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An Ammonia Concentration and Gastric Mucosal Lesions with *Helicobacter pylori* Infection

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— **Article** —

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Abstract Relationship between the ammonia concentration and gastric mucosal lesions with *Helicobacter pylori* (*H. pylori*) infection were investigated. The aim of this study is to evaluate the usefulness of the new method for *H. pylori* infection using ammonia biosensor, and to clarify the ammonia concentration at the gastric mucosa.

The ammonia concentration at the gastric mucosa was measured in 149 patients with/without *H. pylori* using an ammonia biosensor. Also, the ammonia concentration and $^{13}CO_2$ in the expiratory air were measured after spraying ^{13}C- urea over the mucosal surface.

The ammonia concentration in *H. pylori*-positive patients was significantly greater than that in *H. pylori*-negative. In *H. pylori*-positive patients with mild atrophy, the antrum value of ammonia concentrations was significantly greater than in the other areas, in severe atrophy, values tended to be lower than in the mild and moderate atrophy. The ammonia concentration increased markedly after ^{13}C-urea spraying with a positive correlation with the $^{13}CO_2$ concentration.

H. pylori produce ammonia from urea at the gastric mucosa. It appears that *H. pylori* moves from the antrum to the upper body of the stomach with progression of mucosal atrophy. The measurement of ammonia concentration at the gastric mucosal surface is a non-invasive and effective method for the diagnosis of *H. pylori* infection.

Key words — *Helicobacter pylori*, ammonia concentration, ammonia biosensor, new non-invasive test for *Helicobacter pylori*

Introduction

The mechanism of mucosal injuries caused by *Helicobacter pylori* (*H. pylori*) infection has been elucidated[1-4], not yet fully understood. Ammonia produced by *H. pylori* is considered to be one important factor[5 - 9]. Progressive mucosal atrophy caused by *H. pylori* infection may be a risk factor for gastric cancer[10 - 12]. Endoscopy is an essential modality for the detection of *H. pylori* infection, and pathologic examination, culture, and rapid urease tests of biopsy specimens have been used during endoscopy to confirm this diagnosis. However, these tests provide data that are limited to the examined site and not the entire stomach. Serum antibody titers and ^{13}C-urea breath tests have been used to augment this shortcoming, but these methods require time and invasive sampling of mucosal specimens, blood, or expiratory air. We developed a new, non-invasive method for the diagnosis of *H. pylori* infection by the measurement of ammonia, which can be performed during routine endoscopic examination.

The ammonia concentration in gastric juice is considered to be high in patients with *H. pylori* infection[13, 14], and measurement of ammonia confirms the presence or absence of *H. pylori*. It also provides information on bacterial urease activity, as well as

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the distribution and density of *H. pylori* infection. We developed an ammonia biosensor, which was reported its usefulness in a previous study, where a correlation was suggested between ammonia concentrations in the expiratory air and theses in the blood of patients with cirrhosis[15]. We applied during routine endoscopy to measure the ammonia concentration at the mucosal surface of the stomach. This study was conducted not only to evaluate the usefulness of this new instrument but also to clarify the relationship between the ammonia concentration and gastric lesions such as atrophy and peptic ulcer.

Patients and Methods

Measurement of ammonia concentrations:

The ammonia biosensor (Chemical, Co., Tokyo) consists of a pH glass electrode covered by a polymeric membrane which has selective permeability to ammonia. Ammonia penetrating through the membrane causes a reaction with the internal fluid of the biosensor as follows; [$NH_4^+ \leftrightarrow NH_3 + H^+$]. The concentration of ammonia can be indirectly obtained by detecting changes in H^+. A biosensor 3 m in length with an electrode tip of 2.5 mm maximum diameter

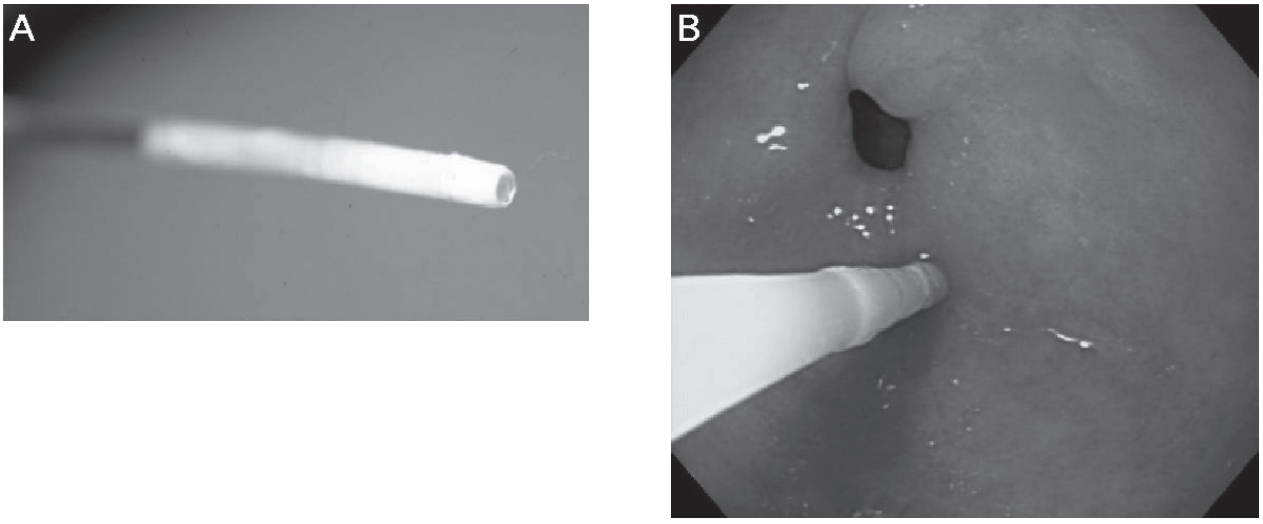

Figure 1: the ammonia biosensor; [A] The electrode tip of the ammonia biosensor protrudes through the forceps channel of the endoscope. [B] The biosensor is advanced to place the tip on the mucosal surface of the stomach.

Table 1: Measurment samples of ammonia concentration, gastric mucosal lesions, medication, and number of patients

measurement samples of ammonia concentration	gastric mucosal lesions	medication	number of patients		
			total	*H.pylori* positive	*H.pylori* negative
in gastric juice	no lesions except atrophy	no medication	62	32	30
at gastric mucosa	no lesions except atrophy	no medication	56	27	29
	peptic ulcer scar	H_2-blocker	26	23	3
	no lesions	no medication	5	1	4

can go through the forceps channel of an endoscope (Q240, Olympus, Tokyo: Fig. 1A). During routine endoscopic examination, the biosensor is advanced from the forceps port to place the electrode tip on the mucosal surface of the stomach (Fig. 1B). The ammonia concentration at the electrode tip is shown on a display and recorded continuously. The mean value averaged over 10 sec was used as the ammonia concentration at the mucosal surface in this study. The electrode can be used at temperatures ranging from 0 to 50°C and for ammonia concentrations ranging from 0.10 to 100 ppm. The concentration of ammonia in gastric juice or at the mucosal surface was measured in 149 patients undergoing upper gastrointestinal endoscopy in Osaka Medical College Hospital (Table 1).

Ammonia concentration in gastric juice and gastric mucosal atrophy:

Subjects consisted of 62 patients undergoing upper gastrointestinal endoscopy who showed no gastric lesions except for atrophy. Antibiotics and proton pump inhibitors were not given within the 7 days before examination. After obtaining informed consent, the ammonia concentration was measured in gastric juice samples taken during endoscopy. Serum anti-*H. pylori* IgG antibody titers were measured using an ELISA (Kyowa-medics, Tokyo) before endoscopy. Titers of 0.8 or higher were considered positive for *H. pylori*. The severity of mucosal atrophy was classified into 3 grades by Kimura-Takemoto classification[16]: mild atrophy (limited to the lesser curvature of the middle body), moderate atrophy (extending to the entire lesser curvature of the body), and severe atrophy (extending from the anterior and posterior walls of the body to the entire greater curvature).

Ammonia concentration at the mucosal surface and gastric mucosal atrophy:

In 56 of the patients with no gastric lesions except atrophy, after endoscopic examination of the stomach, the mucosal surface was washed with water to remove gastric juice. The ammonia concentration on the mucosal surface was then measured using the ammonia biosensor at 4 points (the greater curvature, lesser curvature, anterior wall, and posterior wall) of 3 areas each (the antrum, gastric angle, and upper body).

Ammonia concentration at the mucosal surface with peptic ulcer:

After obtaining informed consent, the ammonia concentration at the mucosal surface was measured during follow-up endoscopy in 26 patients undergoing maintenance therapy with an H_2-blocker after healing of peptic ulcer and in 5 patients with no gastric lesions. A ^{13}C-urea breath test was performed on the same day. After confirming healing of peptic ulcers, the ammonia concentration was measured at the mucosal surface. ^{13}C-urea (100 mg) dissolved in 100 ml water was then injected from the forceps port and sprayed over the entire inner wall of the stomach. The ammonia concentration on the mucosal surface was measured again 5 min after the ^{13}C-urea spray. The measurement was performed around the ulcer scars as well as the points defined above. Expiratory air samples taken before and 20 min after ^{13}C-urea spray were subjected to a measurement of $^{13}CO_2$ concentrations using a mass spectrometer. $\Delta^{13}CO_2$ values of $2.5^{0}/_{00}$ or greater were considered to be *H. pylori*-positive.

Statistical analysis:

Data are expressed as mean ± SE. Statistical analysis was performed using Student's t test for comparison of groups and Fisher's PLSD test for multiple comparisons with a significance level of $p<0.05$.

This study was conducted according to Declaration of Helsinki principles. All patients agreed to the informed consent in written form.

Results

Ammonia concentration in gastric juice and gastric mucosal atrophy:

The ammonia concentration in gastric juice was <0.10 ppm in 30 patients negative for anti-*H. pylori* IgG antibody, irrespective of the severity of gastric mucosal atrophy. The ammonia concentration in gastric juice was 1.62±0.87 ppm on average in 32 patients positive for anti-*H. pylori* IgG antibody, which was significantly higher than in the antibody-negative group. In the antibody-positive group, the ammonia concentration in gastric juice was 3.25±0.52 ppm in patients with mild atrophy (3 men and 1 woman with a mean age of 69.3±11.3 years), 1.46±0.59 ppm in patients with moderate atrophy (9 men and 8 women, 64.2±12.6 years), and 1.28±0.68 ppm in patients with severe atrophy (6 men and 5 women, 68.7±10.6 years). The ammonia concentration in gastric juice tended to be lower in patients with more severe mucosal atrophy (Fig. 2).

Ammonia concentration at the mucosal surface and gastric mucosal atrophy:

The ammonia concentration at the mucosa was <0.10 ppm in 24 patients negative for anti-*H. pylori* IgG antibody, irrespective of the severity of gastric mucosal atrophy. The ammonia concentration at the gastric surface was 2.32±0.14 ppm on average in 27 patients positive for anti-*H. pylori* IgG antibody, which was significantly higher than in the antibody-negative group.

No significant differences were noted in the ammonia concentrations between the greater curvature, lesser curvature, anterior wall, and posterior wall of the antrum, gastric angle, and upper body in patients positive for anti-*H. pylori* antibody. Therefore, the mean ammonia concentrations at the above 4 points were used for analysis. In the mild atrophy group (4 men with a mean age of 64.5±20.3 years), the ammonia concentration was 3.15±0.60 ppm in the antrum, 1.43±0.13 ppm in the gastric angle, and 1.03±0.10 ppm in the upper body, significantly higher

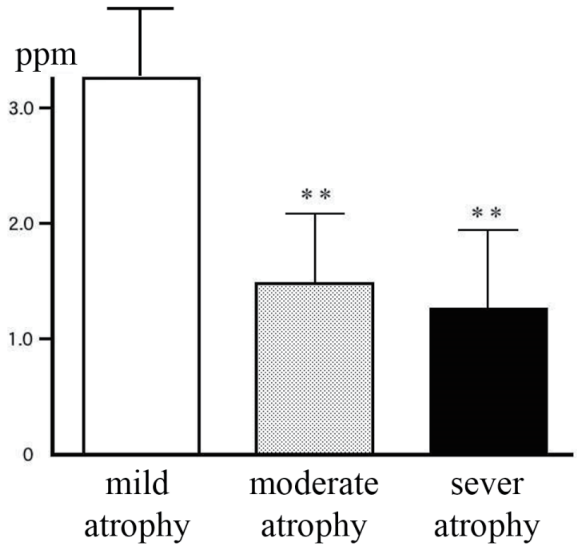

Figure 2: The ammonia concentration in gastric juice by the atrophic degree; The ammonia concentration in gastric juice in the mild atrophy group was significantly higher than in the moderate and severe atrophy groups.
** : $p<0.005$ vs mild atrophy

in the antrum than in the other areas. In the moderate atrophy group (7 men and 6 women, 61.8±10.1 years,) the ammonia concentration was 2.25±0.29 ppm in the antrum, 2.59±0.27 ppm in the gastric angle and 3.39±0.38 ppm in the upper body, significantly higher in the latter. In the severe atrophy group (7 men and 3 women, 75.4±6.9 years), the ammonia concentration was 1.51±0.24 ppm in the antrum, 2.01±0.42 ppm in the gastric angle, and 2.29±0.42 ppm in the upper body; significantly higher in the upper body compared with the antrum. The ammonia concentration tended to be lower in the severe atrophy group than in the mild and moderate atrophy groups (Fig. 3).

Ammonia concentration at the mucosal surface with peptic ulcer:

There were 13 patients (8 men and 5 women, 69.6±10.3 years) with gastric ulcer, 8 patients (4 men and 4 women, 68.6±9.6 years) with duodenal ulcer, 5 patients (3 men and 2 women, 68.6±9.6 years) with gastroduodenal ulcers, and 5 patients (2 men and 3 women, 66.6±8.2 years) without ulcer. The ammonia concentration at the mucosal surface was 2.32±0.14 ppm in the 23 patients positive for *H. pylori* by ^{13}C-urea breath test. The distribution of ammonia concentrations in *H. pylori*-positive patients with gastric ulcer showed a similar pattern as in *H. pylori*-positive patients without gastroduodenal lesions. In contrast, the ammonia concentration at the mucosal surface was 0.12 - 0.22 ppm in 4 patients and <0.10 ppm in 4 patients all negative for *H. pylori* (2 patients with gastric ulcers, one with duodenal ulcer, 1 with gastroduodenal ulcers, and 4 with no peptic ulcer). The ammonia concentration increased markedly to 8.55±1.94 ppm after ^{13}C-urea spray in the *H. pylori*-positive group, while the concentration did not change in the *H. pylori*-negative group. The ammonia concentration after ^{13}C-urea spray was 3.32±0.96 ppm around ulcer scars, which was not significantly higher than at other measurement points in the same area and lower than the maximum value in the stomach. The ammonia concentration around the duodenal ulcer

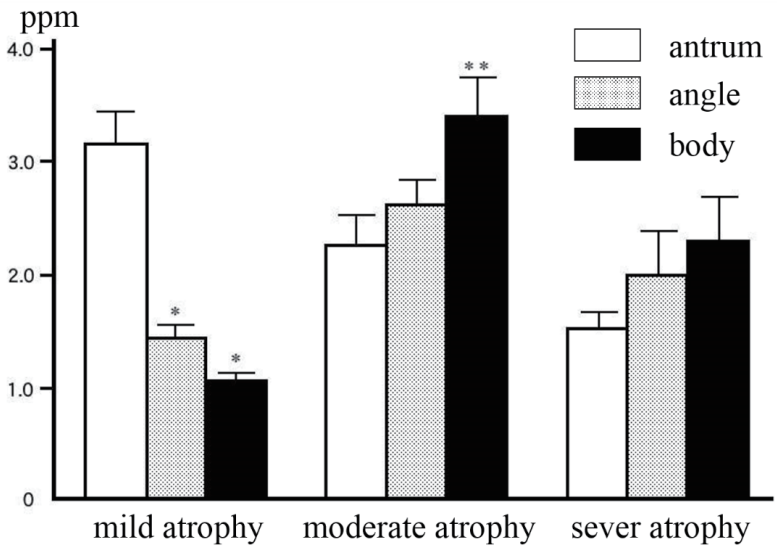

Figure 3: The ammonia concentration at the gastric mucosal surface by the atrophic degree; In the mild atrophy group, the ammonia concentration was significantly higher at the mucosa of the antrum than in other areas. In the moderate atrophy group, it was significantly higher in the upper body. The ammonia concentration tended to be lower in the severe atrophy group than in the moderate atrophy group. *: $p<0.05$, **: $p<0.005$ vs antrum

was <0.10 ppm before and after ^{13}C-urea spray. A positive correlation was noted between the ammonia concentration at the mucosal surface (maximum value) after ^{13}C-urea spray and the $^{13}CO_2$ concentration in the expiratory air (Fig. 4). Recurrence of peptic ulcer was not found in any patients after ^{13}C-urea spray.

Discussion

Studies have suggested that chronic active gastritis and gastric mucosal atrophy are caused by *H. pylori* infection[17], and recurrence of peptic ulcer is markedly reduced after *H. pylori* eradication[18, 19]. Although the development of mucosal lesions due to *H. pylori* infection has been studied by many researchers, a unified concept of the mechanism has not been established. *H. pylori* has strong urease activity which produces ammonia from nitrogen sources to neutralize acidic pH in the stomach and to create a suitable environment for its growth. Among *H. pylori*-related factors, ammonia above a certain concentration is toxic to the gastric mucosa[20 - 22].

The ammonia concentration in gastric juice was 1.62±0.87 ppm in patients with *H. pylori* infection, significantly higher than in those negative for *H. pylori* (<0.10 ppm). It is not clear whether the ammonia concentration in gastric juice reflects the ammonia concentration at the mucosal surface or whether ammonia in gastric juice causes gastric mucosal injuries despite the mucus gel layer on the gastric surface[23, 24].

The endoscopic measurement of local ammonia concentrations with the biosensor has the following advantages: (1) the measurement can be performed non-invasively during routine endoscopic examination, (2) sampling of mucosal specimens, blood, or expiratory air is unnecessary, (3) the false negative rate is low because the measurement is a plane-diagnostic procedure over the entire stomach, rather than a spot test such as biopsy, and (4) the measurement provides a distribution density of *H. pylori* and estimates the severity of mucosal atrophy in the stomach.

The ammonia concentration at the mucosal surface was 2.31±1.25 ppm on average in *H. pylori*-positive

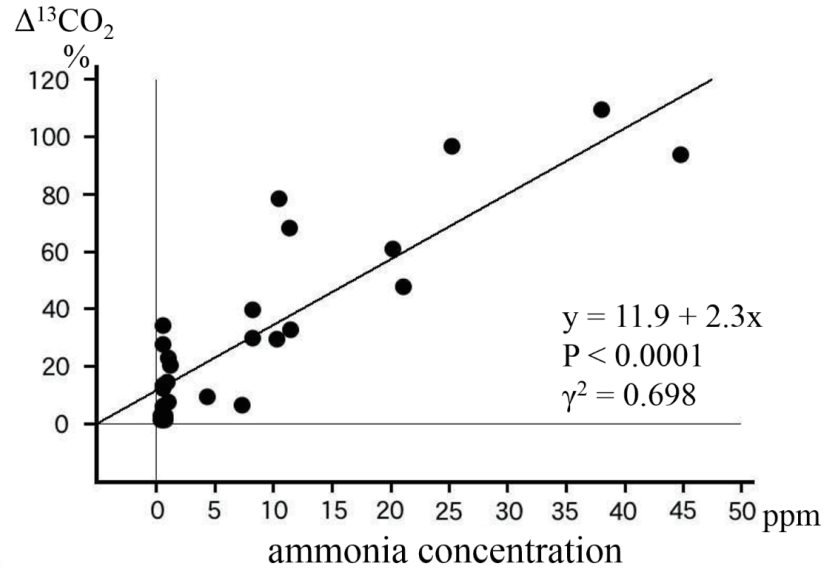

Figure 4: The correlation between the ammonia concentration and the $^{13}CO_2$ concentration; A positive correlation was noted between the ammonia concentration at the mucosal surface after ^{13}C-urea spray and the $^{13}CO_2$ concentration in expiratory air.

patients, significantly higher than the <0.10 ppm value in *H. pylori*-negative patients. Moreover, the concentration significantly increased to 11.30±10.98 ppm after endoscopic ^{13}C-urea spray in the *H. pylori*-positive group, but remained <0.10 ppm in the *H. pylori*-negative group. A positive correlation was noted between the $^{13}CO_2$ concentration in the expiratory air 20 min after ^{13}C-urea spray and the ammonia concentration at the mucosal surface. These findings confirm that ammonia produced by *H. pylori* in the stomach is correctly measured by the biosensor.

The correlation between the severity of mucosal atrophy associated with *H. pylori* infection and the ammonia concentration at the mucosal surface was studied in patients without abnormal findings on gastric endoscopy. The ammonia concentration at the mucosal surface was significantly higher than in those negative for *H. pylori*. In patients positive for *H. pylori*, the ammonia concentration was highest in the antrum, followed by the gastric angle and upper body in the mild atrophy group. In the moderate atrophy group, the concentration was highest in the upper body, followed by the gastric angle and antrum. With advancing severity of atrophy, the highest concentration of ammonia migrated from the antrum to the upper body of the stomach. In the severe atrophy group, the distribution of ammonia concentrations showed a similar pattern as in the moderate atrophy group, although the ammonia concentration at the mucosal surface was rather low in the severe atrophy group.

These findings suggest that *H. pylori* preferentially moves from the antrum to the upper body of the stomach with progression of mucosal atrophy, resulting in a change in the distribution density of the bacteria. The amount of bacteria may even decrease in subjects with severe atrophy, probably because of poorer environment for growth. However, *H. pylori* can still inhabit atrophic mucosa because ammonia concentrations at severely atrophic sites, which were higher than at non-infected sites, rapidly increased after ^{13}C-urea spray. A high ammonia concentration or a high distribution density of *H. pylori* at non-atrophic sites is considered to be a risk factor for progression of mucosal atrophy.

The distribution of ammonia concentrations in *H. pylori*-positive patients with gastric ulcer showed a similar pattern as in *H. pylori*-positive patients without gastric lesions on endoscopy. No significant differences were noted in the ammonia concentrations after ^{13}C-urea spray between mucosa around the ulcer scars and normal adjacent mucosa. The ammonia concentration around the ulcer scars was lower than the maximum ammonia concentration in the stomach. Therefore, the distribution density of *H. pylori*, or the ammonia concentration at the mucosal surface, may not directly relate to the development or recurrence of gastroduodenal ulcers[25].

Pathologic examination of biopsy specimens, culture, and rapid urease tests have been used during endoscopic examination to establish the diagnosis of *H. pylori* infection. As other noninvasive detection of *H. pylori* infection in clinical practice, the breath ammonia measurement after ingestion of unmarked urea may be feasible as a diagnostic test for *H. pylori*, however, its sensitivity and specificity will be not enough[15, 26, 27]. Although a new endoscopic technique (endoscopic urease sensor) to directly measure the urease activity of *H. pylori* is under development[28], our method may be superior because ammonia is the end product of *H. pylori* and its sensitivity is markedly increased after non-labeled urea spray (data not shown). Its sensitivity and specificity are 93 to 99% and 95 to 99% with pathologic examination of biopsy specimens, 77 to 94% and 100% with culture, 86 to 97% and 86 to 98% with rapid urease tests, and 92.9% and 95.7% with the endoscopic urease sensor technique, respectively[29]. When 0.15 ppm is chosen as a cut-off value, the sensitivity was 97.4% and the specificity was 97.0% with the ammonia biosensor method.

In conclusion, it was shown that *H. pylori* produced

ammonia from urea at the gastric mucosa surface. The main distribution of *H. pylori* moved from the antrum to the upper body of the stomach with progression of mucosal atrophy. However, the ammonia concentration at the gastric mucosal surface may not directly relate to the recurrence of peptic ulcers. The measurement of ammonia concentrations at the mucosal surface during routine endoscopic examination with the biosensor is a non-invasive and effective method for the diagnosis of *H. pylori* infection and the measurement of ammonia concentration.

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