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Original Article

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# Identification of Autoantigens in Pediatric Gastric Juices

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## ABSTRACT

**Purpose:** This study aimed to investigate the presence of autoantigens in the gastric juices of children.

**Methods:** Gastric juice and serum samples were obtained from 53 children <15 years of age who underwent gastric endoscopy. Among these, 8, 22, and 23 participants were in the age groups 0–5, 6–10, and 11–15 years, respectively. These samples were analyzed using two-dimensional electrophoresis (2-DE), immunoblot analysis, and matrix-assisted laser desorption ionization-time of-flight mass spectrometry. Furthermore, we reviewed the histopathological findings and urease test results and compared them with the results of 2-DE and immunoblot analysis.

**Results:** There were no statistically significant differences in urease test positivity, grades of chronic gastritis, active gastritis, or *Helicobacter pylori* infiltration of the antrum and body among the three age groups. Three distinct patterns of gastric juice were observed on 2-DE. Pattern I was the most common, and pattern III was not observed below the age of 5 years. Histopathological findings were significantly different among active gastritis ($p$=0.037) and *H. pylori* infiltration ($p$=0.060) in the gastric body. The immunoblots showed large spots at an approximate pH of 3–4 and molecular weights of 31–45 kDa. These distinct, large positive spots were identified as gastric lipase and pepsin A and C.

**Conclusion:** Three enzymes, which are normally secreted under acidic conditions were identified as autoantigens. Further investigation of the pathophysiology and function of autoantigens in the stomach is required.

**Keywords:** Gastric juice; Gastritis; Autoantigens; Children; *Helicobacter pylori*

## INTRODUCTION

Gastric juice is a unique body fluid that contains all the components secreted from the stomach. Therefore, the analysis of molecular markers and protein components in gastric juice continues to enhance the detection rates of gastric cancer and other gastroduodenal diseases, such as *Helicobacter pylori*-associated and atrophic gastritis [1-4].

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**Conflict of Interest**
The authors have no financial conflicts of interest.

Molecular mimicry of human gastric epithelial cell antigen and *H. pylori* antigen has been reported and may be influenced by autoimmune reactions [5,6]. Anti-parietal cell antibody has been detected in approximately 50% of the patients with *H. pylori*-associated chronic gastritis [7]. Anti-gastric autoantibodies play a role in the pathogenesis and outcomes of *H. pylori* gastritis, particularly in the development of gastric mucosal atrophy [8]. Long-standing *H. pylori* infection is associated with atrophic corpus gastritis, including autoimmune-type severe atrophy with vitamin $B_{12}$ malabsorption [9].

Regardless of *H. pylori* infection, no study has identified autoantigens or autoantibodies in the gastric juices of children to date. Therefore, this study aimed to investigate the presence of autoantigens in the gastric juices of pediatric patients using two-dimensional electrophoresis (2-DE), immunoblotting with the patient's pooled sera, and protein identification using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF-MS).

## MATERIALS AND METHODS

### Gastric juice samples from children

Gastric juice samples and serum of children aged 0–15 years who had undergone upper gastrointestinal endoscopy at the Department of Pediatrics, Gyeongsang National University Hospital (GNUH) were obtained after approval by the ethical committee of GNUH (number: GNUH-IRB 2021-05-001). All the gastric juice samples were aspirated immediately after insertion of the endoscope; following this, serum was collected and immediately frozen at -80°C deep freezer of the Biobank of GNUH. The participants were divided into three age groups: 0–5, 6–10, and 11–15 years.

### Histopathologic findings and urease test results

We reviewed histopathological reports according to the updated Sydney system [10]. Hematoxylin and eosin-stained gastric body and antrum specimens were examined by two blinded pathologists. In addition, the urease test results of children who preserved the gastric juice samples and serum were determined.

### Preparation of gastric juices

Gastric juice was collected from patients undergoing endoscopy. The pH of all samples were measured using a pH meter and neutralized to pH 7–8 by adding 1 M Tris base to inhibit protease activity. The samples were centrifuged at 14,000 rpm for 30 minutes at 4°C to remove cells and debris. The supernatant was frozen at –70°C and lyophilized. It was dissolved in the lysis buffer (9.5 M urea, 4% CHAPS, 65 mM DDT, 40 mM Tris-HCl, 0.01% SDS, and 0.5% ampholytes 3/10 (Bio-Rad), and centrifuged at 14,000 rpm for 30 minutes at 15°C. The supernatant was desalted and concentrated using an Amicon centrifugal filter (Amicon® Ultra-15; Merck Millipore) with a molecular weight cutoff of 3 kDa. The protein content in the concentrated samples was measured using the Bradford method.

### 2-DE and image analysis

2-DE sample preparation was performed as described previously [11]. The solubilized protein samples (30–50 μg) were mixed with the rehydration solution, containing 8 M urea, 4% CHAPS, 10 mM DTT, and 0.2% carrier ampholytes (pH 3.0–10.0, Bio-Rad), to a final volume of 130 μL and applied to immobilized pH gradient (IPG) strips (7 cm; Bio-Rad) of pH 3.0–10.0 in a re-swelling tray (Bio-Rad). IEG was performed using a Protean IEF Cell (Bio-Rad) and

three preset programs consisting of a first conditioning step (15 minutes at 250 V), a linear voltage ramping step (3 hours at 4,000 V), and a maximum voltage ramping step of up to 30,000 Vh. Following IEF, the strips were equilibrated with 0.375 M Tris buffer (pH 8.8) containing 6 M urea, 2% SDS, 20% glycerol, 2% DTT, and 0.01% bromophenol blue. The equilibration was repeated with the same buffer supplemented with 2.5% iodoacetamide. The second dimension was separated on 12% SDS-PAGE gel using Mini-PROTEAN Tetra cell (Bio-Rad). The resolved protein spots on the gels were visualized by silver staining [12] and scanned using a Fluor-S MutiImager (Bio-Rad). The spot intensities of each sample were determined and analyzed using the PDQUEST 2-D Gel Analysis Software Version 6 (Bio-Rad) installed on a Magic Station M5660 (Samsung).

### Immunoblot analysis

The equilibrated IPG strips were separated on 12% SDS-PAGE gel and transferred onto a nitrocellulose membrane (0.2 microns; Bio-Rad Laboratories). The membranes were immunostained with the following antibodies: they were put in 1:10 dilution of their serum and pooled sera at 37°C for 30 minutes. An alkaline phosphate-conjugated goat anti-human IgG-Fc antibody (Bethyl Laboratories, Inc.) was diluted at 1:10,000 and incubated at 37°C for 30 minutes. Chemiluminescent detection was performed using BCIP/NBT SOLUTION from Amresco (Amresco E116-100ML).

### Destaining and in-gel digestion of protein spots

Silver-stained spots were excised from 2-DE gels and transferred to microcentrifuge tubes. The spots were destained with fresh chemical reducers in a 1:1 ratio of 30 mM potassium ferricyanide and 100 mM sodium thiosulfate, as described previously [13], with occasional mixing until the brownish color disappeared. The gel pieces were rinsed thrice with distilled water to stop the reaction. Ammonium bicarbonate (500 μL of 200 mM) was added to cover the gels for 20 minutes and then discarded. The gel pieces were dehydrated with 100 μL acetonitrile and dried in a vacuum centrifuge. In-gel digestion was performed as described by O'Connell and Stults [14]. Gel pieces were rehydrated by covering them with a digestion buffer containing trypsin (12.5 ng/mL, Promega) and incubated on ice for 45 minutes. The enzyme solution was replaced with 20 μL of the buffer without enzyme, and digestion was carried out for a minimum of 16 hours at 37°C. The gel pieces were then vortexed vigorously for 30 minutes. The digested solution (20 μL) was transferred into clean Eppendorf tubes and dried in a vacuum. The resulting pellets were dissolved in 2 μL of 0.1% trifluoroacetic acid (TFA).

### MALDI-TOF-MS

The sample solution (10 μL each) and matrix solution (α-cyano-4-hydroxycinnamic acid [40 mg/mL], 50% acetonitrile, and 0.1% TFA) were obtained as described above, were mixed and loaded into the target well, and then dried and washed quickly using deionized water. The plate was dried for 10 minutes at room temperature and subjected to MALDI-TOF-MS analysis using a Voyager Biospectrometry Workstation (PE Biosystems) with the following parameters: Mass spectra were obtained over a mass range of 800–2,500 Da. Peptide mass fingerprints were analyzed using the MS-FIT program of PortinProspect developed by the ICSF Mass Spectrometry Faculty (http://prospector.ucsf.edu/ucsfhtuml/msfit.htm). The NCBI database of proteins was searched to identify the proteins, using monoisotopic peptide masses and allowing a molecular-mass range of 2-DE±15%; a peptide mass window was extended. Pyroglutamic acid modification of N-terminal glutamine, oxidation of methionine, and acrylamide modification of cysteine were considered.

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### Statistical analysis

The data were analyzed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Co.). The urease test positivity and the degree of histopathological findings of the gastric antrum and body in the 2-DE pattern and age groups were evaluated. We determined statistically significant differences in the urease test positivity and the inflammatory changes in the gastric antrum and body regions among age groups and 2-DE patterns by using the $\chi^2$ test. *p*-values of <0.05 were considered as statistically significant.

## RESULTS

Serum and gastric juice samples from 53 children were included in this study (**Table 1**). The median age of the children was 10.4 years (range: 2.4–14.8 years), and the numbers of male and female children were 27 and 26, respectively.



**Table 1.** Urease test results and histopathological findings of the gastric antrum and body according to age group

| Age groups | 0–5 yr (N=8) | 6–10 yr (N=22) | 11–15 yr (N=22) | *p*-value |
|---|---|---|---|---|
| Positivity of urease test | | | | |
| Antrum | 4 (50.0) | 11 (50.0) | 9 (40.9) | 0.733 |
| Body | 7 (87.5) | 11 (50.0) | 13 (59.1) | 0.177 |
| Histopathologic findings antrum | | | | |
| Chronic gastritis | | | | 0.442 |
| Normal | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Mild | 6 (75.0) | 14 (63.6) | 15 (68.2) | |
| Moderate | 1 (12.5) | 8 (36.4) | 6 (27.3) | |
| Severe | 1 (12.5) | 0 (0.0) | 1 (4.5) | |
| Active gastritis | | | | 0.828 |
| Normal | 5 (62.5) | 17 (77.3) | 16 (72.7) | |
| Mild | 2 (25.0) | 2 (9.1) | 3 (13.6) | |
| Moderate | 1 (12.5) | 3 (13.6) | 2 (9.1) | |
| Severe | 0 (0.0) | 0 (0.0) | 1 (4.5) | |
| *H. pylori* infiltration | | | | 0.923 |
| Normal | 6 (75.0) | 15 (68.2) | 16 (72.7) | |
| Mild | 1 (12.5) | 5 (22.7) | 3 (13.6) | |
| Moderate | 1 (12.5) | 2 (9.1) | 3 (13.6) | |
| Severe | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Body | | | | |
| Chronic gastritis | | | | 0.896 |
| Normal | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Mild | 6 (75.0) | 18 (81.8) | 17 (77.3) | |
| Moderate | 2 (25.0) | 4 (18.2) | 5 (22.7) | |
| Severe | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Active gastritis | | | | 0.795 |
| Normal | 6 (75.0) | 17 (77.3) | 19 (86.4) | |
| Mild | 2 (25.0) | 4 (18.2) | 2 (9.1) | |
| Moderate | 0 (0.0) | 1 (4.5) | 1 (4.5) | |
| Severe | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| *H. pylori* infiltration | | | | 0.554 |
| Normal | 6 (75.0) | 17 (77.3) | 19 (86.4) | |
| Mild | 2 (25.0) | 5 (22.7) | 2 (9.1) | |
| Moderate | 0 (0.0) | 0 (0.0) | 1 (4.5) | |
| Severe | 0 (0.0) | 0 (0.0) | 0 (0.0) | |

Values are presented as number (%).
*H. pylori*: *Helicobacter pylori*.


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Among them, 8, 22, and 23 children belonged to the age groups 0–5, 6–10, and 11–15 years, respectively. The urease test results and histopathological findings of the gastric body and antrum were available for 52 children (98.1%).

There were no statistically significant differences in the positivity of the urease test ($p$=0.733) in the antrum, grades of chronic gastritis ($p$=0.442 and $p$=0.896), active gastritis ($p$=0.828 and $p$=0.795), or *H. pylori* infiltration ($p$=0.923 and $p$=0.554) in the antrum and body among the three age groups (**Table 1**).

## 2-DE profiles of gastric juices

Three distinct gastric juice patterns were observed using a silver-stained 2-DE gel (**Fig. 1**). The first pattern (Pattern I) showed several large spots at a pH range of 3.0–5.0 and below the molecular weight of 45 kDa and 1–10 spots above pH 5.0. The second pattern (Pattern II) showed 10 spots above pH 3.0 and in the molecular weight range of 21-68 kDa. The last pattern (Pattern III) showed no spots at pH 3.0, and small and multiple spots above pH 5.0, in the molecular weight range of 14–69 kDa. Pattern I was the most common among the three age groups (**Table 2**). Pattern III was not observed in the 0–5 years group. There was no statistically significant difference in the frequency of patterns according to age group ($p$=0.709).

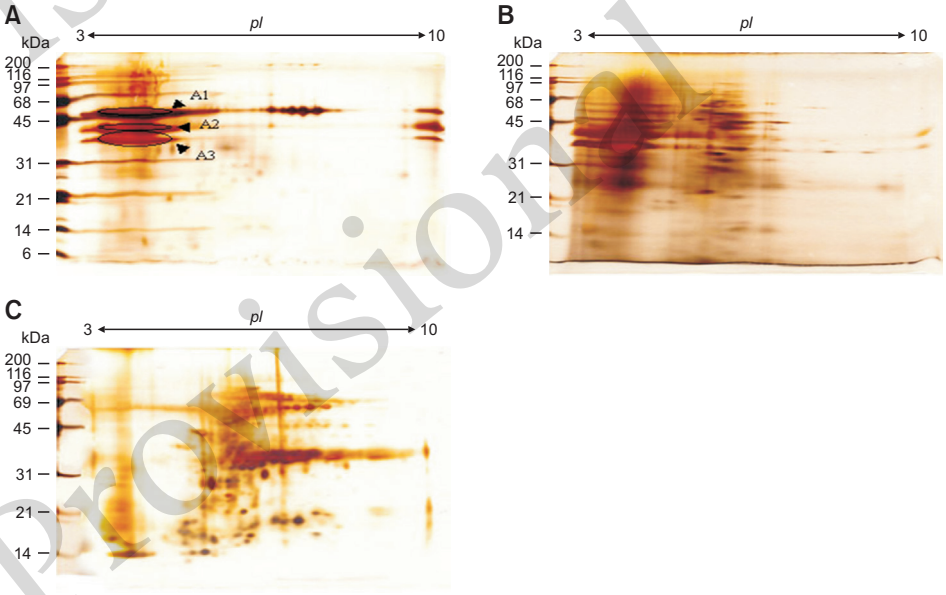


**Fig. 1.** Three distinct patterns of silver-stained 2-DE of gastric juice. (A) Pattern I shows that 1–3 or more large spots are observed in the pH range of 3.0–5.0 and a molecular weight range of 40 kDa, and less than 1–10 spots are observed in the pH 5.0 or higher area. (B) Pattern II shows that more than 10 spots were observed in the pH 5.0 or higher area, similar to the pattern I. (C) Pattern III shows that a molecular weight of approximately 40 kDa, no spots that appeared in the pH range of 3.0–5.0, and more than 20 spots were observed only in the area of pH 5.0 or higher.
2-DE: two-dimensional electrophoresis.

**Table 2.** Abundance of the three patterns obtained using gastric juices on 2-dimensional electrophoresis, according to age groups

| Age groups (yr) | Pattern I | Pattern II | Pattern III |
|---|---|---|---|
| 0–5 | 6 (75.0) | 2 (25.0) | 0 (0.0) |
| 6–10 | 16 (72.7) | 4 (18.2) | 2 (9.1) |
| 11–15 | 19 (82.6) | 2 (8.7) | 2 (8.7) |

Values are presented as number (%).




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The positivity of the urease tests was similar to that of the 2-DE findings ($p$=0.419). Histopathological findings were similar among patients with chronic gastritis, active gastritis, and *H. pylori* infiltration in the gastric antrum but significantly differed among patients with active gastritis ($p$=0.037) and *H. pylori* infiltration ($p$=0.060) in the gastric body (**Table 3**).

## Immunoblot analysis & proteomic analysis

Immunoblotting using participants' sera and pooled serum showed large spots at a pH range of 3–4 and a molecular weight range of 31–45 kDa (**Figs. 2** and **3**). These large spots correlated with the silver-stained spots of patterns I and II of 2-DE. In pattern III of 2-DE, small-sized spots at a pH range of 7–10 and a molecular weight range of 31–86 kDa reacted with the patient's sera and pooled serum; however, no protein spots matched the silver-stained 2-DE (**Fig. 4**).

The three distinct large positive spots in the immunoblotting results were labeled A1, A2, and A3. These were identified as gastric lipase, pepsin A, and pepsin C, respectively (**Table 4**).

**Table 3.** Urease test results and histopathological findings of the gastric antrum and body according to 2-DE patterns and updated sydney system, respectively

| | Pattern I | Pattern II | Pattern III | $p$-value |
|---|---|---|---|---|
| Positivity of urease test | | | | |
| Antrum | 17 (41.5) | 4 (50.0) | 3 (75.0) | 0.419 |
| Body | 24 (58.5) | 4 (50.0) | 3 (75.0) | 0.709 |
| Histopathologic findings antrum | | | | |
| Chronic gastritis | | | | 0.644 |
| Normal | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Mild | 28 (70.0) | 4 (50.0) | 3 (75.0) | |
| Moderate | 10 (25.0) | 4 (50.0) | 1 (25.3) | |
| Severe | 2 (5.0) | 0 (0.0) | 0 (0.0) | |
| Active gastritis | | | | 0.631 |
| Normal | 31 (77.5) | 4 (50.0) | 3 (75.0) | |
| Mild | 4 (10.0) | 2 (25.0) | 1 (25.0) | |
| Moderate | 4 (10.0) | 2 (25.0) | 0 (0.0) | |
| Severe | 1 (2.5) | 0 (0.0) | 0 (0.0) | |
| *H. pylori* infiltration | | | | 0.336 |
| Normal | 30 (87.5) | 5 (62.5) | 2 (50.0) | |
| Mild | 5 (12.5) | 3 (37.5) | 1 (25.0) | |
| Moderate | 5 (12.5) | 0 (0.0) | 1 (25.0) | |
| Severe | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Body | | | | |
| Chronic gastritis | | | | 0.803 |
| Normal | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Mild | 31 (77.5) | 7 (87.5) | 3 (75.0) | |
| Moderate | 9 (22.5) | 1 (12.5) | 1 (25.0) | |
| Severe | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Active gastritis | | | | 0.037 |
| Normal | 32 (80.0) | 7 (87.5) | 3 (75.0) | |
| Mild | 8 (20.0) | 0 (0.0) | 0 (0.0) | |
| Moderate | 0 (0.0) | 1 (12.5) | 1 (25.0) | |
| Severe | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| *H. pylori* infiltration | | | | 0.006 |
| Normal | 32 (80.0) | 8 (100.0) | 2 (50.0) | |
| Mild | 8 (20.0) | 0 (0.0) | 1 (25.0) | |
| Moderate | 0 (0.0) | 0 (0.0) | 1 (25.0) | |
| Severe | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Total number | 40 | 8 | 4 | |

Values are presented as number (%).
2-DE: two-dimensional electrophoresis, *H. pylori: Helicobacter pylori*.

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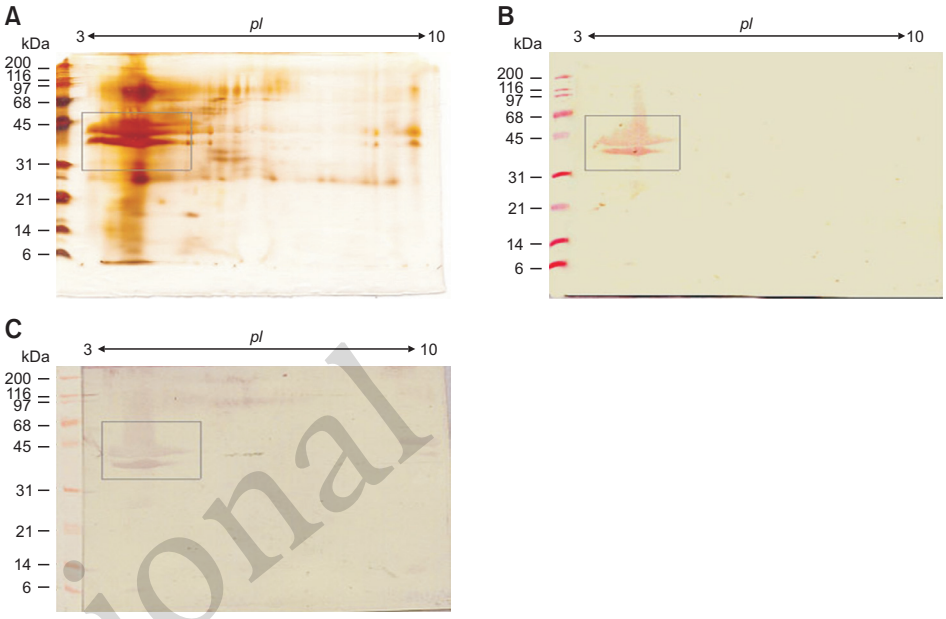


**Fig. 2.** Silver-stained 2-DE and immunoblot analysis using anti-human immunoglobulin G of the proteins of the pattern I in gastric juice. Two large spots in the pH range of 3.0–5.0 and approximate molecular weight 40 kDa (square box) show antigenicity. (A) Silver-stained 2-DE of Pattern I. (B) Immunoblot analysis using own serum. (C) Immunoblot analysis using the pooled serum.
2-DE: two-dimensional electrophoresis.

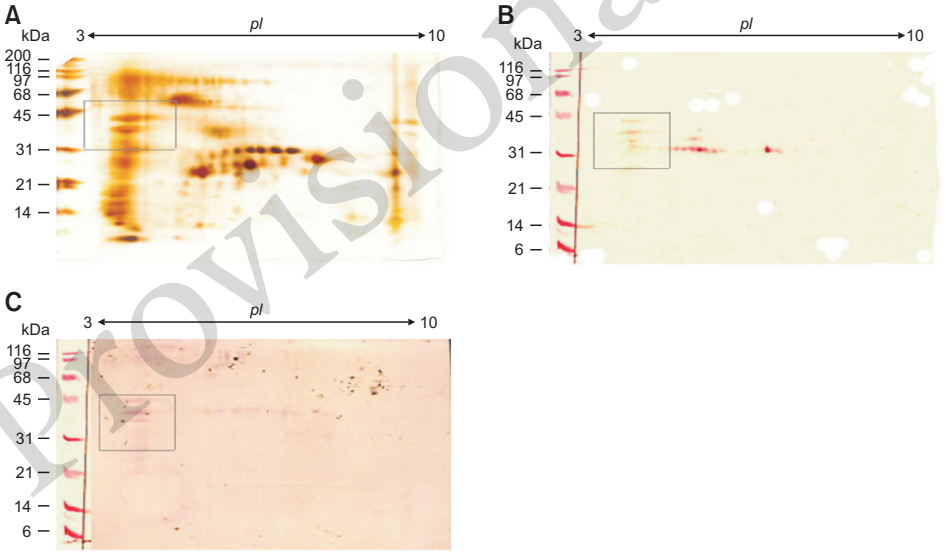


**Fig. 3.** Silver-stained 2-DE and immunoblot analysis using anti-human immunoglobulin G of the proteins of pattern II in gastric juice. More than ten spots at pH 5.0 or higher and molecular weight range of 30–50 kDa (square box) show antigenicity. (A) Silver-stained 2-DE of pattern II. (B) Immunoblot analysis using own serum. (C) Immunoblot analysis using the pooled serum.
2-DE: two-dimensional electrophoresis.




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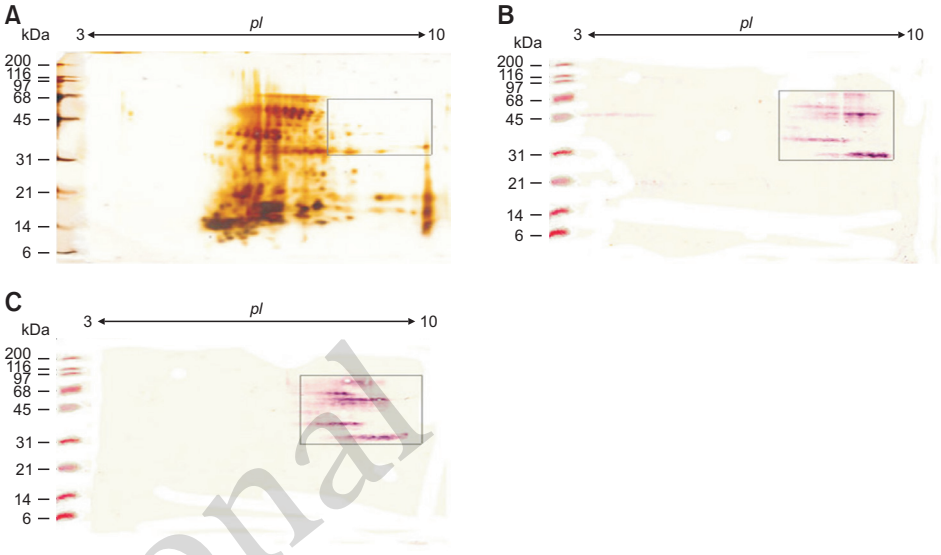


**Fig. 4.** Silver-stained 2-DE and immunoblot analysis using anti-human immunoglobulin G of the proteins of pattern III in gastric juice. Small-sized spots at a pH range of 7.0–10.0 and molecular weight range of 31–86 kDa (square box on B and C) show antigenicity. In silver-stained 2-DE (A), no spot is observed in the square box at the same location. (A) Silver-stained 2-DE of Pattern III. (B) Immunoblot analysis using own serum. (C) Immunoblot analysis using the pooled serum.
2-DE: two-dimensional electrophoresis.

**Table 4.** List of proteins identified from the pattern I of two-dimensional electrophoresis

| Spot | Protein |
|---|---|
| A1 | Gastric lipase |
| A2 | Pepsin A |
| A3 | Pepsin C |

A1, A2, and A3 represent the spots shown in Fig. 1A.

## DISCUSSION

To the best of our knowledge, this is the first study to investigate the presence of autoantigens in the gastric juices of pediatric patients. Analysis of gastric juices using 2-DE resulted in three patterns of protein spots, and the presence of autoantigens in gastric juice was related only to pattern I. The identified autoantigens were gastric lipase, pepsin A, and pepsin C.

Gastric lipases are secreted by the chief cells of the gastric mucosa. It also participates in fat digestion [15]. The lipolytic activity of human gastric lipase showed higher acidity at pH 2.8 compared to that at pH 5.4 [16]. In children, pH does not affect gastric lipase activity [17]. Sarles et al. reported that gastric lipase activity is rarely reduced in children with gastritis [18]. In 37 children aged 16±3 years, the mean activity of gastric lipase in gastric juice in the non-*H. pylori* group (0.247±0.09 mU/L) was significantly lower than in the *H. pylori* group (0.466±0.151 mU/L) and in the healthy control group (0.504±0.191 mU/L) [17]. Pepsin is the principal enzyme involved in protein digestion and the main component of gastric juice [19]. Since the chief cells in the gastric body release pepsin as a zymogen, activation by an acidic environment is necessary. Pepsin requires an acidic environment for protein digestion (pH 1.5 to 2) and remains structurally stable up to a pH of 8.0 [20]. Therefore, gastric lipase and pepsin may remain in hypochlorhydria caused by *H. pylori* infection. The autoantigenic action of pepsin and gastric lipase in gastric juice may be related to active *H. pylori* gastritis of the gastric body. However, the exact mechanisms by which pepsin and gastric lipase act as




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antigens in children remain unknown. Therefore, further studies are needed to investigate the pathogenesis of autoantibodies against these proteins in gastric juice.

In 562 children aged 0–15 years, the median gastric juice pH was similar among the three age groups (gastric pH <2.0) [21]. The proportion of individuals with hypochlorhydria (gastric pH >4.0) increased with age (1.3% in the 0–4 years, 6.1% in the 5–9 years, and 8.2% in 10–15 years) [21]. Chronic and active gastritis was more severe in the hypochlorhydria than in the normal group [21]. These findings suggest that the age of children and degree of gastritis are related to the production of autoantigens in gastric juices. Both gastric lipase and pepsin showed maximal activity in acidic environments (pH <3.0). Hypochlorhydria may be related to the production of gastric lipase and pepsin, which are autoantigens.

In the present study, the proportions of the three patterns of 2-DE in gastric juices varied according to age group. Pattern III was not found in children under 5 years of age; thus, autoantigens were present in children above the age of 5 years, and the degree of active gastritis and *H. pylori* infiltration in the gastric body was associated with the presence of autoantigens in gastric juices. *H. pylori* infection at a young age triggers the development of autoimmune gastritis, which progresses from mild chronic inflammation and oxyntic atrophy to an advanced stage associated with vitamin $B_{12}$ deficiency and pernicious anemia [22]. This result was similar to the findings of a study regarding the role of *H. pylori* infection in pediatric autoimmune gastritis, which suggested the presence of neutrophilic inflammation in 12 of 20 pediatric patients with autoimmune gastritis, seven of whom showed evidence of *H. pylori* infection [23]. Patients with *H. pylori*-positive autoimmune gastritis are more likely to manifest corpus-predominant gastritis than antral-predominant gastritis, which is typically associated with *H. pylori* infection [18]. As *H. pylori* infection progresses to a chronic state, corpus atrophy accelerates. The histopathological distribution of gastric atrophy may depend on the antigenicity of infected *H. pylori* [24]. Another pediatric study found no relationship between *H. pylori* infection and the presence or grade of oxyntic mucosal atrophy or intestinal metaplasia [25]. In Korean adults with atrophic gastritis, the mean gastric juice pH was higher in the *H. pylori*-positive group (N=17) than in the *H. pylori*-negative group (N=29). These results suggest that hypochlorhydria originates from *H. pylori* infection-induced atrophic gastritis and intestinal metaplasia in the gastric body [26].

Evidence supporting the role of *H. pylori* as an autoimmune response triggering the presence of parietal cell antibodies in 30–65% of patients infected with *H. pylori* [27,28]. The etiology of this process is presumed to be antigenic mimicry or cross-reactivity between *H. pylori* and the proton pump, suggesting that *H. pylori* infection may stimulate CD4+ T cells to target parietal cells, inducing similar histopathological and functional changes in the pathogenesis of autoimmune gastritis [6,27]. The three autoantigens identified in gastric juices in this study were proteins produced by gastric chief cells. This finding suggests that *H. pylori* infection of the gastric body is associated with autoimmune reactions.

The present study has several limitations. First, the amount of gastric juice in children is limited, which limits sample availability. Therefore, only 2-DE and immunoblot analyses were performed. Since the number of patients was small, especially in patterns II and II, even a small difference may seem statistically significant. Second, other secretions, including saliva and bile, can enter the stomach. Third, this study had a minimal sample size.

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In conclusion, this study identified three proteins as autoantigens, which are normally secreted enzymes under acidic conditions and are not observed in the gastric juices of children under 5 years of age. Further investigations regarding the pathophysiology and function of autoantigens in the stomach are required.

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