

Original Article



Analysis of Somatostatin-Secreting Gastric Delta Cells according to Upper Abdominal Symptoms and *Helicobacter pylori* Infection in Children

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Conflict of Interest

The authors have no financial conflicts of interest.

ABSTRACT

Purpose: Gastric delta cells (D-cells), which are somatostatin-secreting cells, are the main paracrine inhibitor of acid secretion. The number of D-cells was studied in children presenting with upper gastrointestinal (UGI) disease.

Methods: We retrospectively investigated the number of D-cells in the gastric body and antrum through immunofluorescence examinations according to symptoms, endoscopic findings, and *Helicobacter pylori* infection in 75 children who visited Hanyang University Hospital Pediatrics.

Results: The mean patient age was 12.2±3.3 years. The male-to-female ratio was 1:1.4. The mean D-cell number per high-power field in the antrum and body was 20.5 and 12 in children with substernal pain, 18.3 and 10.3 in vomiting, 22.3 and 6 in diarrhea, and 9.3 and 6 in abdominal pain, respectively ($p>0.05$). According to endoscopic findings, the mean D-cell number in the antrum and body was 14.3 and 6 with gastritis, 14 and 9.3 with reflux esophagitis, 16.7 and 8.7 with duodeno-gastric reflux, 19.3 and 12.7 with gastric ulcer, 16 and 13.7 with duodenitis, and 12.3 and 4 with duodenal ulcer, respectively ($p>0.05$). The D-cell number in the gastric body was 2.7 and 8.7 in children with current *H. pylori* infection and non-infected children, respectively ($p=0.01$), while those in the antrum were 15.5 and 14, respectively, with no statistical significance.

Conclusion: The D-cell number was lower in the gastric body of children with current *H. pylori* infection. Further studies concerning peptide-secreting cells with a control group would provide information about the pathogenic pathways of UGI disorder.

Keywords: Somatostatin-secreting cell; Children; Stomach; *Helicobacter pylori*

INTRODUCTION

Upper gastrointestinal (UGI) diseases in pediatric patients have been documented in detail with the development of pediatric endoscopic devices and active applications in these patients since the early 1980s. The previously serious gastrointestinal (GI) diseases became highly treatable with the advancement of diagnostic tools and clinical methods as well as the development of effective pharmaceuticals. However, very little is understood regarding the basic physiologic and hormonal mechanism of GI disease pathogenesis. The stomach has

many defensive factors, such as the mucus layer, epithelial layer, and blood supply. Gastric mucosal injury-inducing factors include infections, medications, vascular disorders, and allergies [1]. In healthy people, these defenses and injuries are balanced. However, when the injuries overwhelm these defenses, peptic ulceration occurs.

Helicobacter pylori is known as the main pathogen of peptic ulcer disease. With *H. pylori* infection, the inflammatory cells substitute for normal gland cells, resulting in hypochlorhydria or hyperchlorhydria depending on the site of *H. pylori* colonization in the stomach. Long-term *H. pylori* infection in the gastric body and fundus induces hypochlorhydria and poses the risk of gastric cancer. On the contrary, chronic localization in the gastric antrum arouses hyperacidity and duodenal ulcer disease [2,3]. Gastric acid plays a crucial role in the development of UGI diseases. Therefore, it is highly important to control hydrochloric acid levels, and acid-lowering medicines are the main prescription for UGI diseases. Gastric acid secretion is regulated by hormonal, paracrine (histamine, gastrin, and somatostatin), and neural (vagal) factors as well as by diverse endocrine cells, including gastrin cells (G-cells), D-cells, enterochromaffin cells, enterochromaffin-like (ECL) cells, ghrelin cells, and P/D1 cells. G-cells secrete gastrin during ingestion in the antrum of the stomach. Gastrin activates ECL cells to secrete acid by releasing histamine and stimulating parietal cells. Additionally, gastrin can stimulate mucosal growth in the stomach [4] and regulate some important cellular processes, such as proliferation, apoptosis, migration, invasion, angiogenesis, and tissue remodeling [5]. D-cells produce somatostatin, the main paracrine inhibitor of acid secretion and GI motility [6]. Somatostatin influences gastric motility, hence delaying gastric emptying and increasing gastric volume [7], suppresses pepsin and acid secretion; and inhibits gastrin release [8]. Antral somatostatin affects G-cells, but oxyntic somatostatin acts on both ECL cells and parietal cells. Thus, somatostatin inhibits gastric acid secretion via different pathways according to the location within the stomach [9]. To our knowledge, few studies have been conducted on the relationship between D-cell distribution and *H. pylori* infection in children with UGI diseases. Our study aimed to investigate the distribution and number of D-cells in the gastric body and antrum according to *H. pylori* infection, patient symptoms, and endoscopic findings through immunohistochemical study.

MATERIALS AND METHODS

Trial design

We investigated the number and distribution of somatostatin-secreting D-cells in the stomach mucosa according to patient symptoms, GI endoscopic findings, and the presence of *H. pylori* infection. The patients were classified by clinical presentations and endoscopic findings. In addition, we compared the number of D-cells between the *H. pylori*-positive and *H. pylori*-negative groups.

Patients

We conducted a retrospective study in children who had visited the Department of Pediatrics at Hanyang University Hospital presenting with GI symptoms and had undergone UGI endoscopic examinations between June 2016 and September 2017. The study protocol was approved by the Institutional Review Board (2019-04-047). The children presented with symptoms of abdominal pain, vomiting, substernal pain, and diarrhea. *H. pylori* infection was diagnosed by a rapid urease test, stomach mucosal pathologic examination, serum anti-*H. pylori* IgG, and stool *H. pylori* antigen.

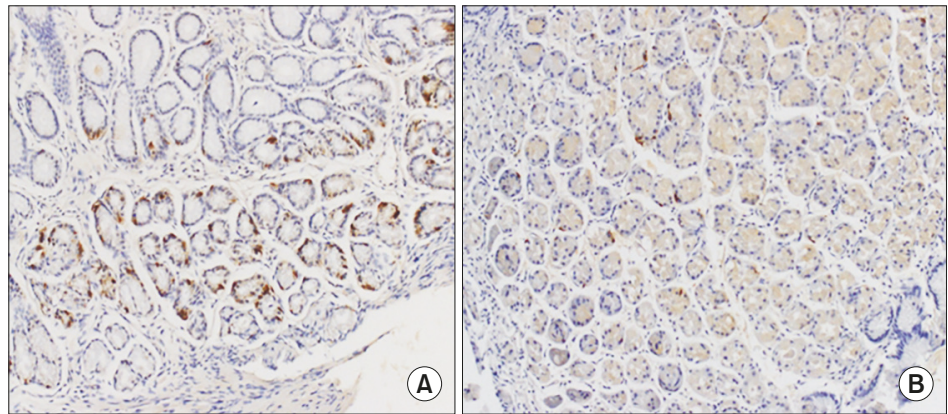


Fig. 1. Immunofluorescence results of somatostatin-immunoreactive D-cells in the antral mucosa (A) and the body mucosa (B) of the human stomach (IHC, somatostatin, $\times 200$).

Immunohistochemistry for D-cell expression

Endoscopic biopsies of the stomach were performed in most patients. Sections of the paraffin blocks were immunostained using the Bond-max Automated Immunohistochemistry (IHC)/in situ Hybridization Stainer (Leica Biosystems, Nussloch, Germany) according to the manufacturer's protocol. Then, 4- μm thick sections were immunostained with a primary rabbit polyclonal antibody against somatostatin (1:200, ab183855; Abcam, Cambridge, UK). Somatostatin-positive cells were counted by two independent pathologists (SP and YK) who were blind to patient clinical outcome. Microscopic images showing positive somatostatin-secreting D-cell staining are shown in **Fig. 1**. We initially obtained various stomach lesions. We investigated the number of D-cells only in the body and antrum of the stomach.

Statistical analysis

We used the nonparametric method to compare differences in D-cell numbers because they did not follow the normal distribution. Two statistical analyses were used depending on the number of groups. The Mann-Whitney U-test was used to analyze differences between two groups, and the Kruskal-Wallis test was used to analyze differences among more than two groups. Data were analyzed using SAS version 9.4 (SAS Institute, Cary, NC, USA). A *p*-value less than 0.05 was considered to be significant.

RESULTS

In total, 75 patients were enrolled. The mean patient age was 12.2 ± 3.3 years. The male-to-female ratio was 1:1.4. The presenting symptoms were abdominal pain (45, 60.0%), vomiting (10, 13.3%), substernal pain (8, 10.7%), and diarrhea (7, 9.3%). The most common endoscopic finding was gastritis (50, 66.7%): acute erosive hemorrhagic gastritis in 21 children, chronic superficial gastritis in 12, nodular gastritis in 7, verrucous gastritis in 2, and combinations of the above-mentioned lesions were present in 8 children. The next common endoscopic findings were reflux esophagitis (21, 28.0%), duodeno-gastric reflux (D-G-R; 11, 14.7%), gastric ulcer (11, 14.7%), duodenitis (8, 10.7%), and duodenal ulcer (5, 6.6%). Active *H. pylori* infection was diagnosed in 8 patients (10.7%). A greater number of D-cells per high-power field (HPF) was found in the antrum than in the body (**Table 1**).

Table 1. Baseline characteristics

Categories	Value (n=75)
Age (yr)	12.2±3.3
Sex (male:female)	31:44 (1:1.4)
Main symptoms	
Abdominal pain	45 (60.0)
Vomiting	10 (13.3)
Substernal pain	8 (10.7)
Diarrhea	7 (9.3)
No symptom	5 (6.7)
Endoscopic findings	
Gastritis	50 (66.7)
Acute erosive hemorrhagic gastritis	21/50
Chronic superficial gastritis	12/50
Nodular gastritis	7/50
Verrucous gastritis	2/50
Combined*	8/50
Reflux esophagitis	21 (28.0)
D-G-R	11 (14.7)
Gastric ulcer	9 (12.0)
Duodenitis	8 (10.7)
Acute erosive duodenitis	4/8
Chronic duodenitis	3/8
Nodular duodenitis	1/8
Duodenal ulcer	5 (6.6)
Current <i>Helicobacter pylori</i> infection	8 (10.7)
Number of D-cells	
Antrum	14 (5–24.7)
Body	6.7 (3.7–13.7)

Values are presented as mean±standard deviation, number only, number (%), or median number (interquartile range). D-G-R: duodeno-gastro reflux.

*There are 2 more than lesions of the above-mentioned.

We compared the number of D-cells per HPF according to the GI symptoms of the patients. In children with substernal pain, 20.5 and 12 D-cells were found in the antrum and body of the stomach, respectively; in the case of vomiting, these values were 18.3 and 10.3, and with diarrhea, they were 22.3 and 6, respectively. The D-cell number was very low in children with abdominal pain, who showed 9.3 in the antrum and 6 in the body. There was no statistical significance ($p>0.05$) (**Table 2**).

Furthermore, we compared the number of D-cells per HPF according to endoscopic findings. The values in the antrum and body of the stomach were found to differ: 14.3 and 6 for gastritis, 14 and 9.3 for reflux esophagitis, 16.7 and 8.7 for D-G-R, 19.3 and 12.7 for gastric ulcer, and 16 and 13.7 for duodenitis, respectively. The number of D-cells was very low in cases with duodenal ulcers, which showed 12.3 and 4 in the antrum and body, respectively, but there was no statistical correlation ($p>0.05$) (**Table 3**).

The mean number (interquartile range) of D-cells per HPF was 2.7 (0.83–5) in the gastric body in children with current *H. pylori* infection and 8.7 (3.7–15.3) in that of the non-infected children ($p=0.01$), but that value in the antrum was 15.5 (9.2–25.7) in those with *H. pylori* infection and 14 (4.3–24.7) in non-infected children; this result showed no statistical significance ($p>0.05$) (**Fig. 2**).

Table 2. The number of D-cell according to main symptoms

Endoscopic finding	Antrum	Body
Abdominal pain	9.3 (3.7–21.3)	6 (4–13.7)
Vomiting	18.3 (10–29)	10.3 (1.7–11.7)
Substernal pain	20.5 (7.7–25.2)	12 (5.5–17.7)
Diarrhea	22.3 (12.3–40.3)	6 (2–12)
No symptom	17.7 (16–35.3)	17.3 (3.7–17.7)

Values are presented as median (interquartile range).

Table 3. The number of D-cell according to endoscopic mucosal findings

Endoscopic finding	Antrum	Body
Gastritis	14.3 (6–22.7)	6 (2–12.3)
Reflux esophagitis	14 (8–21.7)	9.3 (4.7–15.7)
D-G-R	16.6 (12.3–34.7)	8.7 (4–12.7)
Gastric ulcer	19.3 (10–30)	12.7 (9.7–17.7)
Duodenitis	16 (2.7–28.2)	13.7 (7.7–19.3)
Duodenal ulcer	12.3 (8.3–21.3)	4 (3.7–8.7)

Values are presented as median (interquartile range).

D-G-R: duodeno-gastro reflux.

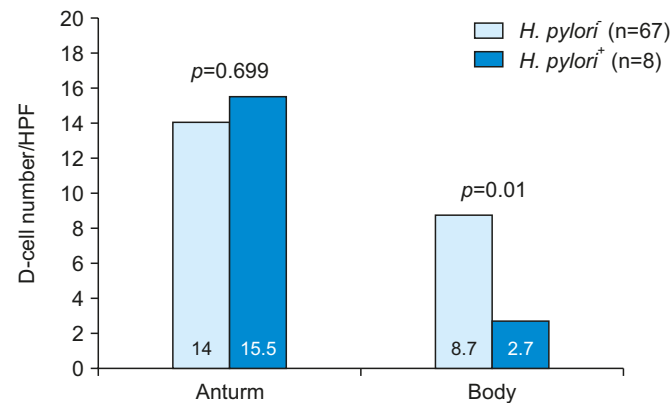


Fig. 2. The mean number of D-cells in the antrum and body of the stomach in children with current *H. pylori* infection. HPF: high-power field, *H. pylori*: *Helicobacter pylori*.

DISCUSSION

Mucosal lesion of UGI is related with acid secretion and cytoprotective mucosal barrier. Even though peptic ulcer disease is developed via multifactorial causes, the action of acid secretion is probably one of the most important pathogenic mechanisms. Authors have experienced several children who did not well controlled the conventional acid management treatment for most UGI disease, mostly peptic ulcer diseases, and have long been thought about what differences may be noticed in the peptide-secreting cells in the stomach according to the symptoms, the different mucosal lesions, and possibly *H. pylori* infections. Acid production in the stomach is regulated collectively by neural, paracrine, and hormonal stimulation as well as intracellular pathways that control the density of proton pumps ($H^+/K^+ATPase$) in parietal cells. These stimulants include acetylcholine from neuronal stimulation, histamine from paracrine ECL cells, and gastrin from hormonal control via the ECL using the ECL cell [10]. Acid secretion by parietal cells in the fundus and body of the stomach promotes protein digestion and micronutrient absorption and lowers the risk of GI infection [11]. Insufficient amounts of gastric acid secretion may result in malabsorption and increase the risk of GI infection. On the other hand, excessive acid secretion induces severe mucosal damage, resulting in intestinal bleeding or perforation. Therefore, tight regulation of gastric acid

production is highly important in the management of UGI diseases. The primary inhibitor of gastric acid production is somatostatin. Several studies have been conducted regarding the correlation between somatostatin and UGI diseases. Antral somatostatin was significantly lower in patients with gastritis or gastric ulcer [12,13]. D-cells closely approximate target cells to show strong suppression of the secretion of acids, histamine, and gastrin by G-cells [10]. An animal study by Schubert [6] showed that the input of acid solution into rat intestines induced a marked increase in somatostatin in the GI lumen and circulating blood, and gastric acid secretion was inhibited.

This study aimed to identify the distribution of D-cells according to patient symptoms, endoscopic findings, and *H. pylori* infection in pediatric groups. The number of D-cells was much lower in both the antrum and body of the stomach in children presenting with abdominal pain than in those with other symptoms; this number was remarkably lower in the antrum and body in children with a duodenal ulcer, which is mainly associated with a hyperacidic environment, although there was no statistical significance. Children with *H. pylori* infection showed a statistically significant decrease in the D-cell number in the body. A follow-up biopsy would enable the analysis of D-cell numbers after treatment to eradicate *H. pylori* and allow for comparisons with the pretreatment status. Most parents of our patients did not agree with repeated endoscopic examination. *H. pylori* eradication was confirmed by a urea breath test, stool *H. pylori* antigen analysis, and the finding of lowered anti-*H. pylori* IgG in the patients.

H. pylori infection is known to influence gastric secretion [14]. D-cells are one of the major endocrine cells in the stomach and play an important role in acid secretion [15]. *H. pylori* infection causes inflammation of the gastric mucosa, which stimulates somatostatin-mediated inhibition of gastric acid secretion to ensure the survival and colonization of *H. pylori* in the acidic environment of the gastric mucosa [10]. Several studies have demonstrated that *H. pylori* infection lowers D-cells in the antral mucosa [16-20]. In an animal study using rats by Zaki et al. [21], acute *H. pylori* infection was shown to inhibit acid secretion by activating intramural CGRP sensory neurons coupled to the enhancement of somatostatin and inhibition of histamine secretion. These previous results were consistent with our finding of a decrease in D-cells; however, the location was not consistent with previous findings, as the decrease in D-cells was found in the gastric body in our study. Currently, the mechanism by which D-cells are decreased with *H. pylori* infection remains unclear. One hypothesis is that *H. pylori* releases TNF- α , which inhibits D-cells, which allow excessive release of gastrin-stimulating acid secretion [22]. Further study might explain the mechanism according to the location.

Our study had some limitations. Firstly, there was no normal control group; this is because it was difficult to perform GI endoscopic examination in healthy children. Secondly, the number of *H. pylori*-infected children was low, probably due to the trend of decreasing *H. pylori* infection nationwide, especially in Korean children, and we could not compare the changes in D-cells pre- and post-eradication treatment of *H. pylori* infection.

Nevertheless, this study was meaningful because we identified a correlation between *H. pylori* infection and the distribution of D-cells in pediatric groups. Additionally, we aimed to investigate the relationship between D-cell distribution, dyspeptic symptoms, and endoscopic findings, although no statistical differences were found. Further study in a larger group of subjects might explain the diverse pathogenesis of *H. pylori* infection and various UGI diseases in pediatric groups.

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