α1-Antitrypsin Precursor in Gastric Juice Is a Novel Biomarker for Gastric Cancer and Ulcer

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Abstract

Purpose: To search for novel disease-specific markers in gastric juice by investigating the protein concentrations and components in gastric juice from patients with various gastroduodenal diseases.

Experimental Design: Protein concentrations and pH values in fasting gastric juice were examined in 120 healthy subjects and 39 gastric ulcer, 38 duodenal ulcer, and 31 gastric cancer patients. The protein components in gastric juice were studied by two-dimensional PAGE and mass spectrometric analysis.

Results: Protein concentrations in gastric juice of patients with gastric ulcers and gastric cancer were significantly higher than those in healthy subjects (1.06 and 2.61 mg/mL versus 0.48 mg/mL; $P = 0.001$ and $P < 0.001$, respectively), and duodenal ulcer patients had lower gastric juice protein concentrations compared with healthy subjects (0.26 versus 0.48 mg/mL; $P < 0.05$). Gastric hypoacidity and advanced age were independent factors affecting the protein concentrations in gastric juice with odds ratios of 32.9 (95% confidence interval, 11.8-90.9) and 3.2 (95% confidence interval, 1.3-8.3), respectively. Each electrophoresis images of gastric juice could be classified into one of three patterns: basic band, specific band, or nonspecific band. The frequencies of specific band pattern in healthy subjects, gastric ulcer, duodenal ulcer, and gastric cancer patients were 6%, 42%, 6%, and 93%, respectively. Proteomic analysis revealed that α1-antitrypsin precursor was the principal peptide in the specific band.

Conclusions: α1-antitrypsin precursor in gastric juice is a novel biomarker for gastric cancer and ulcer. A noninvasive method to obtain gastric juice followed by proteomic analysis may serve as a new tool to screen for gastric malignancies.

Gastric juice contains numerous compounds, including hydrochloric acid, pepsin, lipase, mucin, intrinsic factor, peptides, nucleic acids, and electrolytes (1). Additionally, it may contain salivary constituents due to swallowing, bile due to gastroduodenal reflux, inflammatory mediators or blood from damaged gastric walls, and oncoproteins from gastric cancers (2). Recently, Kasirga et al. (3) showed that gastric juice leukotriene levels were significantly higher in patients with *Helicobacter pylori* infection than in subjects without infection. Marcinkiewicz et al. (4) also showed that gastric mucosa damage by naproxen sodium resulted in profound changes of gastric mucosal barrier, and analysis of residual compounds in gastric juice adequately reflected these changes. Aforementioned findings suggest that analysis of protein components of gastric juice allows accurate discrimination between normal and diseased gastric mucosa.

Recently, a small gelatin capsule containing a pierced plastic cover surrounding a piece of absorbent paper was developed to obtain gastric juice (5). Additionally, a noninvasive string test has also been reported useful to obtain gastric juice samples for the diagnosis of *H. pylori* infection (6). This technique facilitates epidemiologic studies for assessing *H. pylori* status in asymptomatic subjects without the need for endoscopy (7). To date, there are no reliable noninvasive methods to screen for or diagnose gastric cancer. Recent studies indicate that the development of intestinal-type gastric adenocarcinoma is a multistep event progressing from superficial gastritis, atrophic gastritis, intestinal metaplasia, and dysplasia to malignancy through cumulative multiple genetic changes (8–11). With regard to diffuse-type gastric cancer, the loss of adhesion

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molecules, such as E-cadherin, catenin, and Annexin 7, was involved in the carcinogenesis (12–14). Because the sequential changes of gastric foveolar epithelium and glands during the course of cancer development can lead to alterations of the components in gastric juices, proteomic studies of gastric juice hold a great potential to discover novel tumor-specific markers for screening of gastrointestinal malignancies.

This study was therefore designed to investigate the protein concentrations and components in gastric juice of patients with various gastroduodenal diseases in an aim to discover novel disease-specific markers in gastric juice.

Materials and Methods

Subjects. One hundred and twenty consecutive healthy subjects, 39 patients with gastric ulcer, 38 patients with duodenal ulcer, and 31 patients with gastric cancer participated in the study. 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 (15). The patient exclusion criteria included (*a*) the use of proton pump inhibitors, H2-receptor antagonists, or nonsteroidal anti-inflammatory drugs within 4 weeks before the study; (*b*) coexistence of two kinds of gastroduodenal lesions; (*c*) presence of upper gastroduodenal bleeding; and (*d*) coexistence of severe systemic diseases. Gastric cancer was confirmed by histology and classified as intestinal ($n = 19$), diffuse ($n = 9$), and mixed ($n = 3$) according to the Lauren's classification (16). The extent of tumor invasion in gastric cancer patients undergoing surgery ($n = 27$) was further divided into early or advanced gastric cancer according to the criteria proposed by the Japanese Research Society for Gastric Cancer (17). The study was approved by the Medical Research Committee of the Kaohsiung Veterans General Hospital. All patients and controls gave informed consents.

Clinical methods. Endoscopies were done with the Olympus GIF XV10 and GIF XQ200 (Olympus Corp., Tokyo, Japan) 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 the endoscope and collected in a sterile trap placed in the suction line. Routine inspection of the upper gastrointestinal tract was then done, and a biopsy specimen from the antrum of the greater curvature was obtained for rapid urease test to determine *H. pylori* status (18). Additional biopsies for histologic examinations of the stomach were carried out for subjects who provided informed consent for a topographical histopathologic study. Four specimens were taken from the midantrum (pyloric gland area) and middle body (fundic gland area) on both the lesser and greater curvatures (19). The biopsy specimens were fixed in 10% buffered formalin, embedded in paraffin, and sectioned. The sections were stained with a H&E stain. Sections were examined blind to the patient's clinical diagnosis. The activity of gastritis (neutrophil infiltration, mononuclear cell infiltration, glandular atrophy, and intestinal metaplasia) was assessed by the updated Sydney system (20). To adjust clinical characteristics, the following data were recorded for each subject: age, sex, family history of gastric cancer, smoking history, and alcohol consumption.

All the fasting gastric juice samples were collected, processed, and stored identically between the patients and healthy controls. 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 for removal of cell debris and other contaminants. Aliquots of the supernatant were stored at −70°C until assay.

These samples were then analyzed with the use of two-dimensional electrophoresis. Total protein concentration of gastric juice was determined using the Bradford method (21). A fixed amount of protein (50 μg per gel) was analyzed by two-dimensional electrophoresis, and protein spots of interest were identified by mass spectrometric (MS) analysis.

Two-dimensional electrophoresis analysis of protein components in gastric juice. The two-dimensional gel electrophoresis was carried out as described previously (22, 23). The first dimension of gel separation was done using commercially available dedicated apparatus: IPGphor (Amersham Pharmacia Biotech, Uppsala, Sweden). A sample containing 50 μg of protein was precipitated with acetone (1:2, v/v) for 2 h at −20°C, and the mixture was centrifuged at 10,000 × *g* for 10 min at 4°C. The pellet was mixed with rehydration solution (9 mol/L urea, 35 mmol/L Tris, 42 mmol/L DTT, 2% CHAPS, 0.66% SDS, 2% IPG buffer, and trace bromphenol blue) and applied to 11-cm nonlinear pH 4-7 immobilized pH gradient strips (Immobiline DryStrip, Amersham Pharmacia Biotech) for overnight rehydration at 30 V. Proteins were focused sequentially for 1 h at 200 V, 1 h at 500 V, and 1 h at 1,000 V, then a gradient was applied from 1,000 to 8,000 V for 30 min, and focusing was continued at 8,000 V for 8.5 h to give a total of 70 kVh on an IPGphor. For the second dimension, the IPG strips were equilibrated for 12 min in 50 mmol/L Tris-HCl (pH 8.8), 6 mol/L urea, 30% glycerol, 1% DTT, and bromphenol blue and then for 12 min in 50 mmol/L Tris-HCl (pH 8.8), 6 mol/L urea, 30% glycerol, l.2% SDS, 2% iodoacetamide, and bromphenol blue. The strips were applied on top of a 15% gradient acrylamide gel slab (190 × 140 × 1 mm), and electrophoresis was done overnight. The gels were stained with silver nitrate (22). Protein patterns in the gels were recorded as digitalized images using a high-resolution scanner (GS-710 Calibrated Imaging Densitometer, Bio-Rad, Hercules, CA). Gel image matching was done with PDQuest software (Bio-Rad).

In-gel tryptic digestion. The in-gel tryptic digestion was done as described previously (24). Briefly, the gel pieces were digested with porcine trypsin (Promega, Madison, WI) and the peptides were extracted with trifluoric acid and acetonitrile. Before MS analysis, the peptides were purified with Zip Tip_{C18} (Millipore, Bedford, MA) according to the manufacturer's instructions.

Mass spectrometry. For matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS analysis, 1 μL of eluted sample was mixed with 1 μL of matrix solution consisting of 2,5-dihydroxybenzoic acid (50 nmol/μL), acetonitrile (50%), and phosphoric acid (0.4%). Then, 1 μL of the resulting mixture was spotted onto the MALDI stainless steel sample plate and allowed to air dry at room temperature. The protein and peptide mass profiles of eluted samples were generated by an ABI Voyager DE-STR MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA). The spectrometer was run in positive ion mode and with an accelerating voltage of 25 kV. The low mass gate was set at 500 m/z. Protein data base search tool Profound[6] was used to compare the monoisotopic m/z values of the tryptic fragments to those of known proteins in the SwissProt database.

Samples for which MALDI-TOF did not provide unambiguous protein identification were further investigated by electrospray MS/MS. The tryptic digestion supernatant was purified and concentrated with C18 high-performance liquid chromatography and then sprayed from gold-coated glass capillaries using nanoflow electrospray source. MS/MS spectra were then obtained by a nano-high-performance liquid chromatography LTQ FT mass spectrometer (Thermo Electron, Bremen, Germany) and used for database searches using MASCOT amino acid sequencing search engine[7] to check against the SwissProt database for identification.

Statistical analysis. Statistical evaluations were done using the Statistical Package for the Social Sciences program version 10.1 (SPSS, Chicago, IL). The differences in gastric pH and total protein concentrations in gastric juice among healthy subject, gastric ulcer, duodenal ulcer, and gastric cancer groups were determined by a one-way ANOVA

[6] http://www.129.85.19.192/profound_bin/WebProFound.exe
[7] http://www.matrixscience.com

test. The χ^2 test with or without Yate's correction for continuity, and Fisher's exact test when appropriate, was applied to analyze the categorized variables. Differences were considered to be significant at $P < 0.05$. A multivariate analysis with a logistic regression method was carried out to assess the independent factors influencing protein concentrations. Finally, the correlation between the total protein concentrations and the independent factors was analyzed by linear regression.

Results

Table 1 shows the demographic characteristics of healthy subjects and patients with gastric ulcer, duodenal ulcer, and gastric cancer. Patients with gastric cancer were significantly older than healthy subjects (67.5 versus 51.4 years; $P < 0.001$). Additionally, the gastric cancer patient group had a higher male-to-female ratio than the healthy subject group ($P = 0.054$). No significant differences in family history of gastric cancer and history of alcohol consumption were identified between groups. However, the rates of cigarette smoking in gastric ulcer and duodenal ulcer patients were significantly higher than that of healthy subjects ($P = 0.002$ and 0.001, respectively). Furthermore, the rate of *H. pylori* infection in duodenal ulcer patients was also significantly higher than that of healthy subjects ($P = 0.034$).

Characteristics of gastric juice in healthy subjects and various gastroduodenal diseases. The characteristics of gastric juice in healthy subjects and various gastroduodenal diseases are listed in Table 1. The percentages of gastric ulcer and gastric cancer patients with ≥5 mL of gastric fluid in the stomach were significantly higher than that of healthy subjects (44% and 55% versus 22%; $P < 0.01$ and <0.001, respectively). However, no significant differences in the amount of gastric juices were identified between duodenal ulcer patients and healthy subjects.

The mean levels of gastric pH in healthy subjects, gastric ulcer, duodenal ulcer, and gastric cancer patients were 3.0, 4.3, 1.9, and 6.4, respectively. The gastric pH levels in gastric ulcer and gastric cancer patients were significantly higher than that in healthy subjects ($P = 0.001$ and < 0.001, respectively). The duodenal ulcer patients had a lower gastric pH level compared with healthy subjects ($P = 0.001$).

The total protein concentrations in healthy subjects, gastric ulcer, duodenal ulcer, and gastric cancer patients were 0.48, 1.06, 0.26, and 2.61 mg/mL, respectively. Similarly, the protein concentration in gastric cancer patients was the highest of the four and that in gastric ulcer patients was significantly higher than that of healthy subjects ($P = 0.001$). The duodenal ulcer patients had a lower protein concentration compared with healthy subjects ($P = 0.046$). If 0.5 mg/mL (mean + 2 SDs of protein concentrations in the healthy subject without gastric atrophy) was set as the upper limit of normal protein concentration of gastric juice, elevated protein concentrations were noted in 22%, 36%, 8%, and 71% of healthy subjects and

Table 1. Baseline characteristics of healthy subjects and patients with gastric ulcer, duodenal ulcer, and gastric cancer

	Healthy subjects (*n* = 120)	GU (*n* = 39)	DU (*n* = 38)	GC (*n* = 31)
Age (y)	51.4 ± 14.9	57.1 ± 19.1	56.6 ± 16.9	67.5 ± 14.3*
Sex (M/F)	58/62	22/17	25/13	21/10†
Family history of GC, *n* (%)	3 (3)	0 (0)	0 (0)	3 (10)
Smoking, *n* (%)	18 (15)	15 (38)‡	15 (39)§	5 (16)
Alcohol consumption, *n* (%)	11 (9)	4 (10)	5 (13)	2 (6)
H pylori infection, *n* (%)	61 (53)	21 (54)	29 (76)‖	23 (74)‖
Gastric juice, *n* (%)				
Amount (mL)				
<5	94 (78)	22 (56)‡	27 (71)	14 (45)*
≥5	26 (22)	17 (44)	11 (29)	17 (55)
pH level				
pH <2	51 (43)	9 (23)	28 (74)	2 (6)
2 ≤ pH <3	36 (30)	9 (23)	7 (18)	1 (3)
3 ≤ pH <4	9 (7)	5 (13)	3 (8)	3 (10)
4 ≤ pH	24 (20)	16 (41)	0 (0)	25 (81)
Mean	3.0 ± 1.9	4.3 ± 2.6§	1.9 ± 0.6§	6.4 ± 2.3*
Protein concentration (mg/mL), *n* (%)				
Concentration <0.5	94 (78)	25 (64)	36 (95)	9 (29)
0.5 ≤ concentration <1	19 (16)	1 (3)	1 (3)	1 (3)
1 ≤ concentration <2	2 (2)	7 (18)	1 (3)	5 (16)
2 ≤ concentration <3	2 (2)	2 (5)	0 (0)	7 (30)
3 ≤ concentration <4	1 (1)	2 (5)	0 (0)	2 (6)
4 ≤ concentration <5	2 (2)	0 (0)	0 (0)	2 (6)
5 ≤ concentration	0 (0)	2 (6)	0 (0)	4 (12)
Mean	0.48 ± 0.68	1.06 ± 1.52§	0.26 ± 0.21‖	2.61 ± 2.56*

Abbreviations: GU, gastric ulcer; DU, duodenal ulcer; GC, gastric cancer.
*$P < 0.001$, compared with healthy subjects.
†$P = 0.05$, compared with healthy subjects.
‡$P < 0.01$, compared with healthy subjects.
§$P = 0.001$, compared with healthy subjects.
‖$P < 0.05$, compared with healthy subjects.

gastric ulcer, duodenal ulcer, and gastric cancer patients, respectively.

Independent factors influencing the total protein concentrations in the human stomach. Univariate analysis of 11 clinical, bacterial, and gastric juice factors (Table 2) showed that the following five factors were significantly associated with high protein concentrations (≥0.5 mg/mL) in gastric juice: advanced age ($P = 0.001$), family history of gastric cancer ($P = 0.054$), the absence of duodenal ulcer ($P = 0.026$), the presence of gastric cancer ($P < 0.001$), low amount of gastric juice ($P < 0.001$), and gastric hypoacidity ($P < 0.001$). A stepwise forward logistic regression analysis indicated that advanced age and gastric hypoacidity were independent factors influencing the protein concentrations. The odds ratios of the two variables were 3.23 (95% confidence interval, 1.26-8.25) and 32.86 (95% confidence interval, 11.80-90.85), respectively.

Patterns of two-dimensional electrophoresis images of gastric juice from patients with various gastroduodenal diseases. Two-dimensional PAGE of gastric juices was done on gastric juice samples from 47 healthy subjects, 33 patients with gastric ulcer, 33 with duodenal ulcer, and 29 with gastric cancer. The results obtained from two-dimensional protein electrophoresis images were classified into three patterns: basic band, specific band, or nonspecific band. Figure 1A shows the basic band pattern of two-dimensional gel electrophoresis. A major band at acidic isoelectric point (4.0-5.0) was observed. The band had a M_r of 42,000 Da and was indicated by A1 in the figure; it was identified as pepsin A precursor by MALDI-TOF and MS/MS. Figure 1B shows the specific band pattern of a two-dimensional electrophoresis image, in which pepsin A precursors were barely detectable. In contrast, a strong dark band with acidic isoelectric point (4.5-5.5) and M_r of 47,000 Da (spot B1 in Fig. 1B) was very prominent. The tryptic digests of the B1 band from the two-dimensional electrophoresis gel were subjected for MALDI-TOF and MS/MS analysis. The peptide sequences were identified automatically and verified manually using MASCOT amino acid sequencing search engine as described in Materials and Methods. The identified peptide fragments in silver-stained band B1 were summarized in Table 3. The sequence coverage was 18% and the Mascot standard score was 415. These data indicate that the B1 band is α1-antitrypsin precursor (Fig. 1B; Table 3). The two spots B2 and B3 that were consistently shown in the specific band pattern of two-dimensional electrophoresis were also identified as α1-antitrypsin precursors by MALDI-TOF, MS/MS, and MASCOT amino acid sequencing search engine. In addition to α1-antitrypsin precursor, desmoglein-1 precursor (Fig. 1B, spot B5), immunoglobulin κ chain C region (Fig. 1B, spots B6 and B8), neutrophil gelatinase-associated lipocalin precursor, fibrinogen β chain precursor (Fig. 1B, spot B4), α-amylase 2B precursor (Fig. 1B, spot B7), leukocyte elastase inhibitor, lysozyme C precursor selenium-binding protein 1, and desmoplakin were occasionally identified from gastric juice samples with specific band pattern of two-dimensional electrophoresis. Figure 1C presents the nonspecific band pattern of the electrophoresis image. This pattern lacked the basic bands of pepsin A precursors but also specific α1-antitrypsin precursor band. The distributions of protein spots in this group were quite diverse. Some fragments of leukocyte elastase inhibitor, desmoglein-1 precursor, and immunoglobulin κ chain C region were identified from the spots scattering over the gel.

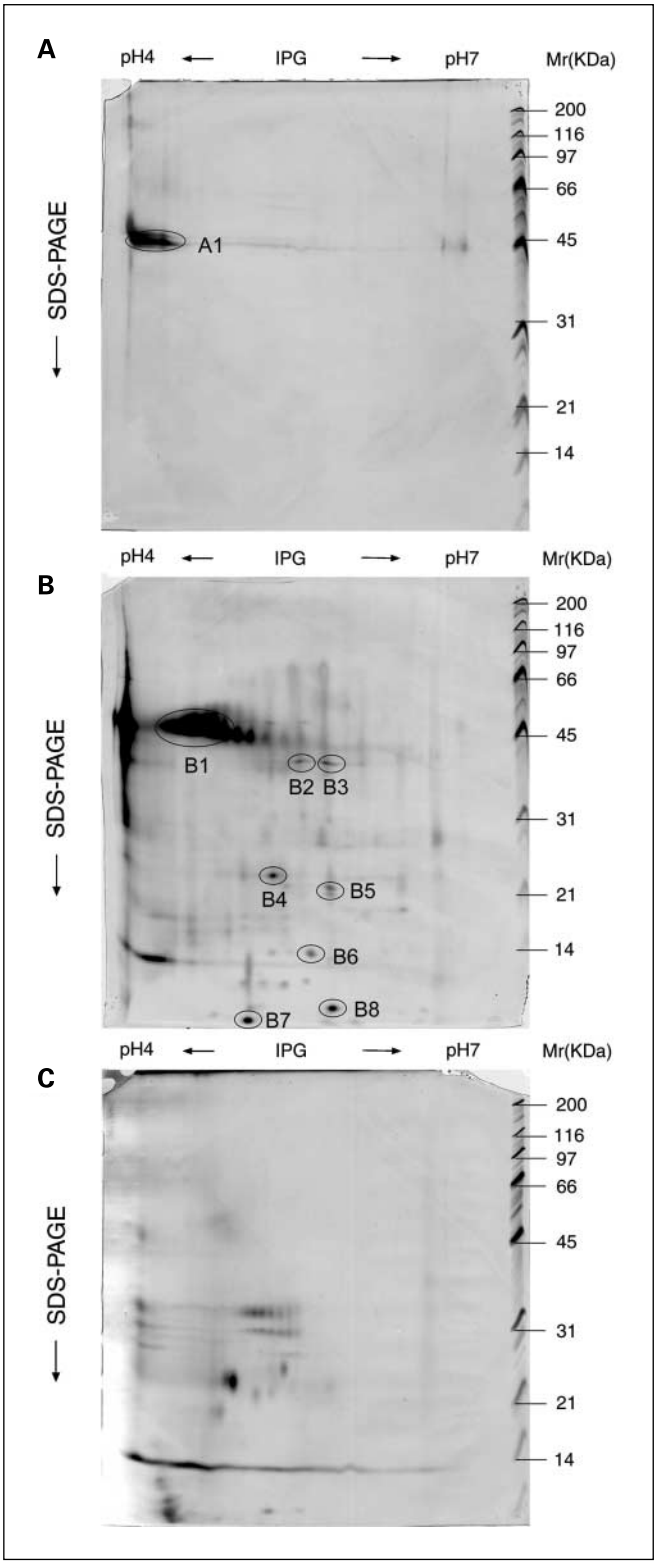
Table 2. Univariate analysis of clinical, bacterial, and gastric juice factors related to high protein concentrations in gastric juice

Principal variables	No. subjects	Rate of high protein concentration (%)	P
Clinical factors			
Age (y)			0.001
<60	135	20.0	
≥60	93	39.8	
Sex			0.435
Female	102	25.5	
Male	126	30.2	
Family history of GC			0.054
(−)	222	27.0	
(+)	6	66.7	
Smoking			0.459
(−)	175	26.9	
(+)	53	32.1	
Alcohol consumption			0.113
(−)	206	29.6	
(+)	22	13.6	
Diseases			
Healthy subjects	120	21.7	—
GU	39	35.9	0.075
DU	38	7.9	0.026
GC	31	71.0	<0.001
Bacterial factor			0.015
(−)	90	18.9	
(+)	133	33.8	
Gastric juice factors			
Amount (mL)			<0.001
<5	157	21.0	
≥5	71	43.7	
Acidity			<0.001
pH <3.5	154	7.1	
pH ≥3.5	74	71.6	

Table 4 displays the two-dimensional electrophoresis patterns of gastric juice in various gastroduodenal diseases. The patterns of electrophoresis images in gastric ulcer and gastric cancer patients differed significantly from those in healthy subjects. The frequencies of the specific α1-antitrypsin precursor band patterns in healthy subjects and gastric ulcer, duodenal ulcer, and gastric cancer patients were 6%, 42%, 6%, and 93%, respectively. The data indicated that α1-antitrypsin precursor in gastric juice was strongly associated with gastric ulcer and gastric cancer (both $P < 0.001$). Interestingly, the specific α1-antitrypsin precursor band was frequently found not only in advanced tumors (90%) but also in early cancer (100%; Table 4). Multivariate analysis revealed that gastric hypoacidity, gastric ulcer, and gastric cancer were independent factors for the presence of α1-antitryspin precursor band ($P < 0.001$, 0.011, and <0.001, respectively). The other factors, including advanced age, sex, and *H. pylori* status, were not related to α1-antitrypsin precursor band.

Table 5 shows the relationships among two-dimensional electrophoresis patterns of gastric juices, atrophic gastritis, and gastric hypoacidity. Glandular atrophy of the corpus and gastric hypoacidity were significantly associated with the presence of the specific α1-antitrypsin band patterns (both $P < 0.001$). The frequencies of the basic band, specific band, and nonspecific band patterns in two-dimensional images of the hypoacidic gastric juices were 0%, 93%, and 7%, respectively.

In the nine healthy subjects who underwent histologic examinations of gastric mucosa, eight without gastric atrophy had a basic band pattern in gastric juice electrophoresis. The other one, who had glandular atrophy in both the antrum and the corpus of the stomach, had a nonspecific pattern in electrophoresis study.



Discussion

This study provides the first evidence of differences in gastric juice protein concentrations among various gastroduodenal diseases. The mean gastric juice protein concentration in gastric cancer patients was significantly higher than that in gastric ulcer patients. Additionally, the gastric juice of gastric ulcer patients had a higher protein concentration compared with that of healthy subjects. In contrast, the gastric juice protein concentration of duodenal ulcer patients was lower than that of healthy subjects. Further analysis by two-dimensional electrophoresis showed that the principal protein components in gastric juice differed significantly among various gastroduodenal diseases. The frequencies of the specific α1-antitrypsin precursor bands in healthy subjects and gastric ulcer, duodenal ulcer, and gastric cancer patients were 6%, 42%, 6%, and 93%, respectively. These findings strongly indicate that measuring protein concentrations of gastric juices combined with analysis of protein constituents may serve as a new diagnostic tool to identify or screen for gastroduodenal diseases.

In this study, we also simultaneously evaluated 11 clinical, bacterial, and gastric juice factors to search for independent variables influencing protein concentrations in gastric juice. Multivariate analysis disclosed that gastric hypoacidity and advanced age were independent factors promoting high protein concentrations in gastric juice with odds ratios of 32.9 and 3.2, respectively. It is well known that pepsin is an active proteolytic enzyme in gastric juice with optimal function at a pH of 1.6, inactive but stable at pH 4 to 6, and irreversibly inactive at a pH of >6 (25, 26). Protein components in gastric juice are prone to be digested by pepsin in acidic gastric acid. Therefore, high protein concentrations in gastric juices with hypoacidity are reasonable. Currently, we have no definite rationale to explain the association between aging process and high protein concentrations in gastric juices, but aging imparts a variety of physiologic changes in the stomach (e.g., decreases in mucosal blood flow and prostaglandin synthesis). Advanced age is also one of the major risk factors for development of gastric ulcer and gastric cancer, probably reflecting increased incidences of *H. pylori* infection, smoking, presence of other diseases, and use of medications (e.g., nonsteroidal anti-inflammatory drug). Further studies are warranted to investigate the mechanisms underlying the relationship between aging and high protein concentrations in gastric juice.

In our study, the two-dimensional electrophoresis images of gastric juice were classified into three groups: basic band, specific band, and nonspecific band. The basic band pattern of

Fig. 1. *A,* basic band pattern in two-dimensional electrophoresis of gastric juice. The sample was obtained from a healthy subject without gland atrophy in the antrum and corpus. A prominent band with M_r of 42,000 Da (*A1*) was observed in this feature. It was identified as pepsin A precursor. *B,* specific band pattern in two-dimensional electrophoresis of gastric juice. Pepsin A precursor bands were barely detectable. In contrast, a strong dark band (*B1*) with acidic isoelectric point (4.5-5.5) and M_r of 47,000 Da was very prominent. The peptide in this specific band was identified as an α1-antitrypsin precursor. The principal peptides identified from B2 and B3 spots were also fragments of α1-antitrypsin precursors. *C,* nonspecific pattern in two-dimensional electrophoresis of gastric juice. This pattern lacked not only the basic bands of pepsin A precursor but also the α1-antitrypsin precursor band. There were numerous nonspecific spots observed in this electrophoresis feature.

Table 3. The silver-stained spots B1, B2, and B3 in the two-dimensional protein electrophoresis image of gastric juice, which was classified as specific band pattern

Source	Match protein	Score	Peptide sequences identified	Peptide score
B1	α1-Antitrypsin precursor	415	FLEDVK	22
			FLEDVKK	46
			KQINDYVEK	25
			QINDYVEK	39
			DTEEEDFHVDQVTTVK	22
			LGMFNIQHCK	32
			FLENEDR	44
			FLENEDRR	35
			RSASLHLPK	36
			SASLHLPK	28
			LSITGTYDLK	56
			SVLGQLGITK	30
B2	α1-Antitrypsin precursor	394	FLEDVK	22
			FLEDVKK	35
			KQINDYVEK	32
			QINDYVEK	53
			DTEEEDFHVDQVTTVK	28
			LGMFNIQHCK	15
			FLENEDR	33
			FLENEDRR	36
			RSASLHLPK	28
			SASLHLPK	19
			LSITGTYDLK	51
			SVLGQLGITK	39
B3	α1-Antitrypsin precursor	323	FLEDVK	21
			FLEDVKK	16
			KQINDYVEK	27
			QINDYVEK	20
			QINDYVEKGTQGK	15
			DTEEEDFHVDQVTTVK	16
			LGMFNIQHCK	10
			FLENEDR	46
			FLENEDRR	39
			SASLHLPK	35
			LSITGTYDLK	52
			SVLGQLGITK	26

NOTE: Peptide fragments were identified by nano-high-performance liquid chromatography LTQ FT-MS and MASCOT amino acid sequencing engine search.

electrophoresis images consisted of a major band formed by pepsin A precursor. This image pattern was predominant in healthy subjects (74%) and duodenal ulcer patients (85%). In contrast, the predominant pattern of electrophoresis images in gastric cancer patients was the specific band type (93%).

Proteomic analysis using a MALDI-TOF mass spectrometer revealed that α1-antitrypsin precursor was the principal peptide in the specific band. Recently, Lee et al. (27) also reported that α1-antitrypsin in gastric juice was associated with gastric cancer. Furthermore, Sudo et al. (28) have shown that the levels of fecal α1-antitrypsin were markedly higher in advanced gastric cancer patients compared with those in healthy control subjects and early gastric cancer patients. Nonetheless, abundant α1-antitrypsin precursor in gastric juice is not cancer specific.

Table 4. Two-dimensional electrophoresis patterns of gastric juices of various gastroduodenal diseases

Gastroduodenal diseases	Two-dimensional electrophoresis			*P*
	Basic pattern, *n* (%)	Specific band pattern, *n* (%)	Nonspecific pattern, *n* (%)	
Healthy subjects (*n* = 47)	35 (74)	3 (6)	9 (19)	—
GU (*n* = 33)	16 (48)	14 (42)	3 (9)	<0.001*
DU (*n* = 33)	28 (85)	2 (6)	3 (9)	0.455
GC (*n* = 29)	1 (3)	27 (93)	1 (3)	<0.001*
Early GC† (*n* = 6)	0 (0)	6 (100)	0 (0)	
Advanced GC† (*n* = 21)	1 (5)	19 (90)	1 (5)	

*Compared with two-dimensional electrophoresis patterns of gastric juice in healthy subjects.
†Gastric cancer patients with confirmed staging by surgery.

Table 5. Relations among two-dimensional electrophoresis patterns of gastric juices, atrophic gastritis, and gastric acidity

Principal variables	Two-dimensional electrophoresis			*P*
	Basic pattern, *n* (%)	Specific band pattern, *n* (%)	Nonspecific pattern, *n* (%)	
Gastric histology				
Antrum				
Atrophy (−) (*n* = 14)	11 (79)	3 (21)	0 (0)	—
Atrophy (+) (*n* = 49)	21 (43)	24 (49)	4 (8)	0.055*
Corpus				
Atrophy (−) (*n* = 40)	29 (73)	9 (23)	2 (5)	—
Atrophy (+) (*n* = 23)	3 (13)	18 (78)	2 (9)	<0.001*
pH				
<3.5 (*n* = 36)	32 (89)	2 (6)	2 (6)	—
≥3.5 (*n* = 27)	0 (0)	25 (93)	2 (7)	<0.001†

*Compared with two-dimensional electrophoresis patterns of nonatrophic group.
†Compared with two-dimensional patterns of low pH group.

In this work, 14 of 33 (42%) gastric ulcer patients had abundant α1-antitrypsin precursors in gastric juice. Previously, a fecal α1-antitrypsin test has also been used to detect protein-losing gastroenteropathy (29, 30). In this study, we well showed that the presence of α1-antitrypsin precursor in gastric juice is strongly associated with glandular atrophy of the corpus and gastric hypoacidity. Additionally, de la Fuente Perucho et al. (31) also found that the levels of α1-antitrypsin in gastric juice correlated with the gastric pH. Based on these analytic results, we propose that excessive α1-antitrypsin precursor, which originates from various inflammatory cells and flows into the stomach through the cancerous or ulcerative lesions, can be protected from rapid proteolysis in the gastric juice with hypoacidity.

It is also important to note that all the eight healthy subjects having a basic band pattern in the electrophoresis study did not have gland atrophy in the stomach. Conversely, the only one with nonspecific band pattern in proteomic analysis of gastric juice had remarkable glandular atrophy in both the antrum and the corpus. These findings implicate that the preservation of pepsin A precursor in gastric juices indicates the absence of gastric atrophy. The healthy subjects with nonspecific band pattern in gastric juice protein analysis may have precancerous conditions in the stomach, such as atrophy and intestinal metaplasia.

In conclusion, this work is the first verifying that the protein concentrations in gastric juices of gastric cancer and gastric ulcer patients are significantly higher than those in healthy subjects, whereas duodenal ulcer patients have lower gastric juice protein concentrations than healthy subjects. By way of this study, it was determined that gastric hypoacidity and advanced age are the independent factors influencing the protein concentrations in gastric juices. It was noted that the protein components in gastric juice of patients with various gastroduodenal diseases are markedly different. α1-Antitrypsin precursor in gastric juice was found to be a novel biomarker for gastric cancer and ulcer. Thus, it can be concluded that a noninvasive method to obtain gastric juice followed by proteomic analysis may serve as a new tool to screen for gastric malignancies.

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