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# Fasting gastric pH of Japanese subjects stratified by IgG concentration against *Helicobacter pylori* and pepsinogen status

Running head: Gastric pH and *H. pylori* antibody titer level

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## *ABSTRACT*

Background: The clinical significance of Helicobacter pylori antibody titer has been controversial, and the association between extent of gastric atrophy or acid secretion and H. pylori antibody concentration has not been elucidated. Materials and methods: Serum pepsinogen and, H. pylori antibody concentration, and fasting gastric pH (as an indicator of acid secretion) were measured in 231 patients undergoing upper gastrointestinal endoscopy. “Atrophic” pepsinogen was defined as pepsinogen-I < 70 ng/mL and pepsinogen-I/II ratio < 3. Other levels of pepsinogen were defined as “normal”. Fasting gastric pH was analyzed in subjects stratified by pepsinogen level and by H. pylori antibody concentration. Results: H. pylori antibody concentration showed no significant relationship with fasting gastric pH when all subjects were analyzed together. In H. pylori-seronegative subjects, fasting gastric pH was within normal range, irrespective of the extent of mucosal atrophy. In H. pylori-seropositive subjects, H. pylori antibody concentration was positively correlated with fasting gastric pH in subjects with “normal” pepsinogen, but inversely correlated in those with “atrophic” pepsinogen. Particularly in subjects with low H. pylori antibody concentration and atrophic mucosa, a group reportedly at high risk of non-cardia cancer, the most impaired acid secretion was shown among subjects with atrophic mucosa. Conclusions: The relationship between acid secretion and H. pylori antibody concentration differs depending on the presence of mucosal atrophy. Our findings provide a possible rationalization for measuring both serum pepsinogen levels and H. pylori antibody concentration in gastric cancer screening.

**Key words:** *Helicobacter pylori*, pepsinogens, atrophic gastritis, acid secretion, antibody concentration

## *Introduction*

*Helicobacter pylori* is a rod-shaped bacterium that commonly infects the human stomach (1). Chronic infection leads to chronic active inflammation of the gastric mucosa, which can progress to extensive atrophic gastritis with intestinal metaplasia, dysplasia, and finally to differentiated-type noncardia gastric cancer (2). Uemura et al. reported that gastric cancer developed in patients with *H. pylori* infection but not in uninfected patients (3). Atrophic gastritis is usually diagnosed from biopsy specimens; however, many previous studies have shown that it can be diagnosed non-endoscopically by assaying the serum levels of pepsinogen, and that reduction of serum pepsinogen I or the ratio of serum pepsinogen I to II is a reliable markers of chronic active gastritis, a high-risk precursor of gastric cancer (4, 5). Both *H. pylori* infection and resultant mucosal atrophy in the stomach are associated with an increased risk of gastric cancer development, particularly differentiated-type noncardia gastric cancer. Hence, the combined analysis of *H. pylori* seropositivity and mucosal atrophy determined by the serum pepsinogen method is considered to be a better non-invasive method of stomach cancer screening than measuring pepsinogen alone (6-9). Gastric cancer incidence can be predicted accurately by the combination of *H. pylori* seropositivity and serum pepsinogen. For example, gastric cancer incidence among subjects negative for *H. pylori* and with pepsinogen-I levels >50 ng/mL (16/100000 person-year) is significantly lower than for those selected by pepsinogen-I levels >50 ng/mL alone (85/100000 person-year). Similarly, gastric cancer incidence among subjects positive for *H. pylori* and with pepsinogen-I levels <30 ng/mL (596/100000 person-year) is significantly higher than for those selected by pepsinogen-I levels <30 ng/mL alone (341/100000 person-year) (8). However, the clinical significance of

measuring IgG titer against *H. pylori* has been controversial. Recently, studies have suggested that a low titer of *H. pylori* antibody with mucosal atrophy determined by pepsinogen status is a stronger predictor of differentiated-type gastric cancer than a high titer (9-12). Tatemichi et al. showed that the odds ratio for gastric cancer in subjects with low *H. pylori* titer was significantly higher than that in those with high *H. pylori* titer (14.1 vs. 6.7). They also found that the odds ratio decreased as IgG titer increased among subjects with *H. pylori* seropositivity and mucosal atrophy (9). Because the IgG titer of *H. pylori* could be a marker for the density of *H. pylori*, the mechanisms underlying this inverse correlation between low *H. pylori* antibody titer and gastric cancer might include the spontaneous decrease in *H. pylori* in severe atrophic gastritis, which would reduce *H. pylori* antibody titer (13-15).

As the degree of mucosal atrophy progresses, a decrease in parietal cell numbers leads to acid in the stomach being neutralized; hence, pH itself becomes a good indicator of mucosal atrophy (16, 17). However, the association between gastric acid secretion and *H. pylori* antibody concentration has not yet been elucidated. In subjects with negative *H. pylori* antibody and high pepsinogen level, which is considered to indicate histologically and functionally normal gastric mucosa without atrophy, stomach acidity is thought to be normal. In subjects with a low titer of *H. pylori* antibody and low pepsinogen level, which has been shown by several cohort studies to be associated with a high risk of differentiated-type stomach carcinoma, acid secretion is thought to be extremely impaired due to atrophic gastritis. However, these are merely hypotheses that have not been examined in detail.

In the present study, we examined the fasting gastric pH (an indicator of acid secretion) of subjects stratified by pepsinogen status and by *H. pylori* IgG antibody concentration to clarify the relationship between gastric acid secretory status and *H. pylori* antibody profile. We also refer to the clinical significance of measuring concentrations of *H. pylori* antibody and serum pepsinogen in detecting subjects with extensive atrophy, a high-risk group for non-cardia gastric cancer.

## *Methods*

### *Subjects*

From 2007 to 2010, we prospectively enrolled 231 subjects aged 21 to 86 years who were attending Tokyo Dental College Ichikawa General Hospital outpatient clinic for routine upper gastrointestinal endoscopy. Exclusion criteria were as described previously (18, 19). Gastric mucosal atrophy was diagnosed according to the endoscopic atrophic border scale of Kimura and Takemoto (20); there were three classifications (1, mild or no atrophy; 2, moderate atrophy; and 3, severe atrophy). All patients had endoscopy in the morning (from 9 am to 12 pm). This study was approved by the Tokyo Dental College Ichikawa General Hospital Ethics Committee, and it was conducted according to the principles of the Second Declaration of Helsinki. All patients provided written informed consent prior to enrolment.

### *Assays for antibody to* H. pylori*, pepsinogen-I, and pepsinogen-II in serum*

A blood sample was drawn immediately before the endoscopic examination and centrifuged at 12 000 g for 5 min at 4°C. The serum was stored at −20°C until assayed. A diagnosis of *H. pylori* infection was based on detection of serum IgG antibodies to *H.*

*pylori* with a specific enzyme-linked immunosorbent assays (ELISA) kit (E Plate Eiken *H. pylori* Antibody, Eiken Chemical Co., Ltd., Tokyo, Japan). In this ELISA kit, the two-graph receiver operating characteristic analysis, a commonly used method to set the cut-off point in ELISA, was used as a tool for selecting cut-off points (21). Briefly, two curves of sensitivity and specificity of the ELISA as a function of the applied cut-off points were constructed, and the intersection point of the two curves was set as the cut-off point for *H. pylori* concentration and defined as 10 U/mL. Sensitivity and specificity were calculated based on results of the 13C-urea breath test as a gold standard. Fujioka and Tokieda have reported a sensitivity of 100% and specificity of 80% for this kit with respect to *H. pylori* culture and rapid urease test in 70 Japanese subjects, and these results are the highest among available kits in Japan (22). Subjects with antibody concentration <10 U/mL were categorized as the infection-negative group; those with a concentration of 10-30 U/mL were designated the low-concentration group; those with a concentration of 30-50 U/mL were classified as the moderate- concentration group; and those with a concentration >50 U/mL were classified as the high-concentration group. Measurement of serum pepsinogen-I, pepsinogen-II, and *H. pylori* IgG antibodies was contracted out to Mitsubishi Chemical Medience Co., Ltd. (Tokyo, Japan) as described previously (18, 19). Pepsinogen was defined as "atrophic" when the criteria of both pepsinogen-I < 70 ng/mL and pepsinogen-I/II ratio < 3 were fulfilled (23). Pepsinogen was defined as "normal" if pepsinogen-I > 70 ng/mL, or if pepsinogen-I < 70 ng/mL and pepsinogen-I/II ratio > 3.

***pH measurement***

Gastric juice samples were collected through a sterile tube during endoscopy, and

their pH was measured as described previously (18, 19). Briefly, specimens were centrifuged at 12000 × *g* for 5 min and the supernatant collected; pH was then determined using a glass electrode (pH meter M-12, Horiba Instruments Ltd., Kyoto, Japan). Because fasting gastric pH is closely associated with basal acid output (correlation coefficient > 0.7) (24), we used fasting gastric pH as an indicator of acid secretion by the stomach.

### *Statistical analyses*

Subjects were classified into eight groups, with two categories for level of mucosal atrophy ("normal" pepsinogen and "atrophic" pepsinogen) and four for IgG concentration against *H. pylori* (negative, low, moderate, and high). Fasting gastric pH in subjects with no mucosal atrophy and negative *H. pylori* IgG was defined as control. These data were expressed as mean ± standard deviation (SD). One-way ANOVA was used to determine statistical significance of differences. Correlations between fasting gastric pH and *H. pylori* IgG concentration were examined by Spearman rank correlation test. A p-value less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed using the Statcel2 software program (OMS Publishing Inc., Saitama, Japan).

## *Results*

### *Clinical features of the patients*

Table 1 shows the demographic and clinical characteristics of the 231 subjects. Their mean age was 59.6 years, and there were 122 men and 109 women. *H. pylori* was

detected in 106 of the subjects. Based on the endoscopic findings, 110 (47.6%) had mild or no atrophic gastritis (atrophic border scale 1), 46 (19.9%) had moderate atrophic gastritis (atrophic border scale 2), and 75 (32.4%) had severe atrophic gastritis (atrophic border scale 3). Eighteen (7.8%) had a gastric ulcer, 21 (9.1%) had a duodenal ulcer, and 4 (1.7%) had both a gastric ulcer and a duodenal ulcer. Endoscopically "severe atrophic gastritis" (atrophic border scale 3) was seen in 81.4% of subjects with "atrophic" pepsinogen (n=43) and 21.2% of those with "normal" pepsinogen (n=188).

***Fasting gastric pH stratified by the presence of mucosal atrophy and ~~titer~~ IgG profile against* H. pylori**

Figure 1 shows fasting gastric pH stratified by pepsinogen level (as an indicator of mucosal atrophy) and further by *H. pylori* IgG concentration with a negative cut-off value of 10 U/mL. With regard to *H. pylori*-seronegative subjects, fasting gastric pH did not differ between subjects with or without mucosal atrophy and remained within normal range irrespective of the extent of mucosal atrophy. In seropositive subjects with no mucosal atrophy ("normal" pepsinogen group), fasting gastric pH increased as IgG concentration increased, although there was no significant difference between the moderate and high concentration groups (Figure 1A). On the other hand, with regard to *H. pylori*-seropositive subjects with mucosal atrophy ("atrophic" pepsinogen group), fasting gastric pH decreased as IgG concentration increased (Figure 1B). It should be noted that subjects with a low IgG concentration against *H. pylori* and mucosal atrophy showed the highest fasting gastric pH.

***Relationship between serum* H. pylori *antibody concentration and fasting gastric pH***

Table 2 shows Spearman correlation coefficients for the correlation between fasting gastric pH and *H. pylori* antibody concentration in *H. pylori*-seropositive subjects stratified by pepsinogen status. *H. pylori* antibody showed no significant relationship with fasting gastric pH when all subjects were analyzed together. However, when the analysis was confined to those with mucosal atrophy ("atrophic" pepsinogen, n=38), *H. pylori* antibody concentration had an inverse relationship with fasting gastric pH, and this correlation was statistically significant (correlation coefficient = −0.331, $p < 0.05$). In subjects without mucosal atrophy (those with "normal" pepsinogen; pepsinogen-I > 70 ng/mL, or pepsinogen-I < 70 ng/mL and pepsinogen-I/II ratio > 3, n=121), *H. pylori* antibody concentration was positively correlated with fasting gastric pH (correlation coefficient = 0.374, $p < 0.01$).

Figure 2 shows the scatter plots of the relationship between fasting gastric pH and *H. pylori* antibody concentration among *H. pylori* seropositive subjects, with "normal" pepsinogen subjects shown in Figure 2A, and "atrophic" pepsinogen subjects in Figure 2B.

Table 3 shows Spearman correlation coefficients for the correlation between fasting gastric pH and endoscopic atrophic grade in *H. pylori*-seropositive subjects. Endoscopic atrophic grade was significantly correlated with fasting gastric pH, irrespective of serum pepsinogen status.

## *Discussion*

A correlation between the quantity of *H. pylori* in gastric mucosa and serum

antibody level has been reported. For example, subjects show a substantial decrease in serum antibody titers 6 months after successful eradication of *H. pylori* infection by antibiotics (25). Spontaneous disappearance of *H. pylori*, as verified histologically or serologically, has been described in some long-term follow-up studies, particularly in subjects with severe atrophy (13-15). A possible mechanism underlying this is that the development of mucosal atrophy and intestinal metaplasia during the worsening of atrophic gastritis renders the gastric conditions inhospitable for *H. pylori*. Thus, low seropositivity of *H. pylori* antibody can originate from two different clinical conditions: 1) non-atrophic gastric mucosa with infection of only a small number of *H. pylori* organisms; and 2) severe mucosal atrophy with disappearance of *H. pylori*. Under the latter conditions, the resultant decrease in parietal cell numbers leads to severe impairment of acid secretion. Several studies in Japan have reported that subjects with negative or low-seropositive *H. pylori* IgG titer and mucosal atrophy determined by pepsinogen level showed a higher risk for gastric cancer than high-titer subjects (8-12).

In the present study, we examined the fasting gastric pH of subjects stratified by pepsinogen status and by concentration of IgG against *H. pylori*, and clearly showed the possibility that stratification of patients by these two parameters may be highly effective for predicting gastric acid secretion. We showed a statistically significant inverse correlation between *H. pylori* antibody concentration ~~titer~~ and gastric pH in subjects with mucosal atrophy, and conversely, a positive correlation in subjects without mucosal atrophy. We also showed that fasting gastric juice pH in subjects with low *H. pylori* concentration and mucosal atrophy, a known subgroup showing high incidence of non-cardia cancer caused by extensive atrophy, was predictably the highest among all

subjects. Our findings suggest that *H. pylori* density, which correlates with *H. pylori* antibody concentration, increases as degree of atrophy progresses up to a certain degree, but decreases with further atrophy. This finding explained why antibody concentration was not associated with either extent of mucosal atrophy or fasting gastric pH when analyzed in all subjects irrespective of mucosal atrophy. This could explain the previous reports suggesting that *H. pylori* antibody titer was not associated with gastric cancer incidence (26, 27).

The other important finding obtained by this study was that fasting gastric pH in *H. pylori*-negative subjects with "atrophic" pepsinogen was normal rather than high, suggesting that atrophy was not severe. This seems to contradict several previous reports that subjects who were negative for *H. pylori* and had severe atrophy were at highest risk for gastric cancer because the severity of the mucosal atrophy had reduced *H. pylori* load to the point where antibodies against the bacterium were negative (8, 28, 29). One possible explanation for this discrepancy is that the specificity of the ELISA we used to detect *H. pylori* antibody was greater than that in previous studies, and hence we could exclude with certainty subjects with *H. pylori* infection. Tatemichi and Sasazuki used the same kit as we did, and the present results are in accordance with their finding that subjects with negative *H. pylori* IgG titer exhibited lower risk for gastric cancer than those with a low titer (9, 30).

Spearman rank correlations between fasting gastric pH and endoscopic atrophic grade were statistically significant as shown in Table 2B. The correlation coefficient in all *H. pylori* seropositive subjects (n=106) was 0.538, stronger than that between

pepsinogen and gastric pH (correlation coefficient = 0.101). The strong correlation between endoscopic atrophy and acid secretion seems reasonable because endoscopists can directly and macroscopically evaluate the mucosal atrophic condition and detect severe mucosal atrophy. It is also in accordance with a previous study (31). We have not demonstrated the superiority of serum screening methods over endoscopy in terms of effectiveness in detecting cancer. Our aim is to rationalize the mechanisms underlying serum screening methods using both pepsinogen and *H. pylori* antibody. Although endoscopy has the highest detection rate of gastric cancer among major screening methods, it is an invasive method with low cost-effectiveness that is unsuitable for population screening and depends heavily on endoscopist skill. Thus, from the viewpoint of mass screening for gastric cancer, endoscopy is likely to be unfeasible even in developed countries.

One weakness of this study is the fact that the fasting gastric pH does not always reflect the acid secretory function of the stomach. However, because there are no reported simple and commercially available screening methods to measure acid secretory function of the stomach, and because fasting gastric pH has been reported to be well correlated with basal acid output (24), it would appear reasonable to use fasting gastric pH as an indicator of acid secretory status in analyses of large study populations such as ours.

In conclusion, this study demonstrated different relationships between *H. pylori* antibody titer and fasting gastric pH depending on gastric mucosal atrophy status in subjects who were seropositive for *H. pylori*. The extent of atrophy, as determined by

fasting gastric pH, showed a **inverse** association with *H. pylori* antibody **concentration** in those with mucosal atrophy, but a **positive** association in those without atrophy. We also demonstrated that subjects with a low *H. pylori* IgG concentration and mucosal atrophy, who are reportedly at high risk for differentiated-type gastric cancer, exhibit severely impaired acid secretion. This suggests severe mucosal atrophy in this subgroup, in accordance with previous reports. These findings seem to provide a rationalization for measuring serum pepsinogen and *H. pylori* antibody concentration simultaneously in gastric cancer screening, particularly in Japan.

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## Table 1

## Baseline characteristics of the study population

| | Total population | Pepsinogen status | |
|---|---|---|---|
| | | "Atrophic" pepsinogen | "Normal" pepsinogen |
| **Number** | **231** | **43** | **188** |
| **Age** | **59.6±13.7** | **66.0±9.0** | **58.2±14.1** |
| **Male%** | **52.8%** | **60.5%** | **51.1%** |
| **Gastric pH** | **2.8±2.0** | **5.2±2.1** | **2.3±1.5** |
| **Pepsinogen-I** | **51.4±24.5** | **32.8±18.4** | **55.6±23.8** |
| **Pepsinogen-II** | **13.0±8.7** | **17.1±6.8** | **12.1±8.9** |
| **Pepsinogen-I/II ratio** | **5.0±2.4** | **1.9±0.7** | **5.7±2.1** |
| **Positive H. pylori antibody %** | **45.9%** | **88.4%** | **36.2%** |
| **Endoscopic artophic border scale** | | | |
| **Mild or no atrophy %** | **47.6%** | **7.0%** | **56.9%** |
| **Moderate atrophy %** | **19.9%** | **11.6%** | **21.8%** |
| **Severe atrophy %** | **32.5%** | **81.4%** | **21.3%** |

Gastric mucosal atrophy was diagnosed according to the endoscopic atrophic border scale of Kimura and Takemoto, with three classifications (mild or no atrophy, moderate atrophy, and severe atrophy).

**Table 2**

**Correlation between fasting gastric pH and IgG antibody concentration against *H. pylori*, stratified by mucosal atrophy, in *H. pylori*-seropositive subjects**

| | Correlation coefficient (rs) | p value (two-sided test) |
|---|---|---|
| **All subjects (n=106)** | **0.101** | **0.303** |
| **Subjects with "atrophic" pepsinogen (n=38)** | **−0.331** | **<0.05** |
| **Subjects with "normal" pepsinogen (n=68)** | **0.374** | **<0.01** |

In *H. pylori* -seropositive subjects, *H. pylori* antibody concentration ~~titer~~ showed no statistically significant relationship with fasting gastric pH when all subjects were analyzed. "Atrophic" pepsinogen was defined as pepsinogen-I < 70 ng/mL and pepsinogen-I/II ratio < 3.0. Other subjects were defined as having "normal" pepsinogen (pepsinogen-I > 70 ng/mL, or pepsinogen-I < 70 ng/mL and pepsinogen-I/II ratio > 3). However, *H. pylori* antibody concentration was inversely correlated with fasting gastric pH in subjects with "atrophic" pepsinogen (n=68), and positively correlated in those with "normal" pepsinogen (n=38).

**Table 3**

**Correlation between fasting gastric pH and endoscopic atrophic border scale, stratified by mucosal atrophy, in *H. pylori*-seropositive subjects**

| | Correlation coefficient (rs) | p value (two-sided test) |
|---|---|---|
| **All subjects (n=106)** | **0.538** | **<0.01** |
| **Subjects with "atrophic" pepsinogen (n=38)** | **0.507** | **<0.01** |
| **Subjects with "normal" pepsinogen (n=68)** | **0.346** | **<0.01** |

Atrophic border scale had three classifications (1, mild or no atrophy; 2, moderate atrophy; and 3, severe atrophy), and correlations between atrophic border scale and gastric pH were determined by the Spearman rank correlation test. Endoscopic atrophic border scale status was significantly correlated with fasting gastric pH, irrespective of mucosal atrophy defined by *H. pylori* antibody and pepsinogen.

***Figure legends***

**Figure 1.**

Fasting gastric pH in *Helicobacter pylori*-seropositive subjects stratified by *H. pylori* antibody titer, in the "normal" pepsinogen group (A) and the "atrophic" pepsinogen group (B).

Data are shown as mean ± standard deviation. Subjects were divided into four groups on the basis of *H. pylori* antibody concentration: negative, antibody concentration < 10; low, 10-30 U/mL; moderate, 30-50 U/mL; and high, >50 U/mL. "Atrophic" pepsinogen was defined as pepsinogen-I < 70 ng/mL and pepsinogen-I/II ratio < 3.0. Other subjects were defined as having "normal" pepsinogen. Among those with normal pepsinogen, fasting gastric pH in subjects with low *H. pylori* antibody concentration was significantly higher than that in those with high antibody concentration (A). In subjects with "atrophic pepsinogen", fasting gastric pH in subjects with low *H. pylori* antibody concentration was significantly lower than that in those with high antibody concentration (B).

*$p<0.05$ compared with the low antibody concentration group, as determined by the Neuman-Keuls test.

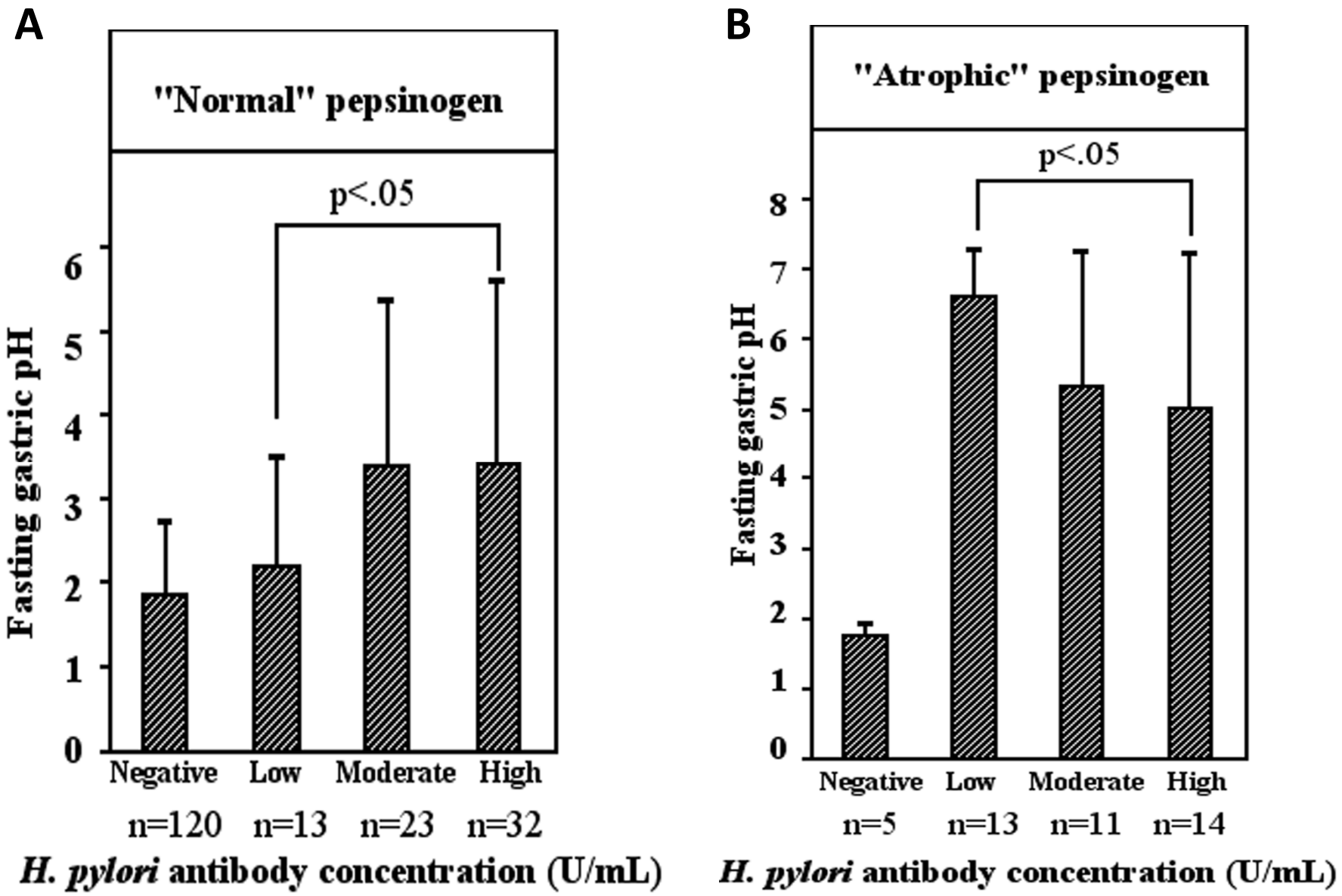
**Figure 2**

**A.** Scatter plot of the relationship between fasting gastric pH and *H. pylori* antibody concentration among *H. pylori* seropositive subjects, in the "normal" pepsinogen group (Spearman rank correlation, $\gamma = -0.331$; $P<0.05$).

**B.** Scatter plot of the relationship between fasting gastric pH and *H. pylori* antibody

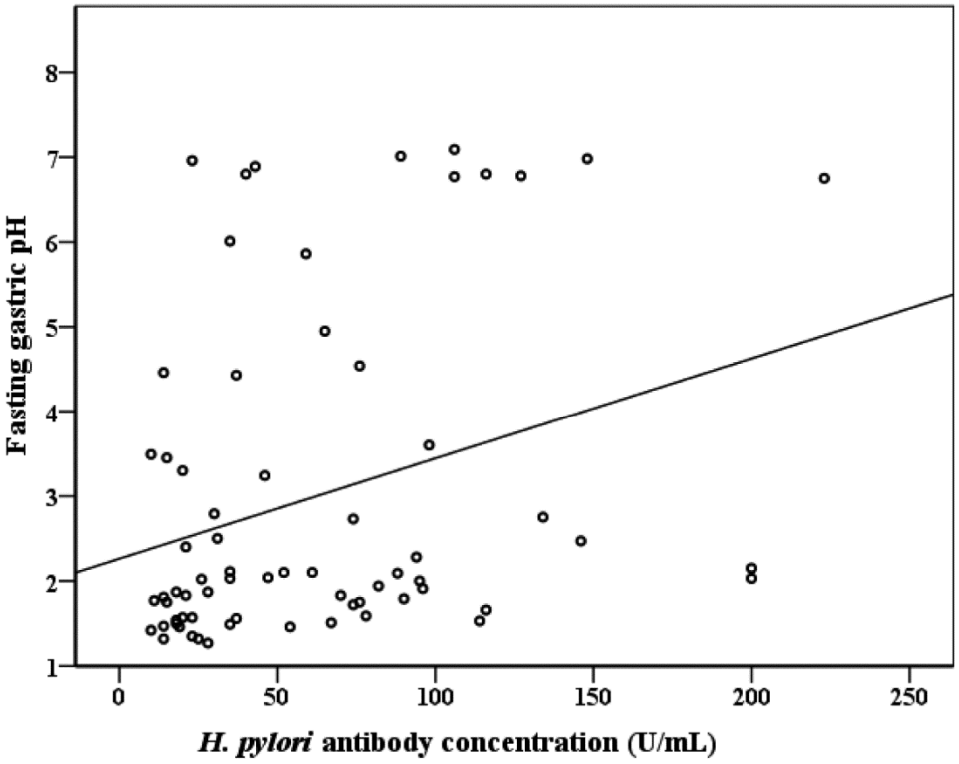
concentration among *H. pylori* seropositive subjects, in the “atrophic” pepsinogen group (Spearman rank correlation, $\gamma = 0.374$; $P<0.01$).

**Figure1.**

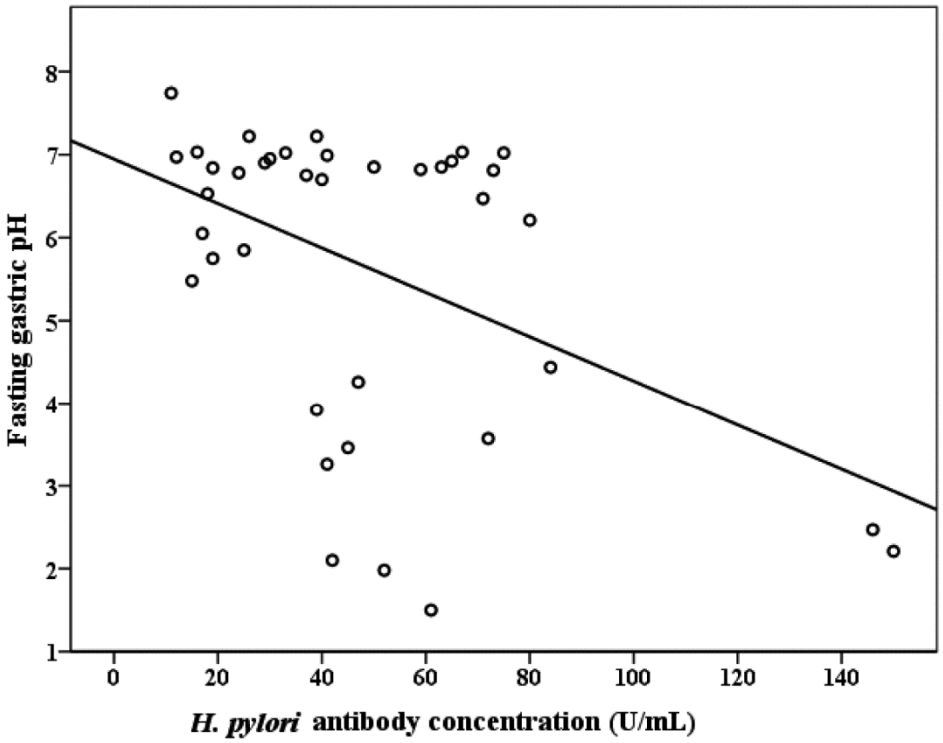

A
"Normal" pepsinogen
p<.05
Fasting gastric pH
0
1
2
3
4
5
6
Negative
Low
Moderate
High
n=120
n=13
n=23
n=32
H. pylori antibody concentration (U/mL)
B
"Atrophic" pepsinogen
p<.05
Fasting gastric pH
0
1
2
3
4
5
6
7
8
Negative
Low
Moderate
High
n=5
n=13
n=11
n=14
H. pylori antibody concentration (U/mL)

# Figure2.

**A**


Fasting gastric pH
8
7
6
5
4
3
2
1
0
50
100
150
200
250
H. pylori antibody concentration (U/mL)

**B**


Fasting gastric pH
8
7
6
5
4
3
2
1
0
20
40
60
80
100
120
140
H. pylori antibody concentration (U/mL)