Differences in Regression Patterns of Complete and Incomplete Intestinal Metaplasia at Ten Years after Helicobacter pylori Eradication

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This study was conducted to reveal the reversibility of subtype of intestinal metaplasia (IM) and Paneth cells after H. pylori eradication (HPE). Among 75 patients, we retrospectively examined the proportions of patients with complete type of IM (CIM), incomplete type of IM (IIM) and Paneth cells in their biopsy specimens obtained from the greater curvature of the antrum (A2) and the greater curvature of the middle corpus (B2) before and during a follow-up period of 10 years after HPE. Immunohistochemistry was used to determine IM type. Compared to before HPE, the proportion of patients with CIM did not decrease significantly during the 10-year follow-up after HPE both in A2 (32% vs. 21.3%, P = 0.13) and in B2 (6.7% vs. 2.7%, P = 0.60). IIM rates in A2 was significantly lower during this time (26.7% vs. 10.7%, P = 0.04), whereas no patients showed IIM in B2 before HPE. The proportion of patients with Paneth cells decreased significantly in A2 after 3, 8, and 9 years of HPE and in B2 after 4, 6 and 9 years of HPE (P < 0.05 for all). Thus, IIM and Paneth cells regressed during a period of 10 years after HPE.

Key words: intestinal metaplasia, Helicobacter pylori, gastric cancer

I. Introduction

Helicobacter pylori (H. pylori) infection is a major etiological factor of gastric cancer (GC) as infection causes atrophy and intestinal metaplasia (IM) in the gastric mucosa and these are considered precancerous or paracancerous lesions [8, 15, 23, 35]. IM is the most common type of gastric metaplasia wherein small or large intestinal mucosa with goblet cells is seen in the places where the gastric mucosa should have existed [16]. IM is classified as either complete IM (CIM) or incomplete IM (IIM) [33], and some studies have reported on the differences between CIM and IIM with respect to the risk of developing GC—specifically, that IIM is regarded as a background of GC [7, 11, 33].

CIM has a brush border appearance and is described as solely intestinal type IM [32], along with the presence of Paneth cells [12, 27], which contribute to appropriate immune responses and homeostasis in the small intestine [26, 38]. In contrast, IIM is characterized by the presence of the gastric foveolar epithelium and pyloric-type mucin, and is, thus, described as the mixed gastric and intestinal type IM [27, 32, 33]. IIM is thought to be a paracancerous lesion because it is found around GC [33]. Previously, IM was classified as type I (CIM) and type II/III (IIM) based on Alcian Blue-periodic acid Schiff staining [10]; however, recently, a new classification using immunohistochemical markers has been proposed in which the brush border of CIM glands is stained with CD10 [33], while IIM glands are stained with MUC5AC or MUC6 because of the pres-
ence of foveolar epithelium and pyloric-type mucin, respectively [25, 33]. Assessing IM subtype is a difficult task and it becomes easier by immunohistochemical staining.

We and others have previously reported that IM is less likely to regress, compared to atrophy, during a period of 10 years after H. pylori eradication (HPE) [14, 20, 21, 31]. However, few reports have addressed the reversibility of IM after HPE, especially after classifying IM as CIM or IIM. Furthermore, the role of Paneth cells in the stomach and their reversibility after HPE remain unclear.

Therefore, the present study examined the reversibility of CIM, IIM, and Paneth cells in IM, both before HPE and during a follow-up period of 10 years after HPE, by histopathological evaluation, and the aims of the study were to define the risk factors for GC development after HPE to identify high-risk patients.

II. Methods

Subjects and study design

We have collected biopsy specimens of patients who were suspected of H. pylori infection at the Oita University Hospital, Japan. If patients accepted to be taken biopsy specimens, they underwent esophagogastroduodenoscopy and they were taken specimens from the greater curvature of the antrum and he greater curvature of the middle corpus. Among 4229 patients who were taken specimens between January 1993 and April 2020 in our institution, we retrospectively reviewed biopsy specimens from 75 patients who were taken biopsy specimens both before HPE and after 10 years of HPE (Fig. 1). Of these 75 patients, 45 were male and 30 were female. Their age before HPE ranged from 28 to 76 years (mean ± SD, 58.6 ± 8.8 years). In addition to before HPE and after 10 years of HPE, they were also taken biopsy specimens at 6 months after HPE and once every year after HPE during a 10-year follow-up period after HPE. However, a few biopsy specimens at 6 months after HPE and between 1–9 years after HPE could not be obtained in some patients.

After providing informed consent, patients underwent a histopathological examination, culture testing, and the rapid urease test (RUT) to identify the presence of H. pylori. Patients with H. pylori infection received HPE therapy that comprised a proton pump inhibitor, amoxicillin, and clarithromycin or metronidazole. After 4 weeks of HPE, a urea breath test (UBT) was performed and patients testing negative were deemed to have been successful in HPE. All 75 patients included in this study had successfully completed HPE and reinfected patients were not included in this study.

Clinical features of all 75 patients

We examined patients’ sex, age, background diseases and endoscopic atrophy to investigate their background factors. Endoscopic gastric atrophy was evaluated with the Kimura-Takemoto classification [13]. Briefly, the grade of endoscopic gastritis atrophy was classified into 7 grades: C0, no atrophy; C1, atrophic change limited to the antrum; C2, atrophic change extending to the distal corpus; C3, atrophic change extending to the proximal corpus; O1, atrophic change in the lesser curvature extending to the cardia; O2, atrophic change extending between lesser and
greater curvatures; O3, atrophic change extending to the greater curvature. Each atrophy status was scored as C0–C3 (0 to 3) and O1–O3 (4 to 6).

**Histopathological analysis**

Biopsy specimens were endoscopically collected from the greater curvature of the antrum and the greater curvature of the middle corpus according to the updated Sydney System (USS), which recommends “A2” and “B2” as the best biopsy sites [4] (Fig. 2). Thus, A2 was located 2–3 cm superior to the pyloric ring while B2 was located 8 cm inferior to the cardia [4]. One biopsy specimen was obtained at each time of biopsy from each of the accurate sites. Specimens in separate vials were immediately fixed in 10% neutral buffered formalin for 24 hr, embedded in paraffin wax blocks, sliced into 3-μm-thick sections on a microtome, and stained with hematoxylin and eosin (H&E) to identify IM and Paneth cells. For each year, we counted the number of patients with Paneth cells in A2 and B2, and calculated the proportion of these patients as CIM or IIM. The proportion of patients with Paneth cells was analyzed using the Mantel-Haenszel test for trend while USS scores were evaluated using the t-test and the results are expressed as the mean ± standard error. All analyses were performed using IBM SPSS Statistics 24 (IBM Corp., Armonk, NY, USA) and P values < 0.05 were considered statistically significant.

**Immunohistochemical analysis**

Immunohistochemical staining was performed when IM was found in H&E sections and anti-CD10, anti-MUC5AC and anti-MUC6 antibodies were used to classify specimens as CIM or IIM. The immunohistochemical procedure used is as follows. Briefly, after deparaffinization and subsequent rehydration to remove xylene, endogeneous peroxidases were inactivated by treatment with 3% hydrogen peroxide solution (Wako, Osaka, Japan), antigen retrieval was performed at pH 6.0, sections were incubated overnight with monoclonal primary antibodies against CD10 (clone 56C6, 1:100; Leica, Newcastle, UK) [24], MUC5AC (clone CLH2, diluted; Leica, Newcastle, UK) [17], and MUC6 (clone CLH5, diluted; Leica, Newcastle, UK) [37], followed by incubation with peroxidase-labeled goat anti-mouse secondary antibody (ENVISION reagent; Dako, Glostrup, Denmark). Sections were developed by incubation with 3,3′-diaminobenzidine for 5 min and stained with hematoxylin, after which they were dehydrated, cleared, and mounted. As a negative control, some sections were incubated with phosphate-buffered saline (PBS) without the addition of the primary antibody, and these control tissues showed no immunoreactivity.

IM glands were classified as CIM or IIM based on Tsukashita’s study [33], i.e., CIM was defined as CD10+/MUC5AC−/MUC6− while IIM was CD10−/MUC5AC+/MUC6− (Fig. 3). A few glands were CD10+/MUC5AC+/MUC6− and we classified them as CIM because they were always found at the periphery of CD10+/MUC5AC+/MUC6− glands (Fig. 3) and also because Tsukashita et al. have reported that all IIM glands are MUC5AC or MUC6 positive [33]. Further, a few glands that were CD10+/MUC5AC−/MUC6− were classified as IIM because they also stained positive with antibodies to gastric mucins, namely MUC5AC and MUC6 and because IIM is characterized as a mixed gastric and intestinal type lesion [32]. Briefly, IM glands with gastric-type mucin were classified into IIM, while IM glands without gastric-type mucin were classified into CIM irrespective of CD10 expression (Table 1). Although MUC2 staining is useful for detecting goblet cells [1], we did not perform MUC2 staining in the present study because we considered that it was not essential for classifying CIM or IIM. We enumerated the number of patients with CIM and IIM in A2 and B2, and calculated the proportion of these patients for each year.

**Statistical analysis**

The proportion of patients with CIM and IIM were evaluated using the Mantel-Haenszel test for trend while the proportion of patients with Paneth cells was analyzed using the chi-square test. USS scores were evaluated using the t-test and the results are expressed as the mean ± standard error. All analyses were performed using IBM SPSS Statistics 24 (IBM Corp., Armonk, NY, USA) and P values < 0.05 were considered statistically significant.

**Statement of Ethics**

We conducted the present study according to World Medical Association Declaration of Helsinki. This study was approved by the research ethics committee at the Oita University Hospital (approval number 908).
III. Results

Clinical features of all 75 patients

All samples obtained from A2 before HPE were categorized into CIM, IIM, or no IM groups; thus, in A2, 24 (24/75, 32.0%) patients had CIM, and 20 (20/75, 26.7%) had IIM. There were 16 patients with both CIM and IIM, and they were counted in both CIM and IIM groups. In B2, 5 patients (5/75, 6.7%) had CIM before HPE while none had IIM; therefore, patients were classified into CIM or no IM groups. Table 2 provides demographic details of the patients in each group. GC after HPE was seen in 4 of the 75 patients, and all of them had IIM in A2 before HPE, while two of them had CIM in this region. Furthermore, all patients without both CIM and IIM in A2 before HPE did not have GC after HPE.

Intestinal metaplasia in the Antrum and the Corpus

In A2, compared to before HPE, the proportion of patients with IIM decreased significantly during the 10-year follow-up period after HPE, i.e., from 26.7% to 10.7% ($P = 0.04$). In contrast, even though there was a decrease in CIM from 32.0% to 21.3%, ($P = 0.13$; Table 3), it was not significant. These results suggest that IIM regresses after 10 years of HPE compared with before HPE whereas CIM does not regress after HPE in A2.

In B2, the reduction in the proportion of patients with

| Table 1. Immunohistochemical classification of IM glands |
|---------------------------------------------|
| Subtype of IM | CD10 | MUC5AC | MUC6 |
| CIM           | +    | −      | −    |
|               | +    | +      | +    |
|               | +    | +      | −    |
|               | +    | −      | +    |
| IIM           | −    | +      | −    |
|               | −    | +      | −    |
|               | −    | −      | +    |

IM; intestinal metaplasia, CIM; complete type IM, IIM; incomplete type IM.
IM glands not stained with gastric-type mucin were classified into CIM.
IM glands stained with gastric-type mucin were classified into IIM.
Table 2. The patients’ features of CIM, IIM and no IM groups in the greater curvature of the antrum (A2) and the greater curvature of the middle corpus (B2)

| Details of patients | CIM group (n = 24) | IIM group (n = 20) | no IM group (n = 47) |
|--------------------|-------------------|-------------------|---------------------|
| Sex (male/female)  | 17/7              | 12/8              | 25/22               |
| Mean age           | 59.6 ± 7.5        | 62.1 ± 6.9        | 57.0 ± 9.6          |
| Background disease before HPE | | | |
| GU                 | 15                | 11                | 9                   |
| DU                 | 4                 | 2                 | 15                  |
| GC                 | 1                 | 1                 | 0                   |
| ITP                | 0                 | 1                 | 4                   |
| MALToma            | 0                 | 1                 | 1                   |
| Background disease after HPE | | | |
| GC                 | 2                 | 4                 | 0                   |
| Mean gastric mucosal atrophy | 3.8            | 4                 | 3.2                 |

IM; intestinal metaplasia, CIM; complete type IM, IIM; incomplete type IM, HPE; *Helicobacter pylori* eradication.

All patients were classified into CIM, IIM and no IM groups according to the presence of IM subtypes before HPE.

In A2, all patients were classified into CIM, IIM and no IM groups. Among them, 16 patients with both CIM and IIM were classified into both CIM and IIM groups in A2.

In B2, all patients were classified into CIM and no IM groups because no patients had IIM before HPE.

Sex and background disease are presented as the number of patients.

Mean age is presented as the mean ± standard deviation.

Gastric mucosal atrophy was scored as, 0- C0; 1- C1; 2- C2; 3- C3; 4- O1; 5- O2; 6- O3.

Table 3. Proportion of patients with IM glands in the greater curvature of the antrum (A2) and the greater curvature of the middle corpus (B2) before and after HPE

| Details of patients | CIM group (n = 5) | IIM group (n = 0) | no IM group (n = 70) |
|--------------------|-------------------|-------------------|---------------------|
| Sex (male/female)  | 4/1               | —                 | 41/29               |
| Mean age           | 64.8 ± 3.3        | —                 | 58.1 ± 8.9          |
| Background disease before HPE | | | |
| GU                 | 2                 | —                 | 24                  |
| DU                 | 0                 | —                 | 19                  |
| GC                 | 1                 | —                 | 0                   |
| ITP                | 0                 | —                 | 5                   |
| MALToma            | 0                 | —                 | 2                   |
| Background disease after HPE | | | |
| GC                 | 1                 | —                 | 3                   |
| Mean gastric mucosal atrophy | 3.8            | —                 | 3.4                 |

IM; intestinal metaplasia, HPE; *Helicobacter pylori* eradication, CIM; complete type IM, IIM; incomplete type IM.

In A2, the proportion of IIM decreased significantly during 10 years after HPE (P = 0.04; Mantel-Haenszel test), whereas the proportion of CIM did not decrease significantly (P = 0.13; Mantel-Haenszel test).

In B2, the proportion of CIM did not decrease significantly during 10 years after HPE (P = 0.60; Mantel-Haenszel test) and IIM did not found before HPE.
CIM (6.7% to 2.7%, $P = 0.60$) or IIM (0% to 0%, $P = 0.37$) during the 10-year follow-up was not significant (Table 3).

Paneth cells in the Antrum and the Corpus

Fig. 4 shows the presence of Paneth cells in IM in H&E sections. Paneth cells were found before HPE in this patient, but these disappeared from the IM glands at 5 and 10 years after HPE. Before HPE, 13 patients (13/75, 17.3%) had Paneth cells in A2, and the proportion of patients with Paneth cells reduced significantly to 4.7%, 5.2%, and 4.8% at 3, 8, and 9 years after HPE, respectively ($P < 0.05$ for all, Fig. 5). Additionally, 5 patients (5/75, 6.7%) had Paneth cells in B2 before HPE, and the proportion of patients with Paneth cells reduced significantly to 0% at 4, 6 and 9 years after HPE ($P < 0.05$ for all, Fig. 5). Taken together, these results suggest that Paneth cells regress after HPE in both A2 and B2.

USS scores in the Antrum and Corpus

Compared to before HPE, atrophy scores were significantly improved in A2 at 1 year after HPE and at 3–10 years after HPE (1st year: $P < 0.05$, 3–10 years: $P < 0.01$, Fig. 6) and in B2 after HPE at all time points tested ($P < 0.01$ for all, Fig. 6). On the other hand, scores of intestinal metaplasia including both CIM and IIM did not significantly improve in both A2 and B2 after HPE at all time points tested compared to before HPE. Additionally, compared to before HPE, inflammation and activity scores were significantly improved in A2 and B2 after HPE at all time points tested ($P < 0.01$ for all, Fig. 7). In A2, the mean score of atrophy was 1.25 in the 4 patients with GC after HPE and 1.33 in the remaining 71 patients ($P = 0.85$). In B2, the mean score of atrophy was 0.50 in the 4 patients with GC after HPE and 0.48 in the remaining 71 patients ($P = 0.95$).

IV. Discussion

In the present study, IIM in A2 regressed after 10 years of HPE whereas CIM in both A2 and B2 did not regressed after HPE. In addition, Paneth cells regressed after HPE in both A2 and B2. IM is regarded as precancerous or paracancerous lesions of GC both before and after HPE [15, 23, 35]. Although GC develops less frequently
A. In A2, the proportions were significantly lower after 3, 8 and 9 years of HPE compared to that before HPE ($P < 0.05$ for all).

B. In B2, the proportions decreased after 4, 6 and 9 years of HPE compared to before HPE ($P < 0.05$ for all).

$P < 0.05$ (vs before HPE, chi-squared test).

Fig. 5. Proportion of patients with Paneth cells in the greater curvature of the antrum (A2) and the greater curvature of the middle corpus (B2) before and after HPE. A: In A2, the proportions were significantly lower after 3, 8 and 9 years of HPE compared to that before HPE ($P < 0.05$ for all). B: In B2, the proportions decreased after 4, 6 and 9 years of HPE compared to before HPE ($P < 0.05$ for all). * $P < 0.05$ (vs before HPE, chi-squared test).

A. The greater curvature of the antrum (A2)

B. The greater curvature of the middle corpus (B2)

Fig. 6. Mean scores of atrophy and intestinal metaplasia including both CIM and IIM in the greater curvature of the antrum (A2) and the greater curvature of the antrum middle corpus (B2) before and after HPE as assessed by USS. A: In A2, atrophy scores significantly improved at 1 and 3–10 years after HPE compared to before HPE (1st year: $P < 0.05$, 3–10 years: $P < 0.01$). B: In B2, atrophy scores significantly improved ($P < 0.01$) after HPE at all time points tested compared to those before HPE. Intestinal metaplasia scores did not significantly improve during a follow-up period of 10 years after HPE in both A2 and B2. Mean ± SE.
after HPE compared to before HPE [5, 8, 18, 19, 29, 30], risk of GC development after HPE needs to be evaluated. As GC development is related to the degree of IM after HPE [22, 28], and IIM especially predisposes to GC development [7, 11, 33], it is possible to identify high-risk patients by understanding histopathological differences in the degree of regression between CIM and IIM after HPE.

In the present study, 4 patients had GC after HPE. All of them had IIM in A2 before HPE, and two of them had CIM in A2 before HPE. On the other hand, all patients without IIM and CIM in A2 did not had GC after HPE. Thus, the presence of IM before HPE may be used to evaluate the risk of GC after HPE. On the other hand, the mean scores of atrophy between patients with GC and without GC after HPE were not different significantly. More numbers of patients are needed to determine atrophy and IM at biopsy specimens before HPE as the risk of GC after HPE because atrophy and IM are considered precancerous or paracancerous lesions [8, 15, 23, 35].

Our study has uniquely classified IM into CIM or IIM based on immunohistochemistry of biopsy specimens collected from A2 and B2 in 75 patients, and these samples were used to evaluate the reversibility of IM during a long follow-up period after HPE. In the present study, the proportion of patients with IIM in A2 decreased significantly during the 10-year up period after HPE. However, IM assessed by USS did not improve significantly in A2. This discrepancy was due to the fact that assessment of IM by USS included both CIM and IIM. Therefore, it is necessary to evaluate CIM and IIM separately. Few studies have described a histopathological classification of IM into CIM and IIM. For e.g., Sánchez Cuén et al. have defined CIM and IIM using H&E and complementary toluidine blue staining and their study showed that CIM regressed by one year after HPE [27]. In contrast, Urakami et al. classified IM using Alcian Blue-periodic acid Schiff staining and they report reversibility of CIM and IIM as follows [36]. IIM was found more frequently before HPE, and HPE converted IIM to CIM. Subsequently, CIM persisted without regression for a mean follow-up duration of 3.6 years after HPE [36]. In contrast, discrepancies among studies on the regression of IM may be partly explained by differences in histopathological staining methods used and the short duration of follow-up. We have examined the reversibility of CIM and IIM for a period of 10 years using immunohistochemical staining. To the best of our knowledge, no reports have described the evaluation of such a large number of CIM and IIM cases for such a long duration using immunohistochemical staining. Therefore, we deem the results of the present study to be more reliable.

We propose that immunohistochemical staining is suitable for classification of IM because differentiating between IIM and CIM using only H&E staining is difficult. One of the reasons for this is that Paneth cells regress after HPE, as seen in the present study, even though CIM did not regress after HPE. This result suggests that Paneth cells were not always features of CIM after HPE because Paneth cell behave independently from the affinity of mucus stain-
ing after HPE. A role for Paneth cells in gastric mucosa and GC has remained unclear. They are mainly found in the small intestine and recent studies have reported a role for them in innate immunity and maintenance of homeostasis in the intestinal mucosa [26]. In the present study, Paneth cells regressed immediately after HPE as the USS scores of inflammation and activity. Thus, in gastric mucosa infected with H. pylori, Paneth cells may contribute to the generation of appropriate immune responses.

Chen et al. have hypothesized that the presence of Paneth cells at the esophagogastric junction is a histopathologic marker for severe and prolonged mucosal injuries because Paneth cells were observed more frequently in patients with long-segment Barrett’s esophagus than short-segment Barrett’s esophagus [3]. They have also stated that patients with Paneth cells were less likely to show regression of dysplasia than those without Paneth cells [3]. Thus, regression of Paneth cells in the stomach may prevent GC development after HPE. In the present study, Paneth cells significantly regressed after HPE but not all Paneth cells disappeared after HPE. Based on these findings, patients with Paneth cells after HPE may be the risk of GC development.

The present study has several limitations. First, the number of patients is small to evaluate the reversibility of IM in B2. IM has been previously reported to be uncommon in B2 [14], and in the present study, only 5 of the 75 patients had IM before HPE in this region. Second, this was a retrospective observational study and some biopsy specimens at 6 months after HPE and between 1 and 9 years after HPE in some patients were not obtained even though specimens were always taken before HPE and at 10 years after HPE in all patients. Third, biopsy specimens may not always reflect the entire lesion because IM has a patchy distribution pattern. Endoscopic methods as methylene blue or Indigocarmine contrast with white light endoscopy, narrow-band imaging (NBI), or linked color imaging (LCI) are useful for evaluating the spatial extent of IM [2, 6, 9, 34]. Nonetheless, because these methods cannot distinguish between CIM and IIM, our study approved that immunohistochemical staining was an appropriate method for classification of IM.

The following conclusions can be drawn from the results presented here. First, IIM before HPE may be the risk of GC after HPE. Second, regression of IIM and Paneth cells may contribute to the prevention of GC after HPE.

V. Declarations

Conflicts of interest/Competing interests

The authors declare that they have no conflicts of interest to declare.

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VII. References

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