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Associations among Gastric Juice pH, Atrophic Gastritis, Intestinal Metaplasia and *Helicobacter pylori* Infection

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Background/Aims: Gastric juice plays a crucial role in the physiology of the stomach. The aim of this study is to evaluate associations among the pH of gastric juice, atrophic gastritis (AG), intestinal metaplasia (IM), pepsinogen, and *Helicobacter pylori* infection. **Methods:** Gastric biopsies and juice were collected from 46 subjects who underwent endoscopies at Seoul National University Bundang Hospital between November 2011 and March 2013. *H. pylori*, AG and IM were evaluated, and pepsinogen I or II, I/II ratio, and interleukin (IL)-1β levels were measured. **Results:** The mean pH of gastric juice was higher in the *H. pylori*-positive group (n=17) than that in the *H. pylori*-negative group (n=29) (4.54 vs 2.46, p=0.002). When patients were divided into pH <3 (n=28) and pH ≥3 (n=18) groups, *H. pylori* was lower in the pH <3 group (21.4%) than in the pH ≥3 group (61.1%) (p=0.007). The pH ≥3 group demonstrated AG and IM more frequently than the pH <3 group in the body (p=0.047 and p=0.051, respectively) but not in the antrum. There were no differences in pepsinogen I or II, I/II ratio, and IL-1β levels between the two groups. **Conclusions:** There is a relationship between chronic *H. pylori* infection and gastric juice pH ≥3, which may originate from AG and IM in the body. **(Gut Liver 2018;12:158-164)**

Key Words: Gastric juice; *Helicobacter pylori*; Gastritis, atrophic; Intestinal metaplasia

INTRODUCTION

One of the important functions of stomach is to regulate and sustain acid secretion to sterilize ingested food.[1] Gastric juice plays a crucial role in the physiology of stomach and gastric acid determining pH of gastric juice. The gastric juice is a strong acid containing active proteolytic and lipolytic enzymes to kill swallowed microorganisms. Since pepsin, a proteolytic enzyme, is optimized only when gastric pH is low,[2] gastric pH is normally kept below 4.0.[3] On the contrary, the bactericidal effect of gastric juice starts to drop dramatically above this pH.[4] Gastric acid secretion is a complex process involving neuronal, hormonal, and endocrine pathways.[5] All of these pathways have one common target: the parietal cell which is the functional machinery for acid secretion sits.

Gastric acid secretion is affected by various factors, but *Helicobacter pylori* is known as the most critical factor. When *H. pylori* infection spreads to oxyntic mucosa, it reduces acid secretion and induces atrophy, hypergastrinemia and dysplasia, or gastric adenocarcinoma.[6] *H. pylori* infection could affect the main pathogenesis of most upper gastrointestinal diseases through this process.[1] The associations between *H. pylori*, atrophic gastritis (AG), intestinal metaplasia (IM), and pepsinogen (PG) have been studied previously.[7-9] There are many articles suggesting that not only *H. pylori* infection induces AG and IM, but also AG and IM could be reversed when it is eradicated.[10] Few studies, however, reported the association between the acidity of gastric juice and *H. pylori* infection.[11,12] Our hypothesis is that the pH of gastric fluid is affected by *H. pylori* infection by the same way of AG and IM. The aim of this study is to evaluate the associations among gastric fluid acidity, AG, IM, PG, and *H. pylori* infection state based on the backgrounds above.

MATERIALS AND METHODS

1. Subjects

Patients who underwent standard endoscopy from November 2011 to March 2013 at Seoul National University Bundang Hospital were screened to enroll. Patients with a history of any

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stomach surgery, *H. pylori* eradication therapy, systemic diseases for which medication was taken for a long period of time, antibiotics use within the last 3 months prior to enrollment or use of antacids, proton pump inhibitor or H2 blocker within 4 weeks of enrollment were excluded from the initial candidates. A total of 106 patients were considered as initial candidates of enrollment.

After applying topical lidocaine spray to the patient, patients were sedated by injecting sedatives intravenously, and the patients were screened for premalignant or malignant gastric mucosal lesions or checked to identify the causes of dyspepsia. The effect of topical lidocaine spray used at the initiation of endoscopy could not been eliminated completely, however, it was applied only into the oropharyngeal cavity and gastric fluid was aspirated from the gastric fundus. Gastric fluid had not been collected when following situation occurred: (1) not clean gastric fluid due to saliva or any foods; (2) not fully sedated patient and the patient complaint about discomfort; or (3) patient refusal of sedative endoscopy due to necessity of driving a car after endoscopy. All patients who were failed to collect gastric fluid were excluded from enrollment, and 46 subjects were finally enrolled (Fig. 1).

This study was approved by the Institutional Review Board of the Seoul National University Bundang Hospital, Korea (B-1112/141-007). Written informed consent was obtained from all of the participants before the study enrollment.

2. Endoscopic examination, histologic examination, *H. pylori* test, and gastric juice sampling

Ten biopsy specimens per subjects were obtained to perform three types of *H. pylori* testing (histology, rapid urease test, and culture) during gastroscopy.[13,14] All biopsies were examined by experienced pathologist who was blind from the patients' information at all. To avoid contamination, the endoscope was washed and disinfected by immersing in a detergent solution containing 7% proteolytic enzymes and 2% glutaraldehyde.[15] Patients were proven to be currently infected with *H. pylori* by the following three methods: (1) histologic evidence of *H. pylori* infection by modified Giemsa staining; (2) a positive rapid urease test (CLOtest; Delta West, Bentley, Western Australia); and (3) a positive *H. pylori* culture test.[13,14] To distinguish past infection from current infection, two additional methods were used; (1) measuring serum *H. pylori* IgG level (Genedia *H. pylori* ELISA; Green Cross Medical Science Co., Eumseong, Korea) and (2) checking the history of *H. pylori* eradication treatment. When all the tests above were proved negative with no history of *H. pylori* eradication treatment, the patient was defined as *H. pylori*-negative. Histologic features of gastric mucosa were recorded according to the updated Sydney scoring system (i.e., 0=no, 1=mild, 2=moderate, and 3=severe) regarding the degree of neutrophil and monocyte infiltration, AG, and IM after hematoxylin and eosin staining.[16]

3. Aspiration of gastric juice and measurement of pH of gastric juice

Approximately 5 mL of gastric juice in the gastric fundus was aspirated gently by the one doctor (N.K.) from all of the subjects. Gastric juice was transported as rapid as possible to the laboratory. Gastric juice was centrifuged (3,000 rpm, 5 minutes), and supernatant was collected; then, pH was determined using a glass electrode (inoLab pH Level 1; WTW, Weilheim, Germany).[17]

4. Serum PG levels

Fasting serum was collected from all subjects on the same day of endoscopy. The samples were centrifuged immediately at 4°C and stored at −70°C. Serum concentrations of PG I and II were measured using a latex-enhanced turbidimetric immuno-

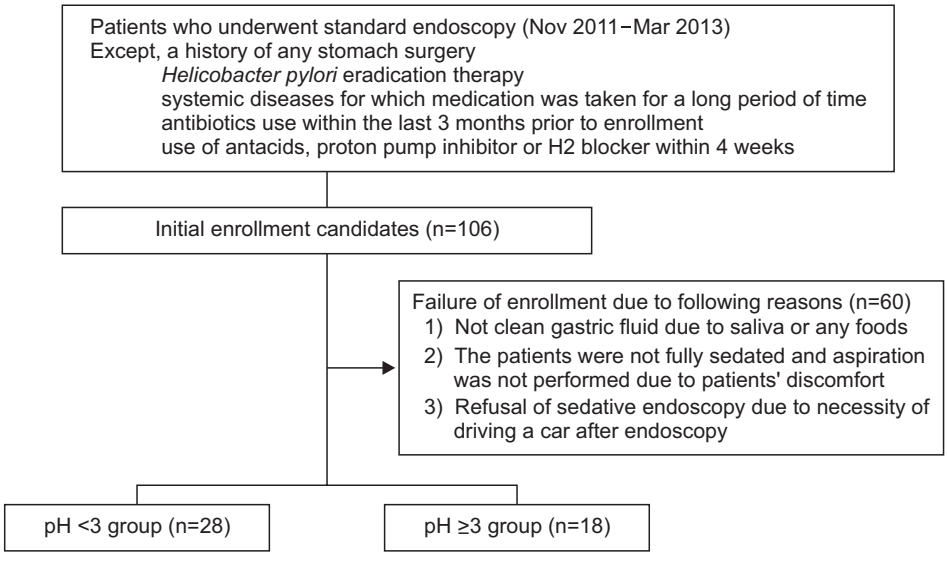

Fig. 1. Diagram of study patient enrollment.

assay (L-TIA; Shima Laboratories, Tokyo, Japan). And PG I to PG II ratios (PG I/II) were calculated.[18]

5. Measurement of gastric mucosal interleukin-1β levels

The biopsy samples stored at −70°C were thawed on ice, and then homogenized at 4°C in 200 μL of phosphate-buffered

Table 1. Baseline Characteristics of the 46 Subjects according to *Helicobacter pylori* Infection Status

Variable	Total (n=46)	*H. pylori*-positive group (n=17)	*H. pylori*-negative group (n=29)	p-value
Age, yr	57.54±12.65	55.53±12.40	58.72±12.86	0.414
Male sex	34 (73.9)	13 (76.5)	21 (72.4)	1.000
Body mass index, kg/m^2	22.69±3.71	23.52±3.33	22.22±3.89	0.256
Alcohol drink				0.465
Never	5 (10.9)	1 (5.9)	4 (13.8)	
Past	8 (17.4)	2 (11.8)	6 (20.7)	
Current	33 (71.7)	14 (82.4)	19 (65.5)	
Smoking				0.842
Never	16 (34.8)	5 (29.4)	11 (37.9)	
Past	20 (43.5)	8 (47.1)	12 (41.4)	
Current	10 (21.7)	4 (23.5)	6 (20.7)	
Atrophic gastritis, antrum*	n=34	n=12	n=22	0.263
No	16 (47.1)	5 (41.7)	11 (50.0)	
Mild	12 (35.3)	4 (33.3)	8 (36.4)	
Moderate	2 (5.9)	2 (16.7)	0	
Severe	4 (11.8)	1 (8.3)	3 (13.6)	
Atrophic gastritis, body*	n=36	n=17	n=19	0.263
No	23 (63.9)	9 (52.9)	14 (73.7)	
Mild	8 (22.2)	6 (35.3)	2 (10.5)	
Moderate	4 (11.1)	2 (11.8)	2 (10.5)	
Severe	1 (2.8)	0	1 (5.3)	
Intestinal metaplasia, antrum	n=46	n=17	n=29	0.949
No	21 (45.7)	7 (41.2)	14 (48.3)	
Mild	11 (23.9)	4 (23.5)	7 (24.1)	
Moderate	9 (19.6)	4 (23.5)	5 (17.2)	
Severe	5 (10.9)	2 (11.8)	3 (10.3)	
Intestinal metaplasia, body	n=46	n=17	n=29	0.593
No	29 (63.0)	10 (58.8)	19 (65.5)	
Mild	4 (8.7)	1 (5.9)	3 (10.3)	
Moderate	9 (19.6)	5 (29.4)	4 (13.8)	
Severe	4 (8.7)	1 (5.9)	3 (10.3)	
pH level of gastric juice	3.23±2.09	4.54±2.16	2.46±1.64	0.002†
Serum pepsinogen level	n=29	n=13	n=16	
Pepsinogen I, ng/mL	66.33±91.15	105.17±157.35	43.54±24.42	0.133
Pepsinogen II, ng/mL	20.24±25.32	34.80±41.62	11.64±3.81	0.068
Pepsinogen I/ II ratio	3.68±1.84	3.06±1.59	3.89±1.92	0.223
	n=38	n=12	n=26	
Serum interleukin-1β, pg/mg‡	22.59±38.99	16.05±13.53	25.60±46.26	0.490

Data are presented as mean±SD or number (%).
*Atrophic gastritis was evaluated according to the updated Sydney scoring system (mild, moderate, and severe) using endoscopic biopsy specimens to assess the degree of neutrophil and monocyte infiltration; †Statistical significance; ‡Interleukin-1β levels were measured using ELISA kits and expressed as picograms of cytokine per milligrams of biopsy protein (pg/mg protein) in the supernatant fluid.

saline (pH 7.4) using a polypropylene micro pestle. The homogenate was then centrifuged at 10,000 *g* for 10 minutes. Total protein was estimated using Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Interleukin-1β (IL-1β) levels were measured using R&D systems ELISA kits (Minneapolis, MN, USA) by following the manufacturer's instructions. IL-1β levels in the supernatant fluid were expressed as picograms of cytokine per milligram of biopsy protein (pg/mg protein).[19]

6. Statistical analysis

Parametric continuous variables are presented as mean± standard deviation (SD). Categorical variables are presented as numbers and percentages. Categorical variables were analyzed by a chi-square test, and continuous variables were analyzed by Student t-test. p-values of less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS software version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

1. Baseline patient characteristics

The mean age of 46 subjects was 57.54 years and 34 patients (73.9%) were male and 12 patients (26.1%) were female (Table 1). After pathologic review of gastric mucosa taken by endoscopic biopsy, AG was evaluated on antrum in 34 patients and on body in 36 patients. According to updated Sydney scoring system, 18 patients (52.9%), and 13 patients (36.1%) were identified mild AG or higher grade of AG on antrum and on body, respectively. IM was examined for all study patients, and 14 patients (30.5%), and 13 patients (28.3%) were verified IM with moderate grade or higher on antrum, and on body, respectively. The mean gastric juice pH was 3.23. Serum PG I and PG II were verified in 29 patients, and mean PG I and PG II level was 66.3 and 20.2 ng/mL, respectively.

2. *H. pylori* infection, AG and IM

Using the updated Sydney scoring system, we checked the distribution of histologic AG, IM and *H. pylori*. The infection rate of *H. pylori* was not statistically correlated with AG in antrum (p=0.263) and body (p=0.263). There was no statistical correlation between *H. pylori* prevalence and IM in antrum (p=0.949) and body (p=0.593) (Table 1).

3. *H. pylori* infection, pH of gastric juice and serum PG

Patients were categorized into *H. pylori*-positive (n=17) and *H. pylori*-negative (n=29) group. The mean pH level of gastric juice is significantly higher in *H. pylori*-positive group (4.54 vs 2.46, p=0.002). Serum PG II level was 34.8 ng/mL in *H. pylori*-positive group, which is higher than 11.6 ng/mL in in *H. pylori*-negative group, but, it did not reach the significance (p=0.068). Serum PG I/II ratio and serum IL-1β level did not show significant differences.

Table 2. Comparison of Clinical Features of Patients according to Gastric Juice pH Level

Variable	pH <3 group (n=28)	pH ≥3 group (n=18)	p-value
pH level	1.79±0.45	5.46±1.58	
Age, yr	58.54±13.65	56.00±11.10	0.513
Male sex	19 (67.9)	15 (83.3)	0.315
Body mass index, kg/m^2	22.53±4.10	22.96±3.11	0.704
Alcohol drink			0.994
Never	3 (10.7)	2 (11.1)	
Past	5 (17.9)	3 (16.7)	
Current	20 (71.4)	13 (72.2)	
Smoking			0.720
Never	10 (35.7)	6 (33.3)	
Past	13 (46.4)	7 (38.9)	
Current	5 (17.9)	5 (27.8)	
Atrophic gastritis, antrum*	n=20	n=14	0.337
No	10 (50.0)	6 (42.9)	
Mild	8 (40.0)	4 (28.6)	
Moderate	0	2 (14.3)	
Severe	2 (10.0)	2 (14.3)	
Atrophic gastritis, body*	n=19	n=17	0.169
No	15 (78.9)	8 (47.1)	
Mild	2 (10.5)	6 (35.3)	
Moderate	2 (10.5)	2 (11.8)	
Severe	0	1 (5.9)	
Intestinal metaplasia, antrum	n=28	n=18	0.717
No	14 (50.0)	7 (38.9)	
Mild	7 (25.0)	4 (22.2)	
Moderate	5 (17.9)	4 (22.2)	
Severe	2 (7.1)	3 (16.7)	
Intestinal metaplasia, body	n=28	n=18	0.117
No	19 (67.9)	10 (55.6)	
Mild	4 (14.3)	0	
Moderate	3 (10.7)	6 (33.3)	
Severe	2 (7.1)	2 (11.1)	
Serum pepsinogen level	n=17	n=12	
Pepsinogen I, ng/mL	57.57±28.72	90.43±168.55	0.434
Pepsinogen II, ng/mL	21.99±28.72	22.08±22.51	0.994
Pepsinogen I/ II ratio	3.90±1.70	2.98±1.86	0.177
	n=23	n=15	
Serum interleukin-1β, pg/mg†	29.80±48.81	11.53±7.57	0.091

Data are presented as mean±SD or number (%).
*Atrophic gastritis was evaluated according to the updated Sydney scoring system (mild, moderate, and severe) using endoscopic biopsy specimens to assess the degree of neutrophil and monocyte infiltration; †Interleukin-1β levels were measured using ELISA kits and expressed as picograms of cytokine per milligrams of biopsy protein (pg/mg protein) in the supernatant fluid.

4. Clinical features of patients according to the pH level of gastric juice

The subjects were categorized into two groups based on pH 3 in gastric juice as below 3 is acknowledged as the normal range of pH of gastric juice.[3] The number of subjects with pH <3 of gastric juice was 28. The average pH value was 1.79±0.45 (mean±SD) (Table 2). On the other hand, the number of subjects with value pH ≥3 was 18, the average value of pH was 5.46±1.58. There was no significant difference in sex, age, body mass index, alcohol, or smoking habits between the two groups.

5. *H. pylori* infection and serum PG according to the pH level of gastric juice

The association between acidity of gastric juice and *H. pylori* infection was studied (Fig. 2). The infection rate with pH <3 group was 21.4%. On the contrary, the infection rate with pH ≥3 group was 61.1% and there was statistical significance (p=0.007).

PG I and PG II with pH <3 on the scale were 57.57±28.72 and 21.99±28.72, respectively. PG I/II ratio with pH <3 was 3.90±1.70. On the other hand, PG I and PG II with pH ≥3 were 90.43±168.55 and 22.08±22.51, respectively and PG I/II ratio with pH ≥3 was 2.98±1.86. There was no statistical difference between regarding pH 3 (Table 2).

IL-1β with pH <3 on the scale were 29.80±48.81 pg/mg. IL-1β with pH ≥3 was 11.53±7.57. There was no statistical difference regarding pH of gastric juice (p=0.091) (Table 2).

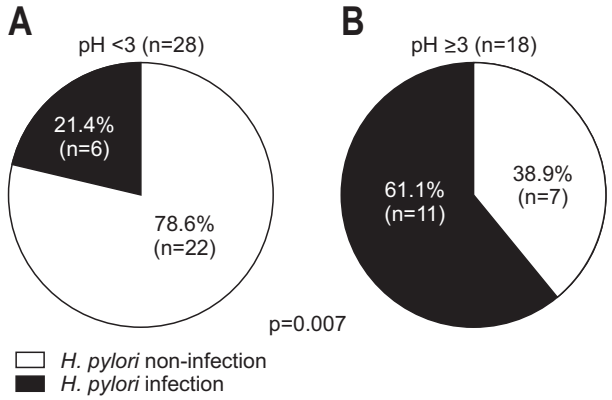

Fig. 2. Association between gastric juice acidity and *Helicobacter pylori* infection. (A) The *H. pylori* infection rate at pH <3 is 21.4%. (B) By contrast, the infection rate at pH ≥3 is 61.1%, which is statistically significant.

6. AG and moderate or severe IM according to the pH level of gastric juice

The relationship between pH of gastric juice and AG was analyzed (Fig. 3). The presence of atrophy in gastric body was 21.1% with less than pH 3 group. On the contrary, the presence of atrophy in gastric body was 52.9% with more than pH 3 group (p=0.047). However, it was not statistically significant in gastric antrum (p=0.738). When distribution of histologic AG was checked using the updated Sydney scoring system, there was no statistical significance in antrum (p=0.337) and body (p=0.169) (Table 2).

The severity of IM between two gastric pH groups was compared (Fig. 4). The presence of moderate or severe IM in gastric body was 17.9% with pH <3 group. On the contrary, the presence of moderate or severe IM in gastric body was 44.4% with pH ≥3 group. It showed marginal significance (p=0.051). On the other hand, there was no statistical significance in gastric antrum (p=0.318).

DISCUSSION

One of the important functions of stomach is to regulate and

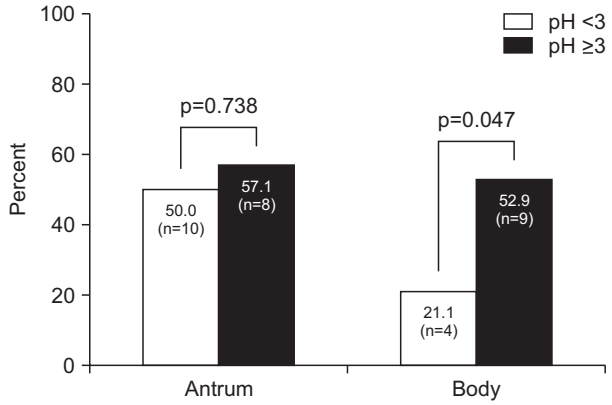

Fig. 3. Association between gastric juice acidity and atrophic gastritis. The presence of atrophic gastritis in the gastric antrum at pH <3 and pH ≥3 is 50.0% and 57.1%, respectively. The presence of atrophic gastritis in the gastric body for patients in the pH <3 and pH ≥3 groups with AG in the gastric antrum are 21.1% and 52.9%, respectively, which is statistically significant.

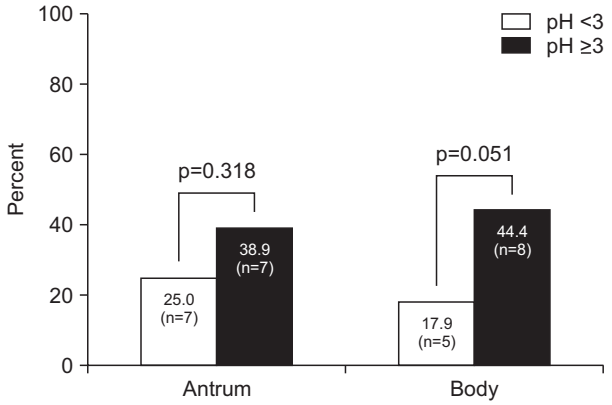

Fig. 4. Association between gastric juice acidity and moderate or severe intestinal metaplasia. The presence of moderate or severe intestinal metaplasia in the antrum in the pH <3 and pH ≥3 groups is 25.0% and 38.9%, respectively. The presence of moderate or severe intestinal metaplasia in the body in the pH <3 and pH ≥3 groups is 17.9% and 44.4%, respectively, which is statistically significant.

sustain acid secretion to sterilize ingested foods and digest them. Thus affecting factors on the gastric acid are very important to understand gastrointestinal diseases. As *H. pylori* colonizes in the stomach for long time it is quite understandable that there is close relationship between *H. pylori* and gastric acid. In the acute infection stage, direct contact between parietal cells and *H. pylori* and several cytokines released from this process reduce the gastric acid secretion.[20,21] Upregulation of IL-1β and tumor necrosis factor-α by *H. pylori* infection strongly suppresses acid secretion[22] and, at the same time, IL-1β reduces secretion of histamine from enterochromaffin-like cells.[23] This makes *H. pylori* thrive in the corpus and destroy parietal cells and finally impair gastric acid secretion. As *H. pylori* infection progresses into chronic state, corpus atrophy is accelerated.[6] In turn, gastric acid secretion diminishes in the result of loss of parietal cells.[24]

Based on the long-term interplay between *H. pylori* and environment in the stomach, we estimated essential factors for gastric acid secretion. Collected gastric juice was categorized into two groups by acid level of pH 3, since previous studies observed below pH 3 as normal gastric acidity range.[3,25] In this study, we found that *H. pylori* infection was significantly associated with gastric acidity. In other words, pH <3 group showed 21.4% of *H. pylori* infection rate, but pH ≥3 group showed much higher infection rate (61.1%, p=0.007). Moreover, there are several other studies reporting the association between *H. pylori* infection and gastric acidity, especially after *H. pylori* eradication. El-Omar *et al*.[26] reported that *H. pylori* eradication improved hypochlorhydria in patients with mild atrophy in gastric body. Similarly, Iijima *et al*.[11] assessed gastrin-stimulated acid output and histologic change prior to eradication and 1, 7 months after eradication and gastric acid secretion over 5 years in 12 patients with hypochlorhydria (<0.6 mmol/10 min). Gastric acid secretion was reversed to normal range in nine of 23 patients (39 %) at 7 months after eradication,[11] and previous studies suggest that eradication of *H. pylori* causes immediate increase of gastric acid secretion.

Apart from direct effect of *H. pylori* on gastric acid, AG and IM in the gastric body were also associated with gastric fluid acidity in this study. Atrophic change in the gastric body appeared more frequently in the group with pH ≥3. The similar tendency of IM with marginal significance was observed in gastric body. The presence of moderate or severe IM in the gastric body was more prominent in the pH ≥3 group, but, there was no association in the antrum. Given that the parietal cells exist mainly in the body, this finding of the study is quite explainable.

Many other previous studies reported that PG can be used as a marker of AG.[27] PG I and II are produced by gastric mucosa.[28] PG I is exclusively produced in chief and mucous neck cells in the fundic glands, while PG II is secreted not only in the these cells and but also in the cells in the pyloric glands and Brunner's glands. As gastritis progresses, both PG I and PG II increase. Since chief cells are replaced by pyloric glands as inflammation becomes aggravated, the level of PG II increases and the level of PG I starts to decrease. Finally, the PG I/II ratio decreases. As low serum PG I and PG I/II ratio reflect gastric atrophy, these markers have been studied as biomarkers for screening high risk group of gastric cancer. In this study, however, there was no statistical significance between gastric fluid acidity and PG I or PG I/II ratio, which might suggest it is more complicated consequences of gastric hormones and vagus nerve stimulation. Unraveled relationship between gastric acidity would be needed to study further.

There are several limitations in the study. First of all, this is related to the difficulty of measuring gastric acidity like other studies. Since gastric fluid is sticky, aspiration of clean gastric juice is technically tough. Other secretions including saliva or bile could move into the stomach, it might be contaminated by them. Although we tried gastric juice aspiration as carefully as possible only during the sedative endoscopy, aspiration could not be performed sometimes due to patients' discomfort such as coughing or belching. In addition, the number of study patients has also been very small for the above reasons. This might lead no significance between acidity of gastric fluid and IL-1β. Secondly, as topical spray anesthetic was used before the initiation of endoscopy, it might influence on gastric juice. Those topical aerosols, but, were sprayed into the oropharynx and applied for all patients, the influence of topical anesthetics on the gastric juice might be minimal. In the future, larger cohort would be expected to reveal the correlation between the environment in stomach and *H. pylori* infection.

In spite of these limitations, we suggest a relationship between chronic *H. pylori* infection and gastric juice pH ≥3 and this result might be originated from AG and IM in the body.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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