<table>
<thead>
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<th>Title</th>
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<tr>
<td>Author(s)</td>
<td>Kishikawa, H; Nishida, J; Ichikawa, H; Kaida, S; Matsukubo, T; Miura, S; Morishita, T; Hibi, T</td>
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<td>Journal</td>
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<tr>
<td>URL</td>
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This is the pre-peer reviewed version of the following article: Digestion. 2011 Apr 14;84(1):62-69., which has been published in final form at http://dx.doi.org/10.1159/000322221
Serum nitrate/nitrite concentration correlates with gastric juice nitrate/nitrite: a possible marker for mutagenesis of the proximal stomach

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Running head: Serum nitrate/nitrite as a marker for gastric cancer

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The authors declare no conflict of interest.
ABSTRACT

Background/ Aims: In the normal acid-secreting stomach, luminally generated nitric oxide, which contributes to carcinogenesis in the proximal stomach, is associated with the concentration of nitrate plus nitrite (nitrate/nitrite) in gastric juice. We investigated whether the serum nitrate/nitrite concentration is associated with that of gastric juice and whether it can be used as a serum marker. Methods: Serum and gastric juice nitrate/nitrite concentration, Helicobacter pylori antibody, and gastric pH were measured in 176 patients undergoing upper endoscopy. Results: Multiple regression analysis revealed that serum nitrate/nitrite concentration was the best independent predictor of gastric juice nitrate/nitrite concentration. On single regression analysis, serum and gastric juice nitrate/nitrite concentration were significantly correlated, according to the following equation: gastric juice nitrate/nitrite concentration (µmol/l) = 3.93 − 0.54 × serum nitrate/nitrite concentration (µmol/l; correlation coefficient = 0.429, p<0.001). In analyses confined to subjects with gastric pH less than 2.0, and in those with serum markers suggesting normal acid secretion (pepsinogen-I >30 ng/l and negative H. pylori antibody), the serum nitrate/nitrite concentration was an independent predictor of gastric juice nitrate/nitrite concentration (p<0.001). Conclusion: Measuring the serum nitrate/nitrite concentration has potential in estimating gastric juice nitrate/nitrite concentration. The serum nitrate/nitrite concentration could be useful as a marker for mutagenesis in the proximal stomach.

Key words: Gastric cancer, Biological markers, Regression analysis, Carcinogenesis, Serum nitrate/nitrite.
INTRODUCTION
Although stomach cancer rates have declined worldwide over the past 2 decades, the disease remains very common and is estimated to be the 2nd most frequent cancer in the world [1]. Recognized risk factors for gastric cancer include *Helicobacter pylori* infection, high dietary intake of salt and nitrates, and hypochlorhydria [2-4]. However, the mechanism by which these risk factors predispose to gastric carcinogenesis is not fully understood. Mirvish [5] found that in subjects with healthy acid secretion, carcinogenic N-nitroso compounds (NOCs) are formed via intraluminal conversion of nitric oxide when nitrites in gastric juice are exposed to acidic conditions, and that these NOCs induce a series of changes in the gastric mucosa that lead to gastric cancer. When nitrite enters the stomach of subjects with a normal gastric pH (gastric juice pH <2.0), it is decomposed to nitric oxide within a few seconds at the gastroesophageal junction, and this contributes to the high incidence of mutagenesis at this anatomical site [6-8]. The prevalence of adenocarcinoma of the gastroesophageal junction in developed countries has been increasing at an alarming rate [9]. The majority of these cancers arise in nonatrophic gastric mucosa and have a diffuse subtype; thus, they cannot be detected by serum markers, including pepsinogen. Hence an additional marker for detecting those at high risk for the diffuse type of cardia cancer is required [10]. Although several epidemiological studies have suggested an association between exposure to high levels of nitrate and an increased incidence of proximal stomach cancer, noninvasive and serological methods of predicting carcinogenic nitric oxide level at the gastroesophageal junction have not been well investigated [3, 11]. Therefore, it is theoretically important to evaluate intragastric nitric oxide concentration in order to assess mutagenic potential at the gastroesophageal junction. However, nitric oxide cannot be measured noninvasively, and its precursor, nitrite, also cannot be estimated accurately in gastric juice because it is rapidly decomposed to various nitrogen oxides at the gastroesophageal junction [6, 7]. Thus, other methods to predict
intragastric nitric oxide are necessary. A useful measure is the concentration of nitrate plus nitrite (nitrate/nitrite) quantified by colorimetric assay based on the Griess reaction, which is considered to be an indicator of nitric oxide activity in vivo [12-14]. In this method, all nitrates are converted to nitrite and total nitrite is measured as an indicator of overall nitrate/nitrite production. The stomach contents usually have an extremely low pH, and under this specific condition, the nitrate/nitrite concentration in gastric juice is almost equal to the nitrate concentration because most nitrite is converted to nitric oxide within a few seconds. The fact that nitric oxide and its derivatives contribute to carcinogenesis has been already established as a consensus of opinion, and the highest concentration of nitric oxide in the human body occurs in the gastric lumen. Hence the apparent relationship between gastric cancer and nitric oxide has generally been accepted [15, 16]. Intragastric nitric oxide is reported to originate from salivary nitrite, the concentration of which is directly proportional to the gastric juice nitrate/nitrite concentration [8, 15, 17]. Meanwhile, approximately 25% of dietary nitrate is absorbed from the upper small intestine into the plasma and actively concentrated within the salivary glands and secreted in saliva - this recirculation of nitrate is called the enterosalivary circulation [18, 19]. Thus there should be a positive correlation between the concentration of nitrate/nitrite in the serum and gastric juice; however, to the best of our knowledge, this relationship has not been investigated in humans. We therefore focused on the relationship between serum nitrate/nitrite concentration and gastric juice nitrate/nitrite concentration in humans. We postulated that if a positive association was obtained between the serum nitrate/nitrite concentration and gastric juice nitrate/nitrite concentration, serum nitrate/nitrite would be associated with intraluminal synthesis of nitric oxide and, thus, might be useful as a marker for stomach carcinogenesis.
MATERIALS AND METHODS

Subjects
We recruited 176 patients undergoing diagnostic upper gastrointestinal endoscopy at the gastroenterology outpatient clinic of Tokyo Dental College, Ichikawa General Hospital. Endoscopy was performed using an Olympus videoscope (XQ240, XQ260; Olympus, Tokyo, Japan) after subjects had fasted for at least 12 h, and a blood sample was drawn immediately before the endoscopic examination. The serum nitrate/nitrite concentration has been reported to recover 72 h after the restriction of nitrate and nitrite in the diet [20]. Although serum and gastric juice nitrate/nitrite are slightly, but inevitably, influenced by daily exposure to dietary nitrate, it is impractical to measure serum and gastric juice nitrate/nitrite under strict dietary nitrate restriction, even though it might be meaningful to exclude subjects with excess nitrate exposure. Therefore, in the present study, we assessed nitrate/nitrite without dietary nitric oxide restriction. The blood sample was centrifuged at 12 000 g for 5 min at 4°C, and serum was stored at −20°C until assayed. Exclusion criteria were as follows: (1) the use of histamine-2 receptor antagonists, proton pump inhibitors, or nonsteroidal anti-inflammatory drugs within the preceding month; (2) the use of H. pylori eradication therapy before the study; (3) a history of gastric cancer or any kind of esophageal or gastric surgery or vagotomy; (4) a history of pernicious anemia or autoimmune gastritis as recalled by the patient; (5) the presence of viral infections such as acute respiratory tract infections; (6) pregnancy or lactation; and (7) the presence of severe systemic diseases including sepsis, diabetes, and renal and/or liver dysfunction. Gastric mucosal atrophy was diagnosed according to the endoscopic atrophic border scale of Kimura and Takemoto [21], with 3 classifications (1, mild or no atrophy; 2, moderate atrophy; and 3, severe atrophy). This study was approved by the Tokyo Dental College Ichikawa General Hospital Ethics Committee, and written consent to participate was obtained from all patients.
Assay for Nitrate/Nitrite in gastric juice
During the endoscopic examination, 1 ml of gastric juice was collected from the stomach through a sterile tube (Specimen Collection Container, Nippon Sherwood Medical Industries Ltd., Tokyo, Japan). The specimen was centrifuged at 12,000 g for 5 min, and 0.9 ml of the supernatant was mixed with 0.3 mL of 1 mol/l NaOH, rendering the sample alkaline. Samples were stored at 4°C, then centrifuged and analyzed the same day on 96-well microplates with a specific enzyme immunoassay (EIA) kit (Nitrate/Nitrite Colorimetric Assay Kit, Cayman, Ann Arbor, Mich, USA) based on a modified Griess reaction. This assay kit measured the total amount of nitrate and nitrite combined.

Assay for Nitrate/Nitrite in serum
After thawing the serum sample, the concentration of nitrate/nitrite was determined by a modified Griess reaction as stated above. Measurement of serum and gastric juice nitrate/nitrite was contracted out to Mitsubishi Chemical Medience Co. Ltd. (Tokyo, Japan).

Assays for Pepsinogen and Antibody to H. pylori in Serum
Diagnosis of *H. pylori* infection was based on detection of serum IgG antibodies to *H. pylori* with a specific enzyme-linked immunosorbent assay (ELISA) kit (E Plate Eiken *H. pylori* Antibody, Eiken Chemical Co., Ltd., Tokyo, Japan). The threshold titers for defining *H. pylori* infection were defined by optical density values according to the manufacturer’s protocol (antibody titer <10 was defined as negative for *H. pylori* infection). The sensitivity and specificity reported by the assay supplier were 100% and 93.8%, respectively. Serum levels of pepsinogen -I and -II were determined using the Architect pepsinogen assay kit (Pepsinogen I/II RIA BEAR kit, Abbott Japan Co., Tokyo, Japan), which is based on an ELISA, in accordance with the manufacturer’s
instructions. Measurement of serum pepsinogen-I, pepsinogen-II, and \textit{H. pylori} IgG antibodies was contracted out to Mitsubishi Chemical Medience Co. Ltd. (Tokyo, Japan) as described previously [22].

\textit{pH measurement}

Gastric juice samples were collected through a sterile tube during endoscopy as described above, and their pH was measured with a glass electrode (pH meter M-12, Horiba Instruments Ltd., Kyoto, Japan) as described previously [22].

\textit{Statistical Analyses}

Data are expressed as mean ± standard deviation (SD) or ± standard error (SE), and a \( p \)-value less than 0.05 was taken to indicate statistical significance. All statistical analyses were performed using the Statcel2 software program (OMS Publishing Inc., Saitama, Japan). We used multiple and single regression analyses to determine the relationships between the investigated variables. The “unstandardized coefficient” is interpreted as the predicted change in the dependent variable (gastric juice nitrate/nitrite concentration, in this study) caused by a change of one unit in an independent variable. The “standardized coefficient” (\( \beta \)) is the estimate to determine which of the independent variables have a greater effect on the dependent variable in a multiple regression analysis. The closer the coefficient to the absolute value of 1, the stronger the effect of that independent variable is on the dependent variable.

\textbf{RESULTS}

\textit{Clinical Features of the Patients}

Table 1 shows the demographic and clinical characteristics of the 176 subjects. Their mean age was 59.5 years, and there were 94 men and 82 women. Seventy-eight of the subjects were \textit{H. pylori}-positive. Based on the endoscopic findings, 92 (52.3\%) had
non-ulcer dyspepsia, 14 (7.9%) had a gastric ulcer, 17 (9.6%) had a duodenal ulcer, and 2 (1.1%) had both a gastric ulcer and a duodenal ulcer. According to the endoscopic atrophic border scale of Kimura and Takemoto [21], 21 (21.4%) of the H. pylori-negative subjects had moderate gastric atrophy and 9 (9.2%) had severe atrophy. In the H. pylori-positive group, 27 (34.6%) had moderate atrophy and 32 (41.0%) had severe atrophy.

**Best Predictors of Gastric Juice Nitrate/Nitrite Using Multiple Linear Regression Analysis**

To determine the best predictors of gastric juice nitrate/nitrite concentration, multiple regression analysis was performed using 5 independent variables (serum nitrate/nitrite concentration, fasting gastric pH, H. pylori antibody titer, age, and gender) and gastric juice nitrate/nitrite concentration as the dependent variable (Table 2). According to standardized coefficients, which reflect the relative strength of each independent variable, fasting gastric pH (β = 0.27) and serum nitrate/nitrite concentration (β = 0.42) were statistically significant predictors of gastric juice nitrate/nitrite concentration, and serum nitrate/nitrite was the best predictor (standardized coefficient closer to the absolute value of 1). Since nitrate and nitrite accumulate in hypochlorhydria, acid secretion was associated with quantity of nitrate/nitrite entering the stomach in people with impaired acid secretion, as reported in previous studies [23].

**Relationship between Gastric Juice Nitrate/Nitrite Concentration and Serum Nitrate/Nitrite Concentration**

Because serum nitrate/nitrite was found to have the greatest influence on gastric juice nitrate/nitrite from multiple regression analysis, single regression analysis was then performed to determine the correlation between the serum and gastric juice nitrate/nitrite concentration. As shown in Table 3, gastric juice nitrate/nitrite
concentration was significantly correlated with the serum nitrate/nitrite concentration (correlation coefficient = 0.407, p<0.001). The equation for this model, calculated using the independent predictor of serum nitrate/nitrite concentration, was as follows: gastric juice nitrate/nitrite concentration ($\mu$mol/l) = 3.93 – 0.54 × serum nitrate/nitrite concentration ($\mu$mol/l). The scatterplot of the relationship between serum and gastric juice nitrate/nitrite is shown in Figure 1. The gastric juice nitrate/nitrite concentration in subjects undergoing PPI treatment was significantly correlated with serum nitrate/nitrite concentration (data not shown, correlation coefficient = 0.699, p<0.001), suggesting that serum and gastric juice nitrate/nitrite are significantly correlated, irrespective of pharmacologically induced acid secretory status.

**Relationship between the Serum and Gastric Juice Nitrate/Nitrite Concentrations for Subjects with Gastric juice pH less than 2.0**

Because the serum nitrate/nitrite concentrations were theoretically correlated with the total amount of nitric oxide synthesized in the stomach in subjects with normal acid secretion, but not those with hypochlorhydra, they should be associated with carcinogenesis in the proximal stomach, as mentioned in the Introduction. To evaluate this association, we performed a single regression analysis to determine the factors influencing gastric juice nitrate/nitrite in subjects with fasting gastric pH less than 2.0 (N = 107).

As shown in Table 4, serum nitrate/nitrite concentration was significantly associated with gastric juice nitrate/nitrite concentration (correlation coefficient = 0.395, p<0.001) in these subjects. The scatterplot of the relationship between serum and gastric juice nitrate/nitrite is shown in Figure 2.

**Relationship Between the Serum and Gastric Juice Concentrations of Nitrate/Nitrite in Subjects with Normal Acid Secretion as Assessed by Pepsinogen-I and H. pylori**
Antibody

Since the serum nitrate/nitrite concentration is postulated to be associated with diffuse-type carcinogenesis only when acid secretion is normal, it is particularly important to identify subjects with normal acid secretion in Japan, a country with a high incidence of gastric atrophy. Those in the normal acid secretion group with a high intragastric nitric oxide level could then be identified by measuring their serum biological markers. The combined use of serum anti-\textit{H. pylori} antibodies and pepsinogen measurement has been proposed as a serological prescreening measure for severe atrophic gastritis in Japan. Yanaoka et al. [24] suggested that high serum pepsinogen-I concentration and negative \textit{H. pylori} antibody can accurately identify subjects without chronic atrophic gastritis.

Reduction in the area of fundic gland mucosa with progression of chronic atrophic gastritis is correlated with decreased acid secretion and with progression of atrophy [25]. These findings are compatible with the model equation determined in our previous study, which showed that negative \textit{H. pylori} antibody and a high pepsinogen level are suggestive of normal fasting gastric pH [22]. We therefore performed a single linear regression analysis of gastric juice and serum nitrate/nitrite concentration confined to subjects with pepsinogen-I >30 ng/l and negative \textit{H. pylori} antibody (n= 80). As shown in Table 5, a significant relationship was found between the nitrate/nitrite concentrations in serum and gastric juice in this subgroup (Correlation coefficient = 0.414, p<0.001). The scatterplot of the relation between serum and gastric juice nitrate/nitrite is shown in Figure 3.

DISCUSSION

Nitrosative stress was first raised as a factor involved in gastric carcinogenesis when Correa [26] suggested that the increase in intraluminal nitrite in hypochlorhydria leads to the formation of carcinogenic NOCs. Mirvish [5] later stressed that these compounds are formed when nitrite is acidified in the healthy acid-secreting stomach. Ascorbic acid
prevents intraluminal NOCs formation from nitrite that has entered the stomach [7]. The mechanisms of carcinogenesis in the proximal stomach were recently clarified [8]. The volatile nitric oxide produced in the lumen readily diffuses into the surrounding epithelium and reacts with oxygen to form nitrosating compounds, and their formation is considered is seen as an explanation for the particularly high incidence of epithelial mutagenesis and neoplasia at the gastroesophageal junction [6-8].

Several serum markers have been proposed as being predictive of gastric cancer, including pepsinogen, total gastrin, and the circulating component of serum gastrin (G-17) [27-29]. Pepsinogen, the most widely used of these biological markers, is used to noninvasively detect atrophic gastritis, a precancerous lesion for gastric cancer. One study showed that when pepsinogen-I <50 ng/ml and pepsinogen-I/II ratio <3 were taken to indicate extensive atrophic gastritis, the percentage of those screened who needed further examination was 19.5%. The cancer detection rate was 0.28%, and 83% of the cancers detected by the pepsinogen test were of the intestinal type [30]. Thus, the pepsinogen test is extremely useful for detecting the well-differentiated histological type (very similar to the intestinal type) of gastric carcinoma in asymptomatic subjects with small, early, intestinal-type cancers, particularly in Japan, where the incidence of gastric atrophy is extremely high.

However, as reported by Parsonnet et al. [31], diffuse-type gastric cancer cannot be effectively detected by the pepsinogen test. The reason for the unreliability of serum markers in detecting diffuse-type cancer is that most of these cancers occur without gastric atrophy, as reported by Inomata et al. [32] This suggests that normal acid secretion may be important for the development of diffuse-type adenocarcinoma, particularly at the gastroesophageal junction. This is compatible with the hypothesis of McColl et al. [6-8] which says that nitric oxide at the gastroesophageal junction is significantly associated with mutagenesis at this site when acid secretion is preserved. This finding suggests that gastric juice nitrate/nitrite concentration reflects the nitric
oxide level and can thus be considered correlated with carcinogenesis of the proximal stomach only when acid secretion is normal.

Measurement of the luminal nitric oxide concentration with a nasogastric sensor seems to be the most accurate method of evaluating mutagenesis of diffuse-type cancer in the proximal stomach. Iijima et al. [8] found that nitric oxide generation is maximal at the gastroesophageal junction and the cardia and suggested that the high incidence of mutagenesis at these sites results from the high nitric oxide concentration. If patients at risk of gastric cancer at the gastroesophageal junction could be screened by measuring serum biomarkers instead of by endoscopy, it would be of great interest and significance.

We therefore investigated whether the serum nitrate/nitrite concentration was significantly correlated with gastric juice nitrate/nitrite concentration, a reliable marker of intraluminal nitric oxide. As shown in Table 3, single linear regression analysis showed a significant correlation between serum nitrate/nitrite concentration and gastric juice nitrate/nitrite concentration (correlation coefficient = 0.407, p<0.01). However, the serum nitrate/nitrite concentration is postulated to be associated with carcinogenesis at the gastroesophageal junction only when acid secretion is normal. Therefore, we investigated these correlations in subjects with healthy acid secretion (fasting gastric pH <2.0). The gastric juice nitrate/nitrite concentration was also positively associated with serum nitrate/nitrite concentration in this subgroup, with a high correlation coefficient of 0.395 (Table 4, Figure 2).

On the basis of these findings, we propose a simplified and practical screening strategy to identify patients at high risk of gastric cancer at the proximal stomach. First, subjects with normal acid secretion should be detected noninvasively by serum markers, using the criteria of pepsinogen-I >30 ng/ml and negative *H. pylori* antibody. In this study, 64% of subjects within fasting gastric pH 2.0 could be correctly detected by these criteria (data not shown). We then need to detect among this population those with high nitric oxide concentrations using the serum nitrate/nitrite concentration. A significant
relationship was found between the nitrate/nitrite concentrations in serum and gastric juice in subjects with pepsinogen-I >30 ng/ml and negative *H. pylori* antibody, as shown in Table 5.

These findings suggest that there is a strong correlation between the nitrate/nitrite concentrations in the serum and gastric juice of subjects with normal acid secretion and that it should be possible to use the serum nitrate/nitrite concentration as a noninvasive marker for cancer of the proximal stomach. *H. pylori* infection tends to produce more severe atrophic gastritis in Japan than in other regions of the world [11, 33, 34]. In most Western countries, where atrophic gastritis is relatively rare, it may be unnecessary to select patients with normal acid secretion, and serum nitrate/nitrite may be directly associated with carcinogenesis in the proximal stomach.

Serum nitrate/nitrite concentration is reportedly associated with several systemic diseases, including sepsis, graft-versus-host disease, scleroderma, and severe diabetes [35-38]. Thus, as nitrate/nitrite produced systemically as well as that produced intragastrically is absorbed from the intestine into the plasma, the serum concentration of nitrate/nitrite is not always associated with gastric juice nitrate/nitrite, and this may explain the relatively low correlation coefficient of serum and gastric juice nitrate/nitrite concentrations in this study. This seems to be a potential limitation of our study; however, the exclusion of subjects with severe systemic disease should have mitigated this.

In conclusion, it might be possible to use serum nitrate/nitrite concentration as a biological marker for mutagenesis at the proximal stomach that has not been detected noninvasively by appropriate serum markers. A high serum nitrate/nitrite concentration in subjects thought to be at low risk of non-cardia gastric cancer and not to have atrophic gastritis suggests the possibility of developing cardia cancer and the need for meticulous endoscopic observation.
Acknowledgments
We would like to thank Forte Inc. (Tokyo, Japan) for assistance with the English in this manuscript.

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important for the development of gastroesophageal junction adenocarcinoma in 
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Table 1. Baseline characteristics of the study population

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<td>Age (years)</td>
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<tr>
<td>Pepsinogen-II (ng/mL)</td>
<td>12.4±8.5</td>
<td>17.4±8.5</td>
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<tr>
<td>Serum nitrate/nitrite (μmol/L)</td>
<td>25.6±15.0</td>
<td>24.5±14.4</td>
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<td>Gastric juice nitrate/nitrite (μmol/L)</td>
<td>18.7±23.2</td>
<td>23.6±30.1</td>
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Data are shown as mean ± SD.
Table 2.
Linear regression analysis for predictors of gastric juice nitrate/nitrite concentration

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<tr>
<th>Independent variable</th>
<th>Unstandardized coefficient</th>
<th>Standardized coefficient</th>
<th>p value</th>
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<tr>
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<td>Age</td>
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<td>0.06</td>
<td>0.39</td>
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Dependent variable: gastric juice nitrate/nitrite concentration (μmol/l)

N=176, R (correlation coefficient) =0.548, R²(coefficient of determination) =0.301, p<0.001, SE of the estimate=19.7

*Significant at the 0.05 level.
Table 3.
Single regression analysis for predictors of gastric juice nitrate/nitrite concentration

<table>
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<th>Unstandardized coefficient</th>
<th>Standardized coefficient</th>
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<tr>
<td>Interception (constant)</td>
<td>3.93</td>
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<td>Serum nitrate/nitrite (μmol/L)</td>
<td>0.54</td>
<td>0.41</td>
<td>&lt;0.001*</td>
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Dependent variable: gastric juice nitrate/nitrite concentration (μmol/l)

n=176, R (correlation coefficient) = 0.407, R² (coefficient of determination) = 0.166, p<0.001, SE of the estimate=18.22

The equation for this model was as follows:

Gastric juice nitrate/nitrite concentration (μmol/l) = 3.93−0.54×serum nitrate/nitrite concentration (μmol/l)

*Significant at the 0.05 level.
Table 4.
Single regression analysis for predictors of gastric juice nitrate/nitrite concentration in subjects with fasting gastric pH under 2.0

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<td>2.64</td>
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<td>Serum nitrate/nitrite (μmol/L)</td>
<td>0.45</td>
<td>0.40</td>
<td>&lt;0.001*</td>
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</table>

Dependent variable: gastric juice nitrate/nitrite concentration (μmol/l)

n=107, R (correlation coefficient) =0.395, R²(coefficient of determination) =0.156, p<0.001, SE of the estimate=14.8

The equation for this model was as follows:

Gastric juice nitrate/nitrite concentration (μmol/l) = 2.64 – 0.45×serum nitrate/nitrite concentration (μmol/l)

*Significant at the 0.05 level.
Table 5.
Single regression analysis for predictors of gastric juice nitrate/nitrite concentration in subjects with PG-I>30 ng/L and negative *H. pylori* antibody

<table>
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<th>Independent variable</th>
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<td>Serum nitrate/nitrite (µmol/L)</td>
<td>0.41</td>
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</table>

Dependent variable: gastric juice nitrate/nitrite concentration (µmol/L)
n=80, R (correlation coefficient) 0.414, R²(coefficient of determination) =0.172, p<0.001, SE of the estimate=12.5

The equation for this model was as follows:

Gastric juice nitrate/nitrite concentration (µmol/l) =2.28−0.41×serum nitrate/nitrite concentration (µmol/l)

*Significant at the 0.05 level.*
Figure 1
Scatter plot of the relationship between the serum and gastric juice nitrate/nitrite concentrations.

Correlation coefficient = 0.407 p < 0.001
Figure 2
Scatter plot of the relationship between the serum and gastric juice nitrate/nitrite concentrations in subjects with fasting gastric pH under 2.0.

Correlation coefficient = 0.395, p < 0.001
Figure 3
Scatter plot of the relationship between the serum and gastric juice nitrate/nitrite concentrations in subjects with PG-I > 30 ng/ml and negative *H. pylori* antibody.

Correlation coefficient=0.414 p<0.001