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Identifying Mast Cells in Gastrointestinal Biopsies in Pediatric Irritable Bowel Patients

*Syed Ahsan Rizvi, †Chukwuemeka Oriala, *Jessina Thomas, †Shuan Li, and *Yamen Smadi

ABSTRACT

Objectives: Mast cells (MCs) have been proposed to be involved in the pathophysiology of irritable bowel syndrome (IBS). Nonetheless, the quantity and distribution of MCs in the gastrointestinal tract of pediatric patients with IBS are not well defined. This study aimed to compare the number of MCs in children with and without IBS and to establish histopathological reference values in pediatrics.
Methods: Forty-nine participants with IBS were prospectively enrolled and classified into IBS with atopy (n = 29) and IBS without atopy (n = 20). As our retrospective control group, we selected 42 individuals with a history of polyposis syndrome or gastroesophageal reflux disease with normal histopathology. Retrospective selection of the control cohort was performed in a manner similar to previously published adult and pediatric studies. MCs were prospectively stained immunohistochemically on specimens from the stomach, duodenum, terminal ileum, and descending colon of both groups.
Results: The IBS group showed significantly more MCs per high-power field (MCs/HPF) in the stomach, duodenum, terminal ileum, and descending colon ($P < 0.001$), irrespective of their atopic status. Optimal MC cutoff values for IBS are ≥20.5 MCs/HPF in the stomach (area under the curve [AUC] = 0.84); ≥23.0 MCs/HPF in the duodenum (AUC = 0.79); ≥33.5 MCs/HPF in the terminal ileum (AUC = 0.82); and ≥22.5 MCs/HPF in the descending colon (AUC = 0.86).
Conclusions: Pediatric patients with IBS showed increased numbers of MCs in the stomach, duodenum, terminal ileum, and descending colon when compared with controls. Further trials are needed to explain the role of MCs in pediatric IBS, which might facilitate the development of targeted therapeutic interventions.

Key Words: irritable bowel syndrome, irritable bowel syndrome with constipation, irritable bowel syndrome with constipation and diarrhea mixed, irritable bowel syndrome with diarrhea, irritable bowel syndrome not otherwise specified, mast cell, MCs per high-powered field

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What Is Known

- Irritable bowel syndrome (IBS) is a disorder of the gut-brain axis with a reported global prevalence of 13.8% in children and adolescents.
- One of the common hypotheses on the pathophysiology of IBS is that persistent low-grade immune activation and neuroimmune interactions within the colonic mucosa lead to sensorimotor dysfunction and symptoms.
- According to meta-analyses, adults with IBS have an increased number of mast cells in the ileum, rectosigmoid, and descending colon with no significant increase in the duodenum or jejunum.

What Is New

- In children with IBS, the number of mast cells (MCs) is significantly increased throughout the GI tract, including the stomach, duodenum, terminal ileum, and descending colon, irrespective of co-existing atopy.
- Optimal MCs/HPF cutoff values for IBS are ≥ 20.5, ≥ 23.0, ≥ 33.5, and ≥ 22.5 in the stomach, duodenum, terminal ileum, and descending colon, respectively.
- Identifying pathologic amounts or frequencies of MCs in pediatric IBS patients may help in diagnosis and therapeutic interventions.

Irritable bowel syndrome (IBS) is a functional disorder of the brain-gut axis that causes frequent symptoms of abdominal pain, and bowel disturbances including diarrhea or constipation, and bloating. IBS has a substantial impact on the daily activities (1–3), education (2–4), and health-related quality of life (5,6) of affected children.

Mast cells (MCs) are best known for their activity in allergic responses, immunoregulation, innate immune response, angiogenesis, wound healing, and tissue remodeling (7). They are distributed throughout the gastrointestinal tract, where they regulate intestinal epithelial and endothelial function, gastrointestinal secretion, intestinal motility, absorption, and contribute to host defenses against pathogens (7–9).

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From the *Center for Digestive Health and Nutrition, Arnold Palmer Hospital for Children, Orlando, FL, and the †Department of Pathology, Orlando Health, Orlando, FL.
Address correspondence and reprint requests to Syed Ahsan Rizvi, MD, Center for Digestive Health and Nutrition, Arnold Palmer Hospital for Children, Orlando Health, 60 W Gore Street, FL 32806 (e-mail: ahsan.rizvi@orlandohealth.com).
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Many studies of adults with IBS have reported increased numbers of MCs in mucosal biopsies (10–14). A recent meta-analysis of mucosal biopsies from 344 patients with IBS and 229 healthy controls revealed significantly higher numbers of MCs in the ileum of IBS patients but no significant differences in the duodenum or jejunum (15). Another meta-analysis in adults, including 22 studies on 706 IBS patients and 401 controls, showed an increased number of MCs in the rectosigmoid and the descending colon (16). The association between MCs and IBS is found not only in adults but also in children. Increased numbers of MCs have been identified in the terminal ileum and the colon of pediatric patients with IBS (17). More specifically, increased nerve growth factor (NGF) produced by MCs, has been reported in the rectal mucosa of pediatric patients with diarrhea-predominant IBS, potentially contributing to the development of visceral hypersensitivity (18). These studies demonstrated a greater density and activity of MCs in the small and large intestines of IBS patients, but their generalizability has been limited due to small sample sizes and methodology variations (17,18).

Our primary goal was to quantify the number of MCs per high-powered field (MCs/HPF) using immunohistochemical staining on tissue biopsies from children newly diagnosed with IBS based on the Rome IV criteria (19,20), and compare the number of MCs/HPF to those in control subjects who did not meet this diagnostic criterion. A secondary objective was to establish normative histopathological MC reference values for the pediatric gastrointestinal (GI) tract and cutoff values for IBS.

MATERIALS AND METHODS

Study Design

Our single-center study included both prospective and retrospective phases. Children between the ages of 8 and 21 who underwent an upper and/or lower endoscopy between January 2020 and August 2021 for chronic abdominal pain with normal histology and met the pediatric ROME IV criteria for IBS (19,20) were prospectively enrolled in the study.

Retrospective selection of the control cohort was performed in a manner similar to previously published adult and pediatric studies (13,16–18,21–23). Controls were children aged 8 to 21 years old with a known diagnosis of polyposis syndrome, rectal bleeding, and/or gastroesophageal reflux who had previously undergone upper and/or lower endoscopies between January 2017 and September 2021 and had normal histology.

The study was approved by the Institutional Review Board at Orlando Health Arnold Palmer Hospital for Children, Orlando, FL.

Study Population

Study Group: Children With IBS

Participants aged 8–21 years with IBS based on ROME IV criteria were included (19,20). Our IBS study group was subdivided into IBS with atopy (IBS + atopy) or without atopy (IBS – atopy). Participants with IBS were further classified into subtypes based on predominant stool pattern: abdominal pain associated with constipation (IBS-C), abdominal pain associated with diarrhea (IBS-D), abdominal pain associated with constipation and diarrhea mixed (IBS-M), and abdominal pain not otherwise specified (IBS-U) (3,19,20).

Subjects were excluded from the study if they had an acute illness (eg, appendicitis, gastroenteritis, pneumonia, etc) or a chronic disease, such as cancer, inflammatory bowel disease (IBD), celiac disease, metabolic disease, *Helicobacter Pylori*, eosinophilic gastroenteritis disease, and eosinophilic esophagitis. Nonsteroidal anti-inflammatory medicines (NSAIDs) and other anti-inflammatory treatments, such as MC stabilizers, immunosuppressants, and steroids, were not used by any of the participants. Patients with prolonged use of antibiotics (>2 weeks) were excluded from the study.

Control Group: Children Without IBS

Due to limited surveillance endoscopy in pediatrics compared to adults, it was challenging to choose a control group to evaluate normal MC distribution. Similar to existing adult and pediatric literature (13,16–18,21–23), we selected patients who underwent upper and/or lower endoscopy performed for reasons other than abdominal pain and changes in bowel habits. Our control group included normal histopathological specimens from participants aged 8–21 years with known diagnoses of colorectal polyps and polyposis syndromes, including familial adenomatous polyposis syndrome, MUTYH-associated polyposis syndrome (MAP), Peutz-Jegher syndrome, familial juvenile polyposis, and PTEN hamartomatous tumor syndromes. The control group also included patients with gastroesophageal reflux disease or reflux esophagitis in the absence of abdominal pain syndrome and bowel habit abnormalities, who had normal histological findings of the stomach and duodenum. There was no evidence of acute or chronic inflammation on microscopic examination of any of the specimens utilized in our control group. None of the control group participants met the Rome IV criteria for functional abdominal pain disorder, specifically IBS.

Clinical Data and Symptom Questionnaire

All the participants' electronic medical records (EMRs) were searched for relevant patient information, such as demographics, medical history, presenting symptoms, laboratory evaluation, and history of eczema, allergic rhinitis, environmental allergies, asthma, and/or food allergies.

The ROME IV Diagnostic Questionnaire was given to all prospective subjects with chronic abdominal pain to determine if they met Rome IV criteria for pediatric IBS (20). Children aged 10 years and older completed the questionnaire independently, whereas caregivers assisted children aged 8–9 years. Subjects and/or caregivers also utilized the Bristol stool form scale (BSFS) (24) to evaluate stool appearance. Stool types 1 or 2 were deemed indicative of constipation, while stool types 6 or 7 were deemed indicative of diarrhea (24). Caregivers and/or study subjects also reported symptom severity using a 10-point scale ranging from 0 (does not bother me) to 10 (most bothersome) of the eight most common GI symptoms: difficulty passing stools, hard stools, loose stools, abdominal pain, vomiting, nausea, early satiety, and inability to finish meals (Figure 1, Supplemental Digital Content, *http://links.lww.com/MPG/C902*).

Histology

MCs in the stomach, duodenum, terminal ileum, and descending colon were identified using routine hematoxylin and eosin (H&E) and immunohistochemical tryptase staining on biopsy samples from both groups (IBS and control). Four micron-thick sections of formalin-fixed, paraffin-embedded tissue (FFPET) blocks were mounted on SuperFrost Plus slides (Cardinal Health) and labeled with a unique barcode containing all protocol information (Ventana BenchMark, Roche). This automated system includes user-defined de-paraffinization and antigen retrieval before commencing antibody staining. Prediluted dispensers of the ultraView Universal DAB Detection Kit (Roche) provided all the reagents required for staining. The primary antibody (Clone G3) was mouse antihuman MC tryptase and the counterstain and postcounterstain were hematoxylin (cat #760-2021) and bluing reagent (cat #760-2037).

MC Quantification

The maximum density of tryptase-positive cells in the lamina propria and submucosal layers were manually counted as MCs/HPF and determined by analyzing five randomized microscopy fields. Positivity was defined as distinct immunoreactivity for tryptase with a clear detectable nucleus and characteristic cytoplasm. Artifacts and reactivity that did not meet all these criteria were considered nonspecific. All slides were reviewed by a pathologist (S.L.) with expertise in gastrointestinal pathology.

Statistical Analysis

Descriptive statistics were performed and are reported as mean (± SD), frequency, and percentage. The Chi-square, Fisher exact, independent t-tests, Mann-Whitney U test, and Pearson r were all used in the statistical analysis. In particular, Pearson's rank test was used to evaluate the correlation between gastrointestinal symptom scores and MCs, while Fisher's exact test was used to analyze the dichotomous data. The predictive probability of using MCs to predict IBS, cutoff values, and sensitivity and specificity were determined by constructing receiver operating characteristic (ROC) curves and calculating the area under the curve (AUC) [0.5–1.0]. Statistical significance was defined as $P < 0.05$ with a confidence interval (CI) of 95%. SPSS version 25.0 (IBM) was used for all statistical analysis.

RESULTS

Study Population and Characteristics

A total of 49 subjects with IBS were enrolled (mean age of 14.18 ± 2.96 SD years; range 8–18 years; 71% female; 55% White/not Hispanic or Latino). Twenty-four participants had a colonoscopy with biopsies taken from the descending colon, but one patient's terminal ileum was not intubated.

A total of 42 control subjects were selected, ranging from 8 to 21 years ($P = 0.65$), with a mean age of 14.07 ± 4.08 SD years. Forty-five percent were female ($P = 0.018$) and 61% were White/not Hispanic or Latino ($P = 0.51$). Thirty-two biopsies were obtained from the stomach and duodenum, 24 from the terminal ileum, and 29 from the descending colon. Demographic and clinical characteristics are shown in Table 1.

To control for atopic disorders, the IBS study group was stratified into IBS with atopy (IBS + atopy) (n = 29) and IBS without atopy (IBS – atopy) (n = 20). Overall, the prevalence of atopic disorders in the IBS group was 59% compared to 39% in the control group ($P = 0.75$) (Table 1).

MC Counts by Diagnosis:

IBS and the Control Group

All four GI tract locations in the IBS group had a higher number of MCs compared to the control group: stomach (28.12 ± 12.19 MCs/HPF vs 16.06 ± 4.4 MCs/HPF; $P < 0.001$); duodenum (34.33 ± 15.94 MCs/HPF vs 19.5 ± 8.31 MCs/HPF; $P < 0.001$); terminal ileum (45.13 ± 22.50 MCs/HPF vs 23.50 ± 8.22 MCs/HPF; $P < 0.001$); and descending colon (33.46 ± 12.88 MCs/HPF vs 18.62 ± 7.39 MCs/HPF; $P < 0.001$), respectively (Table 2). Figure 1 shows photomicrographs of tryptase staining in the duodenum, terminal ileum, stomach, and descending colon.

To evaluate the diagnostic accuracy of MC counts, we plotted ROC curves to assess the sensitivity and specificity of the proposed cutoff values for IBS. Optimal MC cutoff values for IBS are ≥20.5 MCs/HPF in the stomach (sensitivity 74.1%; specificity of 75.9%; 95% CI [0.74–0.94]; AUC of 0.84; $P < 0.001$); ≥23.0 MCs/HPF in the duodenum (sensitivity 72.9%; specificity of 71.8%; 95% CI [0.69–0.89]; AUC of 0.79; $P < 0.001$); ≥33.5 MCs/HPF in the terminal ileum (sensitivity 72.7%; specificity of 91.7%; 95%CI [0.69–0.95]; AUC of 0.82; $P < 0.001$); and ≥22.5 MCs/HPF in the descending colon (sensitivity 79.1%; specificity of 79.3%; CI [0.75–0.96]; AUC of 0.86; $P < 0.001$) (Table 3).

IBS With and Without Atopy and IBS Subtypes

Subanalysis of MCs in the presence or absence of atopy did not significantly differ in the stomach ($P = 0.95$), duodenum ($P = 0.93$), terminal ileum ($P = 0.36$), and descending colon ($P = 0.54$) (Table 2, Supplemental Digital Content, *http://links.lww.com/MPG/C902*). In addition, there were no significant differences in MC counts between the subtypes of IBS based on the predominant stool pattern.

TABLE 1. Demographic, clinical, and laboratory variables for the IBS and control groups

	IBS group (n = 49)	Control group (n = 42)	*P* value
Mean age, y (± SD)	14.18 ± 2.96	14.07 ± 4.08	0.65
Female no. (%)	35 (71.4)	19 (45.2)	0.018
BMI (kg/m^2)	23.30 ± 9.34	22.85 ± 7.09	0.67
Race/ethnicity no. (%)			
White	27 (55.1)	26 (61.9)	0.51
Hispanic	12 (24.5)	10 (23.8)	
Black	2 (4.1)	1 (2.4)	
Other/unknown	8 (16.3)	5 (11.9)	
Medical history no. (%)			
Atopic disorders	29 (59.2)	17 (40.5)	0.75
Asthma	20 (68.9)	7 (41.2)	
Food allergy	8 (27.6)	2 (11.7)	
Allergic rhinitis	13 (44.8)	6 (35.3)	
Eczema	1 (3.4)	1 (5.8)	
Environmental and seasonal allergies	6 (20.7)	8 (47.1)	

IBS = irritable bowel syndrome.

TABLE 2. The difference in average MCs/HPF between the IBS and the control cohorts

Location	IBS group (MCs/HPF)	Control group	*P* value
Stomach	28.12 ± 12.19 (n = 49)	16.06 ± 4.4 (n = 32)	<0.001
Duodenum	34.33 ± 15.94 (n = 49)	19.5 ± 8.31 (n=32)	<0.001
Terminal ileum	45.13 ± 22.50 (n = 23)	23.50 ± 8.22 (n = 24)	<0.001
Descending colon	33.46 ± 12.88 (n = 24)	18.62 ± 7.39 (n = 29)	<0.001

IBS = irritable bowel syndrome; MCs/HPF = mast cells per high-power field.

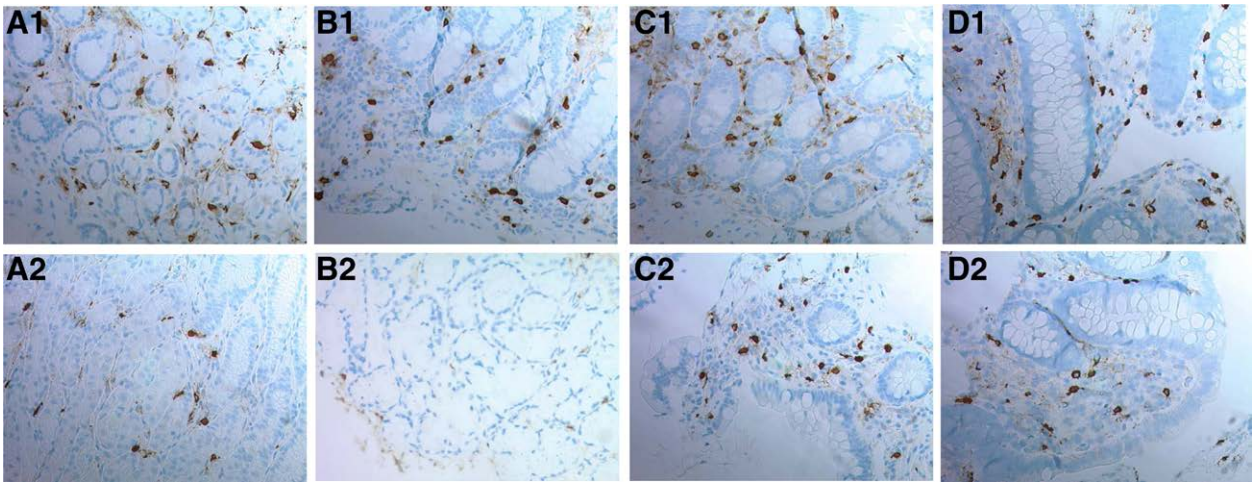

FIGURE 1. Photomicrographs of tryptase staining in the stomach, duodenum, terminal ileum, and descending colon. All images are ×400 original magnification. (A1) Tryptase staining in IBS stomach. (A2) Tryptase staining in stomach control. (B1) Tryptase staining in IBS duodenum. (B2) Tryptase staining in duodenum control. (C1). Tryptase staining in IBS terminal ileum. (C2) Tryptase staining in terminal ileum control. (D1) Tryptase staining in IBS descending colon. (D2) Tryptase staining in descending colon control. IBS = irritable bowel syndrome.

TABLE 3. Receiver operating characteristic curve values of affected IBS and control patients

Location	Cutoff values	Sensitivity, %	Specificity, %	Area under the curve	95% CI	*P* value
Stomach	>20.5	74.1	75.9	0.84	0.74–0.94	<0.001
Duodenum	>23.0	72.9	71.8	0.79	0.69–0.89	<0.001
Terminal ileum	>33.5	72.7	91.7	0.82	0.69–0.95	<0.001
Descending colon	>22.5	79.1	79.3	0.86	0.75–0.96	<0.001

IBS = irritable bowel syndrome.

Correlation of MCs and Symptom Severity

All subjects in the study group experienced abdominal pain, with a mean subjective severity score of 7.13 of 10, and 71.4% (35/49) experienced nausea and/or constipation. The remaining symptoms (difficulty passing stools, hard stools, loose stools, vomiting, early satiety, and not being able to finish meals) had a mean subjective severity score of 3–5 of 10. Common symptoms between our IBS subgroups (IBS + atopy and IBS − atopy) are illustrated in Table 3, Supplemental Digital Content, *http://links.lww.com/MPG/C902*.

MC numbers were observed to be moderately correlated with the subjective abdominal pain severity score (n = 49, $r = 0.614$ and $r^2 = 0.377$, $P < 0.001$); early satiety (n = 49, $r = 0.659$ and $r^2 = 0.434$, $P < 0.001$); and difficulty passing stools (n = 49, $r = 0.600$ and $r^2 = 0.36$, $P < 0.001$). There was a moderate correlation between MC counts and subjective severity scores for both vomiting (n = 49, $r = 0.524$ and $r^2 = 0.274$, $P < 0.001$) and loose stools (n = 49, $r = 0.475$ and $r^2 = 0.225$, $P < 0.001$).

DISCUSSION

IBS is the most common functional GI disorder involving the gut-brain axis that has a multifactorial etiology and significant morbidity (20). The complex interplay between the immune system, low-grade inflammation, and intestinal epithelial permeability mediated by MCs has been proposed to contribute to its pathogenesis (9,25).

This is the largest study examining the distribution of epithelial MCs in the stomach, duodenum, terminal ileum, and descending colon of pediatric IBS patients. To determine the normal reference ranges of MCs in the GI tract, we also examined MC distribution in individuals with reflux disease and polyposis syndromes with

normal histology. Compared to the control group, we observed a statistically significant increase in the number of MCs in each region of the IBS group, with the highest number of MCs/HPF in the terminal ileum. Among the subtypes of pediatric IBS (IBS-C, IBS-D, IBS-U, and IBS-M), no significant difference in the number of MCs was noted based on predominant stool pattern. However, the sample size may not have been large enough to show the difference. Intriguingly, we found that MCs are increased in pediatric IBS irrespective of their atopic status. In our study, most IBS patients reported increased abdominal pain with a mean subjective severity score of 7.13 (range 0 to 10), which was moderately correlated with the total MC count. These results are consistent with prior research demonstrating a correlation between symptoms and MC counts (13,14). Apart from chronic abdominal pain, IBS with atopy and IBS without atopy had similar symptoms (Table 3, Supplemental Digital Content, *http://links.lww.com/MPG/C902*). Furthermore, our results are similar to those of studies in other pediatric populations that have shown a greater density and activity of MCs in patients with IBS compared with controls (17,18). Food-related IgE and non-IgE mechanisms, altered gut microbiota, and intestinal dysmotility are all possible contributors to the increased prevalence of MCs in IBS (26–28).

Our utilization of having subjects with polyps or reflux as our control cohort without any evidence of chronic abdominal pain, changes in bowel habits, and histological inflammation are not unique as these criteria have been used for control cohorts previously in both adult and pediatric literature (16,17,22,23). For instance, Jakate et al selected 50 subjects with non-diarrheal conditions (such as duodenal biopsies for gastroesophageal reflux, *Helicobacter gastritis*, gastric fundic gland polyps, and mucosa adjacent to duodenal adenomatous polyps; and colonic biopsies for melanosis and mucosa adjacent to adenomatous polyps) as their control group. Similar to our results, the mean distribution of MCs in their control group was 13.3 ± 3.5 cells per high-power field (colon, 13.6 ± 3.1 cells; and duodenum, 13.2 ± 3.7 cells) (23). There may be a difference in the normal distribution of MCs between adults and children due to differences in age, dietary habits, and the gut microbiome. Our study provides normative reference values for the distribution of MCs in the gastrointestinal tract (Table 3) in children, which may be useful for diagnosing pathological conditions. However, larger studies are required to determine the effect of age, gender, dietary habits, ethnicity, geographic location, and socioeconomic status on the distribution of MCs in the intestinal tract.

We recognize several limitations in our study, including its single-center design. Due to difficulty in obtaining adequate tissue samples from previous pediatric endoscopies, the study is limited by a small control population. However, our overall sample size was greater than that of previous pediatric studies (17,18). Due to the increased prevalence of IBS in females (29), our IBS group contained significantly more females than the control group. Nevertheless, further analysis revealed no sex differences in MCs in our IBS cohort. Further studies are needed to show an equal distribution of females and men for more representative results. Another pitfall of our study is that not all patients had an equal number of intestinal biopsies. We had a greater number of stomach and duodenum biopsy samples compared to the terminal ileum and descending colon because not all participants underwent colonoscopy. Finally, we only investigated MC distribution in the descending colon and did not explore other segments of the colon. Our decision to examine the descending colon for MC infiltrate was based on a previous meta-analysis of 22 studies, including both the pediatric and adult literature, with a total of 706 IBS patients and 401 controls. In most of these studies, Bashashati et al found a statistically significant increase in MCs in the descending colon and rectosigmoid with no difference in the ascending colon between IBS and the control group (20). Future studies are needed to segmentally explore the MC distribution throughout different segments of the colon, including ascending, descending, and rectosigmoid.

CONCLUSIONS

In conclusion, identifying pathologic amounts or frequencies of MCs in pediatric IBS patients may help in diagnosis and therapeutic interventions. Understanding the precise role of MCs in the etiology of pediatric IBS could result in early diagnosis and the development of more targeted therapies including H1 receptor antagonists, H2 receptor antagonists, and MC membrane stabilizers (such as oral cromolyn sodium and ketotifen) to alleviate symptoms in the future.

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