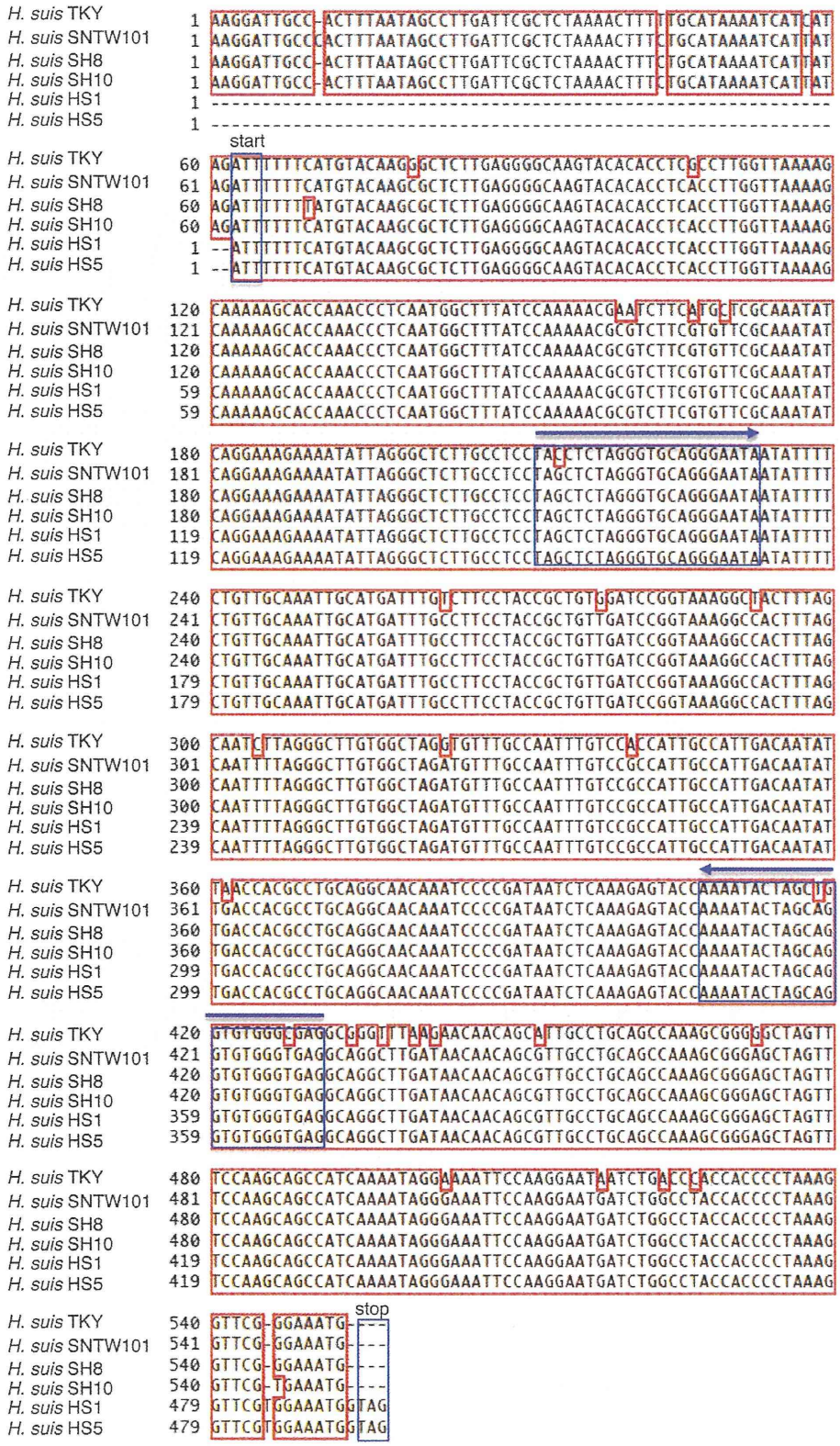
start
stop

Figure 4 Comparison of *Helicobacter suis*-specific arrangements of the *carR* gene. The results of parallel DNA sequencing of the *carR* gene among *H. suis* TKY, *H. suis* SNTW101, *H. suis* SH8, *H. suis* SH10, *H. suis* HS1 (EMBL/GenBank/DDBJ accession no. ADGY01000086.1, contig00086), and *H. suis* HS5 (EMBL/GenBank/DDBJ accession no. ADHO010000186.1, contig00186) are shown. The DNA sequences of *H. suis* TKY, SNTW101, SH8, and SH10 were determined after the PCR amplification using the primer pair carRUP/carRDW (Table 1). For the diagnostic PCR, the primer pair carR2F/carR2R was enclosed and indicated.

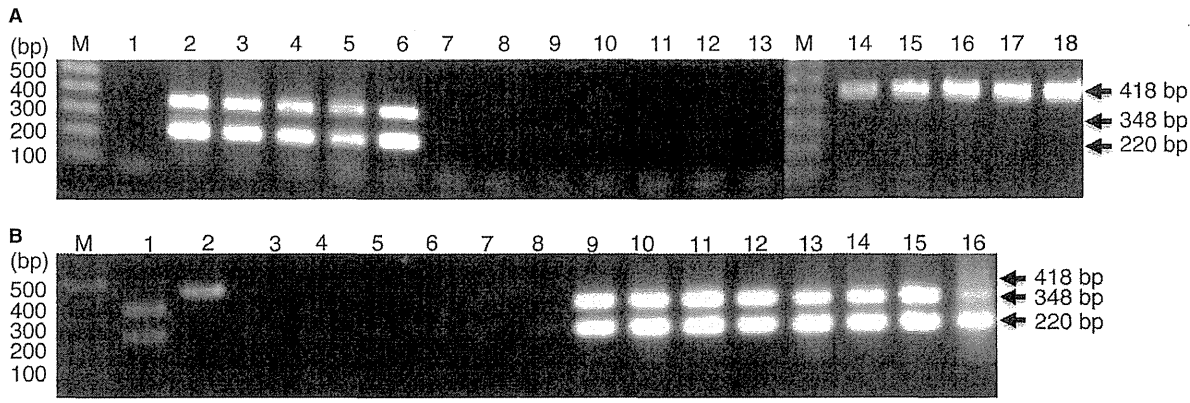

Figure 5 Evaluation of the PCR primers used to amplify the target DNA fragments from *Helicobacter suis*. (*A*) Lane M shows a 100-bp DNA ladder, and lanes 1–18 show the PCR products made from the template DNA, as follows; lane 1, uninfected mouse gastric mucosa (100 ng); lane 2, *H. suis* TKY-infected mouse gastric mucosa (100 ng); lane 3, *H. suis* SNTW101-infected mouse gastric mucosa (100 ng); lane 4, *H. suis* SH8-infected mouse gastric mucosa (100 ng); lane 5, SH10-infected mouse gastric mucosa (100 ng); lane 6, *H. suis* HS5 (10 ng); lane 7, *Helicobacter heilmannii* s.s. ASB1.4 (10 ng); lane 8, *Helicobacter felis* ATCC 49179 (10 ng); lane 9, *Helicobacter baculiformis* M50 (10 ng); lane 10, *Helicobacter bizzozeronii* R1051 (10 ng); lane 11, *Helicobacter cynogastricus* JKM4 (10 ng); lane 12, *Helicobacter mustelae* NCTC 12032 (10 ng); lane 13, *Helicobacter salomonis* R1053 (10 ng); lane 14, *Helicobacter pylori* SS1 (10 ng); lane 15, *H. pylori* TN2GF4 (10 ng); lane 16, *H. pylori* NCTC 11637 (10 ng); lane 17, *H. pylori* ATCC 43579 (10 ng); and lane 18, *H. pylori* RC-1 (10 ng). (*B*) Lane M shows a 100-bp DNA ladder, and lanes 1–16 show the PCR products made from the template DNA, as follows; lane 1, *H. suis* TKY-infected mouse gastric mucosa (100 ng); lane 2, *H. pylori* SS1 (10 ng); lanes 3–8, age-matched uninfected mouse gastric mucosa (100 ng); and lanes 9–16, *H. suis* SNTW101-infected mouse gastric mucosa (100 ng), 20 months post-inoculation. Arrows indicate the amplified DNA bands using the primer pairs VAC3624F/VAC4041R (418 bp, *vacA* gene of *H. pylori*), HH-F5/HH-R4 (348 bp, noncoding region of *H. suis*), and carR2F/carR2R (220 bp, *carR* gene of *H. suis*).

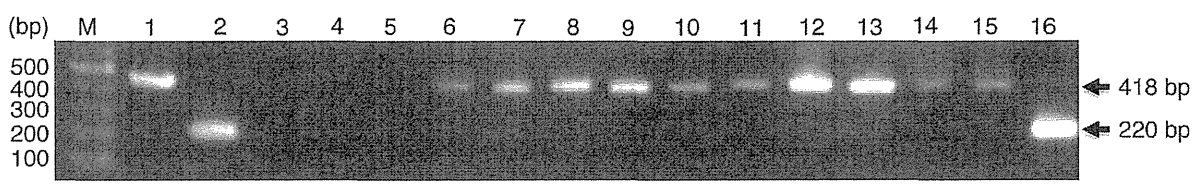

Figure 6 PCR detection of *Helicobacter suis* in gastric biopsy specimens. Diagnostic PCR detection of *Helicobacter* in gastric biopsy specimens of patients with nodular gastritis was carried out using the specific primer pairs Vac3624F/Vac4041R and carR2F/carR2R. Lane M shows a 100-bp DNA ladder, and lanes 1–16 show the PCR products made from the template DNA, as follows: lane 1, *Helicobacter pylori* SS1 (10 ng); lane 2, *H. suis* TKY-infected mouse gastric mucosa (100 ng); and lanes 3–16, prepared from gastric biopsy samples (100 ng). Arrows indicate the DNA bands amplified using the primer pairs VAC3624F/VAC4041R (418 bp) and carR2F/carR2R (220 bp).

U2235R [46]. Recently, a new quantitative real-time PCR method was developed to detect *H. suis* using the *ureA* primer pair of BF_HsuisF1 and BF_HsuisR1 [47]. Indeed, the target DNA fragments of *ureA* genes from *H. suis* strains TKY, SNTW101, SH8, and SH10 were amplified by this PCR system (data not shown). These results led to the conclusion that despite the presence of urease genes, the urease activity in stomach tissue was undetectable in *H. suis* SNTW101. Moreover, not only *H. suis* SNTW101 but also other urease-negative *H. suis* strains have been detected in gastric biopsy specimens from UBT-negative patients [9,16]. So far, there is no clear answer as to how urease-negative *H. suis* strains can colonize in the mouse and human stomachs.

We have been attempting to develop new PCR assay systems for the detection of *H. suis* in gastric biopsy

specimens using *H. suis*-specific primer pairs targeting genes other than 16S rRNA and urease genes. Comparative genomics of the four available *H. suis* genome sequences revealed that *H. suis* TKY, SNTW101, HS1, and HS5 had two promising target DNA sequences for the detection of *H. suis* by PCR amplification which the *H. pylori* strains did not carry (Figs 3 and 4). One was the conserved region of the *H. heilmannii* s.l. genome located within the *H. suis* HS1 contig00090 and HS5 contig00292. Therefore, we designed the *H. suis*-specific primer pairs (HH-F5/HH-R4). The other was a putative *carR* gene (carotenogenesis protein), and the most homologous gene was *carR* of *Azospirillum brasilense*, which codes for a positive regulator of a novel two-component regulatory system for the global expression of carbohydrate catabolic pathways [48]. Using the HH-F5/HH-R4 and carR2F/carR2R primer pairs, the targeting DNA fragments of *H. suis* strains were specifically amplified by PCR (Fig. 5A). Consequently, we also succeeded in detecting the *H. suis* infection in mouse stomachs by PCR using these primer pairs (Fig. 5B). Finally, we succeeded in specifically detecting the *H. pylori* and *H. suis* infections in gastric biopsy specimens by PCR using the VAC3624F/VAC4041R and carR2F/carR2R primer pairs, respectively (Fig. 6).

In the present study, there was a strong association between the gastric colonization of *H. suis* SNTW101 isolated from a patient suffering from nodular gastritis and the formation of gastric lymphoid follicles in the long-term evaluation of the mouse infection model (Fig. 1), whereas in the previous reports, the inoculation with gastric mucosal homogenates containing *H. suis* TKY induced gastric MALT lymphoma in a relatively short-term evaluation of a mouse model [25–28]. In addition, the infection with *H. suis* strains isolated from pigs caused gastric disorders in mice [49,50] and Mongolian gerbils [50]. To determine the difference in virulence among *H. suis* strains, further comparative genome analyses in addition to infection experiments will be needed.

Nucleotide Sequence Accession Numbers

The nucleotide sequences of the noncoding regions (about 590 bp) are available from the EMBL/GenBank/DDBJ database under accession nos. AB849018 (*H. suis* TKY), AB849019 (*H. suis* SNTW101), AB849020 (*H. suis* SH8), and AB849021 (*H. suis* SH10), and the nucleotide sequences of the *carR* genes (about 550 bp) are also available from the EMBL/GenBank/DDBJ database under accession nos. AB849014 (*H. suis* TKY), AB849015 (*H. suis* SNTW101), AB849016 (*H. suis* SH8), and AB849017 (*H. suis* SH10).

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Characteristic Epithelium with Low-Grade Atypia Appears on the Surface of Gastric Cancer after Successful *Helicobacter pylori* Eradication Therapy

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Keywords
Helicobacter pylori, eradication therapy, gastric cancer.

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Abstract

Background: The incidence of gastric cancer after successful *Helicobacter pylori* eradication has been increasing. We previously reported that epithelium with low-grade atypia (ELA) appeared on the surface of gastric cancer after *H. pylori* eradication. Here, we investigate the clinical and biological characteristics of such ELA.

Methods: We studied 27 cases of gastric cancer detected after successful *H. pylori* eradication therapy. We examined the prevalence of ELA among these cases and its significance for endoscopic discovery after *H. pylori* eradication. We additionally investigated the mucus, p53 and Ki67 expressions in ELA.

Results: Epithelium with low-grade atypia that continuous with the gastric tumor was detected in 22 of 27 cases (81%), a significantly greater percentage than that for controls ($p < 0.01$). We found that gastric-type mucin was frequently expressed in this epithelium. Neither p53- nor Ki67-positive cells were found in ELA, irrespective of their expression in tumor tissue. The presence of ELA was positively correlated with the clinical interval between *H. pylori* eradication and gastric cancer detection.

Conclusions: Epithelium with low-grade atypia on gastric cancer tissue, which may develop from gastric cancer cells, is frequently present after successful eradication therapy. This phenomenon could influence the practice of endoscopic diagnosis of gastric cancers.

The association between *Helicobacter pylori* infection and the development of gastric cancer is well established, based on both epidemiological [1,2] and experimental studies [3,4]. Compared to those not infected with *H. pylori*, the odds ratio for gastric cancer is more than 20-fold in persons infected with *H. pylori* [5]. A recent prospective study in Japan showed that the incidence of metachronous gastric cancer is reduced when *H. pylori* eradication therapy is provided following endoscopic resection of early gastric cancer [6]. Therefore, to reduce gastric cancer mortality, we need to focus on the efficacy of *H. pylori* eradication therapy. However, in clinical practice, it has been observed that gastric cancer can develop even after successful *H. pylori* eradication. Indeed, in our previous research, approximately 10% of patients with atrophic gastritis developed clinical gastric cancer following eradication therapy [7].

The form of gastric neoplasms is well known to be influenced by *H. pylori* eradication. For example, hyperplastic gastric polyps are less common following *H. pylori* eradication therapy [8]. Gotoda et al. reported that some gastric adenomas appeared to reduce or disappear after eradication therapy [9]. However, they also suggested that eradication of *H. pylori* could only mask gastric adenomas endoscopically, owing to changes around the gastric mucosa [9]. We previously reported that the macroscopic appearance of both gastric carcinoma and gastric adenoma was altered by eradication therapy [10]. In addition, we demonstrated that characteristic epithelium with low-grade atypia (ELA) often appeared on the surface of gastric cancer tumors that

were detected in patients after *H. pylori* eradication [10]. Along with the increasing use of *H. pylori* eradication therapy, the incidence of gastric cancer after *H. pylori* eradication has also been increasing. It is likely that the alteration brought about by such therapy may affect endoscopic detection of early-stage gastric cancer. This issue could have clinically importance for the post-eradication therapy diagnosis of gastric cancer.

In this study, we investigated the prevalence of ELA that was detected on tumor surfaces following *H. pylori* eradication therapy. We also examined the biological characteristics of this epithelium and discuss its pathogenesis. Further, we discuss the clinical significance of ELA on the endoscopic diagnosis of early gastric cancer among patients who have received successful eradication therapy.

Patients and Methods

Patients

We enrolled 33 consecutive patients with successful *H. pylori* eradication therapy (23 men; mean age: 65.1 years) who had been endoscopically diagnosed as having early gastric cancer at least 1 year after eradication. Our sample was selected from the population of patients who had undergone endoscopic resection at Hiroshima University Hospital between 1998 and 2012. Histopathologic examination was not possible in four cases, poorly differentiated adenocarcinoma was diagnosed in one case, and signet-ring cell carcinoma was diagnosed in one case. After excluding each of these cases, the 27 patients (21 men; mean age: 65.7 years) with differentiated early gastric cancer were enrolled in our study (the AE group). Only one patient in group AE showed synchronous gastric cancer (mucosal adenocarcinoma). Mean follow-up period after eradication was 56.0 months. Before receiving *H. pylori* eradication therapy, corpus atrophic gastritis was detected in all patients, and we confirmed that no neoplasms (including gastric adenoma and carcinoma) were detectable by endoscopic examination. Tumor histology was evaluated according to the Japanese Classification of Gastric Carcinoma, 14th edition [11]. The degree of endoscopic gastric mucosal atrophy was judged according to Kimura–Takemoto's classification [12]. *H. pylori* infection was determined by at least two of the following methods: Giemsa staining, the ^{13}C urea breath test (UBT; Otsuka UBiT-IR200, Tokushima, Japan), and tests for the presence serum IgG antibodies against *H. pylori* (E-plate, Eiken, Tokyo, Japan). Following eradication therapy, all patients received negative test results. To provide a control group, we registered 27 patients with *H. pylori*-positive gastric cancer, matched to the AE group in terms of age, sex, histologic type, and invasion depth. Patients with autoimmune diseases including pernicious anemia, previous gastrectomy, or severe hepatorenal dysfunction were excluded. Fasting sera were collected, and serum levels of gastrin and pepsinogens were evaluated [13]. The Ethics Committee of Hiroshima University approved our protocol.

Definition of Epithelium with Low-Grade Atypia on the Surface of Gastric Tumors

To evaluate ELA in each patient, we examined histologic sections of endoscopically resected gastric cancer tissue after staining with hematoxylin and eosin (HE). ELA was defined according to the following criteria: 1, ELA must lie on the surface of gastric cancer tissue; 2, ELA must be columnar epithelium with spindle or oval nuclei; 3, nuclear polarity must be present in the ELA; and 4, the ELA must be separated and distinguished from the surrounding non-neoplastic mucosa. We subclassified ELA into four grades according to extent: none (score 0), < 5% of the tumor length in the central section (score 1), < 30% of the tumor length (score 2), and more than 30% of the tumor length (score 3). Two specialists (MI and YK) independently judged the ELA score. When these scores did not agree, the final judgment was made unanimously following discussion of the particular case.

Immunohistochemistry

Sections with ELA on the gastric tumor tissue were subjected to immunohistochemical analysis. Immunohistochemistry was performed according to the method described by Sasaki et al. [14] with minor modifications. Four-micrometer sections of fixed tissues were deparaffinized and rehydrated. Endogenous peroxidase was quenched with 0.3% H_2O_2 in methanol for 10 min, followed by rinsing with phosphate-buffered saline (PBS, pH 7.2). Nonspecific binding was blocked with PBS containing 5% skim milk for 20 minutes. The sections were rinsed with PBS and incubated with primary antibodies overnight at 4 °C. Several antibodies were used: anti-human gastric mucin (HGM) monoclonal antibody (Novocastra, Newcastle, UK), anti-HIK antibody (KANTO Reagents, Tokyo, Japan) for gastric mucin, anti-MUC2 (Novocastra), antismall intestinal mucinous antibody for intestinal mucin (Novocastra), and anti-CDX2 antibody (ab76541, Abcam, Cambridge, UK). Previous report demonstrated the staining of HGM/HIK resembled Muc5ac/Muc6 expression pattern [15]. The mucus expression in each section was evaluated according to the methods that we have previously

reported [14]. Anti-p53 (DO7, Dako, Kyoto, Japan) and anti-Ki67 antibodies (MIB-1, Dako) were also used as previously described [10].

Statistical Analysis

All statistical analyses were performed using the Wilcoxon/Kruskal–Wallis test, *t*-test, and Fisher's exact test with JMP software (SAS Institute Inc., Cary, NC, USA). p values of < 0.05 were considered statistically significant.

Results

Clinical Features of Patients with ELA-positive Gastric Cancer

Of the 27 cases of differentiated early gastric cancer that were diagnosed following eradication, ELA (score ≥1) was detected in 22 cases (81%). More than half of gastric tumors in the AE group had an ELA score of 2 or 3, compared with only two cases in the control group. The extent of ELA was significantly higher in the AE group than in the control group (Table 1). A representative image of ELA is presented in Fig. 1. We evaluated the grade of atrophy by endoscopic examination as well as histologic grade of atrophy/intestinal metaplasia in the surrounding non-neoplastic mucosa. There was no difference in these grades between cases in AE group and control.

Mucus Expression Pattern in ELA

Mucus expression in ELA was examined by immunohistochemistry. Results are summarized in Table 2 and Fig. 2. To achieve high-quality staining, we examined 14 tumor sections that were resected by endoscopic submucosal dissection. HGM was specifically expressed in ELA tissue; we found that 71% of ELA samples expressed HGM. On the other hand, intestinal mucin (MUC2 or SIMA) was seldom found in ELA tissue, although eight underlying tumors (8/14; 57%) showed

Table 1 Prevalence of the ELA on gastric cancer after eradication compared with *Helicobacter pylori*-positive gastric cancer

	Grade of the ELA				
	0	1	2	3	
Gastric cancer after eradication	5	7	11	4	] **
Control*	17	8	2	0	] **

*Age, sex, histologic type, and invasion depth matched.
**$p < 0.01$, Wilcoxon/Kruskal–Wallis test.

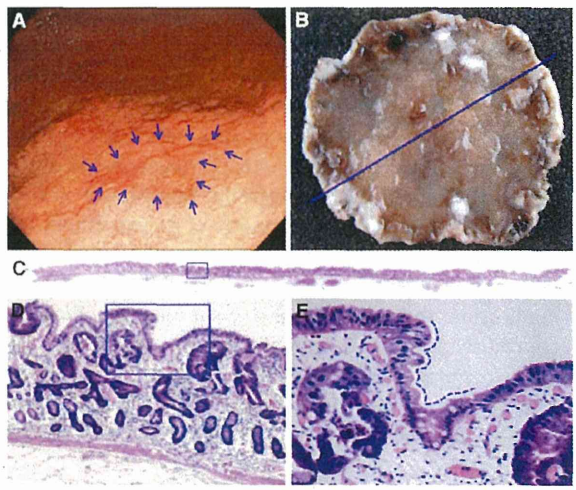

Figure 1 Representative image of ELA. An early gastric cancer (*A*) with superficial depressed type (surrounded by arrows) was treated by endoscopic submucosal dissection (*B*), and a microscopic section (*C*) was made. Microscopic features indicated by box in (*C*) revealed that epithelium with low-grade atypia covered gastric cancer tissue (*D*; ×50). ELA (box area in *D*) was highly magnified and was found as indicated by dotted line (*E*; ×200).

Table 2 Evaluation of the epithelium with low-grade atypia by immunohistochemical staining

	Prevalence of positive expression
HGM	10/14 (71%)
HIK	0/14 (0%)
MUC2	3/14 (21%)
SIMA	0/14 (0%)
CDX2	0/14 (0%)
p53	0/14 (0%)

intestinal differentiation. HGM expression in ELA correlated with neither the mucus pattern of tumor tissue nor that of peripheral non-neoplastic mucosa. Thus, HGM in ELA was found not only on gastric cancer tumors with gastric mucin expression, but also on gastric cancer tumors with the intestinal mucin phenotype. In addition, CDX2 expression was not detected in ELA. Neither p53- nor Ki67-positive cells were found in ELA, irrespective of their expression in tumor tissue (Fig. 3).

Relationship between ELA and Clinicopathological Features, Including Period between Eradication Therapy and Gastric Cancer Detection

Following the examination of mucus expression, we compared the clinicopathological features of high-ELA

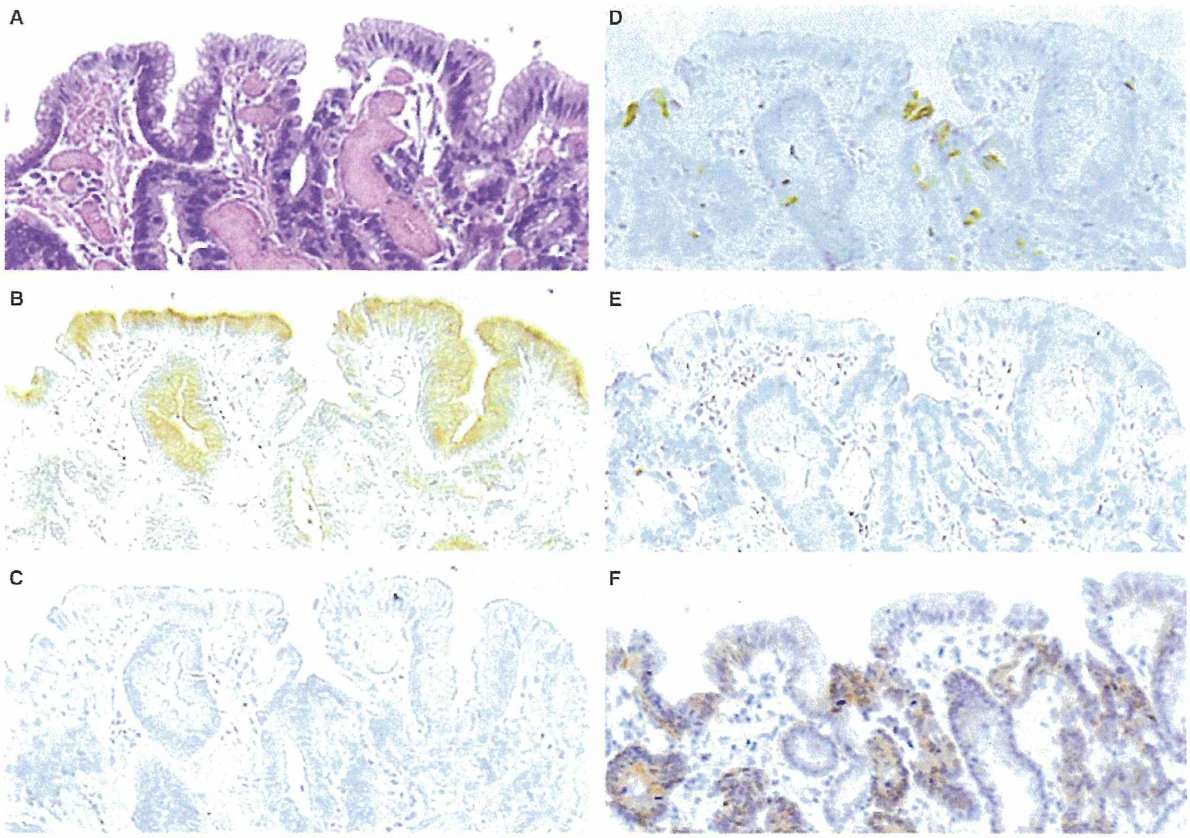

Figure 2 Microscopic feature of ELA and its mucus expression(magnification; ×200). (*A*) HE staining of ELA on gastric cancer tissue, and immunohistochemical staining of HGM (*B*), HIK (*C*), MUC2 (*D*), SIMA (*E*) and CDX2 (*F*).

(Score≥2) and low-ELA (Score≤1) patients with gastric cancer (Table 3). The two groups did not differ with regard to gender, age, location, macroscopic features, or degree of background atrophic gastritis. Additionally, levels of serum markers (gastrin and pepsinogens) did not differ between the two groups. We further examined the mean observation interval between eradication therapy and detection of gastric cancer. After successful eradication, 18 patients of 27 underwent annual endoscopy for cancer surveillance. On average, the interval between eradication therapy and detection of gastric cancer was 59.6 months (range: 12–188 months). We examined the correlation between the presence of ELA and the posteradication interval. As presented in Fig. 4, gastric cancer tumors without ELA tended to be discovered earlier than gastric cancer tumors with ELA. A positive correlation was found between the ELA grade and the duration of the detection interval ($y = 0.155x + 6.158$, $R = 0.448$). Three patients were diagnosed as gastric cancer with submucosal invasion at the time diagnosis. All three had ELA on gastric cancer tissue (grade 2 or 3) and needed long post-eradication interval.

Discussion

In this study, we demonstrated the specific appearance of ELA on gastric cancer tissue after the successful eradication of *H. pylori*. In a previous study, we reported the appearance of normal columnar epithelium on gastric tumors during a short-term follow-up period after eradication therapy [10]. In cases of gastric adenoma, normal foveolar epithelium was found to cover the adenoma tissue after successful eradication therapy; however, epithelium with mild atypia was observed in cases of adenocarcinoma [10]. Therefore, we used the term ELA, or epithelium with low-grade atypia, in this study.

We confirmed that, in clinical practice, ELA frequently appeared on gastric cancer tissue after successful eradication therapy. It is important to determine the

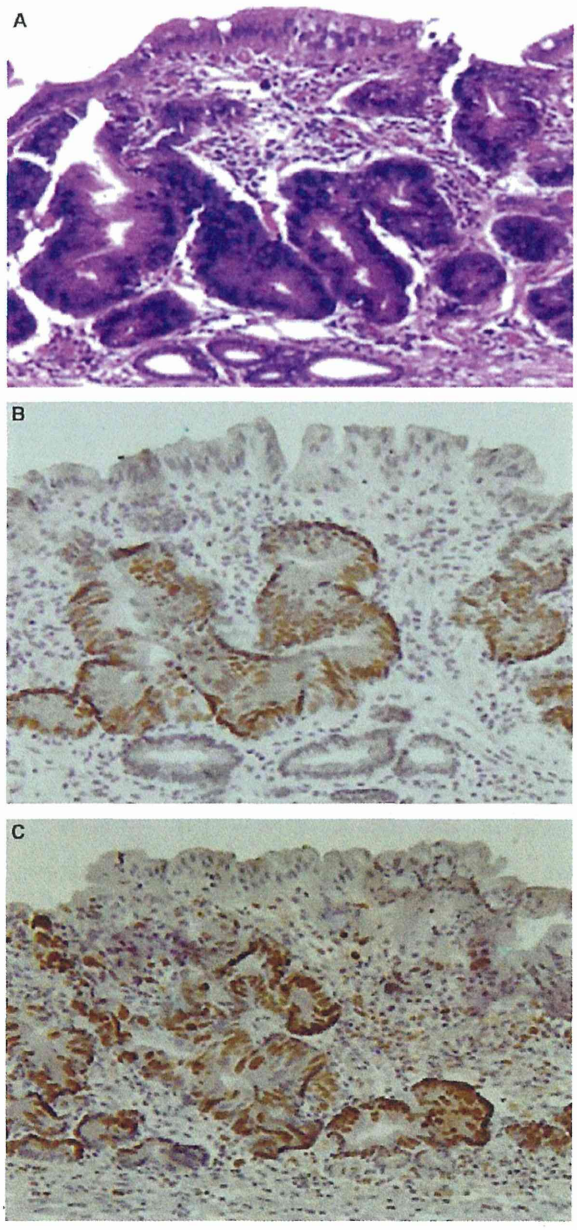

Figure 3 Microscopic feature of ELA and p53/Ki-67 expression (magnification; ×200). (*A*) HE staining of ELA on gastric cancer tissue, and immunohistochemical staining of p53 (*B*) and Ki-67 (*C*).

timing of gastric cancer development in our patients who received eradication therapy. We believe that gastric cancer had already developed prior to eradication therapy, because the follow-up period after eradication was relatively short (56 months). Early gastric cancer generally has a relatively long natural course [16], and Haruma et al.[17] previously reported that the gastric

Table 3 Clinicopathological features of high-ELA (Score≥2) and low-ELA (Score≤1) patients with gastric cancer

Prevalence of the ELA	Score≥2 n = 15	Score≤1 n = 12	*p*-value
Male/Female	13/2	8/4	N.S
Mean age (years)	66.6 ± 2.6	64.4 ± 2.9	N.S
Location (U/M/L)	3/8/4	1/8/3	N.S
Macroscopic type: (elevated/depressed)	2/13	3/9	N.S
Atrophy: (C1,2/C3,O1/O2,3)*	1/8/6	1/5/6	N.S
Gastrin (pg/ml)	126.0 ± 80.7	299.6 ± 94.2	N.S
PGI/PGII	4.98 ± 0.32	4.45 ± 0.38	N.S

*Endoscopic evaluation by Kimura–Takemoto's classification [12]

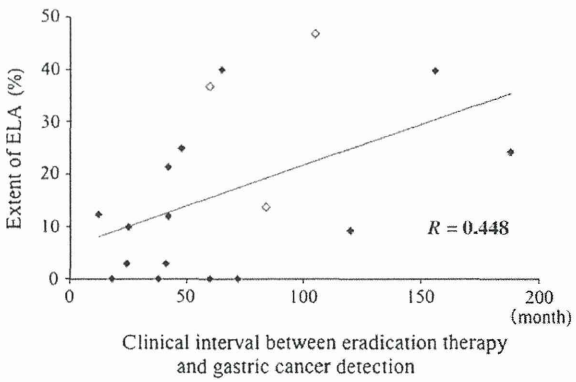

Figure 4 Relationship between ELA and clinical interval between eradication therapy and gastric cancer detection. We examined 18 patients who underwent annual endoscopy for cancer surveillance. Each square represents a patient with mucosal cancer (closed) or gastric cancer with submucosal invasion (open). $y = 0.155x + 6.158$, $R = 0.448$.

mucosal cancer doubling time was approximately 16.6 months. These findings suggest that the time interval between single-cell carcinogenesis and endoscopic diagnosis of a tumor, approximately 10 mm in diameter, is more than 10 years. In our cases, we suspect that ELA appeared on the surfaces of developed gastric cancers that were present but not detectable by endoscopic examination at the time of eradication.

The exact origin of ELA remains unclear. This epithelium had a spotty appearance on tumor tissue and was not continuous with the peripheral non-neoplastic mucosa, suggesting that ELA was not generated from the peripheral mucosa. On microscopic observation of hematoxylin and eosin-stained sections, ELA appeared to be continuous and transformed from neoplastic tissue, suggesting that ELA developed via tumor cell differentiation. Additionally, the characteristics of ELA

were different from those of adjacent tumor cells. Specifically, ELA-expressed gastric-type mucin did not overexpress p53 protein, and lacked proliferative ability, as determined by the absence of Ki67 labeling. These phenomena suggest that gastric tumor cells converted to their original characteristics, typical of mature gastric epithelium. Whether gastric carcinoma cells can reset malignant transformation has not been clarified. However, this situation appears to be similar to the improvements observed in gastric mucosal-associated lymphoid tissue lymphoma after eradication therapy [18]. Decreased levels of some growth factors secreted by inflammatory cells or improvements in stressful environments (such as oxidative stress) might play a role in this phenomenon [19]. Recently, by magnifying narrow-band imaging, Kobayashi et al.[20] reported the gastritis-like appearance of early gastric cancer detected after eradication therapy. The authors referred to this phenomenon as a "maturation" of gastric cancer tissue in patients in whom successful eradication was achieved; this interpretation seems to be consistent with our results of this study. As a next step, additional molecular analyses should be performed to clarify the origins of ELA.

It is important to ascertain whether the appearance of ELA influences the ease of early gastric cancer detected via endoscopic examination. As demonstrated in this study, when covered by ELA, gastric tumors could be detected by endoscopic examination after a relatively long period following eradication therapy. This result could be interpreted in two ways: first, the tumor surface was masked by ELA, making it difficult to detect gastric cancer by endoscopic examination or, second, the ELA appeared gradually over the long-term clinical course. However, because we have already demonstrated the appearance of ELA shortly after eradication therapy (1–2 months), the second hypothesis appears inadequate [10]. If the first hypothesis is correct, clinicians must pay greater attention to patients who have received successful eradication therapy during screening endoscopic examinations. Our systematic review suggested that approximately 10% of patients who had received eradication therapy were diagnosed at an advanced stage of gastric cancer [21]. A previous Japanese prospective study revealed that the prevalence of secondary gastric cancer was reduced when eradication therapy was administered after endoscopic resection of early gastric cancers [6]. It seems likely that eradication therapy could influence the growth rate of gastric cancer. Additionally, the appearance of ELA might mask the tumor surface and partly contribute to the perceived reduced prevalence of secondary gastric cancer.

Currently, the use of *H. pylori* eradication therapy is increasing worldwide. The Japanese government recently (February 2013) approved eradication therapy for patients with *H. pylori*-associated gastritis who are covered by the national health insurance system. Thus, all *H. pylori*-positive Japanese patients can receive eradication therapy after endoscopic examination. As our previous report regarding the rarity of *H. pylori*-negative gastric cancer [22], there has been no further discussion about decreases in the incidence of gastric cancer caused by *H. pylori* eradication. However, in addition to the beneficial aspects of eradication therapy, widespread eradication therapy could introduce another issue. Specifically, if all *H. pylori*-infected patients underwent eradication therapy, all gastric cancers would be classified as "gastric cancer after successful eradication," as was the case in the AE group of our study.

Whether posteradication cancer is similar to regular gastric cancer is still controversial. We previously reported that gastric cancer after eradication has several characteristic features. Specifically, we identified a depressed-type, differentiated histology; gastric-type mucin expression; and low Wnt5a expression [23]. Indeed, Yamamoto et al.[24] also reported high levels of gastric mucin expression in gastric cancers that were discovered after eradication therapy, thus suggesting the same pathophysiology for gastric mucin expression in ELA. The next important clinical issue for the cancer mass survey program in Japan might be the characterization of the biological behaviors of gastric cancers discovered after eradication. To effectively diagnose these new gastric cancers (which might have surface ELA), we must establish useful endoscopic examination methodologies.

In summary, we demonstrated that mature ELA on the surface of gastric cancer tumors is a characteristic feature in patients who have undergone successful eradication of *H. pylori* infection. The possibility that eradication therapy leads to gastric cancer cell differentiation deserves further study and clarification. As a next step, the clinical impact of the appearance of ELA should be examined and discussed in a larger study.

Acknowledgements and Disclosures

Competing interests: the authors have no competing interests.

Author Contributions

YK conducted all analysis and wrote the manuscript. MI was involved in the planning and execution of

study and the laboratory analysis, and advised on the manuscript. MY, OS, ST, and KC were involved in the planning and execution of study and advised on the manuscript. TM and TB advised on the planning of the study, analysis, and manuscript preparation.

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CLINICAL TRIALS STUDY

Value of a new stick-type rapid urine test for the diagnosis of *Helicobacter pylori* infection in the Vietnamese population

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Abstract

AIM: To assess the value of a new test for the diagnosis of *Helicobacter pylori* (*H. pylori*) infection, Rapirun® *H. pylori* Antibody Stick (Rapirun® Stick), in a Vietnamese population.

METHODS: Eligible patients without previous history of *H. pylori* eradication were recruited. Rapid urease test (RUT) and histologic examination were used to diagnose the *H. pylori* infection. Patients were considered *H. pylori* positive when the RUT results were positive and/or the bacteria were detected histologically. Rapirun® Stick tests were performed using urine samples, and the results were compared with the other 2 methods.

RESULTS: We enrolled 200 patients with a mean age of 36 (range, 18-76) years. There were 116 females and 84 males. Of the 200 patients, 111 (55.5%) were diagnosed as being *H. pylori* positive. The sensitivity, specificity, and accuracy of the Stick test were 84.7%, 89.9%, and 87.0%, respectively. There were 17 (8.5%) false-negative patients and 9 (4.5%) false-positive patients.

CONCLUSION: The Rapirun® Stick test has high sensitivity, specificity, and accuracy for the diagnosis of *H. pylori* infection in the Vietnamese population. The test can be clinically applied in Vietnamese populations.

Key words: *Helicobacter pylori*; Urine test; Rapirun® Stick; Vietnamese; Rapid urease test

Core tip: The Rapirun® *Helicobacter pylori* (*H. pylori*) Antibody Stick (Rapirun® Stick) has recently been developed to detect anti-*H. pylori* antibody in urine. This test requires fewer processing steps and provides quicker results. This study attempted to assess the value of this new test for the diagnosis of *H. pylori* infection in a Vietnamese population. The sensitivity, specificity, and accuracy of the Stick test were 84.7%, 89.9%, and 87.0%, respectively. The Rapirun® Stick test has high sensitivity, specificity, and accuracy for the diagnosis of *H. pylori* infection in the Vietnamese population. The test can be clinically applied in Vietnamese populations.

Quach DT, Hiyama T, Shimamoto F, Le QD, Ho LX, Vu NH, Yoshihara M, Uemura N. Value of a new stick-type rapid urine test for the diagnosis of *Helicobacter pylori* infection in the Vietnamese population. *World J Gastroenterol* 2014; 20(17): 5087-5091 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5087.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5087

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection plays an important role in the pathogenesis of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma[1]. Recent studies have demonstrated that a strategy to test and treat *H. pylori* in uninvestigated, dyspeptic patients in primary care is safe and reduces the need for endoscopy[2,3]. In addition, the indications to test and eradicate *H. pylori* have expanded even to subjects who do not have upper gastrointestinal symptoms, including first-class relatives of patients with gastric cancer and patients requiring long-term therapy with aspirin or non-steroidal anti-inflammatory drugs[4]. Therefore, there is an increasing need for non-invasive methods to diagnose *H. pylori* infection.

Several methods to diagnose *H. pylori* infection have been developed, among which the urea breath test (UBT) is currently regarded as the most accurate assay. However, the UBT is still expensive and not widely available in many countries, including Vietnam. An ideal non-invasive diagnostic test should be simple, inexpensive, rapid, and processed without special equipment and expertise but which delivers acceptably accuracy. A rapid urine test based on enzyme-linked immunosorbent assay (ELISA) has been developed for the detection of anti-*H. pylori* antibody in urine. One of these urine-based ELISA kits, the Rapirun® *H. pylori* Antibody Detection Kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), has been reported to have high sensitivity and specificity in several trials in different geographic areas, including Vietnam[5-11]. Recently, a new stick-type rapid urine test, Rapirun® *H. pylori* Antibody Stick (Rapirun® Stick) (Otsuka Pharmaceutical Co., Ltd.), has been developed that requires less labour and which provides results more rapidly than the conventional Rapirun® kit. It takes 15 min to evaluate the result with the Rapirun® Stick, whereas 20 min is required for the conventional Rapirun® kit. This method was reported to have an agreement rate of 98.4% compared with the conventional method in a Japanese population[12]. However, it has not been evaluated in other populations. This study therefore aimed to assess the value of the Rapirun® Stick test for the diagnosis of *H. pylori* infection in a Vietnamese population.

MATERIALS AND METHODS

Patient population

From October 2012 to December 2012, patients undergoing upper gastrointestinal endoscopy at the Department of Endoscopy, University Medical Center in Ho Chi Minh, Vietnam, were recruited. Exclusion criteria for the patients included those with a past history of *H. pylori* eradication therapy or previous gastric surgery and patients taking any type of antibiotics, H_2-receptor blockers, bismuth or proton pump inhibitors in the last 4 weeks before endoscopy. Informed written consent was obtained from all patients participating in the trial. This study was approved by the local ethics committee.

Gastric biopsies

During upper gastrointestinal endoscopy, endoscopic lesions were recorded. Three biopsies were taken from each patient: 2 for histologic examination and 1 for rapid urease test (RUT). The 2 biopsies for histological examination were taken from the greater curvature, one in the antrum and the other in the corpus, and were sent for Haematoxylin and Eosin and Giemsa staining. Tissue specimens were examined by an experienced pathologist (FS) who was blind to all clinical information.

The biopsy for RUT was taken from the greater curvature of the corpus, about 2 cm above the atrophic border. This biopsy location has been reported to optimise the sensitivity of the RUT to detect *H. pylori* in a Vietnamese population[13]. PyloriTek® (Serim Research Co., Elkhart, IN, United States) was used and the colour change was read within 1 h after incubation. This RUT has been validated in several previous studies and has shown very high sensitivity and specificity (90%-98.5% and 97%-100%, respectively)[14-17].

Rapirun® *H. pylori* Antibody Stick test

After endoscopy, urine samples were collected and were processed within 1 h of collection for the detection of antibodies against *H. pylori* using the Rapirun® Stick. The test measures human immunoglobulin G (IgG) antibodies against *H. pylori* in urine using the principle of immunochromatography.

The antigen used is a crude extract from a clinically isolated *H. pylori* strain, the OHPC-040 strain, taken from a Japanese patient with chronic gastritis. A previous report demonstrated that OHPC-040 was the most suitable isolate to detect urinary antibodies to *H. pylori* among 20 clinical isolates extracted from patients with disorders of the upper digestive tract and that OHPC-040 was positive for the *vacA*, *ureB*, and *cagA* genes based on the results of DNA analysis[18]. The test stick contains colloidal gold-conjugated anti-human IgG (Fc) polyclonal antibody (goat). The test line and control line in the evaluation section of the stick are immobilised with *H. pylori* antigen and anti-human IgG polyclonal antibody, respectively.

The Rapirun® Stick test procedure consisted of 2 steps: (1) taking approximately 0.3 mL of the urine sample, as indicated by the measurement guide on the pipette included in the kit, then adding it to a container holding the sample diluent (0.3 mL) and mixing them (an approximately 2-fold dilution); and (2) standing a test stick in the container that holds the mixture of urine and diluent (as described above) with the sample absorption section of the test stick submerged in the diluted sample. After leaving the kit undisturbed for 15 min at room temperature (25 ℃-30 ℃), we then confirmed visually whether any red lines appeared in the evaluation section. The appearance of 2 distinct red bands (one control and one test line) indicates a positive test (Figure 1). The appearance of the control line only indicates a negative result. The absence of a control line indicates an invalid result.

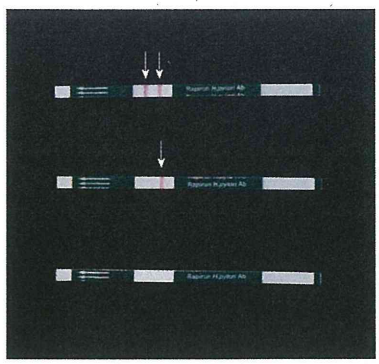

Figure 1 Rapirun® *Helicobacter pylori* Antibody Stick. The sample is considered positive when 2 red bands at the test line and control line (arrows) are observed 15 min later and is considered negative when only the control line is observed. The absence of a control line indicates an invalid result.

Table 1 Demographic characteristics and clinical diagnostics of the patients evaluated

Characteristics	*H. pylori* positive (n = 111)	*H. pylori* negative (n = 89)	Total (n = 200)
Mean age (range) (yr)	35.5 (18-62)	36.6 (18-76)	36 (18-76)
Sex (female/male)	59/52	57/32	116/84
Diagnosis			
Normal gastroduodenal tract	1	6	7
Gastritis and/or duodenitis	83	66	149
Gastric ulcer	4	1	5
Duodenal ulcer	16	1	17
Reflux esophagitis	5	15	20
Reflux esophagitis and peptic ulcer disease	2	0	2

H. pylori: *Helicobacter pylori*.

Definition of H. pylori infection

The results of the RUT and Rapirun® Stick test were read by different researchers who were not aware of the results of the other methods used to diagnose *H. pylori* infection. The definition of *H. pylori* infection in this study required at least one positive test of 2 tests, the RUT and histologic examination. Absence of *H. pylori* infection required both of these tests to be negative. Equivocal tests were excluded from the analysis.

Statistical analysis

Analysis to determine the sensitivity, specificity, positive and negative predictive values, and accuracy of the Rapirun® Stick test was performed with SPSS software for Windows, version 20 (SPSS Inc., Chicago, IL, United States).

RESULTS

We recruited 200 patients in this study. The quality of gastric biopsies for histologic examination to detect *H. pylori* was excellent in all patients. There were no invalid results with the Rapirun® Stick; therefore, we included data from all 200 patients in the analysis.

Table 2 Diagnostic accuracy of Rapirun® Stick test

Rapirun® Stick test	*H. pylori* infection status	
	Positive	Negative
Positive (103)	94	9
Negative (97)	17	80
Total	111	89

Sensitivity, 84.7% (94/111); specificity, 89.9% (80/89); positive predictive value, 91.2% (94/103); negative predictive value, 82.5% (80/97), and accuracy, 87.0% [(94 + 80)/(111 + 89)]. *H. pylori*: *Helicobacter pylori*.

All patients were ethnic Vietnamese. The demographic characteristics of the patients are indicated in Table 1. The mean age of the patients was 36 (range, 18-76) years. There were 116 (58.0%) females and 84 (42.0%) males.

Of the 200 patients, 111 (55.5%) were diagnosed as being *H. pylori* positive: among them, 16 (14.4%) had duodenal ulcer, 5 (4.5%) had reflux esophagitis, 4 (3.6%) had gastric ulcer, and 2 (1.8%) had both gastro-duodenal ulcer and reflux esophagitis. Eighty-nine (44.5%) patients were *H. pylori* negative: among them, only one (1.1%) had gastric ulcer and one (1.1%) had duodenal ulcer, whereas 15 (16.9%) had reflux esophagitis. Of the 24 patients with gastro-duodenal ulcer, 22 (91.7%) had *H. pylori* infection. However, 7 of 22 (31.8%) patients with reflux esophagitis also had the infection.

The sensitivity, specificity, positive and negative predictive values, and accuracy of the Rapirun® Stick test were 84.7%, 89.9%, 91.2%, 82.5%, and 87.0%, respectively (Table 2). There were 17 (8.5%) false-negative patients including 3 with duodenal ulcer, 1 with reflux esophagitis, and 13 with gastritis/duodenitis. Among them, 14 had both a positive RUT and positive histologic examination, 2 had only a positive histologic result, and 1 had only a positive RUT result. There were 9 (4.5%) false-positive patients including 1 patient with reflux esophagitis and 8 with gastritis/duodenitis.

DISCUSSION

To our knowledge, this study is the first to determine the validity of the Rapirun® Stick in a Vietnamese population. The assay is noninvasive, easy to handle, and the cost of using urine as a sample is low. The test can be clinically applied in populations of developing countries such as Vietnam.

Vietnam is one of the countries with a high prevalence of gastric cancer. The mortality rate for gastric cancer is 18.6/100000 for males and 8.4/100000 for females[19]. Among cancer deaths in Vietnam, gastric cancer is the second leading cause followed by lung cancer for males, and fourth, followed by breast, cervix, uterine, and colorectal cancer, for females during 2006 and 2007[20]. The reason of the high mortality from gastric cancer may mainly be the high prevalence of infection from *H. pylori*, a definite carcinogen of gastric cancer. *H. pylori* infection was detected in 65.6% of the hospital-based population (mean age, 42.5 years)[21].

To reduce the incidence of gastric cancer in Vietnam, a nationwide *H. pylori* eradication treatment may be recommendable because *H. pylori* has been regarded as a definite carcinogen, and several studies have shown that its eradication reduces the incidence of gastric cancer development[22,23].

To carry out *H. pylori* eradication treatment, a simple, low-cost, and accurate method is needed to diagnose the infection. There are various methods to detect the infection so far: RUT, bacteriologic culture, histologic examination, UBT, serum antibody assay, and detection of anti-*H. pylori* antibody in urine and *H. pylori* antigen in stool. RUT, bacteriologic culture, and histologic examination require endoscopic biopsy. UBT is regarded as the most accurate assay; however, it requires special apparatus and is expensive to perform. If sensitive screening for *H. pylori* infection were possible using urine samples, it would not only be more convenient in clinical practice but would also be very useful for mass screening. The Rapirun® Stick, a newly developed detection kit for anti-*H. pylori* antibody in urine, is very simple and requires only 15 min to complete. Furthermore, the test does not require technical expertise, special sample handling, or any additional equipment and thus allows considerable savings of diagnosis-related costs. The kit is a candidate test method that would be applicable for use with the Vietnamese population.

The sensitivity, specificity, and accuracy of the conventional Rapirun® kit in a Vietnamese population were reported to be 79.5%, 90.7%, and 84.5%, respectively[11]. In the present study, the sensitivity, specificity, and accuracy of the new Rapirun® Stick test, were 84.7%, 89.9%, and 87.0%, respectively. The values are relatively better in the present study compared with the study using the conventional Rapirun® kit. This may be due to the difference in the populations tested and the methods used to investigate *H. pylori* infection: bacterial culture, histologic examination, and serum ELISA in the study of the conventional Rapirun® kit, and RUT and histologic examination in the present study. Although the antigen used in the Rapirun® Stick is a crude extract from a clinically isolated *H. pylori* strain taken from a Japanese patient, our study clearly demonstrates the usefulness of the Rapirun® Stick in the Vietnamese population. This is truly the first report on the usefulness of the kit external to the Japanese population.

In the present study, 8.5% were false-negative patients. This may be due to the *H. pylori* polymorphism, the host factors in different geographic areas, and the extremely low level of anti-*H. pylori*-specific IgG in the urine of the patients. In contrast, 4.5% were false-positive patients. Graham *et al*[9] reported that 2 patients who had been treated for *H. pylori* infection more than 32 and 42 mo previously, respectively, had positive Rapirun® test results, suggesting that the urine test results may remain positive for an extended time after successful cure of the infection. *H. pylori* in our false-positive patients might have been eradicated intentionally or unintentionally. The reasons for the incidence of the false-positive and false-negative results should be investigated to improve the sensitivity, specificity, and accuracy of the kit.

Evaluation of the diagnostic performance of the conventional Rapirun® kit in various countries, including Japan, Taiwan, South Korea, Vietnam, United States, and European countries (Austria, France, Germany, and Italy), showed a sensitivity of 77.4%-96.7%, specificity of 83.3%-97.4%, and accuracy of 80.4%-96.1%[11,24]. The present study showed high sensitivity, specificity, and accuracy for the new Rapirun® Stick. In addition, the Rapirun® Stick has been reported to have an agreement rate of 98.4% compared with the conventional Rapirun® kit in a Japanese population[12]. Therefore, the Rapirun® Stick can be applicable in many countries, at least in the abovementioned countries.

There are several limitations in the present study. First, the patients were enrolled in only one hospital in Ho Chi Minh, in southern Vietnam. There are reports showing differences in the prevalence of gastrointestinal diseases such as peptic ulcer and gastric cancer and of *vacA*-positive *H. pylori* between Hanoi, in northern Vietnam, and Ho Chi Minh[19]. Therefore, the study population may not be representative of the entire Vietnamese population. Second, RUT and histologic examination were used to diagnose the infection in the present study. In several patients, these methods produced false-negative or false-positive results, leading to the possible misdiagnosis of *H. pylori* infection.

In conclusion, we demonstrated the usefulness of the Rapirun® Stick test for the diagnosis of *H. pylori* infection in a Vietnamese population: the sensitivity, specificity, and accuracy of the Rapirun® Stick test were high. The test can be clinically applied in Vietnamese populations.

COMMENTS

Background

The Rapirun® *Helicobacter pylori* (*H. pylori*) Antibody Stick (Rapirun® Stick) has recently been developed to detect anti-*H. pylori* antibody in urine. This test requires fewer processing steps and provides quicker results. This study attempted to assess the value of this new test for the diagnosis of *H. pylori* infection in a Vietnamese population.

Research frontiers

The Rapirun® Stick was reported to have an agreement rate of 98.4% compared with the conventional method in a Japanese population. However, it has not been evaluated in other populations.

Innovations and breakthroughs

This study is the first to determine the validity of the Rapirun® Stick in a Vietnamese population. The assay is noninvasive, easy to handle, and the cost of using urine as a sample is low.

Applications

The Rapirun® Stick can be clinically applied in populations of developing countries such as Vietnam.

Terminology

Rapid urease test is a rapid test for diagnosis of *H. pylori*. The basis of the test is the ability of *H. pylori* to secrete the urease enzyme, which catalyzes the conversion of urea to ammonia and carbon dioxide.

Peer review

The authors examined the value of new test for the diagnosis of *H. pylori* infection, Rapirun® Stick, in a Vietnamese population. The Stick test has high sensitivity, specificity, and accuracy for the diagnosis. The results are interesting, and suggest that the test can be clinically applied in Vietnamese populations.

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A Computer System to Be Used With Laser-based Endoscopy for Quantitative Diagnosis of Early Gastric Cancer

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Goals: To evaluate the usefulness of a newly devised computer system for use with laser-based endoscopy in differentiating between early gastric cancer, reddened lesions, and surrounding tissue.

Background: Narrow-band imaging based on laser light illumination has come into recent use. We devised a support vector machine (SVM)-based analysis system to be used with the newly devised endoscopy system to quantitatively identify gastric cancer on images obtained by magnifying endoscopy with blue-laser imaging (BLI). We evaluated the usefulness of the computer system in combination with the new endoscopy system.

Study: We evaluated the system as applied to 100 consecutive early gastric cancers in 95 patients examined by BLI magnification at Hiroshima University Hospital. We produced a set of images from the 100 early gastric cancers; 40 flat or slightly depressed, small, reddened lesions; and surrounding tissues, and we attempted to identify gastric cancer, reddened lesions, and surrounding tissue quantitatively.

Results: The average SVM output value was 0.846 ± 0.220 for cancerous lesions, 0.381 ± 0.349 for reddened lesions, and 0.219 ± 0.277 for surrounding tissue, with the SVM output value for cancerous lesions being significantly greater than that for reddened lesions or surrounding tissue. The average SVM output value for differentiated-type cancer was 0.840 ± 0.207 and for undifferentiated-type cancer was 0.865 ± 0.259.

Conclusions: Although further development is needed, we conclude that our computer-based analysis system used with BLI will identify gastric cancers quantitatively.

Key Words: laser-based endoscopy, early gastric cancer, magnifying endoscopy, quantitative analysis

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The authors declare that they have nothing to disclose.

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Features of the microvascular architecture of gastric lesions can be observed under magnifying endoscopy,[1,2] and it has been reported that an irregular microvascular pattern is most useful for distinguishing between gastric cancer and gastritis. Narrow-band imaging (NBI) combined with magnifying endoscopy clearly visualizes the microanatomy and is a reliable imaging technique for characterization and delineation of early gastric cancer.[3]

Intraobserver and interobserver variability is a problem, however, for diagnosis of gastric cancer under magnifying endoscopy. Interpretation of endoscopic findings remains subjective and can vary between individual endoscopists, especially novices. Although clinical training coupled with experience is a realistic approach to predicting histologic diagnoses, a better approach would be objective evaluation, such as computer-aided evaluation. This would allow nonexperts to achieve high diagnostic accuracy. The computer output could also be used as a "second opinion" to avoid oversights during gastrointestinal endoscopy and to assist endoscopists in decision-making and thus avoid unnecessary biopsy.

A new endoscopy system with a light source that makes use of 2 semiconductor lasers has come into recent use. This system allows for the capture of both white-light images and narrow-band light images, that is, blue-laser imaging (BLI), without a customized optical filter typically used for NBI.

We recently devised a software program that can quantitatively identify gastric cancers on images obtained by magnifying endoscopy with BLI. We have attempted to identify flat or slightly depressed, small (< 10 mm), benign reddened gastric lesions, which are difficult to distinguish from cancer by conventional gastrointestinal endoscopy. In this study, we evaluated reliability of the software program for distinguishing cancerous lesions, reddened lesions, and surrounding tissue.

MATERIALS AND METHODS

New Endoscopy System

The new endoscopy system, LASEREO (Fujifilm Medical Co. Ltd., Tokyo, Japan), consists of an LL-4450 light source, a VP-4450HD video processor, and any of a special series of scopes. The LL-4450 light source provides illumination through 2 different lasers with wavelengths of 410 (410 ± 10) and 450 (450 ± 10) nm. The 450-nm-wavelength laser excites the white-light phosphor and produces fluorescent light for standard observations. The 410-nm-

wavelength laser is for BLI, which functions as narrow-band imaging. The lighting setup provides for 3 observation modes (BLI mode, BLI-bright mode, and white-light mode), which can be selected by adjusting the intensity of the 2 lasers. The BLI light is a combination of strong 410-nm laser light, weak 450-nm laser light, and fluorescent light. The BLI-bright light is a combination of strong 410-nm laser light, 450-nm laser light that is stronger than the 450-nm light used for BLI mode, and fluorescent light. The white light is a combination of weak 410-nm laser light, strong 450-nm laser light, and fluorescent light (Fig. 1).

Quantitative Analysis System

For computer-based quantitative analysis of gastric lesions, we apply a newly devised software program that identifies features of endoscopic images and quantifies each image according to corresponding features on training images. We use a bag-of-features representation, wherein an image is represented by a histogram of visual words, which are produced by hierarchical k-means clustering of local features regardless of their location. Bag-of-features representation is a popular technique for image classification inspired by models used in natural language processing. It ignores or downplays word arrangement and classifies on the basis of a histogram of the frequency of visual words. A visual word is a small patch on an image that carries some kind of information related to the features. The analysis system, which has been previously described,[4–6] uses densely sampled scale-invariant feature transform (SIFT) descriptors in a bag-of-features framework. SIFT is an algorithm used to detect and describe local features in images. Densely sampled SIFT descriptors are used as local features. Clustering is performed over all training images to generate k clusters (these clusters are called visual words). SIFT descriptors are then computed at points on a regular grid with dense spacing and also at 2 different scales of local patches centered at each grid point. All descriptors of 128 dimensions are used by the clustering. A regular grid is used because the texture in each BLI image fills the whole image. A support vector machine (SVM) with a linear kernel is used as the classifier.

The recognition process with a bag-of-features is illustrated in Figure 2. Local features are extracted from images and then clustered by means of vector quantization to produce histograms of visual words.

We calculated, with the use of an SVM and logistic regression, the SVM output value for gastric cancer on the validation images.

The simple logistic function was defined by the formula

$$P(t) = 1/(1+e^{-t}),$$

where P denotes the SVM output value for gastric cancer, t denotes the distance from the boundary line for a cancer or surrounding tissue on the training image, and e is the exponential function. The SVM is used for discriminating 2 classes and generating the boundary line that maximizes the distance between the hyperplane and the nearest sample.[7]

Lesions and Endoscopic Procedure

We produced a set of validation images from among images of 100 early gastric cancers (21 superficial elevated-type and 79 superficial depressed-type; 75 differentiated-type and 25 undifferentiated-type) in 95 consecutive patients (73 men, 22 women; mean age, 68.4 y) who were examined by BLI magnification with use of the new video endoscopy system. The VS classifications[3] of the 100 early

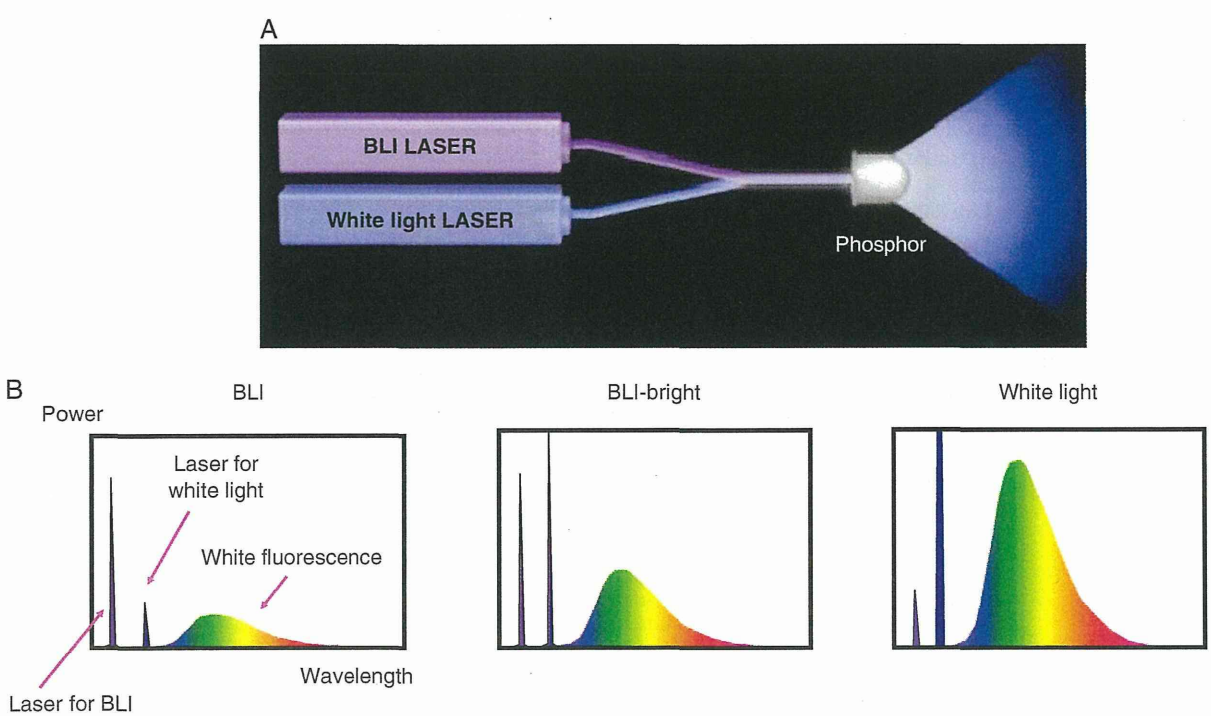

FIGURE 1. A, The LASEREO endoscopy system provides illumination through 2 types of semiconductor lasers and phosphor for white light. One laser (450 nm) is used for white-light imaging, and the other is for blue-laser imaging (BLI) (410 nm). B, Theory of BLI. The combination of a laser for BLI (purple bar), a laser for white light (blue bar), and white fluorescence (rainbow-colored region) provides for BLI, BLI-bright, and white-light imaging. full color online

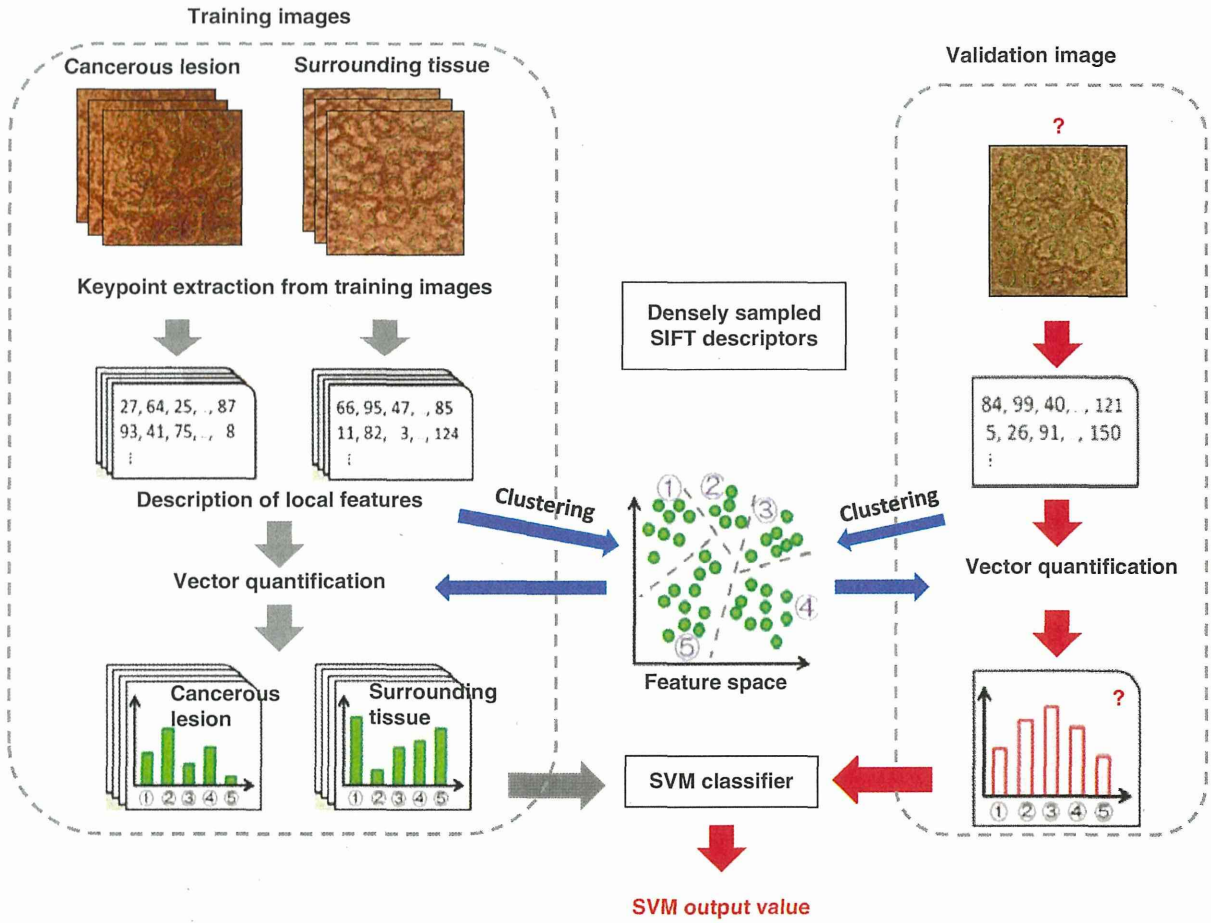

FIGURE 2. Flow diagram of classification by our system. The system uses densely sampled scale-invariant feature transform descriptors in a bag-of-features framework. Images are represented by bag-of-features and then classified by a support vector machine. SIFT indicates scale-invariant feature transform; SVM, support vector machine. full color online

gastric cancers observed under magnifying endoscopy with BLI are shown in Table 1. All examinations were performed at Hiroshima University Hospital between August 2011 and May 2012. When a lesion was found during nonmagnifying observation with white-light imaging, a zoom image up to five sevenths maximum magnification

TABLE 1. Features of the Early Gastric Cancers Depicted by the New Endoscopy System and Categorized According to the VS Classification System*

VS Classification			Demarcation Line	
V Pattern	S Pattern	N (%)	Present [N (%)]	Absent [N (%)]
Regular	Regular	1 (1.0)	0 (0.0)	1 (1.0)
Regular	Irregular	0 (0.0)	0 (0.0)	0 (0.0)
Regular	Absent	0 (0.0)	0 (0.0)	0 (0.0)
Irregular	Regular	4 (4.0)	4 (4.0)	0 (0.0)
Irregular	Irregular	82 (82.0)	82 (82.0)	0 (0.0)
Irregular	Absent	13 (13.0)	13 (13.0)	0 (0.0)
Absent	Regular	0 (0.0)	0 (0.0)	0 (0.0)
Absent	Irregular	0 (0.0)	0 (0.0)	0 (0.0)
Absent	Absent	0 (0.0)	0 (0.0)	0 (0.0)
Total		100 (100)	99 (99.0)	1 (1.0)

Microvascular patterns: regular = mucosal capillaries consistent in size, with regular and symmetrical arrangement and distribution; irregular = vessels varied size, with irregular and asymmetrical arrangement and distribution; absent = a subepithelial microvascular pattern obscured by the presence of an opaque substance, within the superficial part of the mucosa.

Surface patterns: regular = crypt epithelium is constant in width and length, with regular and symmetrical arrangement and distribution; irregular = crypt epithelium varies in width and length, with irregular and asymmetrical arrangement; absent = no epithelial structure visible upon magnifying endoscopy.

*The VS classification system[3] is based on microvascular (V) and microsurface (S) patterns, which are classified as regular, irregular, or absent.