Department of Otolaryngology in the general hospital, where she was evaluated using a laryngoscope and she also underwent a cervical esophagography. The laryngoscope showed no abnormal findings but the cervical esophagography showed a transient esophageal narrowing in the middle to lower esophagus (Fig. 1). The otolaryngologist thought that the esophageal motor abnormality was the cause of this esophageal narrowing and the patient was referred to our hospital. At this time, she was unable to eat a solid meal because of having severe dysphagia.

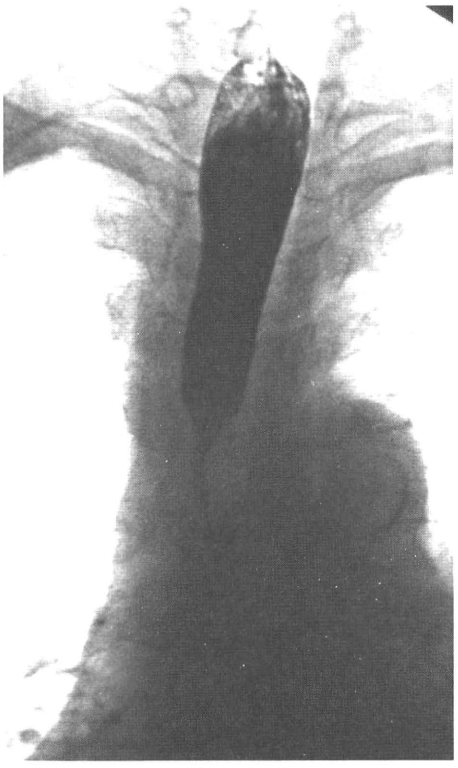

Fig. 1 Barium cervical esophagogram showing narrowing of the esophageal lumen in the middle to lower esophagus

The patient's medical history included diabetes mellitus, which she has had since the age of 50 and which has been treated with insulin [Inolet 30R (12–12–12 unit)] and bronchial asthma, which she has had since childhood, but which was stable from taking theophylline 200 mg (p.o.) and budesonide air inhalation twice per day. The patient did not suffer from any other allergic disease. With regard to the physical examination, mild edema of the lower limbs was observed but there were no other abnormal findings.

Laboratory examinations on the first day of admission showed that peripheral eosinophilia (1279/μl), HbA1C (7.1%), and glucose (175 mg/dl) during a fasting period were high. The immunoglobulin E (IgE) level was normal and 57 types of the antigen-specific IgE and parasitic worm eggs were examined, all of which were negative.

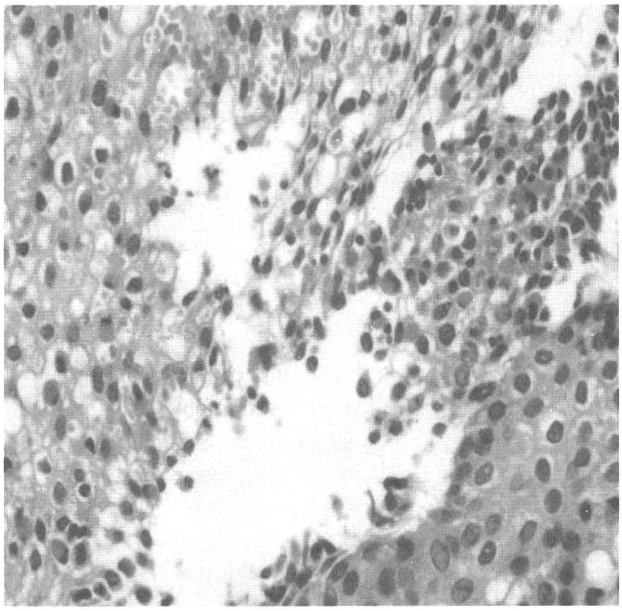

Fig. 3 Histologic findings from the esophageal biopsy. Pathologic features of the esophagus showing >25 eosinophils in the esophageal mucosa (H&E, ×400)

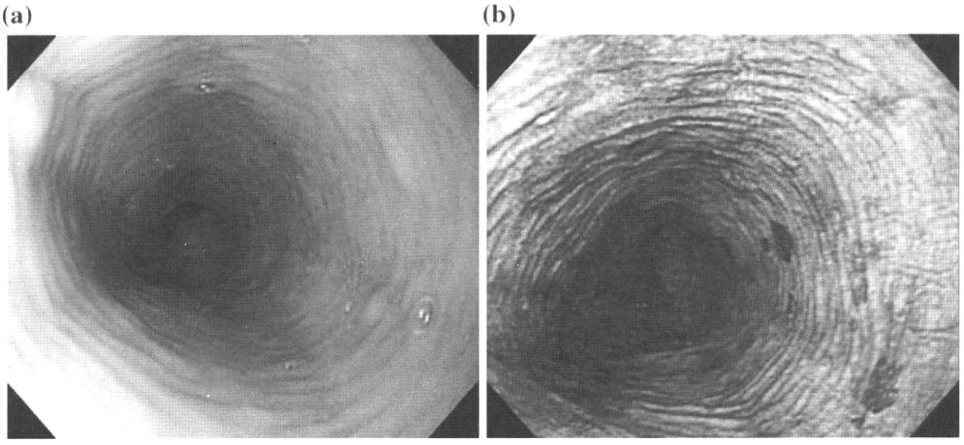

Fig. 2 Esophageal endoscopic findings. **a** Conventional endoscopic finding, **b** dye esophageal endoscopic finding by iodine staining. Circular rings and edematous esophageal mucosa were observed

An esophagogastroduodenoscopy (EGD) was carried out the day after admission and many circular rings appeared immediately after the endoscope was inserted into the esophagus (Fig. 2a). These circular rings disappeared quickly but the esophageal mucosa was edematous. Except for the circular rings and the edematous esophageal mucosa, there were no abnormal findings in the esophagus, stomach or duodenum. Figure 2b shows esophageal endoscopic findings using iodine staining dye where, after spraying with iodine, many circular rings immediately appeared but then quickly disappeared. From these circular rings, EE was suspected and a biopsy was then taken from the middle esophagus. Histologic findings from the esophageal biopsy showed that >25 eosinophils existed per high-power field (HPF) (Fig. 3).

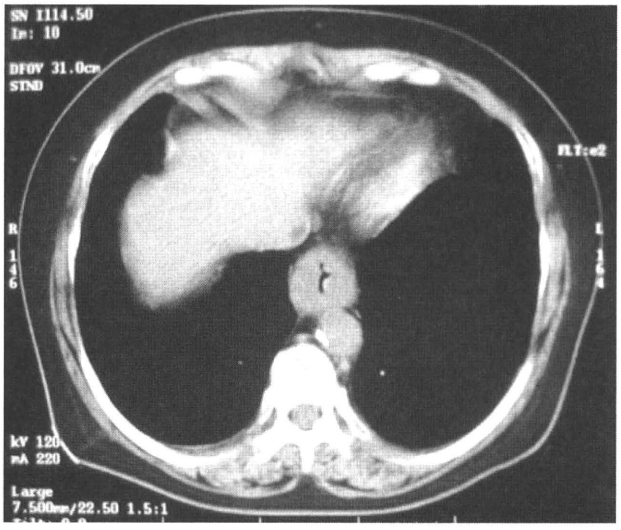

Fig. 4 CT scan of the chest demonstrating the thickened wall of the esophagus

Significant thickening of the esophageal wall was observed on the computed tomography (CT) scan of the chest 3 days after admission (Fig. 4). Except for this, no abnormal findings were observed on the CT scan of the chest or abdomen. Figure 5 shows esophageal contractions after water swallowing (5 days after admission and prior to treatment), where ineffective esophageal peristalsis was observed.

Three elements need to be present for a diagnosis of EE: (a) clinical symptoms of esophageal dysfunction, (b) >15 eosinophils in 1 high-power field, and (c) lack of responsiveness to high-dose PPI or normal pH monitoring of the distal esophagus. All 3 elements were present in this case, therefore the patient was diagnosed with EE.

After the diagnosis of EE, oral corticosteroid (prednisolone 30 mg per day) was administered to the patient and after 3 days of treatment her symptoms had almost disappeared. After 7 days, the symptoms had completely disappeared and the prednisolone was then gradually tapered off. She now takes 2.5 mg prednisolone per day and there has been no recurrence of the symptoms. Figure 6 shows the clinical course of the symptoms, eosinophil and prednisolone treatment.

An esophagogastroduodenoscopy was carried out 4 weeks after the prednisolone treatment. The circular rings had disappeared and it was possible to see the vessels in the esophageal mucosa (Fig. 7). The histologic findings from the esophageal biopsy 4 weeks after the prednisolone treatment, showed only a few eosinophils in the biopsy specimen (Fig. 8).

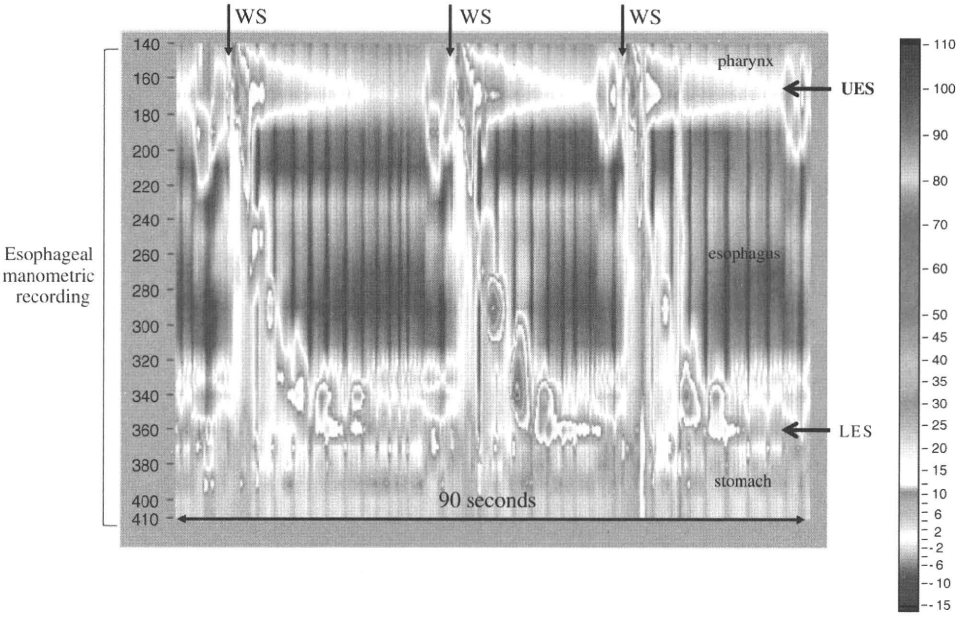

Fig. 5 High-resolution 21-channel perfused manometric recordings in a patient with eosinophilic esophagitis. Time is on the *x*-axis and distance from nares is on the *y*-axis. A computer program was used to code and record pressures over time for each channel, as outlined in vertical color coding on the *right-hand side* of the figure. Anatomical landmarks and motor events are labeled on the figure. Multiple ineffective esophageal contractions after swallowing 5 ml water can be seen. *LES* lower esophageal sphincter, *UES* upper esophageal sphincter, *WS* water swallowing

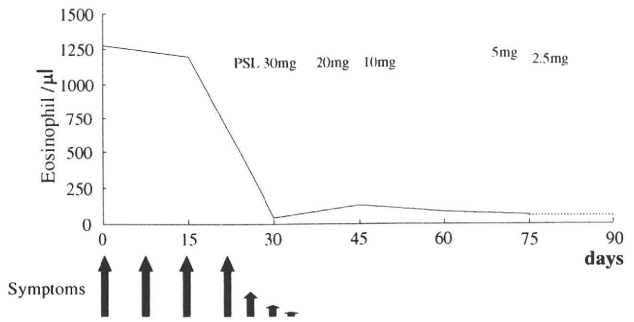

Fig. 6 Clinical course of symptoms, eosinophils and prednisolone treatment

Discussion

EE is a chronic inflammatory disease characterized by an elevated count of eosinophils in the esophagus. Although EE had not been previously been thought to affect adults, in recent times, adult cases have been reported [7]. In fact, there has been a dramatic increase in the diagnosis of EE in Western countries over the last few years, due to both an increased awareness of this disorder as well as an actual increase in prevalence [8, 9]. In Japan, however, to date there are only a few reports [4–6].

The first reported case of EE was by Landres and colleagues in 1978 [10]. Many patients with EE have asthma or other atopic conditions and, in addition, 5–58% of patients with EE are reported to show eosinophilia [11–14]. In 40–73% of patients with EE, increased serum IgE levels, a positive RAST result and a positive skin prick test result have also been reported [11, 12, 14, 15]. Our patient showed increased blood eosinophils but the serum IgE level was normal and she had a history of bronchial asthma. The allergen that causes EE is not known but the antigens which trigger EE may include various ingested food allergens as well as inhaled aeroallergens. It is thought that eosinophils cause mucosal damage through the release of cytotoxic granule proteins, reactive oxygen intermediates and lipid mediators [8]. Cytokines and chemokines such as interleukin (IL)-5 and eotaxin, play an important role in the migration and accumulation of eosinophils and T cells and mast cells may also be involved in the inflammatory response [8, 16].

It has been reported that the predominant symptoms of EE in adults are dysphagia and food impaction, and severe dysphagia and heartburn were also the predominant symptoms in our case. Management of these symptoms in patients with EE is difficult as EE is persistently resistant to PPI therapy. A diagnosis of EE is made more difficult because the symptoms of GERD and EE are often similar, and in the past, patients with EE have been incorrectly diagnosed as suffering from GERD.

EE is a primary esophageal disorder in which there are at least 15 eosinophils per HPF on microscopy [17], without associated eosinophilic infiltration of the stomach or intestine. The essential examination, therefore, in order to correctly diagnose EE, is an upper endoscopy with biopsies. One biopsy has a sensitivity of 50%, while five biopsies increase the sensitivity to 100% [18]. GERD patients may also show an elevated eosinophil count, but this elevation is much lower than that of patients with EE. The eosinophilic changes in GERD are limited to the distal esophagus only, where the density is <5–10 eosinophils per HPF. In the diagnostic guidelines of EE, three criteria need to be present for a diagnosis of EE: (a) clinical symptoms of esophageal dysfunction, (b) >15 eosinophils in 1 HPF, and (c) lack of responsiveness to high-dose PPI or normal pH monitoring of the distal esophagus [17]. Histologic findings from the esophageal biopsy in our case showed that >25 eosinophils existed per HPF. As all 3 criteria were present in our case a diagnosis of EE was made.

An esophagogastroduodenoscopy has been reported to reveal abnormalities in the majority of EE patients, such as absent vascular markings, ridges, furrows, vertical lines, corrugations, rings and adherent whitish plaques [7, 19, 20]. In our case, many circular rings appeared immediately after the endoscope was inserted into the esophagus, but they disappeared quickly. Also in cases with EE, the esophageal mucosa has been reported as being fragile and mucosal tears have been observed in the affected esophagus, which have been reported as occurring spontaneously during endoscopic observation or having been induced by minor trauma during biopsy procedures. In our case, the esophageal mucosa was edematous, but esophageal mucosa fragility or mucosal tears were not clearly evident.

In cases with EE, esophageal motility disturbance has been reported by several investigators and many studies have demonstrated a high degree of ineffective esophageal peristalsis [21]. In our case, ineffective esophageal peristalsis was similarly observed using high resolution manometry. The possibility of EE should therefore be considered when diagnosing patients with abnormal esophageal motor function.

Treatment strategies for EE should include dietary modification and medical therapy [22]. The initial approach to the disorder is often an allergy evaluation in order to identify the allergens which may be present in the diet or in the environment [23]. In our case, however, 57 types of the antigen-specific IgE and parasitic worm eggs were examined, all of which were negative. Systematic and topical corticosteroids effectively resolve acute clinicopathologic features of EE but in our case, topical corticosteroid had already been administered as therapy for bronchial asthma. This therapy was effective for bronchial

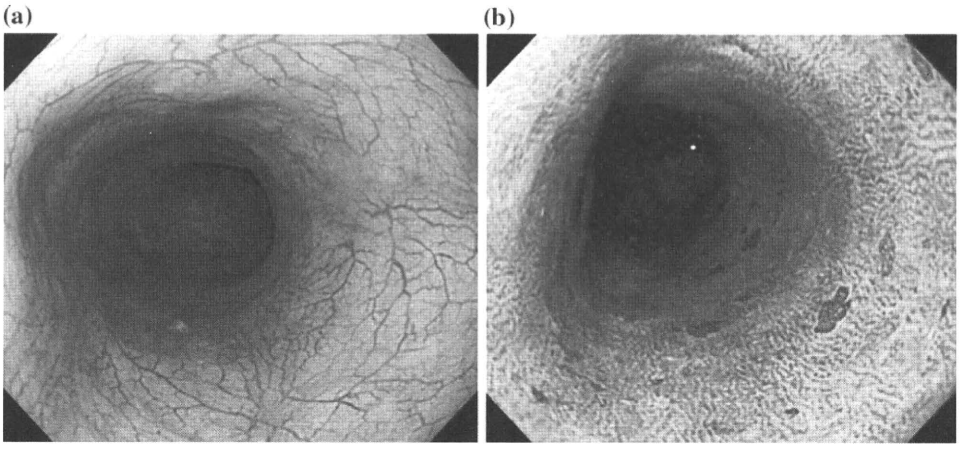

Fig. 7 Esophageal endoscopic findings 4 weeks after prednisolone treatment. **a** Conventional endoscopic findings, **b** dye endoscopic findings by iodine staining. Circular rings disappeared and it was possible to see the vessels in the esophageal mucosa

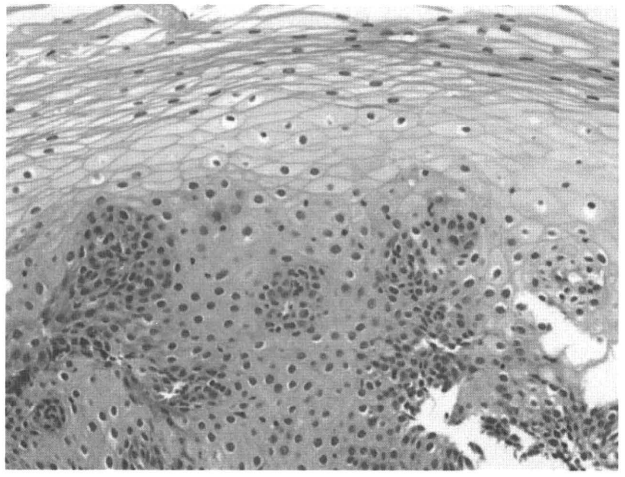

Fig. 8 Histologic findings 4 weeks after prednisolone treatment. Few eosinophils existed (H&E, ×400)

asthma but was not effective for EE. Our case was an emergent case because the patient had severe dysphagia and was not able to eat a solid meal at the time of visiting our hospital. In emergent cases, systematic corticosteroid therapy is recommended [17] and was therefore commenced. The patient started taking prednisolone 30 mg/day and after 3 days of treatment her symptoms had almost disappeared; after 7 days of treatment they had completely disappeared. We then began to gradually taper off the prednisolone. She is now taking 2.5 mg of prednisolone per day with no recurrence of the symptoms.

The prognosis for patients with EE is generally very good. Symptoms usually respond to treatment from corticosteroids, although the need for re-treatment is common. To date, no evidence for any malignant potential of this condition has been identified [12].

Thus we have presented a rare case of EE in Japan. There have been many cases of EE reported in Western countries, but because EE is still rare in Japan, and is a disorder which doctors seldom encounter, its diagnosis is often delayed, sometimes for years. If patients with NERD have dysphagia and heartburn but do not respond to high-dose PPI therapy, EE needs to be considered as a differential diagnosis.

References

1. Nielsen RG, Husby S. Eosinophilic oesophagitis: epidemiology, clinical aspects, and association to allergy. J Pediatr Gastroenterol Nutr. 2007;45:281–9.
2. Blanchard C, Rothenberg ME. Basic pathogenesis of eosinophilic esophagitis. Gastrointest Endosc Clin N Am. 2008;18:133–43.
3. Kelly JK, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA, et al. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. Gastroenterology. 1995;109:1503–12.
4. Furuta K, Adachi K, Kowari K, Mishima Y, Imaoka H, Kadota C, et al. A Japanese case of eosinophilic esophagitis. J Gastroenterol. 2006;41:706–10.
5. Kamimura K, Oosaki A, Sugahara S, Mori S, Morita T, Kimura K. Eosinophilic esophagitis: a case report. Effective treatment with systemic corticosteroids for the relapse of the disease. Clin J Gastroenterol. 2008;1:46–51.
6. Fujiwara H, Morita A, Kobayashi H, Hamano K, Fujiwara Y, Hirai K, et al. Infiltrating eosinophils and eotaxin: their association with idiopathic eosinophilic esophagitis. Ann Allergy Asthma Immunol. 2002;89:429–32.
7. Potter JW, Saeian K, Staff D, Massey BT, Komorowski RA, Shaker R, et al. Eosinophilic esophagitis in adults: an emerging problem with unique esophageal features. Gatrointest Endosc. 2004;59:355–61.
8. Arora AS, Yamazaki K. Eosinophilic esophagitis: asthma of the esophagus? Clin Gastroenterol Hepatol. 2004;2:523–30.
9. Straumann A, Beglinger C. Eosinophilic esophagitis: the endoscopist's enigma. Gastrointest Endosc. 2006;63:13–5.
10. Landres RT, Kuster GGR, Strum WB. Eosinophilic esophagitis in a patient with vigorous achalasia. Gastroenterology. 1978;74:1298–301.
11. Teitelbaum JE, Fox VL, Twarog FJ, Nurko S, Antonioli D, Gleich G, et al. Eosinophilic esophagitis in children: immunopathological analysis and response to fluticasone propionate. Gastroenterology. 2002;122:1216–25.

12. Straumann A, Spichtin HP, Grize L, Bucher KA, Beglinger C, Simon HU. Natural history of primary eosinophilic esophagitis: a follow up of 30 adult patients for up to 11.5 years. Gastroenterology. 2003;125:1660–9.
13. Arora AS, Perrault J, Smyrk TC. Topical corticosteroid treatment of dysphagia due to eosinophilic esophagitis in adults. Mayo Clin Proc. 2003;78:830–5.
14. Croese J, Fairley SK, Massom JW, Chong AK, Whitaker DA, Kanowski PA, et al. Clinical and endoscopic features of eosinophilic esophagitis in adults. Gastrointest Endosc. 2003;58:516–22.
15. Spergel JM, Beausoleil JL, Mascarenhas M, Liacouras CA. The use of skin prick tests and patch tests to identify causative foods in eosinophilic esophagitis. J Allergy Clin Immunol. 2002; 109:363–8.
16. Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, et al. Eotaxin-3 and a uniquely conserved gene expression profile in eosinophilic esophagitis. J Clin Invest. 2006;116:536–47.
17. Furuta GT, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, et al. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology. 2007;133:1342–63.
18. Gonsalves N, Policarpio-Nicolas M, Zhang Q, Rao MS, Hirano I. Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. Gastroint Endosc. 2006;64:313–9.
19. Zimmerman SL, Levine MS, Rubesin SE, Mitre MC, Furth EE, Laufer I, et al. Idiopathic eosinophilic esophagitis in adults: the ringed esophagus. Radiology. 2005;236:159–65.
20. Remedios M, Campbell C, Jones DM, Kerlin P. Eosinophilic esophagitis in adults: clinical, endoscopic, histologic findings, and response to treatment with fluticasone propionate. Gastrointest Endosc. 2006;63:3–12.
21. Martin LM, Vaquero CS, Prudencio SS, Perona JC, Gisbert JP, Otero RM. Eosinophilic esophagitis in the adult-clinical, endoscopic, pH-metric, and manometric findings. Rev Esp Enferm Dig. 2008;100:476–80.
22. Gupte AR, Draganov PV. Eosinophilic esophagitis. World J Gastroenterol. 2009;15:17–24.
23. Kagalwalla AF, Sentongo TA, Ritz S, Hess T, Nelson SP, Emerick KM, et al. Effect of six-food elimination diet on clinical and histologic outcomes in eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2006;4:1097–102.

Migration of Eosinophils and CCR2-/CD68-Double Positive Cells Into the Duodenal Mucosa of Patients With Postinfectious Functional Dyspepsia

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OBJECTIVES: **Recent studies have shown that postinfectious functional dyspepsia (FD) symptoms may persist after elimination of gastrointestinal (GI) infection as well as postinfectious irritable bowel syndrome accompanying colonic inflammation. However, it is unclear whether intestinal chronic inflammation can contribute to clinical symptoms of certain FD patients such as postinfectious FD. To determine the relationship between local inflammation of the duodenum and clinical symptoms, we evaluated the infiltration of several phenotypes of duodenal inflammatory cells as well as gastric motility using ^{13}C urea breath test in postinfectious FD patients.**

METHODS: **We enrolled 136 consecutive patients diagnosed with FD according to Rome III criteria, and 20 healthy controls, after upper GI endoscopy. Gastric motility was evaluated by gastric emptying time (T-max) using the ^{13}C-acetate breath test. Upper abdominal symptoms including epigastric pain, epigastric burning, postprandial fullness, abdominal distension, and early satiety were assessed by questionnaire scores. We obtained biopsy specimens from the stomach and duodenum during upper GI endoscopy. Histological gastritis and duodenitis were assessed as mild, moderate, or severe according to previously described criteria. Characteristics of inflammatory cells and neuroendocrine cells were determined immunohistochemically with antibodies to CD3, CD68, CCR2, Vdelta1 TCR, and serotonin.**

RESULTS: **Endoscopic duodenitis was observed in only 5.7% of postinfectious FD patients. However, the rates of histological duodenitis in duodenal biopsies of postinfectious FD patients were 17% for mild, 26% for moderate, and 57% for severe grades of duodenitis. The degree of histological duodenitis of postinfectious FD patients was significantly greater than that of healthy volunteers. There was a significant correlation between epigastric burning and the degree of duodenitis in postinfectious FD patients. There was no significant difference in histological duodenitis and T-max value in the postinfectious FD patients with or without *Helicobacter pylori* infection. In addition, CD68-positive cell number in postinfectious FD patients was significantly increased compared with the numbers in subjects with epigastric pain syndrome or postprandial distress syndrome and in healthy volunteers. CCR2-/CD68-double positive cell number in postinfectious FD patients was significantly (P=0.009) increased compared with those in healthy volunteers.**

CONCLUSIONS: **Migration of inflammatory cells, in particular, duodenal CCR2-positive macrophages, may have an important function in the pathophysiology of postinfectious FD patients.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg.

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INTRODUCTION

Functional dyspepsia (FD) is divided into two subgroups according to Rome III criteria: epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS) (1). Most FD patients complain of various symptoms related to the intake of meals; however, the pathophysiology of FD remains poorly defined (2,3).

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A number of potentially important abnormalities have been reported in FD patients, including impaired fundic accommodation (4), gastric hypersensitivity to distention (5), abnormal duodenojejunal motility (6,7), duodenal motor and sensory dysfunction (8), duodenal hypersensitivity (9), and *Helicobacter pylori* infection (10). In addition, it has been reported that acid infusion into the duodenal bulb induces dyspepsia in healthy volunteers and more readily induces symptoms in FD patients than in healthy subjects. Increased exposure of the duodenum to acid has a role in the genesis of dyspeptic symptoms in patients with FD with prominent nausea (11). Recent studies have reported mucosal inflammation in the duodenum such as eosinophilia in FD (12,13). Recent advances in the understanding of functional gastrointestinal (GI) disorders suggest that there may be a link to localized derangements in the GI–neural axis, including motor and sensory abnormalities, enhanced sensitivity to luminal contents (6,11), and neuro-immuno dysregulation (14), especially accompanying mucosal inflammation in postinfectious irritable bowel syndrome (IBS) (15). Previous studies have reported accumulation of mucosal neuroendocrine cells and inflammatory cells including T cells in mucosal biopsy specimens from patients with postinfectious IBS (16–18). As symptoms of IBS and FD often overlap with time, we speculated that immune dysfunction might also be involved in the onset of postinfectious FD in adults. However, there are no data currently available concerning sensory abnormalities and mucosal inflammation such as gastritis and duodenitis in postinfectious FD patients defined by Rome III criteria.

Recent studies have shown that postinfectious FD symptoms may persist after elimination of GI infection as well as postinfectious IBS accompanying colonic inflammation (19,20). Immune activation with the release of several mediators, including cytokines, nitric oxide, and histamine, which link with the function of enteric nerves, has been observed in the intestinal mucosa of IBS patients (21). Moreover, immune-mediated activation of mastocytes and macrophages in contact with pain-sensitive endings seems to be involved in the development of hypersensitivity in the experimental models of visceral pain (22). However, it is unclear whether chronic inflammation of gastrointestinal tract can contribute to clinical symptoms of certain FD patients, such as postinfectious FD through upregulation of such sensitizing mechanisms.

To determine the relationship between mucosal inflammation of the gastric and duodenal mucosa and clinical symptoms in postinfectious FD patients, we analyzed infiltration of several phenotypes of duodenal inflammatory cells as well as neuroendocrine cells and also gastric motility using ^{13}C urea breath test in postinfectious FD patients.

METHODS

Patients

Thirty-six patients with EPS and 65 patients with PDS, 35 patients with postinfectious FD patients (postinfectious EPS ($n = 17$) and postinfectious PDS ($n = 18$)), and 20 healthy volunteers were consecutively enrolled according to Rome III criteria (1) after undergoing upper GI endoscopy and abdominal ultrasonography. The criteria for exclusion were severe heart, renal, or pulmonary failure, liver cirrhosis, severe systemic illness, or malignant disease. Patients who had undergone gastroduodenal surgery or who took non-steroidal inflammatory drugs (NSAIDs) or anticoagulants were also excluded. The presence of IBS did not exclude the inclusion of any of these FD patients because coexisting IBS was found to have a minor impact on symptom pattern. Additionally, we asked whether the onset was immediately preceded by symptoms suggestive of an acute gastroenteritis (fever, diarrhea, vomiting, and nausea) identified by use of a questionnaire, when the patient's dyspepsia symptoms had previously been within normal limits. The result of positive stool culture or elevation of C-reactive protein was obtained from the medical records as were any previous reports of postinfectious IBS (23). Only those patients reporting an acute onset and responding to at least one symptom were considered as presumed postinfectious FD patients. *H. pylori* infection was determined by positivity of both urea breath test and histology. Patients had all undergone diagnostic upper GI endoscopy and abdominal ultrasonography for dyspeptic symptoms at the Nippon Medical School Digestive Endoscopic Center and gave their informed consent. The protocol was approved by the Ethical Review Committee of the Nippon Medical School Hospital.

Clinical symptoms

Clinical symptoms of FD were evaluated according to Rome III criteria (1) and must have included at least one of the followings: bothersome postprandial fullness; early satiation; epigastric pain; or epigastric burning. Diagnostic criteria for PDS included bothersome postprandial fullness occurring after ordinary-sized meals and/or early satiation that prevented finishing a regular meal, with either symptom occurring at least several times per week. Diagnostic criteria for EPS included all of the followings: pain or burning that is intermittent, localized to the epigastrium, and of at least moderate severity at least once per week. Diagnostic criteria for PDS and EPS were fulfilled with symptoms occurring for the past 3 months and the onset of symptoms occurring at least 6 months before diagnosis.

Histological examination

Biopsy specimens from the gastric antrum and corpus were fixed in formalin and treated with H&E staining to determine the severity of gastric inflammation. Briefly, the severity of *H. pylori*-infected gastritis was classified into three grades according to the degree of inflammatory cell and neutrophil infiltrations. Neutrophil and mononuclear cell infiltration were each given arbitrary values of 0–3 according to the updated Sydney System (24), representing infiltration levels ranging from none, mild, moderate, and severe, respectively.

In addition, we obtained biopsy specimens from the duodenum (duodenal bulb and second portion of duodenum) during upper GI endoscopy in 35 postinfectious FD (17 postinfectious EPS and 18 postinfectious PDS) patients, 12 EPS patients, and 15 PDS patients. For duodenal pathology, the architecture of villi, presence and grade of acute and chronic inflammation, gastric metaplasia,

pathogens, and presence of erosions were recorded. Histological duodenitis was assessed as mild, moderate, or severe by H&E staining according to criteria of previous studies (25). Mild duodenitis was defined as an expansion of the lamina propria by mild inflammatory cell infiltration. Moderate duodenitis was characterized by partial loss of villi and expansion of the lamina propria by moderate inflammatory cell infiltration. Severe duodenitis was characterized by partial loss of villi and expansion of the lamina propria by severe inflammatory cell infiltration, mainly plasma cells, macrophages, and lymphocytes. Mild to severe duodenal inflammatory cell infiltration was evaluated by the degree (0–3) of mononuclear cell infiltration. Severity of inflammatory cells were classified with 0 = normal, 1 = mild; diffuse superficial infiltration, 2 = moderate; extending into the middle of the mucosa, 3 = severe; transmucosal infiltration according to modified criteria of previous report (26). Histological duodenitis scores in D1 and D2 were combined and mean values of these sums were then calculated. Specimens were evaluated by two experienced pathologists in a blinded manner.

Immunohistochemistry

Biopsy specimens were taken from the duodenum and stomach. Endogenous peroxidase activity was blocked with 5% H_2O_2 in methanol. Characteristics of inflammatory cells in the duodenum were determined immunohistochemically with anti-CD3 (DAKO, Glostrup, Denmark), -CD68 (Santa Cruz Biotechnology, Santa Cruz, CA), -CCR2 (Abcam, Cambridge, UK), -Vdelta1 TCR (Thermo scientific, Waltham, MA), -serotonin antibodies (DAKO). After further washing, bound antibody was detected using the LSAB 2kit (DAKO) with diaminobenzidine as the chromogen. As negative controls, primary antibodies were replaced with isotype-matched immunoglobulin. CD3-, CD68-, CCR2-, Vdelta1 TCR-, and serotonin-producing-positive cell counts were determined in four fields with ×400 magnification. The numbers of positively staining serotonin-producing cells and Vdelta1 T cells per 100 epithelial cells were counted for four fields and the results averaged according to the modified criteria of previous studies (16). The CD3-, CCR2-, and CD68-positive cell counts were expressed per square millimeter. Four microscope fields per biopsy containing mucosal tissue were examined and the results averaged according to previous studies (16). CD3-, CD68-, CCR2-, Vdelta1 T cell, and serotonin-producing cell counts in D1 and D2 were combined and the sum values for each cell type were expressed as the mean for D1 and D2 combined.

Double-label immunofluorescence was used to evaluate the colocalization of immunoreactivities for the following pair of antihuman antibodies: CCR2 goat polyclonal (Abcam) and CD68 mouse monoclonal (Santa Cruz) antibodies. Sections were dewaxed and then microwaved in 0.01 mol/l citrate phosphate buffer (pH 6.0) for antigen retrieval. Sections were incubated overnight at 4°C with a mixture of their primary antibodies and their respective fluorescein isothiocyanatce (FITC)- or Texas red-conjugated secondary antibodies. Specimens were evaluated by two experienced pathologists in a blinded manner.

Measurement of gastric motility

Sodium acetate (water soluble) for emptying of liquids was used as tracer (Cambridge Isotope Laboratories, Cambridge, MA). Probes were analyzed by nondispersive infrared spectroscopy (IRIS; Wagner Analyzentechnik, Bremen, Germany). The subject's own production of 300 mmol CO_2/m^2 body surface and per hour was set as the default. Integrated software solutions caluculated half gastric emptying time ($T_{1/2}$) and the lag phase (T_{lag}) as the point of maximum gastric emptying according to Ghoos *et al.* (27).

Study protocol for gastric emptying of liquids

The liquid test meal consisted of 100 mg of ^{13}C-acetate dissolved in 200 ml of a liquid meal (Racol, 1 ml/1 kcal; Otsuka Pharmacia Company, Tokyo, Japan). Breath samples were collected 0 and 10 sec and 5, 10, 15, 20, 30, 40, 50, 60, 75, and 90 min after ingestion of the test meal at 10:00 AM. Patients were instructed not to drink, eat, or smoke during the test.

Statistical analysis

For statistical evaluation of group data, Students' *t*-test for paired data, and analysis of variance for multiple comparisons, and followed by Scheffe's *F*-test. Mann–Whitney *U*-test was used for analysis of categorical data. A P value of <0.05 was statistically significant.

RESULTS

Characteristics of EPS, PDS, and postinfectious FD patients

The body mass index scores of the various patients groups were not statistically different (**Supplementary Table 1** online). In addition, *H. pylori* positivity rates did not vary significantly among EPS, PDS, and postinfectious FD patients.

T-max value in EPS, PDS, and postinfectious FD patients

We then used the ^{13}C-acetate breath test to calculate the T-max value as a marker of gastric emptying of liquids, and compared gastric motility among EPS, PDS, postinfectious FD patients and healthy volunteers. The T-max value in PDS patients was significantly ($P=0.03$) higher than in healthy volunteers (**Figure 1**). In contrast, T-max values in postinfectious FD patients were similar to those in EPS patients and healthy volunteers (**Figure 1**).

Comparison of degree of gastritis and duodenitis in EPS, PDS, and postinfectious FD patients

To analyze whether there was a difference in duodenal and gastric inflammation among subjects with EPS, PDS, postinfectious EPS, and postinfectious PDS, as well as healthy volunteers, we analyzed the degree of gastritis and duodenitis in duodenal and gastric biopsy specimens. In postinfectious FD, endoscopic evidence of duodenitis with erosions was very rare. The degree of histological duodenitis of postinfectious FD patients was significantly ($P=0.02$) greater than that of healthy volunteers (**Figure 2a**). In contrast, there was no statistically significant difference in the degree of histological duodenitis among EPS, PDS, and healthy volunteers. The rates of histological duodenitis in duodenal

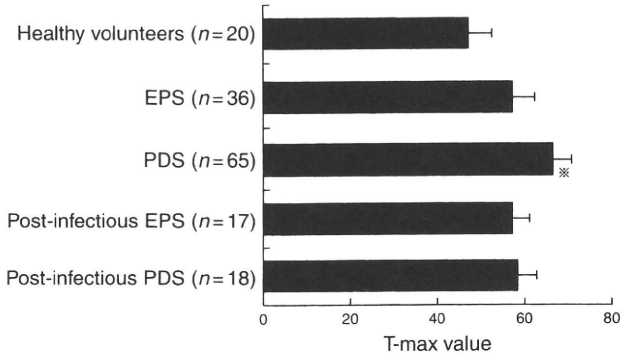


Figure 1. T-max value in epigastric pain syndrome (EPS), postprandial distress syndrome (PDS), and postinfectious functional dyspepsia (FD) patients and healthy volunteers. The T-max value in PDS patients ($n=65$) was significantly higher than in healthy volunteers. *$P<0.05$ vs. healthy volunteers.

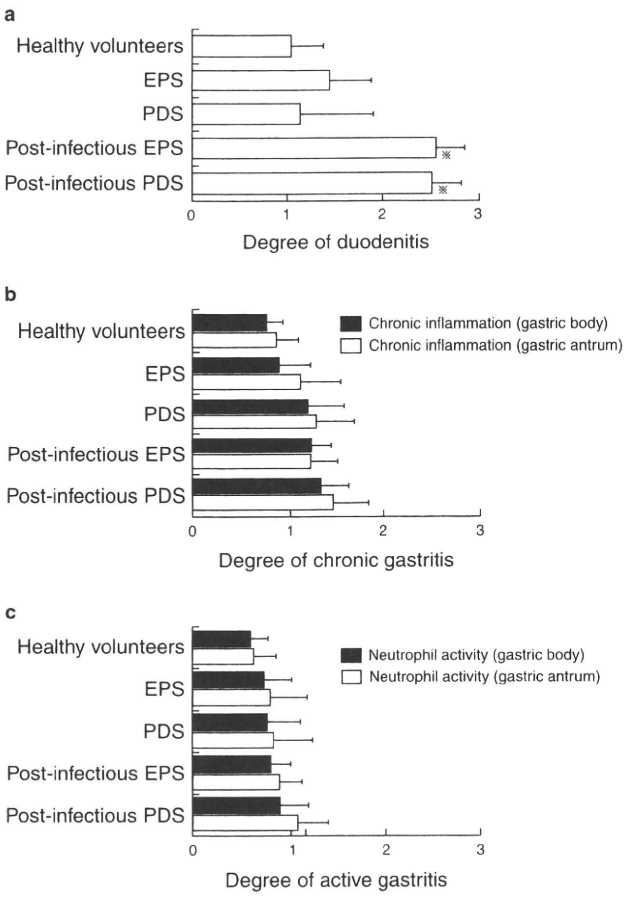


Figure 2. Comparison of the degree of gastritis and duodenitis in epigastric pain syndrome (EPS), postprandial distress syndrome (PDS), and postinfectious functional dyspepsia (FD) patients and healthy volunteers. (**a**) The degree of histological duodenitis in postinfectious EPS ($n=17$) and in postinfectious PDS ($n=18$) was significantly higher than that in healthy volunteers. *$P<0.05$ vs. healthy volunteers. (**b**, **c**) There was no significant difference in the degree of chronic inflammation or neutrophil activity in the gastric body and the gastric antrum among EPS patients ($n=12$), PDS patients ($n=15$), both forms of postinfectious FD patients ($n=35$), and healthy volunteers ($n=20$).

biopsies of postinfectious FD patients were 17% for mild, 26% for moderate, and 57% for severe duodenitis. In contrast, there was no significant difference in the degree of gastritis including chronic inflammation and neutrophil activity between patients with postinfectious FD, EPS, or PDS and healthy volunteers (**Figure 2b** and **c**).

Comparison of histological duodenitis with clinical symptoms in postinfectious FD patients

To analyze whether there was a relationship between clinical symptoms and histological duodenitis in postinfectious FD patients, we compared clinical symptom scores with scores of histological duodenitis in this patient group. We found a significant relationship ($P=0.03$) between epigastric burning and the degree of histological duodenitis in postinfectious FD patients (**Figure 3**). In contrast, there was no significant relationship between clinical symptoms and histological gastritis in postinfectious FD patients (data not shown).

Comparison of T-max value and duodenal inflammation in postinfectious FD patients with or without *H. pylori* infection

To determine whether *H. pylori* infection affects gastric emptying and duodenal inflammatory cell infiltration in postinfectious EPS and postinfectious PDS patients, we compared these two parameters in postinfectious FD patients with or without *H. pylori* infection. There was no significant difference in T-max value and histological duodenitis scores for biopsies from the postinfectious FD patients with or without *H. pylori* infection (data not shown).

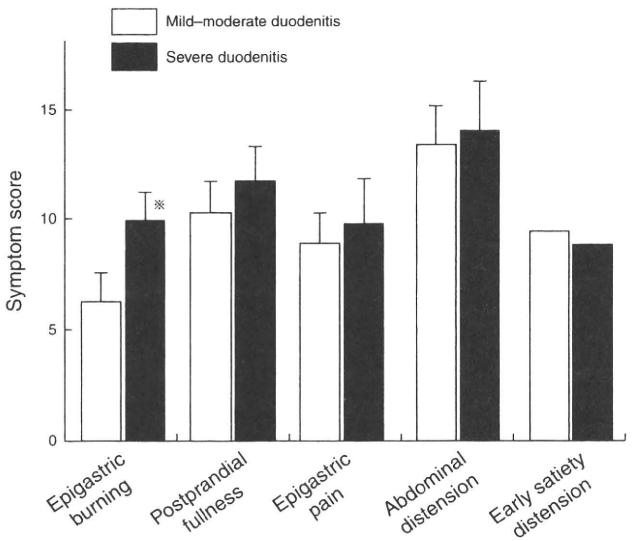


Figure 3. Comparison of histological duodenitis with clinical symptoms in postinfectious functional dyspepsia (FD) patients. There was a significant difference (*$P=0.03$) in epigastric burning reported in patients with mild–moderate histologic duodenitis compared to those with severe histologic duodenitis.

Localization of inflammatory cells and serotonin-producing cells in the duodenum of postinfectious FD

To assess the localization of inflammatory cells and serotonin-producing cells in the duodenum of the postinfectious FD patients, we performed immunostaining using anti-CD3, -CD68, -CCR2, -Vdelta1 TCR, and -serotonin antibodies (**Figure 4a**). We compared numbers of these inflammatory cells and serotonin-producing cells in duodenal biopsies from EPS, PDS, postinfectious FD, and healthy volunteers. CD68-positive cell number in postinfectious FD was significantly ($P=0.04$, 0.02, and 0.01) increased compared to that of EPS, PDS, and healthy volunteers (**Figure 4b**). Numbers of eosinophils in postinfectious FD were significantly ($P=0.03$) increased compared to those of healthy volunteers (**Figure 4b**). However, numbers of serotonin-producing cells, neutrophils, and inflammatory cells such as CD3-positive cells, -Vdelta1 T cells, and CCR2-positive cells in postinfectious FD patients were similar to those in EPS, PDS, and healthy volunteers (**Figure 4b**).

CCR2-positive macrophages in the duodenum of patients with postinfectious FD

We analyzed the numbers of CCR2-positive macrophages (activated macrophages) in duodenal biopsies from postinfectious FD, EPS, PDS, and healthy volunteers. We found that CCR2-positive macrophages had accumulated in the duodenum of the postinfectious FD patients (a representative image is shown in **Figure 5a**). CCR2-positive macrophage counts in postinfectious FD patients were significantly ($P=0.009$) increased compared to those in healthy volunteers (**Figure 5b**). In contrast, no significant differences ($P=0.74$, EPS vs. healthy volunteers; $P=0.69$, EPS vs. PDS; $P=0.59$, PDS vs. healthy volunteers) in CCR2-positive macrophage counts were observed among EPS, PDS, and healthy volunteers (**Figure 5b**).

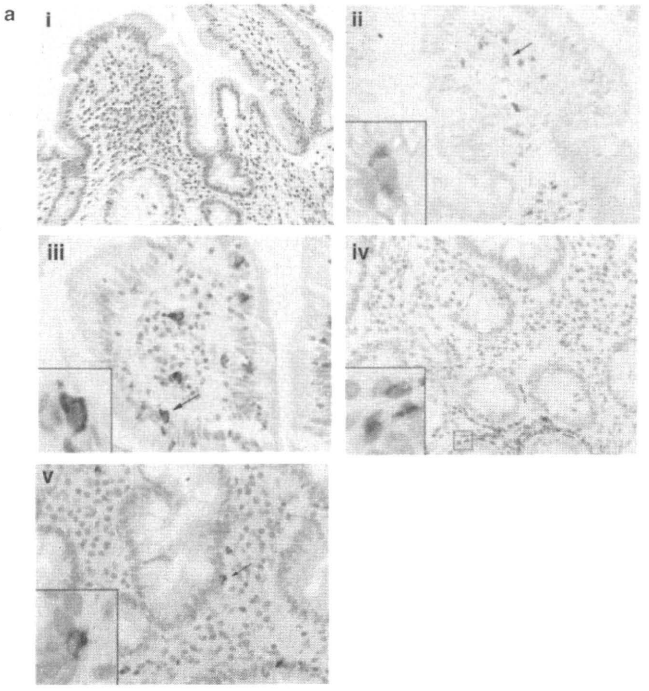
DISCUSSION

Major findings in this study include (i) gastric emptying assessed by ^{13}C-acetate breath test T-max values in patients with postinfectious EPS or postinfectious PDS was similar to results found in EPS patients and in healthy volunteers; (ii) the degree of histological duodenitis in postinfectious FD was significantly higher than that found in healthy volunteers; (iii) a significant correlation between epigastric burning and the degree of duodenitis was found in postinfectious FD patients; (iv) there was no significant difference in histological duodenitis and T-max value in the postinfectious FD patients with or without *H. pylori* infection; and (v) CCR2-positive macrophage and eosinophil counts in postinfectious FD patients were significantly increased compared to those of healthy volunteers.

Gastric emptying is regulated by multiple endogenous and exogenous factors. One of the key clinical features in FD is that symptoms are often meal related, being either induced or exacerbated by food (28). More recently, analogous to findings in IBS, a triggering role for a prior GI infection has been shown in FD patients. Indeed, in a population with an outbreak of Salmonella gastroenteritis, the prevalence of FD was significantly increased up to 1 year after the acute event (19). Tack *et al.* (20) have reported that 25% of the patients with FD reported an acute onset and 17% of FD patients reported an acute onset that was accompanied by signs suggestive of an acute GI infection. These findings suggest that FD, similar to other functional bowel disorders such as IBS or gastroparesis, may occur after an acute intestinal infection (16,29). The nature of the agent causing the presumed acute infection in most instances is unknown. Our data show that T-max values as a marker of gastric emptying in postinfectious PDS were similar to those found in EPS, postinfectious EPS, and healthy volunteers. However, T-max values were significantly higher in PDS patients compared to other groups. We have previously reported that elongation of T-max value is significantly associated with low acylated ghrelin concentrations (30) in PDS patients. Further studies including measurement of acylated ghrelin levels in patients with postinfectious PDS and those without postinfectious PDS will be needed to clarify whether preceding infection affects gastric emptying in postinfectious PDS patients.

In addition, we found that *H. pylori* infection did not have a significant effect on either gastric emptying or histological duodenitis in postinfectious FD. The relationship between *H. pylori* infection and FD patients is still controversial. McColl *et al.* (31) have reported that *H. pylori* eradication therapy was effective for resolution of symptoms in FD patients. On the other hand, Blum *et al.* (32) have reported that in patients with FD, the eradication of *H. pylori* is not likely to relieve symptoms. In some postinfectious FD patients in this study, *H. pylori* eradication was not effective in resolving histological duodenitis. Further studies are needed to clarify whether *H. pylori* eradication therapy is effective in improving clinical symptoms in patients with postinfectious FD.

Although FD is highly prevalent in the general population, very little is known about its underlying mechanisms. Our study was conducted to quantify and characterize duodenal inflammatory cells including eosinophils, CD68-positive cells, CCR2-positive cells, CD3-positive cells, Vdelta1 T cells, and serotonin-producing cells in patients with postinfectious FD, EPS, or PDS, and healthy volunteers. Although Spiller *et al.* (16) have reported that mucosal T cells increase in postinfectious IBS patients, in our study, duodenal Vdelta1 T cell, and CD3-positive T-cell counts did not differ among patients with postinfectious FD, EPS, or PDS, and healthy volunteers. Spiller *et al.* (16) have also reported that enteroendocrine cells increased in postinfectious IBS patients. Almost 90% of 5-hydroxytryptamine (5-HT) is contained in enterochromaffin cells; 5-HT is thought to be linked to the regulation of secretion, motility, and sensory events. However, in this study, there was no significant difference in numbers of serotonin-producing cells of the duodenum among the study groups (EPS, PDS, postinfectious FD, and healthy volunteers). Considering our results, we conclude that the duodenitis of postinfectious FD patients may depend on an accumulation of macrophages and plasma cells with an accompanying partial loss of villi.



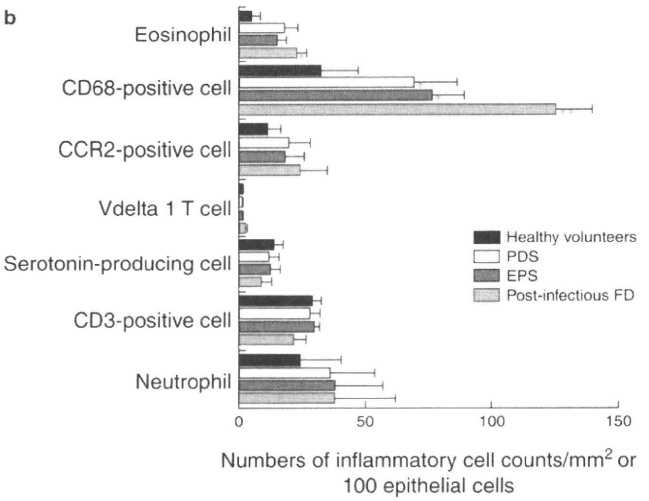


Figure 4. Characteristics of inflammatory cells and serotonin-producing cells in duodenal biopsies from patients with postinfectious functional dyspepsia (FD). (**a**) (i) This representative image (magnification ×250) shows a few serotonin-producing cells in the duodenal biopsy of a patient with postinfectious FD. (ii) Many CD68-positive cells were seen in the duodenal biopsies of postinfectious FD patients (arrow shows a CD68-positive cell, magnification ×400; a higher magnification (×600) of the cell indicated by an arrow is shown in the left lower corner). (iii) A few Vdelta1 T cells are seen in this representative image (magnification ×500; a higher magnification (×750) of the cell indicated by an arrow is shown in the left lower corner). (iv) This representative image shows CD3-positive cells that have migrated into the duodenal mucosa (magnification ×300; a higher magnification (×450) of the boxed area indicated in the photograph is shown in the left lower corner). (v) CCR2-positive cells are seen in the duodenum of a patient with postinfectious FD (arrow shows a CCR2-positive cell, magnification ×500; a higher magnification (×750) of the cell indicated by an arrow is shown in the left lower corner). (**b**) CD68-positive cell number in postinfectious FD duodenal biopsies ($n=35$) was significantly increased compared to those in biopsies from epigastric pain syndrome (EPS) ($n=12$), postprandial distress syndrome (PDS) ($n=15$), and healthy volunteers ($n=20$). Eosinophil counts in postinfectious FD patients were significantly increased compared to those found in biopsies from healthy volunteers. Numbers of CD68-, CCR2-, CD3-positive cells, neutrophil, and eosinophil were evaluated per mm^2 in duodenal specimens. Numbers of Vdelta1 T cell and serotonin-producing cell were counted per 100 epithelial cells. Values shown represent mean±s.e. $^*P<0.05$ vs. healthy volunteers. $^{**}P<0.05$ vs. PDS and EPS patients.

Talley *et al.* (13) and Toukan *et al.* (33) have reported that there is a significant association of the number of migrated eosinophils in the duodenum in subjects with nonulcer dyspepsia. In our study, eosinophil numbers in postinfectious FD were significantly higher than those of healthy volunteers. However, compared with controls, eosinophils were not noted to increase in colonic and rectal biopsies of patients with postinfectious IBS (17). Further studies are needed to clarify whether eosinophils migration into duodenal mucosa is linked to the etiology of postinfectious FD. Kindt *et al.* (34) have reported that macrophage accumulation in the duodenum is increased in postinfectious FD patients. In our study, migration of duodenal macrophages in postinfectious FD patients was significantly increased compared with that seen in biopsies from EPS, PDS, and healthy volunteers. In addition, we found significantly increased levels of eosinophils and CCR2-positive macrophages in the duodenum of postinfectious FD patients compared with healthy volunteers. Previous studies indicate that acute gut infection induces several chemokines and mediators including monocyte chemoattractant protein-1 (MCP-1) and histamine, which might have important functions in accumulation of eosinophils and CCR2-positive macrophages in the duodenal mucosa of postinfectious FD patients (35). In turn, these mediators may impact on sensory-motor dysfunction to produce clinical symptoms such as epigastric burning.

In our study, a significant correlation between epigastric burning and the degree of duodenitis was seen in postinfectious FD patients. The relationship between duodenitis and clinical symptoms such as dyspepsia is still controversial (36,37). We also found that duodenal macrophage accumulation in postinfectious FD was increased compared to that in healthy volunteers in keeping with a previous report (34). Moreover, we showed that CCR2-positive macrophage infiltration in the duodenum of postinfectious FD was significantly increased compared to that in healthy volunteers. Tajima *et al.* (38) have reported that CCR2 expression in macrophages was dependent on prostaglandins. Prostaglandins released from macrophages and sympathetic terminals are thought to have a direct action on receptors located on afferent endings (39). Prostaglandins and other arachidonic acid derivatives increase the sensitivity of nerve terminals to pain-producing substances (39). As such, CCR2-positive macrophages-derived prostaglandins might lead to clinical symptoms through increasing the sensitivity of nerve terminals. In CCR2-deficient mice, macrophage recruitment to sites of neuronal damage is reduced, with a consequent decrease in demyelination (40,41). CCR2-positive macrophage infiltration in the duodenum in postinfectious FD could affect the neural network system through demyelination. Further studies are needed to determine whether duodenal

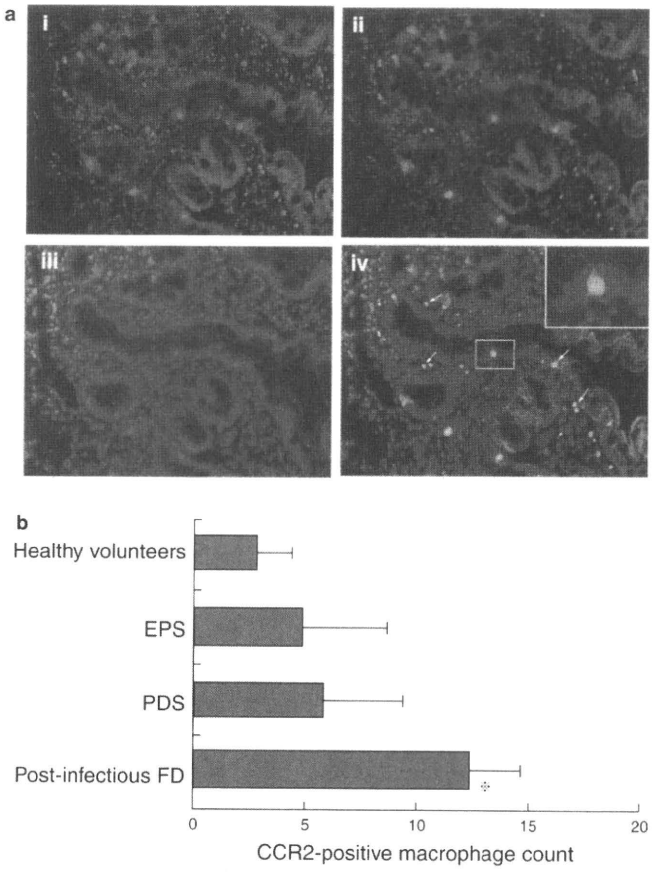


Figure 5. CCR2-positive macrophages in duodenal biopsies from patients with postinfectious functional dyspepsia (FD). (**a**) Immunostaining of duodenal biopsies from postinfectious FD patients showed (i) CCR2-positive cells (red cells; magnification ×300), (ii) CD68-positive cells (green cells; magnification ×300), and (iii) CCR2-positive macrophages (yellow cells) in representative images (magnification ×300; a higher magnification (×450) of the boxed area in the middle of the photograph is shown in the right upper corner). (iv) Negative control in which primary antibodies were replaced with isotype-matched immunoglobulin (magnification ×300). (**b**) CCR2-positive macrophage counts in postinfectious FD patients were significantly increased compared to those in healthy volunteers. Values shown represent mean ± s.e. $P<0.05$ vs. healthy volunteers. EPS, epigastric pain syndrome; PDS, postprandial distress syndrome. *$P= 0.009$.

inflammatory cells infiltration including CCR2-positive macrophages affects sensory nerve cells in the duodenum.

In conclusion, we observed that gastric emptying evaluated by T-max values in postinfectious FD patients was similar to values in EPS patients and healthy volunteers. However, the degree of histological duodenitis of postinfectious FD was significantly greater than that of healthy volunteers. We propose that CCR2-positive macrophages in the duodenum may have an important function in the pathophysiology of postinfectious FD.

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CONFLICT OF INTEREST

Guarantor of the article: Seiji Futagami, MD, PhD.

Specific author contributions: Measuring gastric emptying: Tomotaka Shindo, Tetsuro Kawagoe, and Mayumi Shimpuku; immunostaining: Akane Horie and Katya Gudis; statistical analysis: Takashi Itoh; editing of the paper: Choitsu Sakamoto.

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REFERENCES

1. Drossman DA. The functional gastrointestinal disorders and the Rome III process. Gastroenterology 2006;130:1377–90.
2. Talley NJ, Zinsmeister AR, Schleck CD *et al.* Dyspepsia and dyspepsia subgroups: a population-based study. Gastroenterology 1992;102 (4 Pt 1): 1259–68.
3. Castillo EJ, Camilleri M, Locke GR *et al.* A community-based, controlled study of the epidemiology and pathophysiology of dyspepsia. Clin Gastroenterol Hepatol 2004;2:985–96.
4. Tack J, Piessevaux H, Coulie B *et al.* Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology 1998;115:1346–52.
5. Lunding JA, Tefera S, Gilja OH *et al.* Rapid initial gastric emptying and hypersensitivity to gastric filling in functional dyspepsia:effects of duodenal lipids. Scand J Gastroenterol 2006;41:1028–36.
6. Holtmann G, Goebell H, Talley J. Impaired small intestinal peristalistic refluxes and sensory thresholds are independent functional disturbances in patients with chronic unexplained dyspepsia. Am J Gastroenterol 1996;91:485–91.
7. Wilmer A, Van Cutsem E, Andrioli A *et al.* Ambulatory gastrojejunal manometry in severe motility-like dyspepsia: lack of correlation between dysmotility, symptoms, and gastric emptying. Gut 1998;42:235–42.
8. Samsom M, Verhagen MA, van Berge Henegouwen GP *et al.* Abnormal clearance of exogenous acid and increased acid sensitivity of the proximal duodenum in dyspeptic patients. Gastroenterology 1999;116:515–20.
9. Schwartz MP, Samsom M, Smout AJ. Chemospefic alterations in duodenal perception and motor response in functional dyspepsia. Am J Gastroenterol 2001;96:2596–602.
10. Moayyedi P, Deeks J, Talley NJ *et al.* An update of the Cochrane systematic review of Helicobacter pylori eradication therapy in nonulcer dyspepsia: resolving the discrepancy between systematic reviews. Am J Gastroenterol 2003;98:2621–6.
11. Lee KJ, Demarchi B, Demedts I *et al.* A pilot study on duodenal acid exposure and its relationship to symptoms in functional dyspepsia with prominent nausea. Am J Gastroenterol 2004;99:1765–73.
12. Walker MM, Talley NJ, Prabhakar M *et al.* Duodenal mastocytosis, eosinophilia and intraepithelial lymphocytosis as possible disease markers in the irritable bowel syndrome and fuctional dyspepsia. Aliment Pharmacol Ther 2009;29:765–73.
13. Talley NJ, Walker MM, Aro P *et al.* Non-ulcer dyspepsia and duodenal eosinophilia: an adult endoscopic population-based case-control study. Clin Gastroenterol Hepatol 2007;5:1175–83.
14. Czura CJ, Tracey KJ. Autonomic neural regulation of immunity. J Intern Med 2005;257:156–66.
15. Chadwick VS, Chen W, Shu D *et al.* Activation of the mucosal immune system in irritable bowel syndrome. Gastroenterology 2002;122:1778–83.
16. Spiller RC, Jenkins D, Thornley JP *et al.* Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. Gut 2000;47:804–11.
17. O'Sullivan M, Clayton N, Breslin NP *et al.* Increased mast cells in the irritable bowel syndrome. Neurogastroenterol Motil 2000;12:449–57.
18. Gwee KA, Leong YL, Graham C *et al.* The role of psychological and biological factors in postinfective gut dysfunction. Gut 1999;44:400–6.
19. Mearin F, Perez-Oliveras M, Perello A *et al.* Dyspepsia and irritable bowel syndrome after a Salmonella gastroenteritis outbreak: one-year follow up cohort study. Gastroenterology 2005;129:98–104.
20. Tack J, Demedts I, Dehondt G *et al.* Clinical and pathophysiological characteristics of acute-onset functional dyspepsia. Gastroenterology 2002;122:1738–47.
21. Delvaux M. Alterations of sensori-motor functions of the digestive tract in the pathophysiology of irritable bowel syndrome. Best Pract Res Clin Gastroenterol 2004;18:747–71.

22. Bueno L, Fioramonti J, Delvaux M *et al.* Mediators and pharmacology of visceral sensitivity:from basic to clinical investigations. Gastroenterology 1997;112:1714–43.
23. Spiller RC. Postinfectious irritable bowel syndrome. Gastroenterology 2003;124:1662–71.
24. Dixon MF, Genta RM, Yardley JH *et al.* Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161–81.
25. Elta GH, Appelman HD, Behler EM *et al.* A study of the correlation between endoscopic and histological diagnoses in gastroduodenitis. Am J Gastroenterol 1987;82:749–53.
26. Elta GH, Murphy R, Behler E *et al.* Campylobacter pylori in patients with dyspeptic symptoms and endoscopic evidence of erosion. Am J Gastroenterol 1989;84:643–6.
27. Ghoos YF, Maes BD, Geypens BJ *et al.* Measurements of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. Gastroenterology 1993;104:1640–7.
28. Quigley EMM. Gastric motor and sensory function, and motor disorders of the stomach. In: Feldman M, Friedman LS, Sleisenger MH (eds). Gastrointestinal and Liver Disease, Pathophysiology/Diagnosis/Management. Saunders: Philadelphia, PA, 2002, pp. 691–714.
29. Bityutskiy LP, Soykan I, McCallum RW. Viral gastroparesis: a subgroup of idiopathic gastroparesis. Clinical characteristics and long-term outcomes. Am J Gastroenetrol 1997;92:1501–4.
30. Shindo T, Futagami S, Hiratsuka T *et al.* Comparison of gastric emptying and plasma ghrelin levels in patients with functional dyspepsia and non-erosive reflux disease. Digestion 2009;79:65–72.
31. McColl K, Murray L, El-Omar E *et al.* Symptomatic benefit from eradicating Helicobacter pylori infection in patients with nonulcer dyspepsia. N Engl J Med 1998;339:1869–74.
32. Blum AL, Talley NJ, O'Morain C *et al.* Lack of effect of treating Helicobacter pylori infection in patients with nonulcer dyspepsia. Omeprazole plus clarithromycin and amoxicillin effect one year after treatment study group. N Engl J Med 1998;339:1875–81.
33. Toukan AU, Kamal FM, Amr SS *et al.* Gastroduodenal inflammation in patients with non-ulcer dyspepsia. A controlled endoscopic and morphometric study. Dig Dis Sci 1985;30:313–20.
34. Kindt S, Tertychenyy A, de Hertogh G *et al.* Intestinal immune activation in presumed post-infectious functional dyspepsia. Neurogastroenterol Motil 2009;21:832–e56.
35. Santos J, Guilarte M, Alonso C *et al.* Pathogenesis of irritable bowel syndrome: the mast cell connection. Scand J Gastroenterol 2005;40: 129–40.
36. Gelzayed EA, Biederman MA, Gelfand DW. Changing concepts of duodenitis. Am J Gastroenterol 1975;64:213–6.
37. Villani L, Trespi E, Fiocca R *et al.* Analysis of gastroduodenitis and oesophagitis in relation to dyspeptic/reflux symptoms. Digestion 1998; 59:91–101.
38. Tajima T, Murata T, Aritake K *et al.* Lipopolysaccharide induces macrophage migration via prostaglandin D_2 and prostaglandin E_2. J Pharmacol Exp Ther 2008;326:493–501.
39. Bueno L, Fioramonti J. Visceral perception: inflammatory and non-inflammatory mediators. Gut 2002;51 (Suppl 1): i19–23.
40. Ma M, Wei T, Boring L *et al.* Monocyte recruitment and myelin removal are delayed following spinal cord injury in mice with CCR2 chemokine receptor deletion. J Neurosci Res 2002;68:691–702.
41. Siebert H, Sachse A, Kuziel WA *et al.* The chemokine receptor CCR2 is involved in macrophage recruitment to the injured peripheral nervous system. J Neuroimmunol 2000;110:177–85.

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ORIGINAL ARTICLE—ALIMENTARY TRACT

A Japanese case series of 12 patients with esophageal eosinophilia

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Abstract
Background Eosinophilic esophagitis (EoE) has been a rarely recognized condition in Asian populations, and its clinical manifestation is rarely documented. Our aim was to describe clinically, endoscopically, and pathologically the features of patients with esophageal eosinophilia, including EoE.
Methods Twelve patients histologically proven to have esophageal eosinophilia were investigated. The histological diagnostic cutoff value was defined as a peak of $\geq$15 eosinophils/high-power field (HPF) in esophageal biopsies. Symptoms, endoscopic and pathological findings, and treatment outcome were evaluated.
Results Nine of the 12 patients were male and the 12 patients had a mean age of 47.7 years. Allergic conditions were concurrent in a total of 3 patients. Mild peripheral eosinophilia was observed in only 2 patients. The predominant symptom was solid-food dysphagia, but some patients complained of heartburn, or chest, epigastric, or back pain. Three asymptomatic subjects were also incidentally diagnosed during endoscopic screening. Linear furrows, concentric rings, and white exudates in the esophagus were frequently observed. In 4 of 5 patients who were administered a proton pump inhibitor (PPI), esophageal eosinophilia was histologically decreased or disappeared with symptom relief and endoscopic improvement. In 2 patients unresponsive to PPI, topical steroid therapy, administered by the swallowing of fluticasone propionate, led to symptomatic and histological remission.
Conclusions The endoscopic recognition of linear furrows, concentric rings, and white exudates is important in the diagnosis of eosinophilic esophageal inflammation. In a subset of patients this condition improves clinicopathologically with PPI treatment, and typical EoE, as strictly defined by unresponsiveness to PPI, appears to be a rather rare condition.

Keywords Esophageal eosinophilia · Eosinophilic esophagitis · Proton pump inhibitor · Topical steroid therapy

Abbreviations

EoE	Eosinophilic esophagitis
HPF	High-power field
PPI	Proton pump inhibitor
FP	Fluticasone propionate

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Introduction

Esophageal eosinophilia is a pathological condition defined as eosinophil infiltration within the esophageal mucosa, where eosinophils are normally not found [1]. This condition can result from various causes, and primary or idiopathic esophageal eosinophilia is referred to as eosinophilic

esophagitis (EoE) [2]. It has been reported that EoE, especially in the pediatric population, is closely associated with allergic conditions such as food allergy, bronchial asthma, or atopic dermatitis [2, 3]. EoE has been well described in the pediatric population, but recently has been increasingly recognized in adults [3]. In Western countries, EoE is one of the most common causes of intermittent solid-food dysphagia or food impaction in adults [4–6]. To our knowledge, in Asia, EoE has been recognized only in a few case reports [7, 8], and its epidemiology and pathophysiology have been not well documented. Solid-food dysphagia is the predominant symptom in EoE, but in a subset of patients heartburn or chest pain is the major symptom [9, 10].

It has been reported that most adult EoE patients have a normal esophageal pH, which is one of the definitive criteria noted by a recent diagnostic guideline [2]. However, a substantial overlap of EoE and gastroesophageal reflux disease (GERD) has been documented recently [11, 12]. In addition, it has been shown that acid suppressive therapy with PPIs leads to symptom relief with endoscopic and histological resolution in some patients [13–16]. Consequently, in the clinical setting, there is a possibility that EoE is confused with GERD or given a diagnosis of refractory GERD in patients who are unresponsive to PPI treatment [10]. In a retrospective study conducted in adult EoE patients [17], about 40% of them had not been previously diagnosed with EoE, but with other esophageal disorders such as Schatzki's rings, esophageal stricture, or reflux disease.

In the present report, we retrospectively examined the clinical, endoscopic, and histological features and treatment outcomes of Japanese patients with esophageal eosinophilia, including EoE.

Methods

We reviewed in detail 12 adult patients with esophageal eosinophilia who were diagnosed at our university hospital or affiliated hospitals between October 2006 and November 2009. More than two esophageal biopsies were obtained from any patient with endoscopically suspicious findings of esophageal eosinophilia, such as linear furrows, segmental or transient concentric rings, or white exudates scattered over the mucosal surface [2, 17], regardless of their symptoms. The diagnostic cutoff value for esophageal eosinophilia was defined as a peak of $\geq$15 eosinophils/high-power field (HPF) [2]. One HPF was 0.24 mm^3 in our microscope (40× objective lens and 10× ocular lens; Olympus BX50; Tokyo, Japan). The number of infiltrated eosinophils per HPF was counted in an easily recognizable area of eosinophilia in a low-power field in each sample. Symptoms including a past history of dysphagia or food impaction and concurrent or past allergic conditions were determined by interview or from the medical chart. Peripheral eosinophilia and serum IgE values were also examined in patients from whom blood test data were available. Endoscopic and histological findings and treatment outcome were minutely evaluated. Proton pump inhibitors were primarily prescribed for symptomatic patients for 4–8 weeks, and, in unresponsive cases, topical steroid therapy was admistered by the swallowing of fluticasone propionate (FP) (Flutide®Aerosol, GlaxoSmithKline, Middlesex, UK; 400 μg × 2 times per day) for 4–8 weeks [18, 19]. In practice, patients were instructed to swallow the agent, which was sprayed into the mouth with a metered dosed inhaler without a spacer, and not to eat or drink for at least 30 min after administration. The patients were advised to rinse the mouth out with water to prevent oral candidiasis.

Results

Symptoms, endoscopic findings, histological findings, and treatment outcome are summarized in Tables 1 and 2, in which patients are classified according to responsiveness to PPI therapy. The twelve patients with esophageal eosinophilia consisted of 9 men and 3 women with a mean age of 47.7 years (range 32–68 years). In these 12 patients, the predominant symptom was dysphagia in 6 (50%), epigastric pain and dysphagia in 1, chest pain and back discomfort in 1, heartburn and epigastric and back pain in 1, and no symptoms in 3 (25%). This condition was incidentally diagnosed by endoscopy during cancer screening or during an annual follow-up for chronic gastritis in the 3 asymptomatic patients. Atopic dermatitis, hay fever, or asthma was present in a total of 3 patients. Of the 9 patients whose blood profile was available, 2 male patients had mild peripheral eosinophilia (603 and 1,637/μL). By endoscopy, linear furrows were observed in 10 patients (83%), transient or segmental concentric rings were seen in 10 (83%), and white exudates scattered over the mucosal surface were seen in 9 (75%) (Fig. 1a–c). Erosive esophagitis, corresponding to Los Angeles classification grade A, was seen in one of our patients (case no. 6). As concomitant gastroduodenal lesions, atrophic gastritis was observed in 4 patients, erosive and hemorrhagic gastritis in 3, and duodenitis in one.

One male patient (case no. 8) had almost normal endoscopic findings except for subtly scattered white exudates. Another male patient (case no. 12), in whom the thickness of his esophageal wall was initially noted on by computed tomography, had only a mildly edematous and small-caliber esophagus. His main symptom at the diagnosis of EoE was initially epigastric pain, which was subsequently followed by dysphagia. He used maintenance therapy with an inhaled steroid for asthma and additionally

Table 1 Patient age, gender, symptoms, allergic status, and concomitant GI lesions

Case no.	Age (years)	Gender	Symptom	Allergic condition	Peripheral eosinophilia	Concomitant GI lesions
PPI non-responsive						
1	40	M	Dysphagia	None	–	No findings
2	37	M	Dysphagia	None	–	Multiple scars after EIS
PPI responsive						
3	55	F	Dysphagia	Atopic dermatitis, hay fever	NA	Atrophic gastritis
4	32	M	Dysphagia	None	–	No findings
5	51	F	No symptom	Hay fever	–	Atrophic gastritis
6	43	M	Dysphagia	None	Mild (603/μL)	Erosive esophagitis, hemorrhagic gastritis
7	52	M	Heartburn, epigastric pain, back pain	None	Mild (1637/μL)	Erosive gastritis, duodenitis
Others						
8	57	M	Dysphagia	None	–	No findings
9	55	F	Chest pain, back discomfort	None	–	Atrophic gastritis
10	40	M	No symptom	None	NA	Erosive gastritis
11	35	M	No symptom	None	NA	Erosive gastritis
12	68	M	Epigastric pain, dysphagia	Asthma	–	Atrophic gastritis

GI gastrointestinal, *PPI* proton pump inhibitor, *EIS* endoscopic injection sclerotherapy, *NA* not available

Table 2 Endoscopic and histological findings, and treatment outcomes of the subjects

Case no.	Endoscopic findings			Histological findings		Treatment outcome	
	Linear furrows	Concentric rings	White exudates	No. of biopsies	No. of eosinophils (range, HPF^{-1})	Therapeutic agent	Esophageal eosinophilia
PPI non-responsive							
1	+	+	+	6	3–121	Topical FP	Disappearance
2	+	+	–	3	13–28	Topical FP	Disappearance
PPI responsive							
3	+	+	+	7	7–40	PPI	Disappearance
4	+	+	+	2	25–144	PPI	Disappearance
5	+	+	–	6	66–123	PPI	Disappearance
6	+	+	+	4	35–94	PPI	Decrease
7	+	+	+	2	42–87	PPI	NA
Others							
8	–	–	+	4	0–49	Sodium alginate	NA
9	+	+	+	2	43–70	H2RA	NA
10	+	+	+	3	18–53	No treatment	NA
11	+	+	+	2	25–76	No treatment	NA
12	–	–	–	4	0–16	No treatment	Disappearance

+, observed; –, not observed; *FP*, fluticasone propionate; *PPI*, proton pump inhibitor; *H2RA*, histamine-H2 receptor antagonist; *NA*, not available

took only a PPI for epigastric pain, without systemic steroid therapy. In a follow-up after 4 months, no eosinophilic infiltration was detected on several esophageal biopsies, although the dysphagia and endoscopic findings persisted.

A mean of 3.5 esophageal biopsies (range 2–7, total 42 biopsies) was obtained in each patient. The mean number of infiltrated eosinophils was 36.8/HPF per esophageal biopsy, and wide variation, ranging from 0 to 144/HPF,

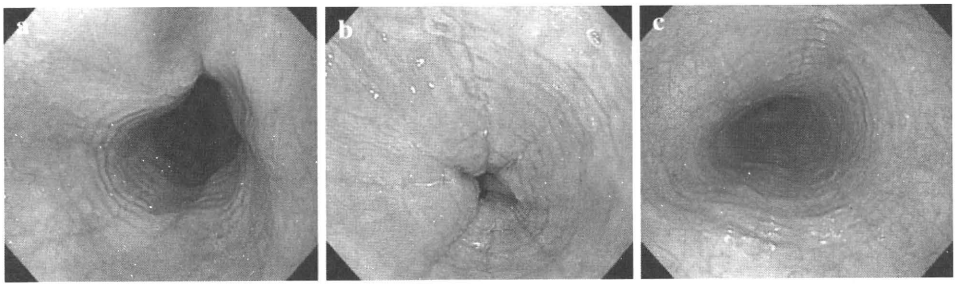

Fig. 1 **a–c** Case no. 1. **a**, **b** Linear furrows and segmental or transient concentric rings are shown. The mucosal surface was edematous, and dendritic vessels inherent to normal esophageal epithelium were not seen. **c** White exudates 1–2 mm in diameter were scattered over the mucosal surface. These exudates were in general subtle as compared with findings for the fungus *Candia albicans*

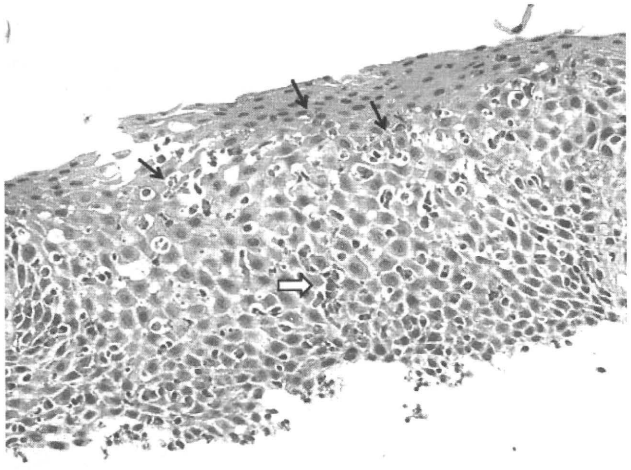

Fig. 2 Case no. 1. Dense eosinophilic infiltration with microabscesses (≥4 eosinophils/high-power field [HPF], *white arrow*), prominent intercellular edema, and degranulation of eosinophils (*black arrows*) were seen in the esophageal epithelium (this biopsy sample was obtained from the mid-esophagus, which had abnormal endoscopic findings). H&E, ×400

was found (a representative histological image of eosinophilic esophagitis is shown in Fig. 2). Mild eosinophilic infiltrations in the gastric and duodenal mucosa were present in only one of 9 patients (case no. 5). She had no gastrointestinal symptoms suspicious of eosinophilic gastroenteritis. Of 7 patients administered a PPI for 4–8 weeks (6 symptomatic patients and 1 who was asymptomatic), 5 patients had almost complete elimination of their symptoms. The infiltration of esophageal eosinophils decreased in 1 patient and completely disappeared in 3 patients after PPI treatment. In 2 patients unresponsive to PPI, topical steroid therapy, administered by the swallowing of FP, led to complete symptomatic relief with endoscopic normalization and histological disappearance of the infiltrated eosinophils. However, in one patient (case no. 1), the dysphagia relapsed, with characteristic endoscopic and histological findings, 8 weeks after the withdrawal of the topical steroid therapy. This patient was treated again by swallowing FP, which led again to remission. In a male patient (case no. 2) with alcoholic liver cirrhosis, characteristic endoscopic findings suspicious of esophageal eosinophilia were noted in his upper esophagus with the development of dysphagia a month after he had had endoscopic sclerotherapy for esophageal varices. Namely, at diagnosis of EoE, linear furrows or concentric rings were seen in his upper esophagus and several mild scars caused by sclerotherapy were seen in his lower esophagus. His symptoms and esophageal eosinophilia were completely resolved by topical FP therapy, although primarily not by PPI. In this patient, allergic reaction to a sclerosant (monoethanolamine oleate) in the esophageal wall or secondarily induced pathological reflux after sclerotherapy may have been a potential trigger of the eosinophilic inflammation [20, 21]. Neither of the two patients who received topical steroid therapy developed oral or esophageal candidiasis. Three patients (case nos. 7, 8, and 9) could not be followed up.

Discussion

In the present study, we described 12 adult patients with esophageal eosinophilia, including EoE. EoE in adults has been increasingly recognized and actively investigated in Western countries, but in Asian populations this condition has been described in only a few case reports [7, 8]. According to the recent diagnostic guidelines established by the American Gastroenterological Association [2], EoE should be diagnosed when clinical symptoms of esophageal dysfunction are present, ≥15 eosinophils in one high-power field (HPF) are detected in 1 or more esophageal biopsies, and high-dose PPI fails to ameliorate the symptoms or normal pH monitoring of the distal esophagus is observed. In our patient group, only two patients (cases no. 1 and no. 2) could be diagnosed as having EoE (although in case no. 2, esophageal eosinophilia might have been caused by hypersensitivity to drugs). Thus, based on our results, typical EoE, as strictly defined by unresponsiveness to PPI, may be rare in Japan, even among those who exhibit both

typical endoscopic findings and histologically confirmed esophageal eosinophilia.

However, recently, a substantial overlap of EoE and GERD has been documented in adult patients, and in a subset of these patients acid suppressive therapy with a PPI leads to symptom relief with endoscopic and histological resolution [11–16]. A mechanistic explanation for the therapeutic effect of PPIs is that the permeability of exogenous allergens to the esophageal wall is attenuated by the curing of the mucosal injury with acid suppression [22]. An anti-inflammatory effect of PPIs, in addition to the acid suppression, may also be associated with their therapeutic efficacy [23, 24]. On the other hand, there is a possibility that eosinophilic inflammation of the esophagus may result in GERD by impairing esophageal motor function [22]. Thus, the causal relationship between EoE and GERD is intricate and controversial [12, 22, 25], and it is difficult to clearly discriminate between these two conditions [22]. There is an opinion that a favorable response to PPI therapy does not preclude a diagnosis of eosinophilic esophagitis [22].

Consistent with the allergic nature of EoE, dietary therapy, topical steroids, systemic steroids, leukotriene receptor antagonists, or some biologic agents have been used for treatment. Of these treatment options, topical steroid therapy administered by the swallowing of fluticasone propionate (FP) has been established as a first-line agent both in children and adults because of its high therapeutic effect and low rate of adverse effects [26]. Recently, Peterson et al. [27] reported that PPI therapy, in addition to topical steroid therapy, yielded similar symptomatic and pathological responses regardless of underlying GERD. Because of their safety and simplicity, PPIs rather than topical steroids may be recommended as first-line agents for esophageal eosinophilia, including EoE, in clinical practice.

In agreement with descriptions in previous Western reports [2, 3], most of our patients were middle-aged and male. Concurrent or past allergic conditions in our patients were unexpectedly few. Although the predominant symptom was dysphagia, 3 asymptomatic subjects were incidentally diagnosed during endoscopic screening. Linear furrows, transient or constant concentric rings, and white exudates scattered over the mucosal surface were frequently observed. As described in many Western reports [2–6, 9–11], such endoscopic findings appeared to be characteristic of eosinophilic esophageal inflammation in our series as well.

Asymptomatic subjects who were incidentally discovered during screening would have remained undiagnosed unless endoscopic examination with esophageal biopsies had been performed. In a recent Swedish study reported by Ronkainen et al. [28], when sampling the esophageal mucosa irrespective of symptoms or endoscopic abnormalities, they found that approximately 1% of the adult general population had definite or probable esophageal eosinophilia. They also noted that none of the patients with definite or probable esophageal eosinophilia had consulted a doctor for their symptoms. Thus, it is suggested that EoE may be an undiagnosed or unrecognized condition, but not uncommon. Although the long-term course of this condition has not been well characterized, about 30% of adult patients with EoE developed esophageal stenosis for which a dilatation procedure was needed after a mean follow-up period of about 7 years [29]. Because endoscopic examination to screen for malignant diseases has been widely performed in the general population in Japan regardless of the presence of symptoms, asymptomatic patients with esophageal eosinophilia may be increasingly detected. The natural course and appropriate clinical management for asymptomatic EoE has yet to be clarified.

Although in the present retrospective report the numbers and sites of esophageal biopsies were not uniform for each patient, there was wide regional variation in the numbers of infiltrated eosinophils within the same patients. In case no. 8, 49 eosinophils/HPF were seen in only one of 4 biopsy samples from the lower esophagus, and no eosinophils were seen in 3 other samples. The endoscopic abnormalities in this patient were much milder than those in the other patients. Due to the patchy distribution of the eosinophilic infiltration, multiple biopsies should be obtained over the entire esophagus. More than 3 biopsies from the proximal to distal esophagus are recommended for improving the diagnostic accuracy for EoE [17, 30].

In summary, the endoscopic recognition of linear furrows, transient and constant concentric rings, and white exudates was useful for the early diagnosis of esophageal eosinophilia including EoE in Japanese patients. A subset of patients with this condition experienced clinicopathological resolution with PPI treatment, and topical steroid therapy administered by swallowing FP was effective for the patients who did not respond to a PPI. EoE, as strictly defined by unresponsiveness to PPI, appears to be rare at present among patients with esophageal eosinophilia in Japan. Because of the increasing prevalence of allergic conditions in Asian populations [31], EoE may increase in future. Further awareness of this unrecognized condition and accumulation of such patients in clinical practice are needed to understand the epidemiology and pathophysiology of EoE in Asian countries.

References

1. Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol. 2006;24:147–74.

2. Furuta GT, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, Bonis P, et al. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology. 2007;133:1342–63.
3. Arora AS, Yamazaki K. Eosinophilic esophagitis: asthma of the esophagus? Clin Gastroenterol Hepatol. 2004;2:523–30.
4. Mackenzie SH, Go M, Chadwick B, Thomas K, Fang J, Kuwada S, et al. Eosinophilic oesophagitis in patients presenting with dysphagia—a prospective analysis. Aliment Pharmacol Ther. 2008;28:1140–6.
5. Pasha SF, DiBaise JK, Kim HJ, De Petris G, Crowell MD, Fleischer DE, et al. Patient characteristics, clinical, endoscopic, and histologic findings in adult eosinophilic esophagitis: a case series and systematic review of the medical literature. Dis Esophagus. 2007;20:311–9.
6. Byrne KR, Panagiotakis PH, Hilden K, Thomas KL, Peterson KA, Fang JC. Retrospective analysis of esophageal food impaction: differences in etiology by age and gender. Dig Dis Sci. 2006;52:717–21.
7. Furuta K, Adachi K, Kowari K, Mishima Y, Imaoka H, Kadota C, et al. A Japanese case of eosinophilic esophagitis. J Gastroenterol. 2006;41:706–10.
8. Lu HC, Lu CL, Chang FY. Eosinophilic esophagitis in an asymptomatic Chinese. J Chin Med Assoc. 2008;71:362–4.
9. Croese J, Fairley SK, Masson JW, Chong AK, Whitaker DA, Kanowski PA, et al. Clinical and endoscopic features of eosinophilic esophagitis in adults. Gastrointest Endosc. 2003;58:516–22.
10. Potter JW, Saeian K, Staff D, Massey BT, Komorowski RA, Shaker R, et al. Eosinophilic esophagitis in adults: an emerging problem with unique esophageal features. Gastrointest Endosc. 2004;59:355–61.
11. Remedios M, Campbell C, Jones DM, Kerlin P. Eosinophilic esophagitis in adults: clinical, endoscopic, histologic findings, and response to treatment with fluticasone propionate. Gastrointest Endosc. 2006;63:3–12.
12. Rodrigo S, Abboud G, Oh D, DeMeester SR, Hagen J, Lipham J, et al. High intraepithelial eosinophil counts in esophageal squamous epithelium are not specific for eosinophilic esophagitis in adults. Am J Gastroenterol. 2008;103:435–42.
13. Dubecz A, Mentrikoski M, Peters JH. Eosinophilic esophagitis with severe GERD. Am J Gastroenterol. 2009;104:527–9.
14. Molina-Infante J, Ferrando-Lamana L, Mateos-Rodríguez JM, Pérez-Gallardo B, Prieto-Bermejo AB. Overlap of reflux and eosinophilic esophagitis in two patients requiring different therapies: a review of the literature. World J Gastroenterol. 2008;14:1463–6.
15. Ngo P, Furuta GT, Antonioli DA, Fox VL. Eosinophils in the esophagus-peptic or allergic eosinophilic esophagitis? Case series of three patients with esophageal eosinophilia. Am J Gastroenterol. 2006;101:1666–70.
16. Desai TK, Stecevic V, Chang CH, Goldstein NS, Badizadegan K, Furuta GT. Association of eosinophilic inflammation with esophageal food impaction in adults. Gastrointest Endosc. 2005;61:795–801.
17. Gonsalves N, Policarpio-Nicolas M, Zhang Q, Rao MS, Hirano I. Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. Gastrointest Endosc. 2006;64:313–9.
18. Basavaraju KP, Wong T. Eosinophilic oesophagitis: a common cause of dysphagia in young adults? Int J Clin Pract. 2008;62:1096–107.
19. Bohm M, Richter JE. Treatment of eosinophilic esophagitis: overview, current limitations, and future direction. Am J Gastroenterol. 2008;103:2635–44.
20. Bordas JM, Feu F, Vilella A, Rodés J. Anaphylactic reaction to ethanolamine oleate injection in sclerotherapy of esophageal varices. Endoscopy. 1989;21:50.
21. Kinoshita Y, Kitajima N, Itoh T, Ishido S, Nishiyama K, Kawanami C, et al. Gastroesophageal reflux after endoscopic injection sclerotherapy. Am J Gastroenterol. 1992;87:282–6.
22. Spechler SJ, Genta RM, Souza RF. Thoughts on the complex relationship between gastroesophageal reflux disease and eosinophilic esophagitis. Am J Gastroenterol. 2007;102:1301–6.
23. Isomoto H, Nishi Y, Kanazawa Y, Shikuwa S, Mizuta Y, Inoue K, et al. Immune and inflammatory responses in GERD and lansoprazole. J Clin Biochem Nutr. 2007;41:84–91.
24. De Jonge PJ, Siersema PD, Van Breda SG, Van Zoest KP, Bac DJ, Leeuwenburgh I, et al. Proton pump inhibitor therapy in gastro-oesophageal reflux disease decreases the oesophageal immune response but does not reduce the formation of DNA adducts. Aliment Pharmacol Ther. 2008;28:127–36.
25. Merwat SN, Spechler SJ. Might the use of acid-suppressive medications predispose to the development of eosinophilic esophagitis? Am J Gastroenterol. 2009;104:1897–902.
26. Moawad FJ, Veerappan GR, Wong RK. Eosinophilic esophagitis. Dig Dis Sci. 2009;54:1818–28.
27. Peterson KA, Thomas KL, Hilden K, Emerson LL, Wills JC, Fang JC. Comparison of esomeprazole to aerosolized, swallowed fluticasone for eosinophilic esophagitis. Dig Dis Sci. 2010;55:1313–9.
28. Ronkainen J, Talley NJ, Aro P, Storskrubb T, Johansson SE, Lind T, et al. Prevalence of oesophageal eosinophils and eosinophilic oesophagitis in adults: the population-based Kalixanda study. Gut. 2007;56:615–20.
29. Straumann A, Spichtin HP, Grize L, Bucher KA, Beglinger C, Simon HU. Natural history of primary eosinophilic esophagitis: a follow-up of 30 adult patients for up to 11.5 years. Gastroenterology. 2003;125:1660–9.
30. Shah A, Kagalwalla AF, Gonsalves N, Melin-Aldana H, Li BU, Hirano I. Histopathologic variability in children with eosinophilic esophagitis. Am J Gastroenterol. 2009;104:716–21.
31. Leung TF, Wong GW. The Asian side of asthma and allergy. Curr Opin Allergy Clin Immunol. 2008;8:384–90.