Original Article

Analysis of Gastric and Duodenal Eosinophils in Children with Abdominal Pain Related Functional Gastrointestinal Disorders According to Rome III Criteria

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Background/Aims
Abdominal pain-related functional gastrointestinal disorder (AP-FGID) is common in children and adults. However, the mechanism of AP-FGID is not clearly known. Recently, micro-inflammation, especially eosinophilia in the gastrointestinal tract, was suggested in the pathophysiology of AP-FGID in adults. The aim of this study was to evaluate the association of gastric and duodenal eosinophilia with pediatric AP-FGID.

Methods
In total, 105 pediatric patients with AP-FGID were recruited and classified into 4 subgroups based on the Rome III criteria. Eosinophil counts in the gastric and duodenal tissues of children with AP-FGID were compared to those from normal pathology references or those of children with *Helicobacter pylori* infection. Tissue eosinophil counts were also compared among the 4 subtypes of AP-FGID.

Results
Eosinophil counts in the gastric antrum and body were significantly higher in children with AP-FGID than normal reference values. Duodenal eosinophil counts were higher in children with AP-FGID, but not significantly when compared with normal reference values. There were no significant differences in eosinophil counts of the stomach or duodenum among the 4 subtypes of AP-FGID. Eosinophils counts in the gastric antrum and body were significantly higher in children with *H. pylori* infection than in those with AP-FGID. Duodenal eosinophilia was prominent in cases of *H. pylori* infection, but not statistically significant when compared with AP-FGID.

Conclusions
Our study revealed that gastric eosinophilia is associated with AP-FGID in children, regardless of the subtype of functional abdominal pain. This suggests some contribution of gastrointestinal eosinophils in the development of pediatric AP-FGID.
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Key Words
Abdominal pain; Child; Eosinophils; Functional gastrointestinal disorder

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Introduction

Abdominal pain-related functional gastrointestinal disorder (AP-FGID) is common in children, with a 16.5% worldwide pooled prevalence rate based on the Rome III criteria.[1] A recent meta-analysis showed that prevalence rates of functional abdominal pain disorder were higher in South America (16.8%) and Asia (16.5%) than in North America (13.4%) and Europe (10.5%).[1] Children with AP-FGID tend to have a lower quality of life compared to healthy children, and AP-FGID can commonly cause absences from school.[2,3] AP-FGID is one of the most common gastrointestinal problems seen by pediatric gastroenterologists; however, no medications have been approved to treat this disorder in children.[4]

To date, the mechanism of AP-FGID in the pediatric population is not clearly disclosed.[5] Several possible mechanisms for the observed symptoms have been suggested, including psychosocial disturbance, abnormal brain-gut interaction, autonomic dysfunction, disordered gastrointestinal motility, visceral hypersensitivity, and immune activation.[6-10] Recent studies suggest that mild inflammation of the intestines is essentially involved in the pathophysiology of functional gastrointestinal disorder (FGID).[11-16] Adult studies on FGID, especially functional dyspepsia (FD) and irritable bowel syndrome (IBS),[13,14] have found that increased infiltration of eosinophils, mast cells, and intraepithelial lymphocytes represent low-grade mucosal inflammation in the gastrointestinal tract. In adult FD patients, gastric eosinophil counts tend not to be significantly increased, but the duodenal eosinophil count and duodenal degranulation is prominently increased.[13,16-21] On the other hand, several studies also show increased infiltration and degranulation of gastric and duodenal mast cells in adult FD patients.[16,22-24] In adult patients with IBS, most studies demonstrate increased infiltration of mast cells in all parts of the duodenum,[14] jejunum,[25] ileum,[26] and colon,[12,27-31] with the exception of a few studies.[32,33] Previous studies also show an increase in T lymphocyte infiltrates.[12,14,25,29,30,33] Conversely, there appears to be no significant differences in tissue eosinophil counts of patients with IBS.[12,14,31,34] However, histopathological studies in pediatric patients with AP-FGID are relatively scarce, moreover, the results of some studies are discrepant from those of studies on adults. Friesen et al found that duodenal eosinophil density and degranulation was increased in children with FD.[35,36] Another study showed that antral eosinophils and mast cells were significantly activated, and that elevated mast cell density of the antrum was associated with delayed gastric emptying and in-

creased preprandial dysrhythmia in children with FD.[37] Schurman et al[38] discovered that nocturnal abdominal pain was associated with higher duodenal mast cell density and antral T lymphocyte counts in children with FD. Mahjoub et al[39] detected increased mast cell numbers in the gastric antrum of children with recurrent abdominal pain, which was not classified by the Rome III criteria. To our knowledge, there have been no studies that have compared tissue eosinophil counts in children by considering subtypes of AP-FGID based on the Rome III criteria.

Therefore, the aim of this study was to evaluate the association of gastric and duodenal eosinophils with pediatric AP-FGID, while considering the subtypes of AP-FGID based on the Rome III criteria.

Materials and Methods

Subjects

From January 2009 through February 2015, children who visited the pediatric gastroenterology outpatient clinics of our tertiary medical center for chronic recurrent abdominal pain, and who were identified as having AP-FGID, were retrospectively enrolled in this study. In total, 105 pediatric patients with AP-FGID were recruited and classified, based on the Rome III criteria, into 4 subtypes: FD, IBS, abdominal migraine (AM), and functional abdominal pain or syndrome (FAPS). Patients were excluded from this study if they had a significant allergy, including food allergy, asthma, atopic dermatitis, and allergic rhinitis, or if they had underlying organic diseases that can cause gastrointestinal symptoms. Laboratory tests, including complete blood cell counts, erythrocyte sedimentation rate, highly sensitive C reactive protein, liver function tests, serum amylase and lipase, serum electrolytes, urinalysis, and stool examinations for occult blood and cultures for bacteria and parasites, as well as abdominal X-ray and ultrasonography, were evaluated in all study patients, which revealed normal results in all subjects. All enrolled children also underwent esophagogastroduodenoscopy with biopsies from the gastric antrum, body, and duodenum. Children who had any gross abnormalities, including mucosal nodularity, erosion, or ulceration on endoscopic examination, were also excluded from the study.

Age- and sex-matched healthy children were not recruited as normal controls for this study because healthy children without any gastrointestinal symptoms would not be indicated for esophagogastroduodenoscopy with biopsy, thus they could not undergo esophagogastroduodenoscopy for the study voluntarily. Instead, we

adopted previously published normal pediatric pathology references as substitution for the data of normal controls.[40] Additionally, a total of 27 pediatric patients with biopsy-proven *Helicobacter pylori* infection were also retrospectively recruited as the disease controls. Of the children with *H. pylori* infection, patients with gastric or duodenal ulcers were excluded, whereas patients with mucosal nodularity and/or erythema were included. The Institutional Review Board of Seoul National University Bundang Hospital approved this study protocol.

Endoscopic Biopsy and Histopathology

Endoscopic biopsies were taken from the gastric antrum, gastric body, and duodenum. Biopsy tissues were immediately fixed in formalin and processed to paraffin wax. Sections were cut at 3 μm, and stained with hematoxylin and eosin, and the Giemsa stain for *H. pylori*. Eosinophils were counted in 5 randomly selected high power fields (HPF). Quantification of eosinophils was performed using an Axioskop40 microscope (Mirax-Carl Zeiss, Oberkochen, Germany) at × 400 magnification. Cell counting was performed by 2 pathologists, who were blinded as to the status of the patient, and the average value of the 5 HPF was calculated for each subject. In the stomach, the grades of gastric tissue inflammation was also evaluated according to the severity of mononuclear cell or neutrophil infiltration: absent (grade 0), mild (grade 1), moderate (grade 2), and marked (grade 3), based on the updated Sydney classification.[41] *H. pylori* infection was identified by histology and rapid urease testing on gastric biopsy tissues.

Histopathological findings and tissue eosinophil counts in the gastric and duodenal tissues from pediatric AP-FGID cases were compared to those from normal pathology references (normal controls), and to those from children with *H. pylori* infection (disease controls). Tissue eosinophil counts were also compared among the 4 subtypes of AP-FGID. Antral and gastric body eosinophil counts were compared according to degree of antral and gastric body histologic inflammation based on the updated Sydney classification.

Statistical Methods

All statistical analyses were carried out using PASW statistics software (version 22.0, SPSS Inc, Chicago, IL, USA). Continuous variables are presented as mean and 95% CI, and categorical variables as a percentage of the total number. Eosinophil counts between normal controls and AP-FGID cases were compared by independent *t* test. Comparisons of eosinophil counts between each subtype of AP-FGID and normal controls were performed with independent *t* tests. Differences in tissue eosinophil counts were also evaluated among the 4 subtypes of AP-FGID by the Kruskal-Wallis test. Pair-wise comparisons of tissue eosinophil counts among the subtypes of AP-FGID were done by the Mann-Whitney U test. Comparisons of eosinophil counts between the AP-FGID and *H. pylori* gastritis group were performed with the Mann-Whitney U test. Antral and gastric body eosinophil counts were compared, according to the degree of histologic inflammation in the antrum and gastric body, by Spearman's correlation analysis. For all statistical analyses, a two-sided *P*-value of < 0.05 was considered statistically significant.

Results

Patient Characteristics

A total of 105 children fulfilling the Rome III criteria for AP-FGID (54 boys and 51 girls; median age, 12.6 years; range, 4.5-19.6 years) were recruited and classified as FD (n = 40), IBS (n = 36), AM (n = 3), FAPS (n = 21), FD + IBS (n = 2), FD + AM (n = 1), and IBS + AM (n = 2). Normal controls included 19 children according to the references cases: 8 boys and 11 girls; median age, 12 years (range, 2-17 years). Disease controls with *H. pylori* infection included a total 27 children: 11 boys and 16 girls; median age, 12.2 years (range, 3.9-18.6 years). There were no significant differences among the AP-FGID group, the normal control group,

Table 1. Demographic Characteristics of the Subjects

	AP-FGID (n = 105)	Normal reference [Normal control] (n = 19)	*H. pylori* gastritis [Disease control] (n = 27)	*P*-value
Gender (M:F)	54:51	8:11	11:16	0.517[a]
Age (mean ± SD, yr)	12.2 ± 3.1	10.6 ± 4.2	11.5 ± 3.9	0.150[b]

[a]Chi-square test.
[b]ANOVA.
AP-FGID, abdominal pain-related functional gastrointestinal disorder; M, male; F, female; *H. pylori*, *Helicobacter pylori*.

A

Gastric antrum

B

Normal control

AP-FGID
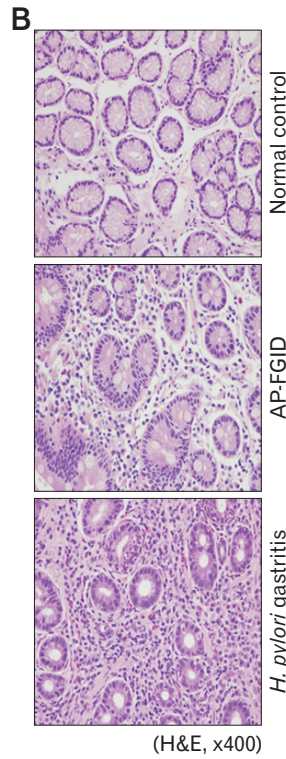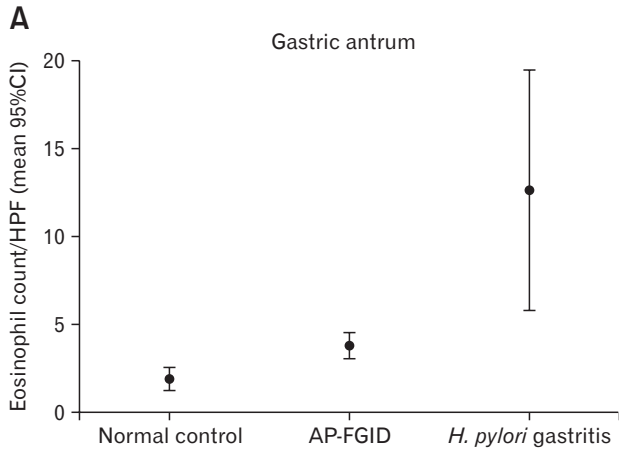
H. pylori gastritis

(H&E, x400)

Figure 1. Eosinophil count in the gastric antrum for normal control, abdominal pain-related functional gastrointestinal disorder (AP-FGID), and *Helicobacter pylori* (*H. pylori*) infection. (A) Eosinophil count in the gastric antrum expressed as mean and 95% CI, 1.9 (1.3-2.5)/high power field (HPF) in normal controls, 3.9 (3.1-4.6)/HPF in AP-FGID, and 12.7 (5.8-19.7)/HPF in *Helicobacter pylori* gastritis (magnification, ×400). (B) Photographs of hematoxylin and eosin staining for eosinophils in the gastric antrum from each representative specimen for normal controls, AP-FGID, and *H. pylori* infection.
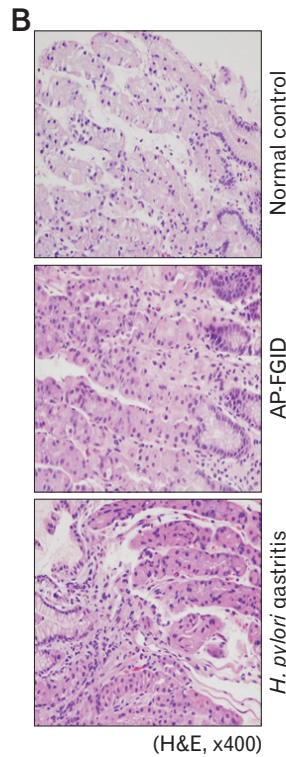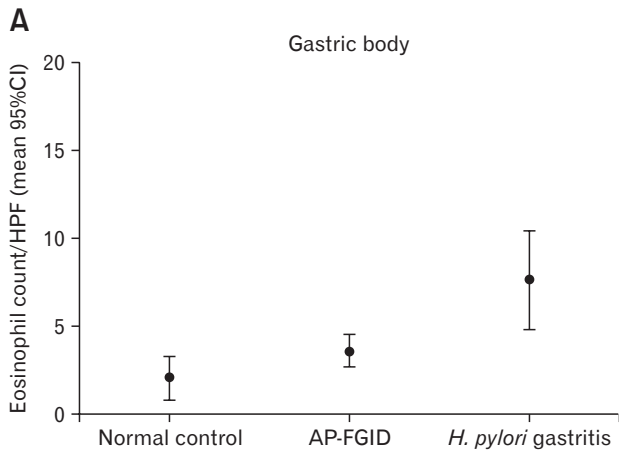
A

Gastric body

B

Normal control

AP-FGID

H. pylori gastritis

(H&E, x400)

Figure 2. Eosinophil count in the gastric body for normal control, abdominal pain-related functional gastrointestinal disorder (AP-FGID), and *Helicobacter pylori* (*H. pylori*) infection. (A) Eosinophil count in the gastric body expressed as mean and 95% CI, 2.1 (0.8-3.4)/high power field (HPF) in normal controls, 3.7 (2.7-4.6)/HPF in AP-FGID, and 7.7 (4.9-10.5)/HPF in *Helicobacter pylori* gastritis (magnification, ×400). (B) Photographs of hematoxylin and eosin staining for eosinophils in the gastric body from each representative specimen for normal control, AP-FGID, and *H. pylori* infection.

and the disease control group in gender distribution ($P = 0.517$) or in age ($P = 0.150$) (Table 1).

Gastric and Duodenal Eosinophil Counts in Abdominal Pain-related Functional Gastrointestinal Disorder and Normal Control

Eosinophil counts in the gastric antrum and body were both significantly higher in children with AP-FGID than the normal pediatric reference value (mean 3.9 [95% CI, 3.1-4.6] eosinophils/HPF vs mean 1.9 [95% CI, 1.3-2.5]) eosinophils/HPF, $P < 0.001$ for the antrum; mean 3.7 [95% CI, 2.7-4.6] eosinophils/HPF vs mean 2.1 [95% CI, 0.8-3.4] eosinophils/HPF, $P = 0.045$ for

the gastric body, respectively) (Fig. 1 and 2; Table 2). Duodenal eosinophil counts were high in children with AP-FGID, but not significantly when compared with normal reference values (mean 12.3 [95% CI, 10.5-14.1] eosinophils/HPF vs mean 9.6 [95% CI, 6.5-12.7] eosinophils/HPF, $P = 0.127$) (Fig. 3 and Table 2).

When tissue eosinophil counts were compared between each subtype of pediatric AP-FGID identified by the Rome III criteria and the normal control, eosinophil counts of the gastric antrum were significantly different in most subtypes except for AM ($P = 0.003$ in FD vs normal control, $P = 0.009$ in IBS vs normal control, $P = 0.015$ in FAPS vs normal control, and $P = 0.147$ in AM vs normal control, respectively). In contrast, there were no

Table 2. Comparison of Gastric and Duodenal Eosinophil Counts Between Children with Abdominal Pain-related Functional Gastrointestinal Disorders and Normal Controls

	AP-FGID (n = 105)		Normal control (n = 19)		P-value
	Mean	95% CI	Mean	95% CI	
Antrum	3.9	3.1-4.6	1.9	1.3-2.5	< 0.001
Body	3.7	2.7-4.6	2.1	0.8-3.4	0.045
Duodenum	12.3	10.5-14.1	9.6	6.5-12.7	0.127

AP-FGID, abdominal pain-related functional gastrointestinal disorder.
The mean number and 95% CI of eosinophils/high-power field for each anatomical region of the upper gastrointestinal tract.
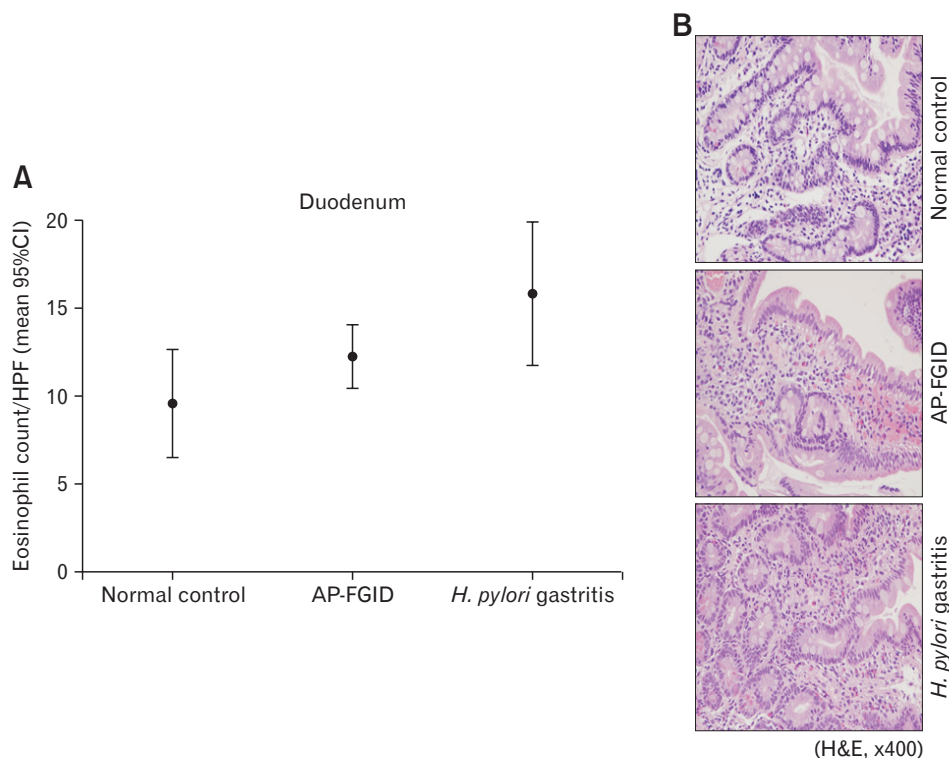P-value was calculated by independent t test.

Figure 3. Eosinophil count in the duodenum for normal control, abdominal pain-related functional gastrointestinal disorder (AP-FGID), and *Helicobacter pylori* infection. (A) Eosinophil count in the duodenum expressed as mean and 95% CI, 9.6 (6.5-12.7)/high power field (HPF) in normal controls, 12.3 (10.5-14.1)/HPF in AP-FGID, and 15.9 (11.8-20.0)/HPF in *Helicobacter pylori* (*H. pylori*) gastritis (magnification, ×400). (B) Photographs of hematoxylin and eosin staining for eosinophils in the duodenum from each representative specimen for normal controls, AP-FGID, and *H. pylori* infection.

significant differences in eosinophil counts of the gastric body and duodenum when comparing each AP-FGID subtype with the normal control (Table 3).

Gastric and Duodenal Eosinophil Counts Among the Subtypes of Abdominal Pain-related Functional Gastrointestinal Disorder in Children

There were no statistically significant differences in tissue eosinophil counts for the gastric antrum, gastric body, and duodenum

Table 3. Comparison of Tissue Eosinophil Counts Between Each Subtype of Abdominal Pain-related Functional Gastrointestinal Disorder and Normal Controls

Biopsy site	AP-FGID (n = 105)		Normal control (n = 19)		P-value
	Mean	95% CI	Mean	95% CI	
H2a. Functional dyspepsia (n = 43)					
Antrum	4.3	2.9-5.7	1.9	1.3-2.5	0.003
Body	4.0	2.5-5.4	2.1	0.8-3.4	0.057
Duodenum	13.4	10.6-16.2	9.6	6.5-12.7	0.066
H2b. Irritable bowel syndrome (n = 40)					
Antrum	3.7	2.5-4.9	1.9	1.3-2.5	0.009
Body	3.3	1.8-4.8	2.1	0.8-3.4	0.217
Duodenum	12.1	9.1-15.0	9.6	6.5-12.7	0.236
H2c. Abdominal migraine (n = 6)					
Antrum	4.8	0.6-9.0	1.9	1.3-2.5	0.147
Body	3.8	−1.3-8.9	2.1	0.8-3.4	0.417
Duodenum	7.6	4.2-11.0	9.6	6.5-12.7	0.305
H2d. Functional abdominal pain syndrome (n = 21)					
Antrum	4.0	2.4-5.6	1.9	1.3-2.5	0.015
Body	3.8	1.7-5.8	2.1	0.8-3.4	0.154
Duodenum	12.2	6.9-17.4	9.6	6.5-12.7	0.390

AP-FGID, abdominal pain-related functional gastrointestinal disorder.
The mean number and 95% CI of eosinophils per high-power field for each anatomical region of the upper gastrointestinal tract.
P-value was calculated by independent t test.

Table 4. Comparison of Tissue Eosinophil Counts Among the 4 Subtypes of Abdominal Pain-related Functional Gastrointestinal Disorder in Children

Biopsy site	AP-FGID subtype	Median (range)	Mean ± SD	P-value
Gastric antrum	FD	3.0 (0-15)	3.9 ± 4.1	0.852
	IBS	2.0 (0-15)	3.2 ± 2.7	
	AM	3.0 (1-10)	4.7 ± 4.7	
	FAPS	4.0 (0-14)	4.0 ± 3.4	
Gastric body	FD	1.0 (0-20)	3.8 ± 4.9	0.888
	IBS	2.0 (0-21)	3.3 ± 4.6	
	AM	2.0 (1-10)	4.3 ± 4.9	
	FAPS	2.0 (0-15)	3.8 ± 4.4	
Duodenum	FD	10.5 (1-42)	12.9 ± 8.4	0.438
	IBS	9.0 (1-40)	11.6 ± 8.3	
	AM	6.0 (5-7)	6.0 ± 2.0	
	FAPS	9.5 (0-50)	12.2 ± 11.2	

AP-FGID, abdominal pain related functional gastrointestinal disorder; FD, functional dyspepsia; IBS, irritable bowel syndrome; AM, abdominal migraine; FAPS, functional abdominal pain or syndrome.
Eosinophils per high power field for each anatomical region of the upper gastrointestinal tract.
P-value was calculated by Kruskal-Wallis test.

Table 5. Pair-wise Comparison of Tissue Eosinophil Counts Between the Subtypes of Abdominal Pain-related Functional Gastrointestinal Disorder in Children

	Antrum (P-value)	Body (P-value)	Duodenum (P-value)
FD vs IBS	0.983	0.973	0.349
FD vs FAPS	0.644	0.658	0.432
IBS vs FAPS	0.384	0.598	0.820
FD vs AM	0.561	0.474	0.131
IBS vs AM	0.584	0.572	0.267
FAPS vs AM	0.895	0.795	0.388

FD, functional dyspepsia; IBS, irritable bowel syndrome; FAPS, functional abdominal pain or syndrome; AM, abdominal migraine.
P-value was calculated by Mann-Whitney U test.

Table 6. Comparison of Tissue Eosinophil Counts Between Children with Abdominal Pain-related Functional Gastrointestinal Disorder and Those with *Helicobacter pylori* Gastritis

	AP-FGID (n = 105)		*H. pylori* gastritis (n = 27)		P-value
	Mean	95% CI	Mean	95% CI	
Antrum	3.9	3.1-4.6	12.7	5.8-19.7	< 0.001
Body	3.7	2.7-4.6	7.7	4.9-10.5	0.002
Duodenum	12.3	10.5-14.1	15.9	11.8-20.0	0.057

AP-FGID, abdominal pain-related functional gastrointestinal disorder; *H. pylori*, *Helicobacter pylori*.
The mean number and 95% CI of eosinophils/high-power field for each anatomical region of the upper gastrointestinal tract.
P-value was calculated by Mann-Whitney U test.

among the 4 subtypes of AP-FGID (Table 4). In addition, there were also no significant differences in eosinophil counts of the stomach and duodenum in pair-wise comparisons between the subtypes of AP-FGID (Table 5).

Gastric and Duodenal Eosinophil Counts in Abdominal Pain-related Functional Gastrointestinal Disorder and *Helicobacter pylori* Infection Group

In children with *H. pylori* infection, mean eosinophil counts in endoscopic biopsies were as follows: 12.7 (95% CI, 5.8-19.7) eosinophils/HPF in the gastric antrum, 7.7 (95% CI, 4.9-10.5) eosinophils/HPF in the gastric body, and 15.9 (95% CI, 11.8-20.0) eosinophils/HPF in the duodenum. The gastric antrum and gastric body eosinophil counts were significantly higher in children with *H. pylori* infection than in those with AP-FGID ($P < 0.001$ in the antrum, $P = 0.002$ in the gastric body, respectively) (Fig. 1 and 2; Table 6). Duodenal eosinophil counts were high in children with *H. pylori* infection, but not significantly when compared to those with AP-FGID ($P = 0.057$) (Fig. 3 and Table 6).

When eosinophil counts of the gastric antrum and body were compared according to the degree of histologic inflammation based on the Sydney classification at the same site between children with AP-FGID and those with *H. pylori* infection, significant correlations between tissue eosinophil counts and histologic inflammation in the gastric antrum and body were noted (Spearman's correlation coefficient $r = 0.364, r^2 = 0.292, P < 0.001$ and $r = 0.264, r^2 = 0.076, P = 0.003$ for eosinophils vs neutrophils in the antrum and body, respectively; $r = 0.410, r^2 = 0.186, P < 0.001$ and $r = 0.343, r^2 = 0.115, P < 0.001$ for eosinophils vs mononuclear cells in the antrum and body, respectively).

Discussion

The aim of this study was to evaluate the association of gastric and duodenal eosinophilia with pediatric AP-FGID, so as to better understand its pathogenesis. The mechanism of AP-FGID is not clearly known yet, and multidisciplinary interpretation has been suggested through a biopsychosocial model.[10] Recent studies suggest that micro-inflammation of the intestines is essentially involved in the pathophysiology of FGID.[16] Adult studies on FGID, especially FD and IBS,[13,14] have found that increased infiltration of eosinophils, mast cells, and intraepithelial lymphocytes represent low-

grade mucosal inflammation in the gastrointestinal tract.

It is not clear how low-grade inflammation occurs, but it has been suggested that gut barrier dysfunction facilitates the penetration of antigens through the gastrointestinal mucosal epithelium in IBS and inflammatory bowel disease, which provokes an immune response.[42,43] For instance, perturbation of luminal contents, such as dietary antigens or gastrointestinal pathogens, through an abnormally increased intestinal permeability may facilitate an inappropriate stimulation of the host immune response in post-infectious IBS patients.[12,44] Increased infiltration of the lamina propria T cells, mast cells, and enterochromaffin cells have also been observed in the colon or ileum of patients with post-infectious IBS.[30] The mediators released by these activated cells types may stimulate sensory afferent pathways and induce visceral hyperalgesia.[18] A recent study demonstrated decreased duodenal mucosal integrity, increased permeability, and low-grade inflammation caused by increased infiltration of mast cells, intraepithelial cells, and eosinophils in patients with FD.[13] In the aforementioned study, the researchers hypothesized that impaired barrier function could facilitate the passage of luminal antigens through the epithelium to the lamina propria, thereby low-grade inflammation develop and persists.[13]

Eosinophils are activated by an inflammatory reaction that is initiated by internal and external triggers.[16,45] Internal triggers, such as anxiety and stress, impinge on gut physiology through the brain-gut axis to induce an inflammatory response, whereas external triggers, such as microbes and allergens, stimulate inflammation via highly-conserved, pathogen-associated molecular patterns.[16,46] With the release of cytokines, such as IL-4, IL-5, and IL-13 from type 2 T helper cells during the inflammatory response, activated eosinophils are ready for massive degranulation.[11,16,20,47] Degranulation contents, such as the nerve growth factor, have a direct influence on sensory nerves, and major basic proteins can induce vagal nerve muscarinic receptor 2 dysfunction.[19,20] Platelet aggregating factor, leukotrienes, and IL-13 are also able to act directly on the smooth muscle cells, thereby increasing contractility and reactivity.[19,20] Thus, the accumulation and degranulation of eosinophils gives rise to neural stimulation and smooth muscle contraction, which consequently produces gastrointestinal symptoms, such as abdominal cramps, abdominal discomfort, and flatulence.[16,19,20,48]

According to a study by Schappi et al,[49] children with atopic FD demonstrated increased eosinophils and mast cells within the antrum, and these cells degranulated rapidly after a cow's milk challenge unlike in children with non-atopic FD. Duodenal eosinophilia can be associated with an allergy,[20] and eosinophils in the duodenum can be activated in patients with allergy-related disorders, including asthma and atopic eczema, which can induce gastrointestinal symptoms in these groups of patients.[11,50,51] To eliminate the factors which can affect eosinophil activation in the gastrointestinal tract, we excluded in the present study patients who had a significant allergy, including food allergy, asthma, atopic dermatitis, and allergic rhinitis.

We hypothesized that micro-inflammation might be the main pathogenesis that gives rise to gastrointestinal symptoms in AP-FGID, as such, we expected inflammatory cell densities in the stomach and the duodenum of the AP-FGID group to be higher than those of normal controls without any symptoms, and lower than those of the H. pylori infection group with definite inflammation. Previous studies show that an H. pylori infection results in significantly higher tissue inflammatory cell densities, including lymphocytes, plasma cells, neutrophils, mast cells, and eosinophils.[52-55] Likewise, in the present study, eosinophil counts of the gastric antrum and body were significantly high in the order of the H. pylori infection group, AP-FGID group, and normal controls; however, duodenal eosinophil counts were not significantly different. Although the difference between mean duodenal eosinophil counts in patients with AP-FGID and that of normal controls was not statistically significant, it might be notable that duodenal eosinophil counts in AP-FGID patients in our study were still more than 10 cells/HPF in most cases, and duodenal eosinophilia have previous been defined as > 10 cells/HPF for children.[20,56]

There were no statistically significant differences in tissue eosinophil counts of the gastric antrum, gastric body, and duodenum among the 4 subtypes of AP-FGID in this study. Further study, that targets a large number of children within each subtype of pediatric AP-FGID, may still be required to evaluate if tissue eosinophil infiltration is different in the entire gastrointestinal tract according to the subtype of pediatric AP-FGID.

Previously published studies demonstrated that eosinophil density in the duodenum was higher in adult patients with FD than in healthy controls, but this was not applied to the density of gastric eosinophils.[16,21] In our study, eosinophil counts of the gastric antrum were significantly higher in children with FD than in normal controls, and duodenal eosinophil counts were high in children with FD; but this difference was not significant when compared with the normal reference value. Histopathological findings of the present study, confirming that antral eosinophils were significantly activated in children with FD, are consistent with the results of a previous study.[37] The results of this present study is different with that of previous studies except for Friesen et al's study, in the way that gastric eosinophil count is associated with AP-FGID. The exact cause that

can account for this different result is not proven and further succeeding studies are still needed to explain the significance of gastric eosinophilia in pediatric AP-FGID. In our study, all children with AP-FGID underwent esophagogastroduodenoscopy with biopsy due to the red flag signs along with chronic abdominal pain; hence, our patients might indicate a more severe disease status and raise the likelihood of overestimation. Nevertheless, eosinophil densities of children with FD in our study was relatively lower in comparison to those from two previous studies, which report a mean eosinophil density of 7.1 ± 5.5/HPF in the antrum of children with FD, and 10.3 ± 10.1/HPF in the antrum and 21.2 ± 11.5/HPF in duodenal bulb of children with FD.[36,37]

In addition, in the present study, we graded the intensity of neutrophil and mononuclear cell infiltration in the stomach using the updated Sydney classification, and compared gastric eosinophil counts with infiltrates of neutrophil and mononuclear cells in order to determine if the degree of eosinophil infiltration correlated with that of other inflammatory cell infiltrations. Accordingly, we were able to verify the association of tissue eosinophil counts with histologic inflammation in the gastric antrum and body, because our study revealed significant correlations between tissue eosinophil counts and histologic inflammation in the gastric antrum and body.

Nevertheless, the present study also has some limitations that remain to be addressed. First, this study did not enroll healthy individuals as part of the normal control group because ethical considerations do not justify endoscopic examination with biopsy sampling for eosinophilic infiltration evaluation in otherwise healthy children. Therefore, as a substitute for recruiting a normal control group, we derived normal reference values based on a previously published study, which evaluated endoscopic biopsies from each region of the gastrointestinal tract for pediatric patients with no identified organic gastrointestinal disease based on histological criteria, endoscopic findings, and final clinical assess.[40] This reference group, however, was not comprised of Korean children and therefore might not be able to represent a precise normal control; this group also included some patients with functional abdominal pain, and some patients with allergies, such as asthma, eczema, and rhinitis. As a result, the normal reference values used in this study were likely to reflect elevated tissue eosinophil counts than that of truly normal individuals. Second, this study incorporates a retrospective analysis and the sample size of this study is relatively small, particularly with regards to the normal control and *H. pylori* infection groups. As such, further prospectively designed studies targeting a large number of pediatric patients and including additional cell types, such as mast cells or intra-epithelial lymphocytes are still needed to confirm low-grade inflammation in pediatric AP-FGID.

In conclusion, gastric antrum and gastric body eosinophil counts were significantly higher in children with AP-FGID in comparison to normal reference values, and lower than those with *H. pylori* infection in our study; whereas, duodenal eosinophil counts of AP-FGID were not significantly different from normal controls or those with an *H. pylori* infection. In addition, there were no significant differences in eosinophil counts of the stomach or duodenum among the 4 subtypes of AP-FGID, based on the Rome III criteria in children.

The present study reveals that gastric eosinophilia, rather than duodenal eosinophilia might be associated with AP-FGID in children. Therefore, the results of our study suggest that there might be some contributions of gastrointestinal eosinophils to the development of pediatric AP-FGID.

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