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Helicobacter pylori infection correlates with severity of reflux esophagitis: with manometry findings*

Tohko Shirota[1], Motoyasu Kusano[1], Osamu Kawamura[1], Tsutomu Horikoshi[1], Masatomo Mori[1], and Toshikazu Sekiguchi[2]

[1]First Department of Internal Medicine, School of Medicine, Gunma University, 3-39-15 Showamachi, Maebashi 371-0034, Japan
[2]Sekiguchi Medical and Dental Clinic, Ohta, Japan

Editorial on page 649

Abstract: The role of *Helicobacter pylori* infection in the development and exacerbation of reflux esophagitis was investigated. The prevalence of *Helicobacter pylori* infection, the severity of atrophic gastritis, and esophageal motility (determined by esophageal manometry by an infusion catheter method) were assessed in patients with mild ($n = 46$) and severe ($n = 27$) reflux esophagitis and subjects without reflux ($n = 28$). Compared with the prevalence of *Helicobacter pylori* infection in the non-reflux group, the prevalence in the mild and severe reflux groups (60.7%, 47.8%, and 14.8%, respectively) was significantly ($P < 0.05$) lower. Atrophic gastritis was milder in both reflux groups than in the non-reflux group. The degree of gastritis was also milder in the severe reflux group than in the mild reflux group. The esophageal sphincter pressure was significantly ($P < 0.05$) lower in the reflux groups than in the non-reflux group, and the amplitude of primary peristalsis was significantly ($P < 0.05$) lower in the severe reflux group than in the non-reflux group. There were no significant differences between reflux patients with and without *Helicobacter pylori* infection in the parameters of esophageal manometry. These data imply that a low prevalence of *Helicobacter pylori* infection may result in a milder grade of atrophic gastritis, and consequently, exacerbate reflux esophagitis.

Key words: *Helicobacter pylori*, reflux esophagitis, gastric mucosal atrophy, esophageal manometry, lower esophageal sphincter pressure

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Introduction

Helicobacter pylori infection was first reported by Warren and Marshall in 1983.[1] It is known that *H. pylori* infection is closely involved in the progression of chronic atrophic gastritis[2] and the development of peptic ulcer.[3] Recently, it was also suggested that *H. pylori* infection plays a role in gastric cancer,[4,5] but only a few studies have assessed the relationship between reflux esophagitis (RE) and *H. pylori* infection. RE is defined as esophageal mucosal lesions caused by reflux of the gastric contents, and gastric acid and various other factors[6] are thought to be involved in the etiology. In atrophic gastritis, gastric acid secretion is influenced by gastric mucosal atrophy. We therefore investigated the prevalence of *H. pylori* infection in relation to the severity of RE and the severity of atrophic gastritis, as well as the effect on esophageal motility, in order to elucidate the role of *H. pylori* in the development and progression of RE.

Subjects and methods

Subjects

The subjects were 73 patients (27 men and 46 women) with RE diagnosed by endoscopic examinations, and 28 controls (7 men and 21 women) with no mucosal lesions in the upper gastrointestinal tract. The subjects were randomly selected from among patients who underwent upper gastrointestinal endoscopy at Gunma University Hospital between November 1994 and March 1997. The severity of RE was graded by the Savary and Miller classification:[7] stage I, one or more supravestibular, nonconfluent mucosal lesions with erythema or exudate or superficial erosions; stage II, confluent erosive and exudative mucosal lesions that are not circumferential; stage III, circumferential erosive and exudative lesions

without stricture; and stage IV, chronic lesions (ulceration, fibrosis, stricture formation, esophageal shortening, and scarring with columnar epithelium).

Forty-six patients (20 men and 26 women) with stage I and II disease were assigned to the mild RE group, and 27 patients (7 men and 20 women) with stage III and IV disease were assigned to the severe RE group. Fifty-five subjects (18 men and 37 women) also underwent assessment of esophageal motility in order to investigate the possibility that disturbed esophageal motility may be related to the severity of RE and *H. pylori* infection. All the subjects gave their informed consent prior to participation in the study, which was performed in compliance with the Helsinki Declaration.

Demonstration of H. pylori

To investigate *H. pylori* infection, two endoscopic biopsy specimens were obtained, at the greater curvature of the gastric antrum and in the upper gastric body. Each biopsy specimen was placed in transport medium (Transport *H. pylori*; BML, Tokyo, Japan) and kept ice-cold during transport, followed by homogenization. The homogenate was layered onto Belo-Horizonte Medium (BHM) (Nikken, Kyoto, Japan) for culture within 24 h after collection of the specimen. Culture was done for 5–7 days under microaerophilic conditions at 37°C and 90% humidity. For identification of *H. pylori*, organisms were tested for the presence of oxidase and urea decomposition, and the morphology was also examined microscopically after gram staining.

The urease test was also performed, with a campylobacter like organism (CLO) test kit (Delta West, Canning Vase, Australia). Two specimens were obtained (at the same points in the stomach as noted above) for culture and were placed onto a test slide immediately after biopsy. Changes in color were observed for 2 h at room temperature or at 37°C in an electrical incubator. When the color changed from red-orange to purple-red, the specimen was considered to be positive for *H. pylori* infection.

The serum titer of *H. pylori*-IgG antibody was measured. A test kit (EPI; Enteric Products New York, NY, USA) was used for measurement, with purified high-molecular weight cell-associated protein (HM-CAP) specific to *H. pylori*.[8] The serum was taken as being positive for *H. pylori* infection when the titer was above a value of 2.2 on enzyme-linked immunosorbent assay (ELISA).

Patients were considered to be infected with *H. pylori* if more than two of these three tests had positive results.

Evaluation of atrophic gastritis

Serum pepsinogen (PG) levels were measured with a pepsinogen I/II radio immunoassay (RIA) bead kit (Dinabot, Tokyo, Japan) for evaluation of gastric mucosal atrophy. Atrophic gastritis was judged to be mild if the PG I/II ratio was >6.0, according to Samloff et al.[9] The severity of atrophic gastritis was also evaluated endoscopically, based on the Kimura-Takemoto classification,[10] in which the border of atrophy was classified into one of six grades (C1, C2, C3, O1, O2, and O3). Atrophic gastritis was classified into three grades: mild (C1 + C2), moderate (C3 + O1), and severe (O2 + O3).

Esophageal manometry

To evaluate esophageal motility, intraluminal pressure in the esophagus was measured in the interdigestive period by an infusion catheter method, with a Dent sleeve sensor (Dent Sleeve, Adelaide, SA, Australia) for the lower esophageal sphincter (LES). The catheter sensor, with an outer diameter of 4.8 mm, consisted of eight polyvinyl tubes with an inside diameter of 0.8 mm. With this catheter, intraluminal pressure 3, 10, and 17 cm proximal to the sleeve for the LES, as well as 6 and 16 cm distal to the stomach was measured. The infusion pump was a pneumohydraulic capillary infusion type (Arndorfer Medical Specialities, Greendale, WI, USA), and distilled water was continuously infused at a rate of 0.6 ml/min. The pressure transducer was a Model CP-01 (Star Medical, Tokyo, Japan), which was connected to an eight-channel polygraph (RMP-6008; Nihon Kohden, Tokyo, Japan). The sensor was inserted nasally under fluoroscopic guidance and was positioned so that the sleeve was at the LES. The subjects remained supine.

Statistical analysis

Quantitative data values are expressed as means ± SD. Analysis of variance (ANOVA) was used to test differences among the groups according to age and pepsinogen level. The χ^2 test was used to test the relationship between the severity of RE and the prevalence of *H. pylori*, and atrophic gastritis. ANOVA and Tukey's method were used to test the relationship between the severity of RE and LES pressure, and the amplitude of primary peristalsis. The Mann-Whitney *U*-test was used to test the effect of *H. pylori* infection on LES pressure and on the amplitude of primary peristalsis. The accepted probability value for statistical significance was defined as $P < 0.05$ with a two-tailed test.

Results

Age and prevalence of H. pylori infection

The mean age of the subjects was 57.8 years in the control group, 60.0 years in the mild RE

group, and 64.5 years in the severe RE group, showing no significant differences among the three groups.

Positive results for *H. pylori* culture, the CLO test, and serum *H. pylori*-IgG were obtained in 16, 15, and 18 of the 28 patients in the control group; in 21, 20, and 21 of the 46 patients in the mild RE group; and in 3, 3, and 10 of the 27 patients in the severe RE group, respectively. The prevalence of *H. pylori* infection was 60.7% (17/28) in the control group, which was the highest level among the three groups, followed by 47.8% (22/46) in the mild RE group. The prevalence of *H. pylori* infection was 14.8% (4/27) in the severe RE group. There was a significant difference among the groups ($P < 0.01$) in the prevalence of *H. pylori* infection (Fig. 1).

Serum pepsinogen level

Although there were interindividual variations in the serum pepsinogen I and II levels and the pepsinogen I/II ratio in all groups, the pepsinogen I level was significantly ($P < 0.05$) higher in the severe RE group than in the other two groups. All three groups had similar pepsinogen II levels, the pepsinogen I/II ratio being significantly ($P < 0.05$) higher in the severe RE group than in the mild RE group (Table 1), and also significantly higher in the Mild RE group than in the non-RE group ($P < 0.05$).

Atrophic gastritis

The percentage of patients with mild atrophic gastritis (C1 + C2) was 36% in the control group, 57% in the mild RE group, and 78% in the severe RE group, increasing with the progression of RE. The percentage of patients with severe atrophic gastritis (O2 + O3) was 61% in the control group, 15% in the mild RE group, and 7% in the severe RE group, decreasing with the progression of RE (Fig. 2).

Esophageal manometry

The LES pressure was significantly ($P < 0.05$) lower in the RE patients than in the control group (Fig. 3a). There was no significant difference in the LES pressure between the RE patients with and without *H. pylori*

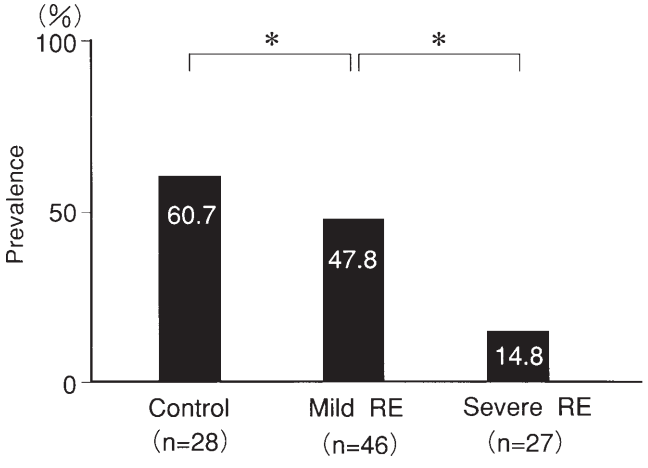

Fig. 1. Prevalence of *H. pylori* infection in control and reflux esophagitis (*RE*) groups. *Numbers in bars* are percentages. * $P < 0.01$ (χ^2-test)

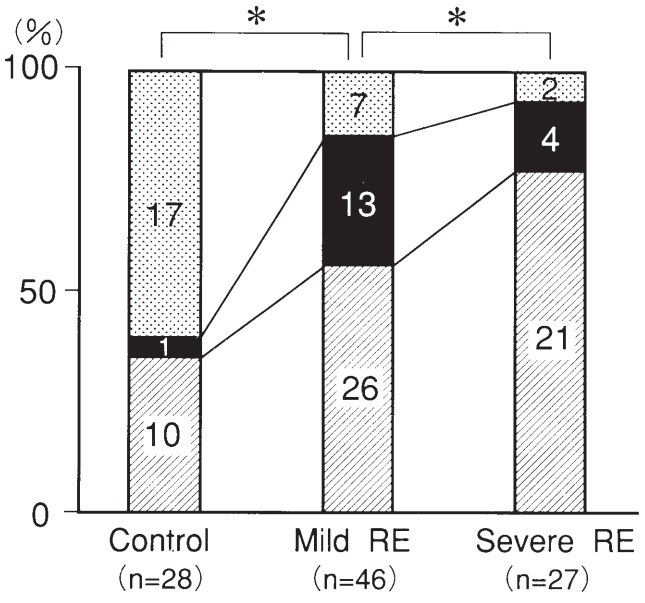

Fig. 2. Pattern of gastric mucosal atrophy in control and reflux esophagitis (*RE*) groups. *O2* and *O3*, severe atrophy (*dotted bars*); *C3* and *O1*, moderate atrophy (*black bars*); *C1* and *C2*, mild atrophy (*striped bars*). *Numbers in bars* are numbers of subjects. * $P < 0.01$ (χ^2-test)

Table 1. Serum pepsinogen I and II levels and pepsinogen I/II ratio

	Pepsinogen I (ng/ml)	Pepsinogen II (ng/ml)	Pepsinogen I/II ratio
Control	54.11 ± 25.48	17.97 ± 11.32	3.66 ± 2.01
Mild RE	64.60 ± 26.48	14.41 ± 8.10	5.14 ± 1.79**
Severe RE	111.64 ± 85.75*	17.74 ± 11.83	6.40 ± 2.75*

* $P < 0.05$ versus mild RE and control; ** $P < 0.05$ versus control
Values are expressed as means ± SD; Statistical analysis was performed by Tukey's method after comparison by analysis of variance (ANOVA)

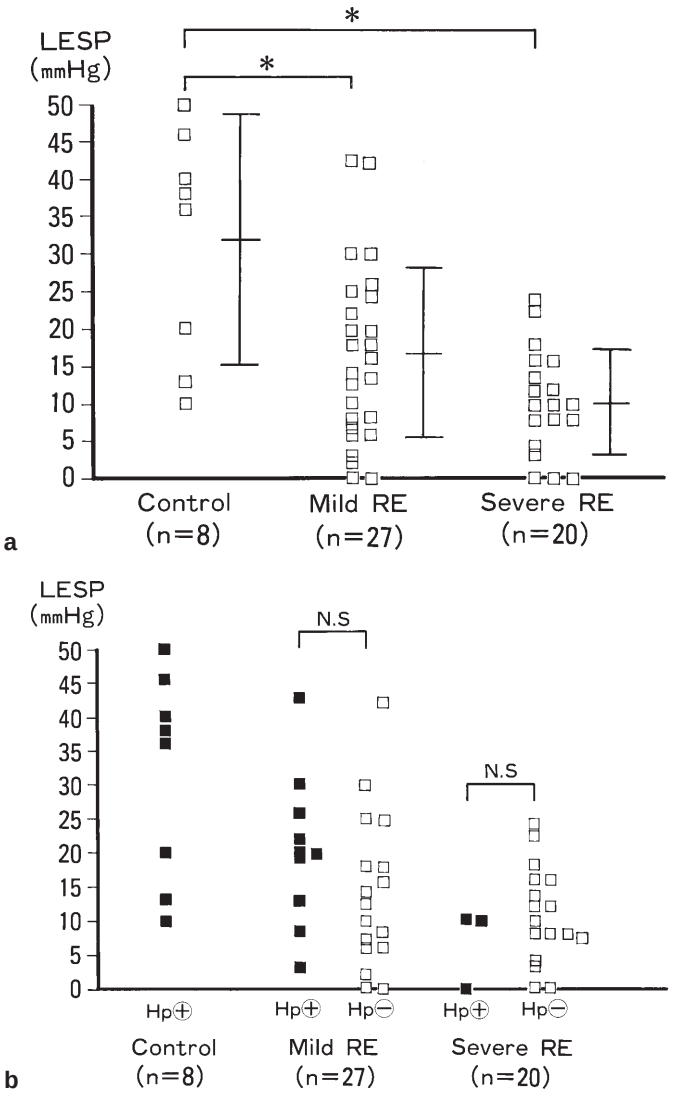

Fig. 3. **a** Lower esophageal sphincter pressure (*LESP*) in control and reflux esophagitis (*RE*) groups. $*P < 0.05$ (Tukey's method after comparison by analysis of variance [ANOVA]). **b** LESP in control and the RE groups with (*Hp+*) and without (*Hp−*) *H. pylori* infection. *N.S.*, No significant difference (Mann-Whitney *U*-test)

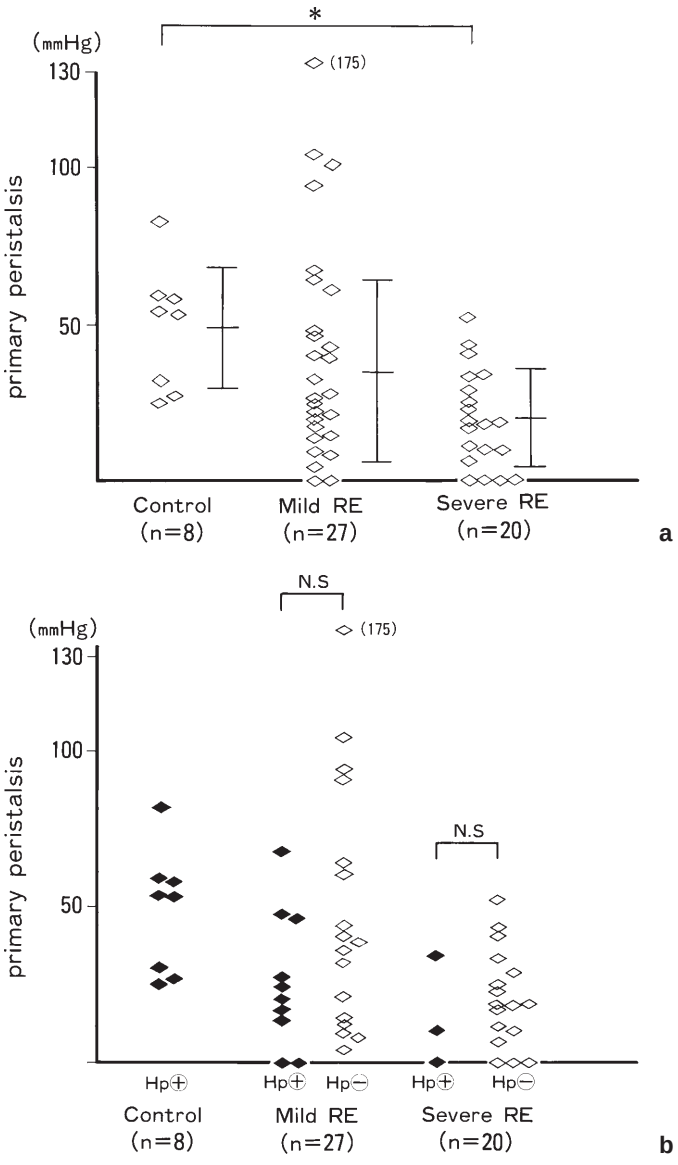

Fig. 4. **a** Amplitude of primary peristalsis in control and reflux esophagitis (*RE*) groups. $*P < 0.05$ (Tukey's method after comparison by ANOVA). **b** Amplitude of primary peristalsis in control group and reflux esophagitis groups with (*Hp+*) and without (*Hp−*) *H. pylori* infection. *N.S.*, No significant difference (Mann-Whitney *U*-test)

infection (Fig. 3b). The amplitude of primary peristalsis in the lower esophageal body was significantly ($P < 0.05$) lower in the severe RE group than in the control group (Fig. 4a). RE patients with *H. pylori* infection showed no significant differences from RE patients without *H. pylori* infection in regard to primary peristalsis (Fig. 4b).

Discussion

H. pylori provokes acute gastritis after it reaches the gastric mucosa,[11] and chronic infection is common.[12,13] Accordingly, *H. pylori* has been closely linked with various gastroduodenal diseases. *H. pylori* infection also plays a critical role in atrophic gastritis, and chronic inflammation arising from long-term infection is thought to be the primary etiology of gastric mucosal atrophy.[14,15] With regard to the prevalence of *H. pylori* infection in Japan, Asaka et al.[16] measured the serum titer of *H. pylori* IgG antibody in asymptomatic individuals and reported that the prevalence of infection increased sharply after the age of 40 years, reaching 70% in the population above this age.

Regarding esophageal *H. pylori* infection, there are arguments for its association with reflux esophagitis, although *H. pylori* was detected in Barrett's epithelium.[17] O'Connor[18] investigated 93 patients with gastroesophageal reflux disease (GERD) and reported that there was no significant correlation between the severity of esophagitis and the presence of *H. pylori* infection, although chronic gastritis was histologically detected in the majority of patients with such infection. On the other hand, McCallum et al.[19] reported that histological gastritis was significantly more common and the prevalence of *H. pylori* infection was significantly higher in patients with gastroesophageal reflux disease than in healthy volunteers, and that gastric acid secretion was greater in patients with *H. pylori* infection than in individuals without infection. They therefore suggested that *H. pylori* infection may be involved in the development of GERD.

In the present study, *H. pylori* infection was found in only about 36% of the patients with RE aged over 40 years and in about 15% of the severe RE group. In contrast, there was *H. pylori* infection in about 61% of the subjects in the control group, and the prevalence of *H. pylori* infection was about 54% among the control subjects aged over 40 years. The prevalence of *H. pylori* infection was therefore significantly higher in the control group than in both the severe and mild RE groups, although the infection rate in the control group was somewhat lower than that reported by Asaka et al.[16] Since the prevalence of *H. pylori* infection was also significantly higher in the mild RE group than in the severe RE group, there was a very low level of *H. pylori* infection in the patients with severe RE.

It has been reported that RE has a tendency to be exacerbated in old age.[20,21] In our study, in the comparison of the three groups, the average age in the severe RE group was the highest, but there was no significant difference among the three groups.

In the present study, to evaluate the severity of atrophic gastritis, serum pepsinogen levels and endoscopic changes were employed. Both parameters indicated that the severity of gastric mucosal atrophy decreased in inverse proportion to the severity of RE. In a comparison among the three groups in regard to the severity of atrophic gastritis, in the severe RE group, 21 patients (78%) were classified as C1 + C2 (mild) and only 3 of them were *H. pylori*-positive. In the same group (2 patients) were classified as O2 + O3 (7%) (severe), and one of them was *H. pylori*-positive. Therefore it was presumed that most of the severe RE patients had got rid of *H. pylori* infection, and consequently the progress of atrophic gastritis had been delayed. These endoscopic findings were also reflected in the pepsinogen I/II ratio, which was significantly higher in the severe RE group than in the other two groups.[22]

Since the pepsinogen I/II ratio is correlated with the maximum acid output,[22] this would suggest that gastric acid secretion is better maintained in patients with severe RE than in patients with mild RE and non-RE individuals. Haruma et al.[23] also investigated the relationship between *H. pylori* infection and gastric mucosal atrophy in RE. They also found that the prevalence of *H. pylori* infection was significantly lower in RE patients than in non-RE individuals and that RE patients had milder gastric mucosal atrophy than non-RE individuals, results consistent with those of the present study.

In addition to the decreased gastric acid secretion related to mucosal changes arising from *H. pylori* infection, a possible direct effect of *H. pylori* on acid secretion also needs to be considered. The direct effect of *H. pylori* infection is still controversial: it was shown to promote acid secretion;[24] but on the other hand, to not alter acid secretion.[25]

LES pressure was lower in our RE patients than in our control group. Both LES pressure and the amplitude of primary peristalsis were significantly lower in the patients with severe RE. It is known that esophageal motility becomes more severely impaired with progression of the severity of RE. Kahrilas et al.[26] stated that the mean amplitude of primary esophageal peristalsis was significantly lower in patients with esophagitis than in healthy individuals, and that LES pressure was significantly lower in patients with severe RE than in healthy individuals. Based on this report, it seems that RE is aggravated by the reduction in both LES pressure and the amplitude of primary peristalsis. Which are factors defending against reflux.

Nevertheless, the amplitude of primary peristalsis and the LES pressure were not significantly different in the RE patients with and without *H. pylori* infection, so that this study provided no support for the hypothesis that *H. pylori* infection has an impact on esophageal motility in RE.

It is still unclear what role gastric acid secretion plays in the development or exacerbation of RE. Whereas some reports[27,28] indicate that gastric acid secretion is increased in RE, other studies have found no correlation between the severity of RE and maximum acid output,[29] or have noted a hypoacidic state in severe RE compared with findings in healthy individuals.[30] Such findings would appear to be inconsistent with the results of this study, but gastric acid is generally accepted to be an important factor promoting RE,[31] and inhibition of gastric acid secretion has been confirmed to be useful in treating this condition.

Epidemiologically, there is a lower prevalence of RE in Japan than in Europe and the United States,[32] and this may be closely related to the high prevalence of atrophic gastritis in Japan,[33] which is usually associated

with *H. pylori* infection. Middle-aged to elderly Japanese commonly have *H. pylori* infection and there are few elderly individuals with high acid secretion, and this may help to inhibit the development or progression of RE. Nevertheless, *H. pylori* infection is not the only important factor involved in the extension of atrophic gastritis. There is a report, in a twin study,[34] that genetic effects influence the acquisition of *H. pylori* infection.

Fontham et al.[35] reported that vitamins and carotenoids play a role in preventing gastric atrophy. Satoh et al.[36] reported that not only *H. pylori*, but also other factors, such as aging and genetic and environmental factors are important in the chronological extension of atrophic gastritis. Kinoshita et al.[37] reported that gastric acid secretion has increased over the past 20 years from the 1970s in the Japanese population, irrespective of *H. pylori* infection, suggesting the presence of factors which increase gastric acid secretion other than the decreased *H. pylori* infection rate. They pointed out that the increased consumption of fat in Japan is a possible factor that influences the change in acid secretion. This is an interesting report, but a study of more subjects will be needed. The development of atrophic gastritis has multiple causes after all, and all *H. pylori* infection does not lead to the development of atrophic gastritis; however, it cannot be asserted that *H. pylori* infection is not related to the development of atrophic gastritis. We assume that *H. pylori* infection is a leading factor in the development of atrophic gastritis because there have been many studies indicating the relationship between *H. pylori* infection and atrophic gastritis.[2,14,15]

Although we did not measure acid secretion directly, since the extension of atrophic gastritis means a reduction in fundic glands, it seems that the severe RE group had maintained acid secretion.

Exposure to gastric acid because of abnormal esophageal motility could play a critical role in the development of RE, and mild atrophic gastritis may contribute to the exacerbation of RE because the function of acid secretion is maintained. The etiology of RE is therefore basically accounted for by abnormal gastroesophageal motility, and the absence of *H. pylori* infection also contributes to its development and progression.

Nowadays, the eradication of *H. pylori* is indicated not only for the treatment of peptic ulcer[38] but also for chronic gastritis.[39] If the prevalence of *H. pylori* infection in Japan is decreased by eradication therapy or as a result of Westernization of the lifestyle, it is possible that exacerbation of RE or an increase in its prevalence may occur.

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