Diagnosis of Gastric Malignancy Using Gastric Juice α1-antitrypsin

Ping-I Hsu1, Chung-Hsuan Chen3, Michael Hsiao3, Deng-Chyang Wu2, Ching-Yi Lin1, Kwok-Hung Lai1, and Pei-Jung Lu4

Abstract

No accurate, inexpensive, and noninvasive test for gastric cancer screening is currently available. Our recent study identified α1-antitrypsin as a potential biomarker of gastric cancer in gastric juice. The aim of this study was to develop a novel noninvasive modality for detecting gastric cancer by measurement of α1-antitrypsin concentration in gastric juice. The work consisted of two parts: (a) investigating the differences in gastric juice α1-antitrypsin concentrations between gastric cancer patients and controls, and (b) screening gastric cancer using string test to obtain gastric juice followed by immunoassay for α1-antitrypsin concentration.

The data showed that gastric juice α1-antitrypsin concentration was markedly higher in gastric cancer patients than in healthy subjects, gastric ulcer patients, and duodenal ulcer patients (all P < 0.001). The area under the receiver operating characteristic curve for identifying gastric cancer cases was 0.96 (95% confidence interval, 0.93–0.99; P < 0.001). The sensitivity and specificity of gastric juice α1-antitrypsin concentration were 96% and 92%, respectively. Gastric juice α1-antitrypsin assay through string test was validated in 93 consecutive patients for gastric cancer screening. The sensitivity and specificity of gastric juice α1-antitrypsin string test at 85% accuracy were 74% and 88%, respectively. The area under the receiver operating characteristic curve for identifying gastric cancer was 0.84. In conclusion, gastric juice α1-antitrypsin concentration in gastric cancer patients markedly exceeds those in healthy subjects and patients with benign gastrointestinal diseases. A noninvasive α1-antitrypsin string test may serve as a new screening tool for identifying gastric cancer patients. Cancer Epidemiol Biomarkers Prev; 19(2); 405–11. ©2010 AACR.

Introduction

Gastric cancer is the second leading cause of cancer death worldwide, killing more than one million people each year (1). The major reason for the high cancer-related mortality is delayed diagnosis, as early cancers are typically asymptomatic. Currently, endoscopic examination with biopsy remains the test of choice for the diagnosis of gastric cancer, but its invasiveness precludes widespread use as a screening tool (2). Serum tumor markers, such as carcinoembryonic antigen, CA 19-9 and, CA 72-4, have shown little benefit as an approach for screening for gastric cancer in the general population because of their low sensitivity in detecting early primary gastric tumors (3, 4). Although gastric cancer screening by photofluorography has been recognized to be useful, it also has several problems, such as limited accuracy, low cost effectiveness, and the risks associated with X-ray exposure (5, 6). A serum pepsinogen assay has recently been developed to screen gastric cancer because many gastric cancers develop from atrophic gastric mucosa (7-9). However, the serum pepsinogen assay would overlook cancer patients without atrophic gastritis or with mild atrophic gastritis (10). Additionally, the positive predictive value of this test for gastric cancer screening is only 1.4% (8).

Our recent study established that α1-antitrypsin is a potential biomarker for gastric cancer in gastric juice (11). In addition, several other peptides, such as pepsinogen, leucin zipper protein, and albumin fragments, are also novel candidates in gastric juice for cancer screening (12). However, sampling of gastric juice for biomarker examination often requires endoscopic examination or insertion of nasogastricduodenal tubes. The invasiveness and costs of these procedures represent major limitations for development in this field.

Various noninvasive methods have recently been developed for obtaining gastric juices (13-15). For example, a noninvasive string test has been reported to get gastric juice samples for the diagnosis of Helicobacter pylori...
infection (13, 14). Additionally, a capsule method containing a pierced plastic cover and a piece of absorbent paper has also been developed to get gastric juice (15). We therefore designed this study to investigate the differences of \( \alpha_1 \)-antitrypsin concentrations in gastric juice between patients with gastric cancer and benign gastro-duodenal diseases, and to develop a novel noninvasive gastric juice \( \alpha_1 \)-antitrypsin test for cancer screening using a string test to obtain gastric juice.

Materials and Methods

Study Design

The study consisted of two parts: (a) investigating the differences in gastric juice \( \alpha_1 \)-antitrypsin concentrations between gastric cancer patients and controls, and (b) screening gastric cancer using a string test to obtain gastric juice followed by an immunoassay for \( \alpha_1 \)-antitrypsin concentration. In the first part, gastric juice \( \alpha_1 \)-antitrypsin concentrations in gastric cancer patients were compared with those in healthy subjects and patients with gastric ulcer and duodenal ulcer. In the second part, gastric malignancies were screened by \( \alpha_1 \)-antitrypsin immunoassay of gastric juices that were obtained by a noninvasive method, a string test.

Gastric Juice \( \alpha_1 \)-Antitrypsin Immunoassay in the Diagnosis of Gastric Cancer

Gastric juice samples from 30 consecutive healthy subjects, 30 patients with gastric ulcer, 30 patients with duodenal ulcer, and 22 patients with gastric cancer were collected for this study to investigate gastric juice \( \alpha_1 \)-antitrypsin concentrations in gastric cancer and benign gastro-duodenal diseases. The healthy subjects that were recruited from our health examination clinics had no clinical history of gastrointestinal diseases, and their endoscopic findings were normal or showed only mild gastritis. The diagnosis of gastric ulcer and duodenal ulcer was confirmed by endoscopic examination. A peptic ulcer was defined as a circumscribed mucosal break 5 mm or more in diameter, with a well-defined ulcer crater (16). The patient exclusion criteria included (a) the use of proton pump inhibitors or histamine-2 receptor antagonists within 4 wk before endoscopy, (b) the coexistence of two kinds of gastroduodenal lesions, a (c) the coexistence of severe systemic diseases. Gastric cancer was confirmed by histology and classified as intestinal and diffuse types according to the Lauren's classification (17). The extent of tumor invasion in gastric cancer patients was further divided according to the tumor-node-metastasis staging system (18). The study was approved by the Medical Research Committee of

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy subjects (n = 30)</th>
<th>GU (n = 30)</th>
<th>DU (n = 30)</th>
<th>GC (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>57.2 ± 2.0*</td>
<td>61.7 ± 2.1</td>
<td>54.5 ± 3.0*</td>
<td>66.7 ± 3.2</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>15/15</td>
<td>14/16</td>
<td>15/15</td>
<td>14/8</td>
</tr>
<tr>
<td>( H. ) pylori status</td>
<td>13 (43%)</td>
<td>18 (60%)</td>
<td>22 (73%)*</td>
<td>14 (67%)</td>
</tr>
<tr>
<td>Gastric juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH level</td>
<td>2.9 ± 0.4†</td>
<td>3.1 ± 0.4†</td>
<td>2.7 ± 0.3‡</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>Protein concentration (mg/dl)</td>
<td>53 ± 1†</td>
<td>72 ± 7§</td>
<td>53 ± 2‡</td>
<td>117 ± 12</td>
</tr>
<tr>
<td>( \alpha_1 )-antitrypsin level (μg/dl)</td>
<td>36 ± 7†</td>
<td>562 ± 92‡</td>
<td>90 ± 23‡</td>
<td>1,560 ± 7</td>
</tr>
</tbody>
</table>

Abbreviations: GU, gastric ulcer; DU, duodenal ulcer; and GC, gastric cancer.

*\( P < 0.05 \) compared with gastric cancer patients.
†\( P < 0.01 \) compared with gastric cancer patients.
‡\( P < 0.001 \) compared with gastric cancer patients.
§\( P = 0.001 \) compared with gastric cancer patients.

Figure 1. The distribution of gastric juice \( \alpha_1 \)-antitrypsin levels in healthy subjects (HS) and patients with gastric ulcer (GU), duodenal ulcer (DU), and gastric cancer (GC).
the Kaohsiung Veterans General Hospital. All patients and controls gave informed consents.

Endoscopies were done with the Olympus GIF XV10 and GIF XQ200 (Olympus Corp.) after patients had fasted overnight. Immediately after insertion of the scope into the stomach, 5 mL of gastric fluid were aspirated through the suction channel of endoscope and were collected in a sterile trap placed in the suction line for protein assay. Routine inspection of the upper gastrointestinal tract was then performed, and biopsy was taken for lesions suspected gastric cancer or ulcer. All the fasting gastric juice samples were collected, processed, and stored. The pH of gastric juice was measured just after collection with a glass-electrode pH meter. Gastric juice was centrifuged at 10,000×g for 10 min at 4°C to remover cell debris and other contaminants. Aliquots of the supernatant were stored at −70°C until assay. The protein concentration of gastric juice was determined using the Bradford method (19). Additionally, gastric juice α1-antitrypsin levels were determined by using a commercial ELISA kit (α1-antitrypsin ELISA kit, Immunodiagnostik AG). This assay uses the “sandwich” technique with two selected polyclonal anti-

bodies that bind to human α1-antitrypsin. Briefly, 100 μL of standards and samples were pipetted into the microtiter plates that had been coated with polyclonal antibodies specific for human α1-antitrypsin. The mixture was incubated at room temperature for 1 h. After washing away unbound proteins, 100 μL of α1-antitrypsin conjugate were added to the wells to sandwich the α1-antitrypsin. Finally, substrate solution was added for color development. The absorbance of the samples was measured by a spectrophotometer set at 450 nm. Each test was run blindly in duplicate. The mean values were taken as the final concentration. The intra-assay variation was 4% to 10%.

**Gastric Juice α1-antitrypsin String Test for the Diagnosis of Gastric Cancer**

Consecutive patients presenting with dyspeptic symptoms were invited to participate in an α1-antitrypsin string test before or within 3 d following panendoscopy. Patients had fasted overnight and then underwent string test (GASTRO-TEST, HDC Corp.) to obtain gastric juice (14). The string test consists of a weighted gelatin capsule with a 70-cm coil of a highly absorbant cotton floss. One end of the floss protruded through a hole in the cap whereas the other was loosely attached to the capsule. The patient was seated on the examination table and swallowed the capsule with 300-mL of water while the free end of the line was held outside the mouth. The capsule generally descended into the stomach immediately. The patient then lay on the table for 1 h to allow the gastric juice to moisten the cotton floss. The string was then removed with a fairly quick but gentle pulling motion. The floss fragment at distal 30-cm of string was cut, added into 1.5 mL microcentrifuge tube with 100 μL iced-cold distilled water. The mixture was then rotated constantly at 4°C for 1 h for protein extraction. After 14,000 rpm centrifugation at 4°C for 10 min, the supernatant was removed to a new microcentrifuge tube followed by the protein concentration determination using the Bradford method (19) and the α1-antitrypsin concentration determination using a commercial ELISA kit as described.

**Statistical Analysis**

Statistical evaluations were done using the SPSS program (version 10.1). The differences in gastric juice α1-antitrypsin concentrations between gastric cancer patients and healthy subjects, and patients with other

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**Table 2. Efficacies of gastric juice α1-antitrypsin levels for the diagnosis of gastric cancer**

<table>
<thead>
<tr>
<th>Cutoff value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>618 μg/dl</td>
<td>21/22 (96%)</td>
<td>82/90 (91%)</td>
<td>21/29 (72%)</td>
<td>82/83 (99%)</td>
<td>103/112 (92%)</td>
</tr>
<tr>
<td>717 μg/dl</td>
<td>21/22 (96%)</td>
<td>83/90 (92%)</td>
<td>21/28 (75%)</td>
<td>83/84 (99%)</td>
<td>104/112 (93%)</td>
</tr>
<tr>
<td>806 μg/dl</td>
<td>20/22 (91%)</td>
<td>83/90 (92%)</td>
<td>20/27 (74%)</td>
<td>83/85 (98%)</td>
<td>103/112 (92%)</td>
</tr>
</tbody>
</table>
gastrointestinal diseases were assessed by Mann-Whitney U test. All data were expressed by mean ± SEM. The $\chi^2$ test with or without Yate’s correction for continuity and the Fisher’s exact test when appropriate were applied to analyze the categorized variables. Differences were considered to be significant at $P < 0.05$. Receiving operating characteristic (ROC) curves were constructed by calculating the sensitivities and specificities of gastric juice $\alpha_1$-antitrypsin concentrations at different cutoff points for differentiating gastric cancer patients from nonmalignant subjects.

**Results**

**Gastric Juice $\alpha_1$-antitrypsin Immunoassay in the Diagnosis of Gastric Cancer**

Table 1 shows the demographic characteristics of healthy subjects and patients with gastric ulcer, duodenal ulcer, and gastric cancer. There were no differences in age between gastric cancer and gastric ulcer patients, but gastric cancer patients were older than healthy subjects and patients with duodenal ulcer (both $P < 0.05$). All study groups were comparable in gender. The frequency of *H. pylori* infection in duodenal ulcer patients was higher than that of healthy subjects ($P < 0.05$). Gastric cancer patients had significantly higher pH levels of gastric juice than healthy subjects, gastric ulcer, and duodenal ulcer patients ($5.1$ versus $2.9$, $3.1$, and $2.7$, respectively; $P < 0.01$, $< 0.01$, and $< 0.001$, respectively). Gastric juice protein concentration in gastric cancer patients also markedly exceeded that in healthy subjects and patients with gastric ulcer or duodenal ulcer ($1.17$ mg/dl versus $0.53$, $0.72$, and $0.53$ mg/dl; $P < 0.001, 0.001$, and $< 0.001$, respectively). Figure 1 shows the distribution of gastric juice $\alpha_1$-antitrypsin levels in gastric cancer patients and other study groups. Gastric juice $\alpha_1$-antitrypsin level in gastric cancer, healthy subjects, gastric ulcer, and duodenal ulcer patients were $1560$ μg/dl (range, $468-3257$ μg/dl), $36$ μg/dl (range, $1-156$ μg/dl), $562$ μg/dl (range, $12-1902$ μg/dl), and $90$ μg/dl (range, $2-571$ μg/dl), respectively (Table 1). Gastric cancer patients had much higher gastric juice $\alpha_1$-antitrypsin levels than healthy subjects, gastric ulcer patients, and duodenal ulcer patients (all $P < 0.001$).

To evaluate the diagnostic value of gastric juice $\alpha_1$-antitrypsin concentration, the ROC curve was constructed based on the sensitivities and specificities of the biomarker at different cutoff points differentiating gastric cancer patients from nonmalignant subjects (Fig. 2). The area under the ROC curve of gastric juice $\alpha_1$-antitrypsin concentration for identifying gastric cancer was $0.96$ (95% confidence intervals, $0.92-0.99$; $P < 0.001$). The best cutoff value of gastric juice $\alpha_1$-antitrypsin for identifying gastric cancer cases was $717$ μg/dl. Using this cutoff

### Table 3. Effect of tumor-node-metastasis staging on gastric juice $\alpha_1$-antitrypsin levels and the sensitivity for the detection of gastric cancer patients

<table>
<thead>
<tr>
<th>Stage</th>
<th>$\alpha_1$-antitrypsin concentration (μg/dl)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I ($n = 5$)</td>
<td>$1516 ± 260$</td>
<td>$5/5 (100%)$</td>
</tr>
<tr>
<td>Stage II ($n = 2$)</td>
<td>$2292 ± 966$</td>
<td>$2/2 (100%)$</td>
</tr>
<tr>
<td>Stage III ($n = 4$)</td>
<td>$1438 ± 480$</td>
<td>$3/4 (75%)$</td>
</tr>
<tr>
<td>Stage IV ($n = 11$)</td>
<td>$1583 ± 141$</td>
<td>$11/11 (100%)$</td>
</tr>
</tbody>
</table>

### Table 4. Gastric juice $\alpha_1$-antitrypsin string tests in consecutive 93 dyspeptic patients

<table>
<thead>
<tr>
<th>Diagnosis of patients</th>
<th>No of patients</th>
<th>pH level</th>
<th>Protein concentration (mg/dl)</th>
<th>$\alpha_1$-antitrypsin concentration (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonmalignant diseases</td>
<td>74</td>
<td>$2.9 ± 0.3^*$</td>
<td>$1.7 ± 0.4^{†}$</td>
<td>$0.61 ± 0.36^{‡}$</td>
</tr>
<tr>
<td>Functional dyspepsia</td>
<td>25</td>
<td>$3.2 ± 0.4$</td>
<td>$1.7 ± 0.6$</td>
<td>$0.52 ± 0.28$</td>
</tr>
<tr>
<td>Gastric erosion</td>
<td>7</td>
<td>$2.9 ± 0.8$</td>
<td>$1.2 ± 0.7$</td>
<td>$0.46 ± 0.39$</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>21</td>
<td>$3.0 ± 0.5$</td>
<td>$2.3 ± 1.1$</td>
<td>$1.09 ± 1.00$</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>11</td>
<td>$2.8 ± 0.7$</td>
<td>$1.5 ± 0.7$</td>
<td>$0.55 ± 0.37$</td>
</tr>
<tr>
<td>Erosive esophagitis</td>
<td>6</td>
<td>$1.5 ± 0.5$</td>
<td>$0.6 ± 0.6$</td>
<td>$0.00 ± 0.00$</td>
</tr>
<tr>
<td>Gastric hyperplastic polyp</td>
<td>4</td>
<td>$2.5 ± 1.5$</td>
<td>$1.3 ± 1.3$</td>
<td>$0.00 ± 0.00$</td>
</tr>
<tr>
<td>Malignant diseases</td>
<td>19</td>
<td>$4.9 ± 0.5$</td>
<td>$17.0 ± 10.3$</td>
<td>$13.17 ± 6.39$</td>
</tr>
<tr>
<td>Gastric adenocarcinoma</td>
<td>18</td>
<td>$4.9 ± 0.6$</td>
<td>$17.7 ± 10.9$</td>
<td>$12.57 ± 6.72$</td>
</tr>
<tr>
<td>Gastric lymphoma</td>
<td>1</td>
<td>6</td>
<td>5.3</td>
<td>$24.00$</td>
</tr>
</tbody>
</table>

$^*P = 0.001$ compared with gastric malignant diseases.

$^{†}P < 0.01$ compared with gastric malignant diseases.

$^{‡}P < 0.001$ compared with gastric malignant diseases.
value, the sensitivity, specificity, and accuracy were 96%, 92%, and 93%, respectively (Table 2).

We next examined whether the gastric juice α1-antitrypsin concentrations correlated with the histologic type of gastric cancer. According to the Lauren’s classification, gastric cancers were classified as intestinal-type (n = 13) and diffuse-type (n = 9) tumors. There were no differences in gastric juice α1-antitrypsin concentrations between the two types of tumors (1.627 μg/dl versus 1.463 μg/dl, P = 0.587). Additionally, there were no significant differences in gastric juice α1-antitrypsin concentrations among different stages of tumors according to the tumor-node-metastasis classification (Table 3). The frequencies of elevated gastric juice α1-antitrypsin concentrations in stage I, II, III, and IV gastric cancers were 100%, 100%, 75%, and 100%, respectively.

**Gastric Juice α1-antitrypsin String Test for the Diagnosis of Gastric Cancer**

Ninety-three consecutive patients receiving endoscopic examination for dyspeptic symptoms were invited to undergo a gastric juice α1-antitrypsin string test. The diagnoses of these patients included functional dyspepsia (n = 25), gastric erosion (n = 7), gastric ulcer (n = 21), duodenal ulcer (n = 11), erosive esophagitis (n = 6), gastric polyp (n = 4), gastric adenocarcinoma (n = 18), and gastric lymphoma (n = 1). The frequencies of H. pylori infection in these patients were 52%, 57%, 62%, 73%, 50%, 25%, 56%, and 100%, respectively. Gastric juice α1-antitrypsin concentrations were 0.51 μg/dl (range, 0.00-5.60 μg/dl), 0.46 μg/dl (range, 0.00-2.80 μg/dl), 1.09 μg/dl (range, 0.00-21.00 μg/dl), 0.55 μg/dl (range, 0.00-3.90 μg/dl), 0.00 μg/dl (all, 0.00 μg/dl), 0.00 μg/dl (all, 0.00 μg/dl), 12.57 μg/dl (range, 0.00-92.00 μg/dl), and 24.00 μg/dl, respectively (Table 4). Patients with malignant gastric diseases had significantly higher gastric juice α1-antitrypsin levels than those with nonmalignant diseases (P < 0.001). The area under the ROC curve for identifying gastric malignancies was 0.84 (95% confidence interval, 0.72-0.95; P < 0.001; Fig. 3). The best cutoff values of gastric juice α1-antitrypsin string test for identifying gastric malignancy (gastric adenocarcinoma and lymphoma) was 0.85 μg/dl. The sensitivity, specificity, and accuracy at this cutoff value were 74%, 88%, and 85%, respectively (Table 5). The frequencies of elevated gastric juice α1-antitrypsin concentrations in stage I (n = 7), II (n = 8), III (n = 2), and IV (n = 1) gastric cancers were 75%, 63%, 100%, and 100%, respectively.

**Discussion**

This study developed a novel noninvasive modality for detecting gastric malignancy using string test to obtain gastric juice, followed by immunoassay for α1-antitrypsin. The first part of this work provided the first evidence of differences in gastric juice α1-antitrypsin concentrations among various gastroduodenal diseases. The gastric cancer patients had markedly higher mean gastric juice α1-antitrypsin concentration than healthy subjects, gastric ulcer patients, and duodenal ulcer patients (1,560 μg/dl versus 36, 562, and 90 μg/dl). The area under the ROC curve of gastric juice α1-antitrypsin concentration for identifying gastric cancer cases was 0.96 with the sensitivity and specificity of 96% and 92%, respectively. In the second part, gastric malignancies were screened with an α1-antitrypsin immunoassay of gastric juices obtained by a string test. The sensitivity, specificity, and accuracy of the gastric juice α1-antitrypsin string test for detecting gastric malignancy were 74%, 88%, and 85%, respectively. This work explored the potential of the noninvasive diagnosis of gastric malignancy by measuring biomarker concentrations in gastric juice.

![Figure 3. ROC curve analysis of gastric juice α1-antitrypsin string test for the detection of gastric malignancies in 93 consecutive dyspeptic patients.](image-url)

**Figure 3.** ROC curve analysis of gastric juice α1-antitrypsin string test for the detection of gastric malignancies in 93 consecutive dyspeptic patients.

<table>
<thead>
<tr>
<th>String test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-antitrypsin</td>
<td>14/19 (74%)</td>
<td>65/74 (88%)</td>
<td>14/23 (61%)</td>
<td>65/70 (93%)</td>
<td>79/93 (85%)</td>
</tr>
</tbody>
</table>

![Table 5. Efficacies of gastric juice α1-antitrypsin string test for the detection of gastric malignancies in 93 consecutive dyspeptic patients](table-url)
Previously, we looked for peptide biomarkers from gastric juice samples using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry. Gastric cancer patients had higher frequencies of α1-anti-trypsin and albumin peaks and lower frequencies of leucine zipper protein and pepsinogen peaks than healthy subjects (12). Another study (11) showed a prominent band of α1-anti-trypsin in the two-dimensional gastric juice electrophoresis image of gastric juice samples from gastric cancer patients. However, the α1-anti-trypsin band seemed not to be cancer specific because a small α1-anti-trypsin band was also identified in the gastric juices from 42%, 6%, and 6% of gastric ulcer patients, duodenal ulcer patients, and healthy subjects, respectively. We therefore hypothesized that measuring gastric juice α1-anti-trypsin levels can further distinguish gastric cancer patients from the subjects without malignancies. This study clearly showed that gastric cancer patients had higher gastric juice α1-anti-trypsin levels than gastric ulcer patients. The percentages of gastric cancer, gastric ulcer, duodenal ulcer patients, and healthy subjects with gastric juice α1-anti-trypsin concentrations beyond 717 μg/dl were 96%, 23%, 0%, and 0%, respectively. The experimental results indicate that quantitative assay is better than qualitative analysis for gastric juice α1-anti-trypsin in distinguishing gastric cancer from benign gastroduodenal diseases.

However, there was a significant overlap of gastric juice α1-anti-trypsin concentrations between gastric cancer and gastric ulcer patients (Fig. 1), although the mean gastric juice α1-anti-trypsin level in the former was higher than that of the latter. In clinical practice, it might be difficult to differentiate gastric cancer from gastric ulcer in some patients with mild elevation of gastric juice α1-anti-trypsin concentrations. Our previous study has shown that leukocyte elastase inhibitor, desmoglein-1 precursor, and immunoglobulin κ chain C region were other potential biomarkers for gastric cancer in gastric juices (11). A scoring system using the combination of different measurements, such as the gastric juice pH, protein concentration, α1-anti-trypsin, and other biomarker levels, could potentially give more accurate results than a single gastric juice biomarker for the discrimination of gastric cancer and merits further investigations.

Theoretically, there is an advantage in gastric cancer detection by using gastric juice samples in which the concentration of cancer-related peptide/protein is higher than that in blood. The major limitation of gastric juice analysis for cancer screening is the invasiveness of endoscopy or nasogastric tube insertion to obtain specimens. The current study tested the feasibility of detecting gastric malignancy using a noninvasive method followed by quantitative assay for α1-anti-trypsin. The data revealed that gastric juice α1-anti-trypsin level in gastric malignant diseases and nonmalignant diseases were $13.17 \pm 6.39 \mu g/dl$ and $0.61 \pm 0.30 \mu g/dl$, respectively. The former markedly exceeded the latter ($P < 0.001$). Notably, the only case with gastric lymphoma also exhibited a very high concentration of α1-anti-trypsin in gastric juice, implying that gastric juice α1-anti-trypsin string test can detect both gastric adenocarcinoma and lymphoma. Overall, the best cut-off value of gastric juice α1-anti-trypsin assay through string test for identifying gastric malignancy was $0.85 \mu g/dl$. With the cut point, the sensitivity, specificity, and accuracy of gastric juice α1-anti-trypsin concentration were 74%, 88%, and 85%, respectively, and area under the ROC curve was 0.84. The results indicate that the noninvasive test for screening for gastric malignancy is feasible. However, the current data cannot totally assess the efficacy of measuring gastric juice biomarkers for cancer screening because only one marker was tested. It merits further study to screen gastric cancer by measuring multiple gastric juice biomarkers following string test.

Currently, the reasons for abundant α1-anti-trypsin in the gastric juice of gastric cancer patient remain unclear. α1-anti-trypsin is the main serine proteinase inhibitor in human plasma. It plays major roles in physiologic and pathologic processes such as angiogenesis, intravascular fibrinolysis, wound healing, and tumor invasion, and metastasis (20, 21). Apart from its synthesis in the liver, α1-anti-trypsin is also synthesized by and secreted from human macrophage, neutrophil and epithelial cells of stomach, intestine, pancreas, and respiratory tracts (22-24). Additionally, it can be produced by certain cancer cells, including gastric cancer, colon cancer, and lung cancer (25, 26). Tumor cells synthesize and release not only a native form of α1-anti-trypsin, but also a variety of cleaved and/or degraded forms of α1-anti-trypsin. These protease inhibitors have multiple effects on tumor cell viability and play diverse roles in tumorigenesis (27). An immunohistochemical study (28) indicated that α1-anti-trypsin is a tissue tumor marker of well-differentiated gastric adenocarcinomas and is related to the invasive growth of the tumors. We therefore propose that the abundant α1-anti-trypsin in the gastric juice of gastric cancer patients may originate from the tumor cells, inflammatory cells, and occult blood leaking from ulcerative tumor tissues. It is important to investigate the relationships between the α1-anti-trypsin levels in gastric juice and α1-anti-trypsin expression in the tumors and gastrointestinal pathologies in the future.

Early detection and treatment of gastric cancer is an important way to decrease cancer death. Currently, a simple, noninvasive and sensitive test for gastric cancer screening is still unavailable. Various tumor markers such as carcinoembryonic antigen and CA19-9 have been evaluated for the early diagnosis of gastric cancer, but their low sensitivity (16-31%) precludes the clinical use (3, 4). The measurement of serum pepsinogen is a popular noninvasive screening test for gastric cancer in Japan. However, it fails to detect gastric cancer without gastric
Gastric Cancer Diagnosis by α1-antitrypsin

atrophy, and its positive predictive value is extremely low (8). Our gastric juice α1-antitrypsin string test possesses acceptable sensitivity, specificity, positive predictive value, and negative predictive value as a novel screening modality for gastric cancer. In this study, the α1-antitrypsin level of gastric juice obtained from endoscopic method was much higher than that from string test. The decreased α1-antitrypsin concentrations in the string test were most likely due to the dilution of gastric juice by the water drunk by the patients when they swallowed the capsules of strings. Another possible reason for this could lie in the extraction method, which further diluted the gastric juice from the string by adding water.

In conclusion, gastric cancer patients have higher gastric juice α1-antitrypsin levels than healthy subjects and patients with benign gastrointestinal diseases. A novel α1-antitrypsin string test may serve as a new screening tool for identifying gastric cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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