth factors for tumour cells.[19] In the present cases all were confirmed to have *H. pylori*-induced chronic gastritis in the background mucosa. Ohkusa *et al.* demonstrated that, after eradication, gastric inflammation had decreased by 1–3 months.[20] We found that ssDNA expression was mainly detected in the deeper area of the gastric tumour in three lesions at posteradication, and all three showed indistinct appearance. In other specimens, ssDNA expression was uniformly detected, and no lesions showed luminal side-dominant pattern in ssDNA expression. These suggest the importance of the growth inhibitory signals from the mucosal side (as opposed to those from the luminal side). This indicates the importance of gastric inflammation in the gastric mucosa rather than *H. pylori* itself on the luminal side. In this study, ssDNA expression was examined only in lesions after eradication, this should be examined at pre- and post-eradication and should be compared in the next step.

Gastrin is known to be an important gut-related hormone and a growth factor for gastric cancer cells[21, 22] and gastric tumour cells have been shown to contain its receptor. Reports have indicated that, after eradication therapy of *H. pylori*, a decreased level of several cytokines such as interleukin (IL)-1, IL-2, tumour necrosis factor-α and interferon-γ, in the gastric mucosa as well as increased acid output results in the decreased level of serum gastrin.[23, 24] However, our results showed that the decrease of the gastrin level is not so obvious after eradication, and alteration of the tumour lesion was not correlated with the serum gastrin level. In our protocol, the observation period is short and that may be a reason for the incomplete depression of the gastrin level. It is unlikely that our new findings of the morphological changes were induced by a gastrin-related system.

It was a surprising finding that a normal columnar epithelium appeared over the tumour tissue after successful eradication therapy. The reason for the alteration is still unknown but we can suggest two possibilities. First, *H. pylori* may directly affect the differentiation of gastric epithelial cells and its eradication could modify this effect although we could find little evidence to support this possibility. Secondly, the appearance of normal epithelium was induced as a regenerative change against injured tumour tissue.

After eradication therapy, it seems likely that gastric acid output increases in patients with atrophic gastritis.[25] In the cases we examined, most patients showed atrophic changes in the corpus suggesting low acid output and recovery after eradication therapy. This may lead to surface injury of the tumour lesion and thus induce regenerative changes. Indeed, we found surface erosion on the tumour lesion in four cases after eradication therapy (data not shown). We also confirmed that the mucosal injury by gastric biopsy before endoscopic resection did not correlate with the appearance of normal foveolar epithelium.

Recently, it has been a topic of discussion as to whether eradication therapy of *H. pylori* influences the reduction of gastric carcinogenesis or not. Previously published data indicated a reduced rate of second cancer discovery in patients who received an endoscopic mucosal resection for the first cancer.[7] Recently, a Chinese group has published data that conflict with previous findings.[26] They demonstrated, with a randomized-controlled trial, that *H. pylori* eradication eliminated cancer incidence in patients with no precancerous lesions upon presentation compared with infected subjects. There was a concurrent 37% relative decrease in cancer incidence in the overall population, but this difference did not reach a level of statistical significance.[26] The only way to study the degree of gastric carcinogenensis is through endoscopic discovery. If eradication therapy itself has an influence on the morphological change of the gastric tumour, this therapy must have an influence on cancer discovery rate. In the present study, we demonstrated the flattened and indistinct appearance of the gastric tumour after eradication even after a short time. Generally, the morphological feature of elevation is the most important characteristic required to find out the gastric neoplasms. Even if the true incidence of cancer was not affected by eradication, the incidence of cancer discovery would be decreased by successful eradication therapy in cases where there is an elevated tumour feature. Moreover, the appearance of normal foveolar epithelium must make it difficult to detect the gastric cancer by endoscopic observation. This must contribute to the reduction in the rate of cancer discovery after successful eradication therapy.

Taken together, this is the first report that has described the typical morphological changes of gastric adenoma or carcinoma tissue over a short period. However, the question still remains as to why only a part of the tumour tissue showed these alterations. It should be clarified as to what is the typical appearance of a gastric tumour that has been affected by *H. pylori* eradication therapy. Moreover, it should also be discussed as to whether eradication therapy can truly diminish the occurrence of gastric cancer and reduce the gastric cancer induced mortality rate of the population.

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