J Neurogastroenterol Motil, Vol. 25 No. 2 April, 2019
pISSN: 2093-0879 eISSN: 2093-0887
https://doi.org/10.5056/jnm18176
Journal of Neurogastroenterology and Motility

Original Article

Degranulated Eosinophils Contain More Fine Nerve Fibers in the Duodenal Mucosa of Patients With Functional Dyspepsia

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Background/Aims
Functional dyspepsia (FD) is characterized as chronic recurrent upper gastrointestinal symptoms in the absence of any organic disorder. We hypothesized that duodenal low-grade inflammation activates superficial afferent nerve sprouting, thereby contributing to hypersensitivity in patients with FD.

Methods
A prospective case-control study was conducted in a tertiary referral center. FD was defined using the Rome III criteria. Standardized endoscopic biopsies were performed in the stomach and duodenum. Hematoxylin and eosin staining and immunohistochemical staining for major basic proteins were performed to detect granulated eosinophil-derived granules, and S-100 staining was performed to detect fine nerve fibers.

Results
A total of 51 patients with FD (82% female; mean age 35.8 ± 13.4 years) and 35 controls were enrolled. Activated eosinophil counts in the duodenum were significantly higher in patients with FD than in controls (41.4% vs 17.1%, $P = 0.005$). Microscopic duodenitis was more frequently detected in patients with FD than in controls. Fine nerve fibers were more abundant in patients with FD than in controls (45.1% vs 11.4%, $P = 0.029$). The abundance of fine nerve fibers highly correlated with the degree of activated eosinophils.

Conclusion
Duodenal low-grade inflammation, such as mucosal eosinophilic accumulation with degranulation, promoted mucosal enteric nerve fiber density and sprouting in patients with FD.
(J Neurogastroenterol Motil 2019;25:212-221)

Key Words
Duodenum; Dyspepsia; Eosinophils; Inflammation; Peripheral nervous system

Received: October 30, 2018 Revised: December 9, 2018 Accepted: January 8, 2019

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Introduction

Functional dyspepsia (FD) is a symptom complex that includes chronic and recurrent epigastric pain, epigastric soreness, early satiation, and postprandial fullness without any underlying structural or biochemical disorder. FD is common in the general population, with a prevalence of 8.1-25.0% in the United States, European, and Asian populations.[1-5] The pathophysiology of FD is not completely understood, and diverse mechanisms have been suggested. Motor disturbances, such as abnormal gastric accommodation and delayed gastric emptying, low-grade inflammation, visceral hypersensitivity, brain-gut interactions, autonomic nervous disorders, and psychiatric comorbidities, may be involved in the development of FD.[6]

Low-grade inflammation in patients with post-infectious irritable bowel syndrome (IBS) may provoke localized derangements in the gastrointestinal (GI)—neural axis, including motor and sensory abnormalities, and may enhance neuro-immuno-dysregulation.[7] Mast cells in the GI mucosa increase irritability of the visceral nervous system by secreting granules containing histamine, serotonin, heparin, or tryptase, which consequently increase the frequency and severity of abdominal pain in patients with IBS.[8,9] Eosinophils are present in low levels in the GI tract, particularly in the lamina propria of the stomach, small intestine, cecum, and colon.[10,11] Interestingly, infiltration of eosinophils has only been associated with eosinophil degranulation in the GI tract, and eosinophil granules, such as major basic protein (MBP), eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase constituents, are toxic to a variety of tissues, including the intestinal epithelium.[12] Combined administration of a histamine inhibitor H1/H2 antagonist and a mast cell stabilizer improves dyspepsia and stomach ache in 89% of dyspeptic children,[13,14] suggesting that the inflammatory response, including duodenal eosinophilia, may be associated with FD; however, studies on this subject are limited.[15]

Although it is believed that the symptoms of patients with FD are related mainly to the stomach, recent studies have shown sensorimotor and structural duodenal abnormalities in patients with FD. Duodenal mast cell hyperplasia has been linked to IBS and eosinophilia with FD.[16,17] Eosinophils have been shown to cause GI dysmotility and impaired gastric relaxation in an animal study.[18] Dietary antigens induce visceral hyperalgesia related to mucosal infiltration of mast cells and eosinophils in patients with food allergic colitis, and these cells are co-localized with neuronal markers, such as neurofilament protein, neuron-specific enolase, and nerve growth factor receptor.[19]

This study aim to identify the association of duodenal eosinophils and/or infiltration of mast cells with dyspeptic symptoms and to exam the fine nerve fibers in patients with FD, which in turn may suggest a novel diagnostic approach in some patients with FD.

Materials and Methods

Study Subjects

The study subjects were patients with FD who satisfied the Rome III criteria, sampled from the outpatient clinic of the referral center. Subjects who were > 18 years old and able to answer a self-administered questionnaire for the symptom survey were included. Exclusion criteria were (1) patients with organic diseases such as reflux esophagitis, peptic ulcer, erosive gastroduodenitis, and malignancies that could cause upper GI symptoms similar to FD; (2) patients with dementia, apoplexy, or mental disease who could not complete the Korean version of the bowel disease questionnaire; (3) patients with uncontrolled diabetes, end-stage renal failure, decompensated liver cirrhosis, or terminal cancer that might induce upper GI symptoms; (4) patients who had undergone major abdominal surgery except appendectomy; (5) those with a bleeding tendency or taking warfarin, aspirin, or antiplatelet drugs; (6) those with a parasitic infection, allergy, urticaria, atopic disease, or asthma that may induce eosinophilia or those taking medications for such diseases; (7) patients with a history of idiopathic eosinophilic GI disease or overt hypereosinophilic syndrome; (8) those who had taken, within the past month, medications such as iodine, sulfonamides, nitrofurantoin, angiotensin-converting enzyme inhibitors, or cephalosporin that may induce eosinophilia; and (9) patients who had taken an antibiotic and non-steroidal anti-inflammatory drugs or been administered an acid secretion inhibitor within the past month.

The control group was sampled among those who did not have any GI symptoms, had received endoscopy for anemia or a screening test, and provided their consent to participate in the study, by applying the same inclusion and exclusion criteria as those for the subjects.

Ethical Considerations

This study was conducted after approval by the Institutional Review Board of Ewha Womans University Mokdong Hospital (IRB No. ECT 189-16). All methods in the present study were carried out in accordance with relevant guidelines and regulations. We received all participant written "informed consent" to study participation in this study and publishing identifying information. All

authors have declared no conflict of interest.

Data Collection

Bowel disease questionnaire and history

We surveyed the demographics and social and medical histories of all participants using a self-administered bowel disease questionnaire.[20] This questionnaire has been validated for evaluation of treatment effects, has high reliability (kappa value = 0.78; 95% confidence interval [CI], 0.52-1.00), and has adequate construct validity for outpatients.[21] The Korean version of the questionnaire was translated from the original questionnaire according to the accepted linguistic validation guidelines. The reliability of the questionnaire was confirmed by the test-retest reliability test, with a median kappa value of 0.74 (95% CI, 0.36-1.00).[22]

The study patients had FD that satisfied the Rome III criteria for the past 3 months, with symptom onset at least 6 months prior to diagnosis. The diagnostic criteria for FD included one or more of the following: (1) early satiety, (2) postprandial fullness, (3) epigastric pain, or (4) epigastric soreness. In this case, early satiety and postprandial fullness were defined as experiencing symptoms at least 2 to 3 times/week, and epigastric pain or epigastric soreness were defined as experiencing symptoms at least once/week.[23] FD was divided into 2 subtypes depending on the symptoms. Epigastric pain syndrome (EPS) is associated with epigastric pain or epigastric soreness that satisfies the Rome III criteria, and postprandial distress syndrome (PDS) is associated with early satiety or postprandial fullness. IBS was defined by the Rome III criteria

Upper gastrointestinal endoscopy and *Helicobacter pylori* test

Endoscopy was conducted by an experienced endoscopy specialist (H.K.J.) with a digestive endoscopy specialist license and > 20 years clinical experience. Upper GI endoscopy was performed using the Olympus CV-260 (Olympus, Tokyo, Japan). Four endoscopic biopsy samples from the normal mucosa of the lesser/greater curvature of the gastric antrum, duodenal bulb, and second portion were stained with hematoxylin and eosin (H&E) followed by immunohistochemical staining. *Helicobacter pylori* infection was determined using the rapid urease test or histology. Two additional biopsies were performed at the gastric antrum and duodenal bulb for electron microscopy (EM).

Microscopic examinations to determine eosinophil infiltration in the duodenum

H&E staining was performed to count the total number of eosinophils and examine microscopic changes. An experienced pathologist (S.P.) evaluated the histology via a blind review of the clinical information. The eosinophil count was performed in hot spots of 5 high-power fields (HPFs) across the biopsy sample. Data are expressed as the mean number of eosinophils/HPF (Olympus BX50, UPlan Fl 40×, ocular magnification ×10; area of the microscopic field 0.55 mm^2) (Fig. 1A).

Low-grade inflammation, mostly lymphoplasmacytic infiltration, not neutrophil infiltration, was assessed semi-quantitatively on the H&E-stained slides in a blinded manner according to the following grading system: grade 1, normal mucosa; grade 2, mild to moderate erosions without eosinophilic degranulation; and grade 3, severe erosions with infiltration of degranulated eosinophils (Fig. 1A).

Immunohistochemistry

Eosinophil activation was determined by counting the degranulated eosinophils. We stained immunohistochemically for MBP (1:100 dilution; BMK13; Millipore, Billerica, MA, USA), which is the predominant protein in eosinophil granules.[24,25] The cytoplasmic membranes of activated or degranulated eosinophils are disrupted, and MBP-positive granules are present in the lamina propria of the duodenal mucosa during activation (Fig. 1B). The degree of eosinophilic degranulation was measured semi-quantitatively: grade 0, no or few extracellular granules; grade 1, < 10% eosinophils degranulated; grade 2, 10-50% eosinophils degranulated; and grade 3, > 50% eosinophils degranulated (Fig. 1B).

Mast cells were stained for c-KIT (1:50 dilution; oncoprotein T595; Novocastra, Wilmington, DE, USA). Mast cells were counted in hot spots of 5 HPFs across the biopsy sample. Data are expressed as the mean number of mast cells/HPF (Olympus BX50, UPlan Fl 40×, ocular magnification ×10; area of the microscopic field 0.55 mm^2). In H&E stain, ganglia can be seen with the naked eye but fine nerve fibers are not seen. Therefore, microscopic examination distinguishes between glial cell and nerve filaments, and the fine filament structures stained in s-100 observed in this study are nerve sprouts. S-100 staining (1:1000 dilution, rabbit polyclonal, Novocastra) was performed to detect fine nerve fibers (Fig. 2A). Fine nerve fiber abundance in the duodenal mucosa was categorized into 3 groups: no or little detection, slightly increased abundance in < 50% of the mucosa, and highly increased abun-

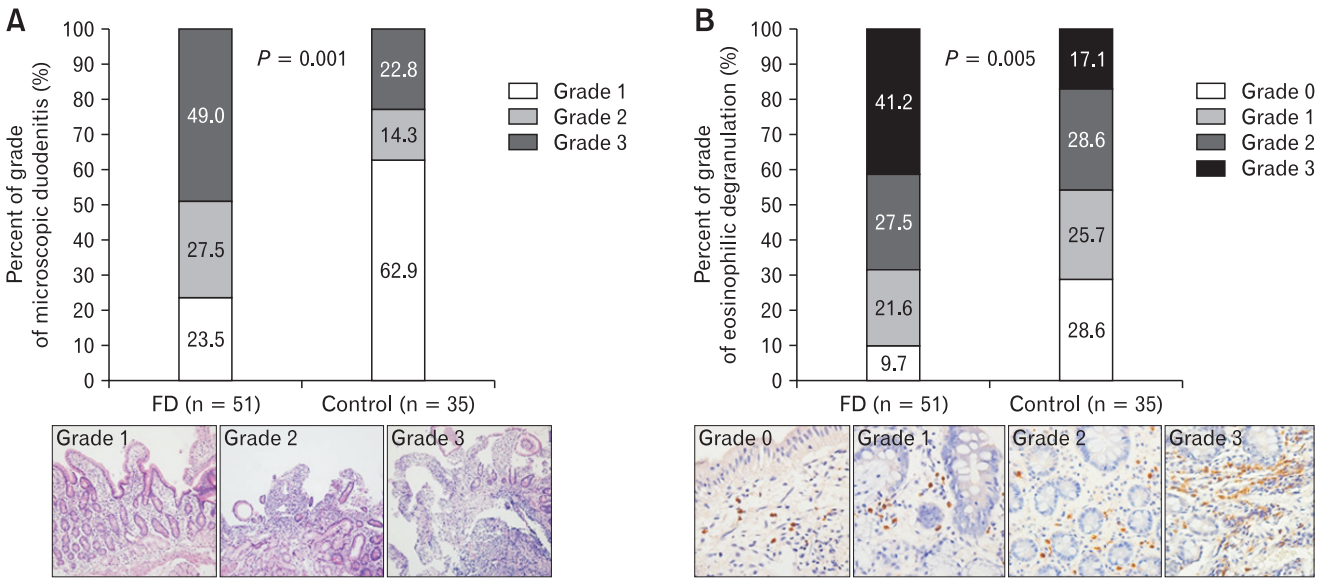

Figure 1. Microscopic grading of duodenal eosinophilia. (A) Grade of microscopic duodenitis in the functional dyspepsia (FD) group (×100 high power field [HPF]). (B) Semi-quantitative grading of eosinophilic degranulation in specimens stained immunohistochemically for major basic protein (×400 HPF).

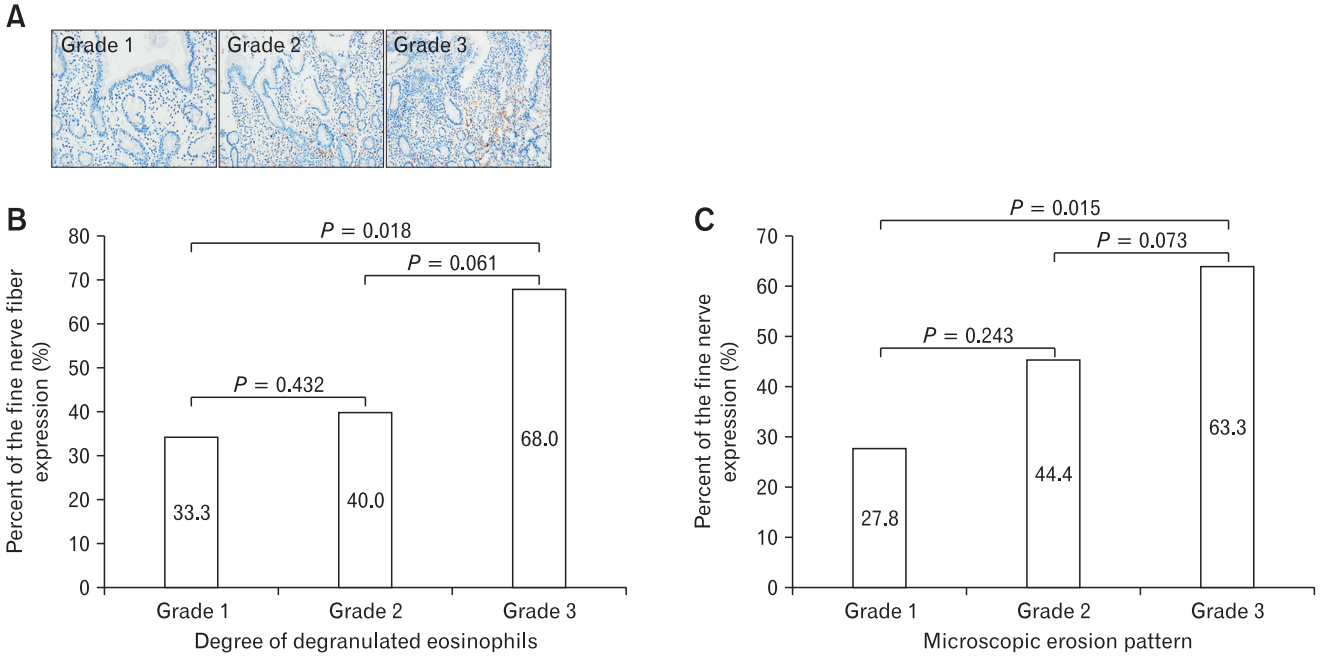

Figure 2. Fine nerve fiber abundance in the duodenal mucosa (S-100 staining, ×200) (A). Increased abundance of fine nerve fibers was highly correlated with the degree of activated eosinophils (B) and microscopic erosion (C).

dance in > 50% of the mucosa.

Electron microscopy to identify activated eosinophils

We examined the characteristics of degranulated eosinophils using EM. Two pieces of extracted tissue were pre-immobilized for 2 hours on 2.5% glutaraldehyde adjusted with 0.1 M phosphate buffer solution (pH 7.4) and then washed with the same buffer solution 3 times for 30 minutes each. After 1 hour, the tissue was

immobilized on 1% osmium tetroxide (0.1 M phosphate buffer solution, pH 7.4). The post-mobilized sample was washed with the same buffer solution, dehydrated in ethanol, which was substituted with propylene oxide twice for 15 minutes each, and embedded with Epon812. The embedded sample was polymerized and cut cross-sectionally at a 1 μm thickness using an ultramicrotome (Reichert Technologies, Buffalo, NY, USA), and the slice was stained with 1% toluidine blue. The regions infiltrated with eosinophils were identified by optical microscopy, and 60-70 nm ultrathin sections were prepared and double stained. The sections were examined by EM using the H-7650 Hitachi microscope (Tokyo, Japan). Figure 3 shows an electron micrograph in which the core matrix density of the secondary eosinophil granules changed and became transparent.

Statistical Methods

The degree of eosinophil infiltration as a continuous variable was compared between the FD and control groups using the Student's *t* test. Associations were identified by calculating odd ratios with 95% CIs, and significance was determined by the chi-square test. *P*-values < 0.05 were considered significant.

Results

Patient Characteristics

A total of 86 subjects were included (51 patients in the FD group and 35 in the control group). Approximately 82% of the subjects were female in the 2 groups. No differences were detected in body mass index, smoking habits, alcohol use, prevalence of diabetes mellitus, hypertension, or chronic viral hepatitis, or medication history between the 2 groups. However, the mean age of the FD group was significantly lower than that of the controls (35.8 ± 13.4 years vs 44.8 ± 8.0 years, $P = 0.001$) (Table). Five patients with FD were presumed to have post-infectious FD, and 11 presented with overlapping IBS (6 patients with diarrhea-predominant IBS, 3 with constipation-predominant IBS, and 2 with mixed IBS).

The mean peripheral eosinophil counts were not different between the groups (193.4 ± 27.4/mm^3 in FD group vs 184.8 ± 36.5 /mm^3 in controls, $P = 0.847$). The presence of *H. pylori* infection was detected in 39.2% of the patients with FD and 37.1% of the controls ($P = 0.842$).

Eosinophilic Infiltration and Its Activation in Duodenal Mucosa of Functional Dyspepsia Patients

The total eosinophil count in the duodenum in the FD group was significantly higher than that in the controls (42.1 ± 27.7/HPF vs 26.4 ± 23.0/HPF, $P = 0.016$) (Fig. 1A). However, no difference in the total eosinophil count was detected in the stomach between the 2 groups (24.3 ± 23.0/HPF vs 18.7 ± 17.3/HPF, $P = 0.232$).

The degree of activated eosinophils in the duodenum was divided into 4 grades (Fig. 1B). The highest grade of activated eosinophils (grade 3) was detected in 41.2% of the FD group, and the lowest grade (grade 0, no activated eosinophils) was detected in 9.7%; however, grade 3 was detected in 17.1% and grade 0 in 28.6% of the controls. Activated eosinophils were significantly more

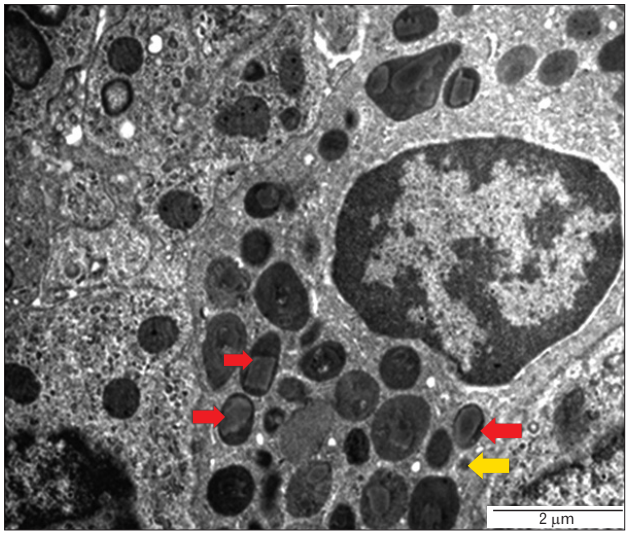

Figure 3. Electron microscopy of eosinophils at the site of inflammation in patients with functional dyspepsia. The loss of granule cores in the absence of granule exocytosis are suggestive of activated eosinophils. Scale bar = 2 μm.

Table. Baseline Characteristics of the Study Participants

Variables	Functional dyspepsia group (n = 51)	Control group (n = 35)
Age (yr)	35.8 ± 13.4[a]	44.8 ± 8.0
Female	42 (82.4)	29 (82.9)
Comorbidities		
Diabetes mellitus	1 (2.0)	1 (2.9)
Hypertension	1 (2.0)	1 (2.0)
Hematocrit (%)	37.9 ± 4.9	30.5 ± 6.5
Eosinophils (10^3/L)	193.4 ± 27.4	184.8 ± 36.5
Helicobacter pylori	20 (39.2)	13 (37.1)

[a]$P = 0.001$.
Data are presented as mean ± SD or n (%).

frequent in the FD group compared with the controls ($P = 0.005$). The difference in the grade of eosinophil activation in the stomach was not significant between the 2 groups ($P = 0.493$). EM was performed in patients with FD to detect activated eosinophils. Eosinophils at inflammatory sites demonstrated loss of granule cores in the absence of granule exocytosis, suggesting that eosinophilic granular proteins were released through piecemeal degranulation (Fig. 3).

Intraepithelial infiltration of eosinophils in the duodenum was observed in 11.4% of the controls and 25.5% of the patients with FD ($P = 0.172$). No difference in intraepithelial eosinophil infiltration was detected in the stomach between the 2 groups ($P = 0.729$).

Total eosinophil counts in the duodenum were 33.5 ± 24.6/HPF in the *H. pylori*-negative group and 39.3 ± 37.0/HPF in the *H. pylori*-positive group ($P = 0.033$); In the *H. pylori*-negative group, total duodenal eosinophil counts in FD were significantly higher compared to controls, however, no difference in eosinophil counts were detected between the FD and control groups in the *H. pylori*-positive group (Fig. 4). No difference in eosinophil infiltration was observed in the stomach according to the presence of *H. pylori* infection ($P = 0.465$). Mast cell counts in the duodenum were 57.7 ± 24.5/HPF in the FD group and 48.1 ± 22.1/HPF in the control group ($P = 0.613$). Those in the stomach were 33.4 ± 12.9/HPF in the FD group and 27.9 ± 13.7/HPF in the control group ($P = 0.874$).

Low-grade Inflammation Associates With Nerve Fibers in the Duodenum of Functional Dyspepsia Patients

The microscopic degree of low-grade inflammation in the duodenal mucosa was divided into 3 grades. Grade 3 inflammation, indicating microscopic erosions with activated eosinophil infiltration, was significantly more frequent in the FD group compared with the controls (49.0% vs 22.8%, $P = 0.001$) (Fig. 1A). Only 23.5% of patients in the FD group had a normal duodenal mucosa, whereas 62.9% of the controls had a normal duodenal mucosa ($P = 0.001$).

Fine nerve fibers were detected more frequently in the duodenum of the FD group than the controls (45.1% vs 11.4%, $P = 0.031$). The degrees of eosinophil activation and microscopic low-grade inflammation were significantly correlated with the grade of fine nerve fiber sprouting ($P = 0.018$ and $P = 0.015$, respectively).

Different Pattern of Duodenal Microscopic Findings According to the Subtype of Functional Dyspepsia

FD was divided into the EPS (n = 15) and PDS subtypes (n = 11). We analyzed the data according to the presence of EPS or PDS because of overlap of EPS with PDS. Microscopic low-grade inflammation in the duodenum of patients with EPS was significantly more frequent compared with that in the controls (86.7% vs 37.1%, $P = 0.001$). The rates of the highest grades of eosinophil activation (60.6% vs 17.1%, $P = 0.002$) and fine nerve fiber abundance (66.7% vs 25.0%, $P = 0.020$) were significantly different between the EPS group and non-EPS group. However, no differences were found in microscopic low-grade inflammation ($P = 0.122$),

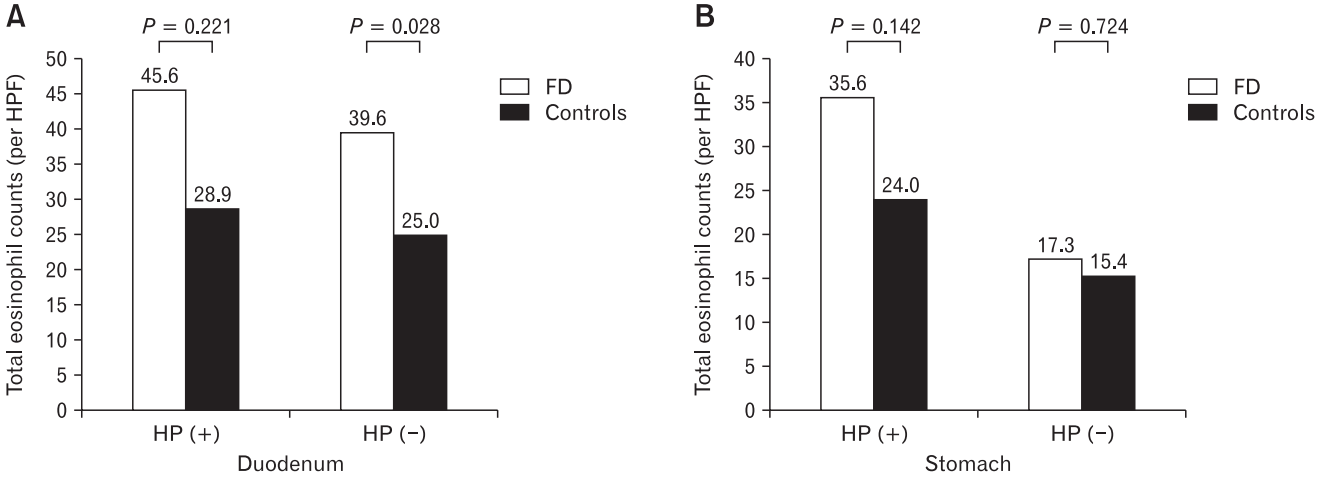

Figure 4. Comparison of total eosinophils count between functional dyspepsia patients (FD) and controls according to the presence of *Helicobacter pylori* (HP) infection. (A) Duodenum. (B) Stomach. HPF, high power field.

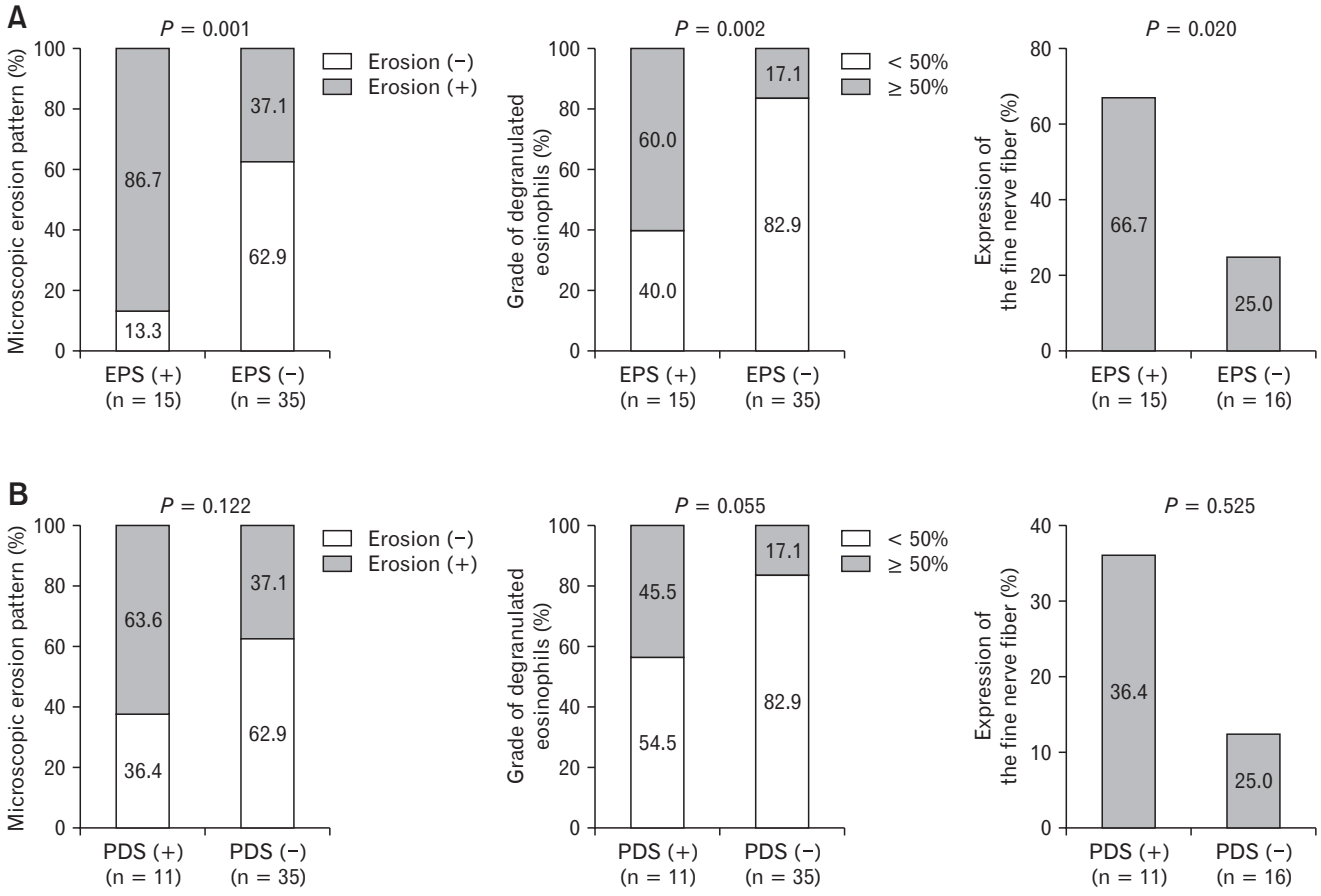

Figure 5. Microscopic findings according to the subtype of functional dyspepsia. (A) Microscopic erosions and degranulated eosinophils were more frequent in patients with epigastric pain syndrome (EPS). (B) Compared with controls; however, these features were not detected in patients with post-prandial distress syndrome (PDS).

the degree of activated eosinophils ($P = 0.055$), or fine nerve fiber abundance ($P = 0.525$) between patients with PDS and non-PDS group (Fig. 5). In EPS group, the grade of the fine nerve fiber expression well correlated with the degree of microscopic duodenitis ($r = 0.569$, $P = 0.027$), but not with degranulation of duodenal eosinophils ($r = 0.518$, $P = 0.061$). In PDS, the degree of S-100 staining well correlated with microscopic duodenitis and activation of eosinophils ($r = 0.421$, $P = 0.012$; $r = 0.354$, $P = 0.045$).

Discussion

This study demonstrated a significant association of duodenal eosinophils and microscopic low-grade inflammation in patients with FD. Moreover, fine nerve fibers in the duodenum were significantly associated with eosinophil activation and degree of low-grade inflammation. In particular, fine nerve fiber abundance correlated with duodenal eosinophil infiltration in patients with EPS rather than PDS.

The pathophysiology of FD is not completely understood and is considered multifactorial. Since the description by Toukan et al[24] of infiltration of the duodenum by eosinophils in patients with FD, several studies have demonstrated significant infiltration of eosinophils into the upper GI tract in patients with FD.[16,26-29] In a Swedish population-based study, the degree of infiltration of eosinophils and mast cells into the duodenum was sampled randomly in 51 patients with FD and 41 patients with IBS. The results suggested that the number of eosinophils, but not mast cells, is increased significantly in the duodenum of patients with FD, and that the number of mast cells are increased significantly in patients with IBS.[16] These findings are consistent with ours and support the hypothesis that infiltration of mast cells and eosinophils accounts for the different symptom patterns in the upper versus lower GI tract.

Activated eosinophils in patients with FD can be identified by immunohistochemical staining or EM. The peak number of acti-

vated duodenal mucosal eosinophils confirmed by EM was related to the generation of dyspeptic symptoms in *H. pylori*-negative children with FD.[17] No universally accepted criterion or method to diagnose eosinophil-related GI disease has been proposed; therefore, a direct comparison of our study with other studies is difficult. Less than 9 eosinophils/HPF are detected in the lamina propria, and generally, less than 5/HPF or no intraepithelial eosinophils are found.[30] We counted the number of eosinophils in hot spots of 5 HPF in the specimens to determine whether eosinophils were activated, and we found moderate to severe eosinophil degranulation in 68.7% of FD cases and 45.7% of controls, but no eosinophilic aggregation or microabscesses, which were frequently detected in eosinophilic enterocolitis, were found in the FD specimens. Unlike overt ulcer or erosion, microscopic duodenitis did not show the acute phase findings and showed the appearance of low-grade chronic inflammation, and is readily missed if the pathologist is not watching with interest. In the present study, microscopic duodenitis is an independent nature from gastritis in *H. pylori*-infected patients and it was detected in 76.5% in FD and 37.1% in controls. Two previous studies also reported similar findings.[27,31] They divided microscopic duodenitis among the FD patients into semiquantitative 4 grades, and microscopic duodenitis was detected in 65.5% of FD patients, but no controls in these studies. Taken together, microscopic duodenitis and eosinophil infiltration observed in FD patients are presumed to represent overlap expression by one-third of controls because these inflammatory changes might be very subtle.

A previous study reported similar findings, in that microscopic duodenitis was detected in 66.3% of FD specimens and was related mainly to *H. pylori* infection.[32] In our study, the eosinophils infiltration was significantly higher in the *H. pylori* positive group compared to the *H. pylori* negative group, but not different between the FD and control groups. In the *H. pylori* negative group, microscopic duodenitis was significantly higher in FD compared to controls.

In the present study, five patients with FD could have been categorized as post-infectious FD, defined by self-reported symptom onset after acute gastroenteritis. No differences were found among any of the microscopic findings or potential post-infectious FD cases (unpublished data); however, the possibility of a type II error cannot be ruled out completely. Previous studies have demonstrated that an acute gut infection induces the secretion of several chemokines and mediators, such as histamine and monocyte chemoattractant protein-1, which could induce the accumulation of eosinophils and C-C chemokine receptor type 2 (CCR2)-positive (activated) macrophages in the duodenum of patients with post-infectious FD.[33] CCR2 macrophages were significantly activated in patients with epigastric burning. Prostaglandins released from macrophages during periods of inflammation are thought to have a direct sensitizing effect on afferent sensory nerve terminals and may produce dyspeptic symptoms. Intriguingly, we demonstrated the presence of sprouting small nerve fibers in the duodenal mucosa of patients with FD compared with that in controls. These nerve fibers were highly abundant and extended into the upper one-half of the duodenal mucosa in cases with microscopic duodenitis, which was not typically found in the control group. These finding are significantly highly expressed in EPS compared with PDS. In another cohort, the duodenal eosinophilia was related with early satiety/fullness, not epigastric pain,[27,34] however, other recent studies showed no significant differences between EPS and PDS.[29,35] In the latter, a significant amount of eosinophils and mast cells infiltrated the submucosal layer combined with altered submucosal ganglionic architecture and accompanied by decreased calcium responses to depolarization in FD.[35] The contribution of the submucosal nerve plexus to normal gastric motility is probably minimal because gastric submucosa contains few intrinsic primary afferent neurons and the intrinsic reflexes are poorly developed.[36] However, mucosal nerves in the small intestine are mainly sensory terminals of the intrinsic primary afferent neuron.[37] In patients with IBS, several studies have demonstrated increased nerve fiber density and sprouting in mucosal tissues of patients. Mucosal extracts obtained from the colon of patients with IBS and transferred to animal or human tissues increased intestinal neuron excitability, mesenteric sensory nerve activity, and visceral or somatic sensitivity.[38-41] Although the effects of these changes on the pathogenesis of FD remain unknown, they could be implicated in the development of visceral hypersensitivity.[42] These structural alterations change neuronal electric activity, leading to impaired motility or secretion and reduced sensory thresholds, resulting in visceral hypersensitivity and pain. Future studies that compare mucosal findings to functional motility and sensory studies will elucidate the role of these mucosal nerve fibers in FD.

There were limitations to this research. Women accounted for 82% and the control group was older than the FD group. The basic characteristics of the controls and FD were not homogeneous because of the difficulty of recruiting controls who had no GI symptoms and had to have no significant endoscopic abnormality. However, there is no clear evidence that the level of GI eosinophils infiltration in adults is changed in age or by sex.[30] Second, the primary endpoint of this study was the difference between FD and controls, of which the sample size was small for subgroup analysis

according to EPS or PDS. For this analysis, further studies including more subjects are required.

In conclusion, infiltration and activation of duodenal mucosal eosinophils increased significantly in patients with FD and related to microscopic duodenitis and mucosal fine nerve sprouting. Our results suggest that low-grade inflammatory changes may induce long-lasting neuroplastic changes in patients with FD, particularly during generation of pain-related symptoms.

Financial support: This research was conducted with Ewha Womans University research funds (Ewha 1-2008-2920-001-1).

Conflicts of interest: None.

Author contributions: Hye-Kyung Jung, Ko Eun Lee, Yeung-Chul Mun, and Sanghui Park acquired data; Min Jin Lee, Hye-Kyung Jung, and Ko Eun Lee analyzed and interpreted data; Min Jin Lee and Hye-Kyung Jung prepared figures, drafted, and revised the manuscript; Hye-Kyung Jung and Sanghui Park designed and supervised the study; and all authors read and approved the final manuscript.

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