Original Article

Determination of Relative Frequency of Mast Cells and Eosinophils in Gastric Mucosa Biopsies in Children with Recurrent Abdominal Pain and Normal Endoscopy

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Abstract

Background

Numerous disorders can cause recurrent abdominal pain. Many of these cases underwent endoscopy and clinician encounters patients with history of recurrent abdominal pain and normal endoscopy. We evaluated these biopsies with regard to their eosinophils and mast cell densities to find out a potential relationship between recurrent abdominal pain with eosinophils and mast cell densities in children.

Materials and Methods

This is a retrospective cross-sectional study. Fifty eight children with recurrent abdominal pain were evaluated following endoscopy. Two forceps biopsy was taken from each patient and then following routine histological evaluation, eosinophils and mast cells counts were performed. A questionnaire was filled for each patient, including clinical, endoscopic and pathologic findings. Data collected were statistically analyzed using SPSS, version 16.

Results

Fifty eight patients (31 girls and 27 boys) fulfilled the entrance criteria (ages7.19±3.1 years). The mean eosinophils density in 1-6 year-old group was more than 7-12 year-old group(3.52±4.67 vs 1.59±1.9)(p=0.04).The mean mast cells density in 7-12 year-old group was more than 1-6 year-old group(6.48±5.17 vs 3.9±3.14)(p=0.02). In boys the mean eosinophils density was higher than that of girls (3.56±5.07 vs 1.6±1.22) (p=0.05). With regard to microscopic findings, the greatest eosinophilic density belonged to normal histology (2.66±4.13).

Conclusion

Increased gastric eosinophils and mast cell densities are a common finding in children with abdominal pain and normal endoscopy. However, further studies are needed to determine the significance of eosinophils and mast cells activation in the disease process or symptom generation.

Key words

Abdominal pain, Endoscopy, Eosinophils, Basophils.

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Introduction

Numerous disorders can cause recurrent abdominal pain. Many of these cases underwent endoscopy and clinician encounters patients with history of recurrent abdominal pain and normal endoscopy. Abdominal pain is perhaps the most common painful health problem in school-aged children. J Apley, a British pediatrician, studied abdominal pain among children extensively and observed that approximately 10% of school aged children have recurrent episodes of abdominal pain. He named this symptom complex as recurrent abdominal pain(RAP) syndrome and defined it as "at least three episodes of abdominal pain, severe enough to affect their activities over a period longer than three months" (1).

RAP is reported in 10-12% of school aged children in developed countries (1, 2). Epidemiological studies in Asia have reported similar prevalence. Traditionally, 90% of cases of RAP have been considered functional disorders. However, with the advent of new technologies, more recent studies have described an increasing proportion of organic causes (3,8). The most challenging task for the clinician, seeing a child with RAP, is to determine whether a specific organic cause may be present and what the most appropriate investigations should be undertaken (9). Recently, a number of basic studies have identified potential roles for eosinophils and mast cells as important participants in the inflammatory cascade within the gastrointestinal microenvironment. Eosinophils are multifunctional leukocytes implicated in the pathogenesis of numerous inflammatory processes, including parasitic helminthes infections and allergic diseases (10,12) and were described by Paul Ehrlich in 1879(13)The gastrointestinal tract is the main non hematopoietic organ where eosinophils reside in the healthy state. Eosinophils are normally present in the lamina propria, but the number of eosinophils regarded as pathological for various sites along the gastrointestinal tract is debated; the highest concentrations are found in the cecum and appendix. Within the gastrointestinal tract, the esophageal epithelium is unique in being devoid of eosinophils under non inflammatory conditions (14). The gastrointestinal tract is a rich source of mast cells with an enormous surface area that permits a high degree of interaction between the mast cells and intestinal luminal contents. The active metabolic products of the mast cell influence gastrointestinal secretion, absorption, and motility through paracrine effects of local mast cell degranulation and also cause systemic effects through the release of cellular products into the blood stream. There is increasing evidence that mast cell-nerve interactions are critical for the initiation of the disturbance of muscle electrical activity (15).Both eosinophils and mast cells have been implicated in the generation of abdominal pain. Since we evaluated gastric biopsies with regard to their eosinophils and mast cell densities to find out a potential relationship between recurrent abdominal pain with eosinophils and mast cell densities in children.

Materials and Methods

This study was retrospective cross-sectional study performed on children with RAP and normal endoscopy who admitted to endoscopy units at Shahid Sadoughi hospital, Yazd-Iran. Sampling was done by census. Fifty eight children ranging in age from 1to 12 years (mean7.19±3.1 years) were evaluated. There were 27 males and 31 females. Clinically-significant RAP was defined as at least three episodes of upper abdominal pain during the three consecutive months preceding the study that was severe enough to affect their normal activities and required medical attention. All patients were

undergone upper endoscopy for the evaluation of abdominal pain .All patients had normal gross endoscopic examinations, without ulceration, erosions, and nodularities or masses. Endoscopy was performed at endoscopy ward under sedation, using Olympus GIF N30 and XP20 endoscopes. All patients had two grasp mucosal biopsies obtained from gastric mucosa. Biopsies were fixed in 10% formalin for standard processing and hematoxylin and eosin and Giemsa staining. The specimens were evaluated for abnormal histologic findings and H.Pylori microorganism. In addition a minimum of 5 high power field were evaluated for eosinophils and mast cell counts on gastric biopsies and the sum was calculated for each case. Eosinophils and mast cell counts and histological evaluation were performed by a single observer. Collected data were analysed using SPSS 16. Pearson correlation coefficients were determined between cell types within diagnostic group.

Results

Biopsies revealed chronic superficial gastritis in 2 patients .Chronic non-specific gastritis (CG) consistent with published criteria was defined as a mononuclear infiltrate with reactive epithelial changes and/or mucin depletion. CG was present in 12 patients. Biopsies were otherwise considered normal in 41 cases and H. Pylori chronic gastritis was found in 3 patients. The mean eosinophils density was 2.55 ±3.66 (table I).The mean eosinophils density in 1-6 year-old group was more than 7-12 year-old group(3.52±4.67 vs 1.59±1.9) (p=0.04).According to pv=0.04 it seems that there is a correlation between age and eosinophils density. The mean mast cell density was 5.19 ±4.43 (table II).The mean mast cells density in 7-12 year-old group was more than 1-6 year-old group(6.48±5.17 vs 3.9±3.14)(p=0.02).According to pv=0.02 it is apparent that there is a correlation between mast cell density and age. In boys the mean eosinophils density was more than girls(3.56±5.07 vs 1.6±1.22) Result shows that there is a correlation between sex and eosinophils density(p=0.05).With regard to microscopic findings the greatest eosinophilic density belonged to normal histology(2.66±4.13) and the least eosinophilic density(2±1.41)belonged to chronic superficial gastritis (table III).According to p. value =0.9 there is no correlation between eosinophilic density and mucosal inflammation. On the other hand ,the greatest mast cell density (6±4.77) belonged to normal histology group and the least mast cell (2±1)density belonged to H.Pylori gastritis (table III).According to pv=0.1,there is no correlation between mast cell density and mucosal inflammation.

Table I: The frequency of eosinophil distribution in the evaluated specimens.

Eosinophil	count	percent
0	10	17.2
1	19	32.8
2	12	20.7
3	6	10.3
4	4	6.9
5	3	5.2
9	2	3.4
19	2	3.4
sum	58	100

This table shows that the mean eosinophils density is 2.55 ±3.66.

Table II:The frequency of mast cell distribution in the evaluated specimens

Mast cell	count	percent
0	6	10.3
1	8	13.8
2	7	12.1
3	9	15.5
4	2	3.4
5	2	3.4
6	5	8.6
7	3	5.2
9	1	1.7
10	8	13.8
11	1	1.7
12	1	1.7
13	2	3.4
15	3	5.2
sum	58	100

This table shows that the mean mast cell density was 5.19 ±4.43.

Table III: The mean eosinophil and mean mast cells counts in the evaluated specimens according to microscopic findings

Microscopic findings		number	SD	max	min	Mean eosinophil count	Mean mast cell count
normal	Mean eosinophil count	41	4.13	19	19	2.66	6
	Mean mast cell count	41	4.77	15	15		
Chronic non-specific gastritis	Mean eosinophil count	12	2.57	9	9	2.33	3.50
	Mean mast cell count	12	3.14	11	11		
H.Pylori gastritis	Mean eosinophil count	3	1.52	4	4	2.33	2
	Mean mast cell count	3	1	3	3		
Chronic superficial gastritis	Mean eosinophil count	2	1.41	3	3	2	3.50
	Mean mast cell count	2	2.12	5	5		

Sum	Mean eosinophil count	58	3.66	19	19	2.55	5.19
	Mean mast cell count	58	4.43	15	15		

Shows that the greatest eosinophilic density belongs to normal histology (2.66±4.13) and the least eosinophilic density (2±1.41)belongs to chronic superficial gastritis (table I).According to p. value =0.9 there is no correlation between eosinophilic density and mucosal inflammation.On the otherhand the greatest mast cell density (6±4.77) belongs to normal histology group and the least mast cell (2±1)density belonged to H.Pylori gastritis .According to pv=0.1,there is no correlation between mast cell density and mucosal inflammation.

Discussion

Recurrent abdominal pain is a common complaint among school-age children that affects up to 15% at any given time. It represents the most common chronic pain entity in paediatric patients. These patients frequently are found to have dyspepsia defined as upper abdominal pain or discomfort. (16).Until a decade ago 'functional gastrointestinal disorder' was a label used for the conditions with uncertain etiology, and was a diagnosis of exclusion .When Rome criteria were defined to diagnose functional gastrointestinal disorders (FGID), it became an important positive diagnosis. According to Rome II criteria, abdominal pain in children related to conditions were classified into five categories; functional dyspepsia, irritable bowel syndrome, abdominal migraine, aerophagia and functional abdominal pain (16). Even though functional bowel diseases are considered as a cause of RAP in children (9). so far, very few studies have been done to detect their prevalence among affected children (17,18,19) .With the advent of paediatric endoscopy and motility studies, more studies have reported organic causes of RAP(3,8). Hyams and Hyman identified certain clinical signs (such as night pain, weight loss, hematemesis, haematochezia, fever, arthritis, delayed puberty, and family history of inflammatory bowel diseases) that should alert the physician to a likely organic cause of RAP (20). Inflammation of the upper gastrointestinal tract may cause dyspepsia and inflammation has been implicated in the development of functional gastrointestinal disorders (21) .Among the inflammatory cells, mast cells and eosinophils are especially important. An increase in mucosal mast cells has been reported with dyspepsia and irritable bowel syndrome (22,23,24). Duodenal eosinophilia has been associated with functional dyspepsia in adults (25). Eosinophils and mast cells communicate with T lymphocytes in the gut mucosa (26). Eosinophils may be activated by a variety of cytokines and inflammatory mediators which include (but are not limited to) platelet activating factor, anaphylatoxins (e.g., C5a), RANTES (regulated on activation normal T cell expressed), MCP (monocyte chemotactic protein)-2, MCP-3, MCP-4, and eotaxin (27). Activation of eosinophils may be associated with gastric dysmotility. Abnormalities in gastric emptying and electrogastrography have been reported with gastrointestinal allergy (28, 29). The eosinophils, eotaxin, and Th-2 cytokines are important in pathogenesis of this disease entity. Recently, a number of basic studies have identified potential roles for eosinophils as important participants in the inflammatory cascade within the gastrointestinal microenvironment. Mast cells have been shown to be increased in IBS in the jejunum (30) ileum (31) and cecum (24) with close proximity to enteric nerves in colonic mucosa (32). Others have

observed mast cell degranulation in both IBS and health (33). There is increasing evidence that mast cell-nerve interactions are critical for the initiation of the disturbance of muscle electrical activity (15) .Animal studies have demonstrated that activated mast cells release mediators that increase excitability of enteric neurons leading to abnormal gut sensory and motor function (34,35). There is only little data in literature concerning the number of eosinophils and mast cells in gastric mucosa of children with gastrointestinal complaints and existing data have rather conflicting results. Lowichik and Weinberg (36) reported frequent eosinophilia on duodenal specimens from autopsies with counts > 20/hpf in 18% of specimens. It is somewhat difficult to interpret those findings as patients could not be interviewed for current gastrointestinal symptoms or followed to see if biopsy findings predisposed to symptoms. Kokkonen et al (37) has defined mucosal eosinophilia of 6-10 eosinophils/hpf defined as mildly increased and >10/hpf as highly increased. PF Whitington and GL Whitington reported <10 eosinophils/hpf in all of 180 mucosal biopsies from control patients (38). There is also little data in literature concerning the number and role of mast cells in children complain of RAP. The purposes of this study were to determine the density of gastric eosinophils and mast cells in pediatric dyspepsia. In this study, we evaluated biopsy specimens of gastric mucosa collected from pediatric patients who had RAP and normal endoscopy with regard to their eosinophils and mast cell densities to find a potential relationship between recurrent abdominal pain and eosinophils and mast cell densities. The current study has this strength that it evaluates both mast cells and eosinophils densities and we were unable to find any similar study in the literature. The mean eosinophils density was 2.55 ± 3.66. The mean mast cell density was 5.19 ±4.43. These figures are less than previously reported studies. Tissue eosinophilia is a central feature of major chronic diseases involving airway and gastrointestinal mucosa. Local eosinophilia has also been used increasingly to diagnose major allergic and inflammatory diseases. Eosinophil granules contain cytotoxic proteins which, when released in the tissue by degranulation, cause injury and pathophysiological effects. (39). The degree of degranualtion may be a better indicator of the disease process rather than density. We were unable to evaluate the eosinophilic activation. In some investigations, researchers have found evidence of moderate to extensive eosinophils degranulation even in biopsies of dyspeptic children with normal mucosal eosinophil densities (40). In addition, there is only little data in literature concerning the number and role of mast cells in children with gasterointestinal complaints and existing data have rather conflicting results. In one study from Iran (41), the authors have evaluated biopsy specimens of gastric mucosa collected from paediatric patients who had normal histology except increase in mast cells and they proposed the term "mast cell gastritis" as a new and different term to be included in classification and grading of gastritis, in cases with normal histology but mast cell count above 30/25 mm^2. As noted in our study, the greatest eosinophils and mast cell densities were seen in group with normal histology and this shows that in this group increased eosinophils and mast cells or their activation may be critical in abdominal pain generation. In conclusion, increased gastric eosinophils and mast cell densities is a common finding in children with abdominal pain and normal endoscopy and histology.

Conclusion

Future and further studies are needed to determine the significance of eosinophils

and mast cells activation in the disease process or symptom generation. Electro-microscope may have value as a modality to study neuroimmune interactions in children with abdominal pain. We hypothesize that a gastric biopsy with increased eosinophils and mast cell counts is helpful in the diagnosis of RAP. Our results suggest that mast cell hyperplasia and eosinophilia characterizes functional dyspepsia, but further study is needed to confirm these novel observations.

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Conflict of interest

The authors have no conflict of interest.

Reference

1-APLEY J, NAISH N. Recurrent abdominal pains: a field survey of 1,000 school children. Arch Dis Child. 1958 ; 33(168):165-70.

2-Huang RC, Plamer LJ, Forbes DA. Prevalence and pattern of childhood abdominal pain in an Australian general practice. J Paediatr Child Health.2000; 36(4):349-53.

3-Ament ME, Christie DL. Upper gastrointestinal fiberoptic endoscopy in pediatric patients. Gastroenterology 1977; 72(6):1244-8.

4-Mavromichalis I, Zaramboukas T, Richman PI, Slavin G. Recurrent abdominal pain of gastro-intestinal origin. Eur J Pediatr 1992; 151(8):560-3.

5-Roma E, Panayiotou J, Kafritsa Y, Van-Vliet C, Gianoulia A, Constantopoulos A.Upper gastrointestinal disease, Helicobacter pylori and recurrent abdominal pain. Acta Paediatr. 1999; 88(6):598-601.

6-Størdal K, Nygaard EA, Bentsen B. Organic abnormalities in recurrent abdominal pain in children. Acta Paediatr. 2001;90(6):638-42.

7-Di Lorenzo C, Hyman PE, Flores AF, Kashyap P, Tomomasa T, Lo S, et al. Antroduodenal manometry in children and adults with severe non-ulcer dyspepsia. Scand J Gastroenterol. 1994;29(9):799-806.

8-Cucchiara S, Riezzo G, Minella R, Pezzolla F, Giorgio I, Auricchio S. Electrogastrography in non-ulcer dyspepsia. Arch Dis Child. 1992;67(5):613-7.

9-Thiessen PN. Recurrent abdominal pain. Pediatr Rev. 2002;23(2):39-46.

10-Gleich GJ, Loegering DA. Immunobiology of eosinophils. Annu Rev Immunol.1984;2:429-59.

11-Weller PF. Eosinophils: structure and functions. Curr Opin Immunol. 1994;6(1):85-90.

12-Rothenberg ME. Eosinophilia. N Engl J Med 1998 28;338(22):1592–600.

13-Jay V. Paul Ehrlich. Arch Pathol Lab Med. 2001;125(6):724-5.

14-Straumann A, Simon HU. The physiological and pathophysiological roles of eosinophils in the gastrointestinal tract. Allergy. 2004;59(1):15-25.

15-Wood JD, Alpers DH, Andrews PL. Fundamentals of neurogastroenterology. Gut.1999;45 Suppl 2:II6-II16.

16-Rasquin-Weber A, Hyman PE, Cucchiara S, Fleisher DR, Hyams JS, Milla PJ,et al. Childhood functional gastrointestinal disorders. Gut. 1999;45 Suppl 2:II60-8.

17-Devanarayana NM, de Silva DG, de Silva HJ. Aetiology of recurrent abdominal pain in a cohort of Sri Lankan children. J Paediatr Child Health. 2008;44(4):195-200.

18-Walker LS, Lipani TA, Greene JW, Caines K,Stutts J, Polk DB, et al. Recurrent abdominal pain: symptom subtypes based on the Rome II Criteria for pediatric functional gastrointestinal disorders. J Pediatr Gastroenterol Nutr. 2004;38(2):187-91.

19-Schurman JV, Friesen CA, Danda CE, Andre L,Welchert E, Lavenbarg T, *et al*. Diagnosing functional abdominal pain with the Rome II criteria:parent, child, and clinician agreement. J Pediatr Gastroenterol Nutr. 2005 ;41(3):291-5.

20-Hyams JS, Hyman PE. Recurrent abdominal pain and the biopsychosocial model of medical practice. J Pediatr. 1998;133(4):473-8.

21-Collins SM. The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. Gastroenterology. 1996 ;111(6):1683-99.

22-Matter SE, Bhatia PS, Miner PB Jr. Evaluation of antral mast cells in nonulcer dyspepsia. Dig Dis Sci. 1990;35(11):1358-63.

23-Weston AP, Biddle WL, Bhatia PS, Miner PB Jr. Terminal ileal mucosal mast cells in irritable bowel syndrome. Dig Dis Sci. 1993;38(9):1590-5.

24-O'Sullivan M, Clayton N, Breslin NP, Harman I, Bountra C, McLaren A, O'Morain CA. Increased mast cells in the irritable bowel syndrome. Neurogastroenterol Motil. 2000;12(5):449-57.

25-Talley NJ, Walker MM, Aro P, Ronkainen J, Storskrubb T, Hindley LA, Harmsen WS, Zinsmeister AR, Agréus L. Non-ulcer dyspepsia and duodenal eosinophilia: an adult endoscopic population-based case-control study. Clin Gastroenterol Hepatol.2007;5(10):1175-83.

26-Moqbel R, Coughlin JJ. Differential secretion of cytokines. Sci STKE. 2006 6;2006(338):pe26.
27-Elsner J, Kapp A. Activation of human eosinophils by chemokines. Chem Immunol 2000; 76:177-207.
28-Ravelli AM, Tobanelli P, Volpi S, Ugazio AG. Vomiting and gastric motility in infants with cow's milk allergy. J Pediatr Gastroenterol Nutr. 2001 ;32(1):59-64.
29-Sabra A, Bellanti JA. Delayed gastric emptying, gastroesophageal reflux, and dyspeptic symptoms: The pathogenetic role of food allergy. Ped Gastroenterol Nutr 2000; 31:S206.
30-Guilarte M, Santos J, de Torres I, Alonso C, Vicario M, Ramos L,et al. Diarrhoea-predominant IBS patients show mast cell activation and hyperplasia in the jejunum. Gut. 2007;56(2):203-9.
31-Weston AP, Biddle WL, Bhatia PS, Miner PB Jr. Terminal ileal mucosal mast cells in irritable bowel syndrome. Dig Dis Sci. 1993;38(9):1590-5.
32-Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology. 2004; 126(3):693-702.
33-Talley NJ, Butterfield JH. Mast cell infiltration and degranulation in colonic
mucosa in the irritable bowel syndrome. Am J Gastroenterol. 1996;91(8):1675-6.
34-Liu S, Hu HZ, Gao N, Gao C, Wang G, Wang X,et al. Neuroimmune interactions in guinea pig stomach and small intestine. Am J Physiol Gastrointest Liver Physiol. 2003;284(1):G154-64.
35-Reed DE, Barajas-Lopez C, Cottrell G, Velazquez-Rocha S, Dery O, Grady EF,et al. Mast cell tryptase and proteinase-activated receptor 2 induce hyperexcitability of guinea-pig submucosal neurons. J Physiol. 2003 1;547(Pt 2):531-42.
36-Lowichik A, Weinberg AG. A quantitative evaluation of mucosal eosinophils in the pediatric gastrointestinal tract. Mod Pathol. 1996;9(2):110-4.
37-Kokkonen J, Ruuska T, Karttunen TJ, Niinimäki A. Mucosal pathology of the foregut associated with food allergy and recurrent abdominal pains in children.Acta Paediatr. 2001;90(1):16-21.
38-Whitington PF, Whitington GL. Eosinophilic gastroenteropathy in childhood. J Pediatr Gastroenterol Nutr. 1988;7(3):379-85.
39-Erjefält JS, Greiff L, Andersson M, Adelroth E, Jeffery PK, Persson CG. Degranulation patterns of eosinophil granulocytes as determinants of eosinophil driven disease. Thorax. 2001;56(5):341-4.
40-Friesen CA, Andre L, Garola R, Hodge C, Roberts C. Activated duodenal mucosal eosinophils in children with dyspepsia: a pilot transmission electron microscopic study. J Pediatr Gastroenterol Nutr. 2002;35(3):329-33.
41-Mahjoub FE, Farahmand F, Pourpak Z, Asefi H, Amini Z. Mast cell gastritis:children complaining of chronic abdominal pain with histologically normal gastric mucosal biopsies except for increase in mast cells, proposing a new entity. Diagn Pathol. 2009 3;4:34.