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Histopathological changes in the gastroduodenal mucosa of children with functional dyspepsia

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ABSTRACT

Introduction and Objective: Functional dyspepsia (FD) is a functional gastrointestinal disorder that affects a significant number of children presenting with chronic abdominal pain. A high proportion of these children undergo endoscopy to obtain mucosal biopsies which, by standard criteria, generally do not identify a clear explanation for symptoms. We undertook this study of children diagnosed with FD to elucidate the histopathological changes of gastroduodenal mucosa and to describe mast cell and eosinophil densities.

Methods: In this retrospective study, we evaluated 114 FD subjects and 10 control subjects from whom gastric antral and duodenal biopsies were available as formalin-fixed paraffin embedded tissue. We reviewed the H&E stained slides and performed immunohistochemistry for tryptase, to determine eosinophil and mast cell densities, respectively.

Results: We found that the duodenal mucosa showed no evidence of inflammation in 86% of subjects, a median peak eosinophil count of 24 and a median peak mast cell count of 22. The histopathological features of the gastric antral mucosa comprised no evidence of inflammation in 52% of subjects, mild chronic inflammation in 41% of subjects, a median peak eosinophil count of 11.5 and a median peak mast cell count of 18.

Conclusions: A significant proportion of children with FD do not show chronic or active inflammation, but have increased mast cell density and eosinophil density in the stomach and duodenum mucosa. Our study adds functional dyspepsia to the list of various abnormalities that have increased gastroduodenal mucosal elevations of eosinophils and/or mast cells.

1. Introduction

Chronic or recurrent abdominal pain is estimated to affect up to 19% of the pediatric population [1]. It is generally accepted that a pathological condition accounting for pain will be identified in less than 10% of these children and adolescents; however, the majority will report symptoms that fit into specific diagnoses under the broader heading of functional gastrointestinal disorders (FGIDs) as defined by Rome criteria [2,3]. There are four FGIDs related to abdominal pain with the two most common being functional dyspepsia (FD) and irritable bowel syndrome (IBS). The most recent Rome criteria, designated Rome IV, were released in 2016 and significantly altered the definition of FD in children and adolescents [2]. FD is defined by the presence of epigastric pain unrelated to stools, early satiety, and/or postprandial fullness. Two subsets of FD are recognized including postprandial distress syndrome (PDS; presence of postprandial bloating and/or early satiety which prevents finishing a normal size meal) and epigastric pain syndrome (EPS; where the pain is localized only to the epigastrium). Endoscopy with biopsy of the esophagus, antrum, and duodenum is a common part of the evaluation of youth with FD such that pathologists commonly evaluate mucosal specimens from these patients in their practice [4].

Like all FGIDs, FD is probably best understood through a biopsychosocial model which states that symptoms are the result of varying contributions from, and interactions between, biological/physiological factors (e.g. inflammation, dysmotility, hypersensitivity), psychological factors (e.g. anxiety, depression), and social factors (e.g interactions with peers, parents, and teachers). Within the biologic aspects of the model, the emphasis is not on diagnosing a pathologic condition but with identifying biologic contributors to the emergence and maintenance of pain. This conceptualization has implications for an evolving role for pathologists in the evaluation of these patients.

Inflammation, particularly as related to eosinophils and mast cells, has received considerable attention in FGIDs, including FD [5]. A role

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for duodenal eosinophils has generally been established while a role for antral and duodenal mast cells is being increasingly recognized. Studies of adults with FD have consistently demonstrated an elevation of duodenal eosinophil density with active degranulation [6–11]. Elevated duodenal eosinophil density has also been reported in children with FD and we have previously demonstrated significant degranulation of duodenal eosinophils in this patient group [12,13]. In adults, FD has been associated with elevated gastric mast cell density [14]. Increased duodenal mast cell density has been associated with adult FD in 2 studies in contrast to an association with IBS in another study [6,7,9]. Wang et al. [9] also reported increased duodenal MC degranulation. We have previously demonstrated active antral MC degranulation in children with FD [15].

There is a paucity of data in the pathology literature regarding the significance and utility of assessing eosinophil and mast cell densities in the gastroduodenal mucosa in the context of FD, particularly in children and adolescents [8,9]. No previous studies have evaluated youth with FD as defined by Rome IV. While mucosal eosinophils and mast cells are increased in FD as compared to controls, the prevalence of increased densities of these cells in FD has not been well established in children and adolescents.

In this study of children and adolescents with FD as defined by Rome IV, we describe histopathological changes in the gastroduodenal mucosa along with mast cell and eosinophil densities, specifically assessing the prevalence of increased cell densities. We also assess eosinophil and mast cell correlations.

2. Material and methods

2.1. Study design

After seeking Institutional Review Board approval, a large study attempting to validate Rome IV criteria for functional abdominal pain was initiated. The division of gastroenterology at our Institution maintains a database of patients diagnosed with FD who were originally diagnosed following Rome III criteria. From that database, we selected a number of patients with FD who conformed to the criteria laid out in Rome IV and elected to correlate the histopathological findings. We evaluated a convenience sample of 114 consecutive patients who fulfilled FD criteria and who had been followed for at least 2 years after the biopsies. We reviewed the hematoxylin and eosin (H&E) stained slides of the upper gastrointestinal mucosal biopsies which were obtained from these subjects as part of routine medical care. The review served to confirm the original histopathological diagnoses. A control group of “normal” children who had underwent endoscopic biopsies at our Institution was created after seeking a separate Institutional Review Board approval. To be placed in the “normal” control group, subjects were identified by searching pathology records for keywords “no diagnostic abnormality” in stomach and duodenum samples and “constipation.” A board-certified Gastroenterologist then reviewed the medical records to exclude FD and endoscopic abnormalities of stomach and duodenum. Utilizing the above strategy, 10 subjects were included in the control group. There were 6 girls and 4 boys in the group with a median age of 10 years (range 5–15 years). One investigator (MS) assessed the eosinophil and mast cell density in the mucosa and also determined if there were increased numbers of intra-epithelial lymphocytes. A second investigator (VS) reviewed and assessed the eosinophil and mast cell density on all cases that had elevated counts. Any significant differences in counts between the investigators were resolved by joint review. An immunohistochemical stain for tryptase was performed on the antral and duodenal biopsies of all study subjects. A mouse monoclonal antibody, anti-human mast cell tryptase, from Dako (clone AA1) was used at a dilution of 1:2000 on the Bond automated immunostainer following routine immunohistochemistry protocol for the stainer.

2.2. Eosinophil and mast cell density

To determine eosinophil density, sections were initially scanned at a low magnification ($\times$10 objective magnification) to determine areas of maximal density. Using an Olympus CH30 microscope with a combination of 40$\times$ objective and 10$\times$ eyepiece, eosinophils were counted in five consecutive high-power fields (hpf). Likewise, positively staining mast cells in the tyrptase-immunostained sections were counted in five consecutive hpf. Both cell types were counted only in the lamina propria of the mucosa. For the eosinophils, only cells demonstrating its nucleus were enumerated. For the mast cells, both spindled and epithelioid forms showing a nucleus were enumerated. Peak and mean cell densities were determined for the eosinophils and mast cells, respectively, in both the antrum and the duodenum. Based on the eyepiece and 40$\times$ objective parameters, the area of field of view of one hpf was calculated to be 0.196 square millimeters. Thus, the area of 5 hpf would be approximately 1 square millimeter.

2.3. Correlation

Following tabulation of the counts, correlation between average (mean) cell counts versus peak density, and peak density in duodenum versus gastric mucosa was undertaken. Differences in cell densities were assessed for individual symptoms and for FD/IBS overlap by the student’s *t*-test Differences in cell counts were compared between PDS, EPS, and PDS/EPS overlap by one way ANOVA.

3. Results

The original pathological diagnoses of the 114 subjects are shown in Table 1. A single case of increased intraepithelial lymphocytes in duodenal mucosa was noted, where the patient’s duodenal mucosal biopsy had > 25 lymphocytes per 100 enterocytes but no villous atrophy or crypt hyperplasia. There were no Giardia or other microorganisms in the duodenum.

3.1. Mast cells

The peak mast cell density in the gastric antrum of FD subjects ranged from 3 to 34 mast cells per hpf with a median of 18; whereas in controls it ranged from 8 to 16 mast cells per hpf with a median of 13. Peak antral mast cell density in the FD subjects was $\geq$15/hpf in 71%, $\geq$20/hpf in 40%, and $\geq$30/hpf in 4%. The average of peak and mean density of mast cells in the gastric and duodenal mucosa of all subjects

Table 1
Original diagnoses of subjects’ endoscopic biopsies (N = 114).

	No diagnostic abnormality	Mild Chronic inflammation	Increased mucosal eosinophils	Acute Inflammation	Increased Intraepithelial lymphocytes
Gastric Mucosa	59 (51.8%)	47 (41.2%)	8 (7%)	2 (1.8%)	None
Duodenal Mucosa	98 (86%)	5 (4.4%)	7 (6.1%)	3 (2.5%)	1 (0.9%)