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Original Article

Quantitative Evaluation of Mucosal Mast Cells in the Colon in Patients with Irritable Bowel Syndrome

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Abstract

Background: Recent studies have shown that mast cells play an important role in the pathogenesis of irritable bowel syndrome as they release variable mediators which alter enteric nerve and smooth muscle function. The aim of this study was to determine whether mucosal mast cells were increased in the colonic mucosa of IBS patients compared to controls.

Materials and Methods: This case-control study was conducted in the Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University, Dhaka, during the period from April 2016 to March 2017 on cases of IBS and comparison group. We recruited 25 IBS patients and 25 healthy controls for this study. Colonoscopic biopsy was taken from the caecum, ascending colon, descending colon, and rectum. Tissue was stained with Giemsa, and then quantitative evaluation of mast cells was performed. Mast cells counts were compared between the two groups of patients.

Results: Mast cells were significantly higher in the caecum, ascending colon, descending colon, and rectum in all subtypes of IBS patients compared to control (10.40±2.10, 6.76±1.83, 8.08±2.19, and 9.16±2.46 vs. 4.20±1.01, 3.32±0.69, 3.04±0.84, and 3.84±1.07 per HPF, respectively). Among four sites, mast cells were significantly higher in the caecum in IBS patients. Mucosal mast cells were relatively elevated in IBS-D patients compared to IBS-C and IBS-M patients, but this was not statistically significant.

Conclusion: Mast cells were significantly increased in the caecum, ascending colon, descending colon, and rectum of the patients with IBS compared to controls. These findings suggest that mast cells may play an essential role in the pathogenesis of IBS.

Keywords: Mast cell, Irritable bowel syndrome

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Introduction

Irritable bowel syndrome (IBS) is a common gastrointestinal functional disorder in clinical practice. It is characterized by symptoms of abdominal pain, bloating, and change in stool frequency and consistency in the absence of an organic cause, with a relapsing and remitting natural history.1 No specific diagnostic procedures identify IBS, and the diagnosis is dependent on

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symptoms and exclusion of organic causes.2 According to the predominant stool pattern experienced by the patient, the condition is often sub-classified into diarrhea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C), and mixed or alternating bowel habits IBS (IBS-M).1 The prevalence of IBS ranged from 1.1% to 29.2% in the general population[3] and occurred more commonly in women than men.4 In Bangladesh, its prevalence is reported at 20.6% in men and 27.7% in women.5 IBS may present at any age with peak prevalence in the third and fourth decades.6 Although this syndrome is not life-threatening, it leads to considerable morbidity, causing a clear restriction in daily life. Not only by medical consumption but also through indirect cost by sick leave, the economic impact is enormous.7 This economic burden adds to the importance of accurately diagnosing and managing IBS in both primary and secondary care.

The pathophysiological mechanism of IBS is complex and poorly understood. Over the years, several theories have been proposed to explain its pathophysiology, including increased intestinal permeability, visceral hypersensitivity, gastrointestinal dysmotility, intestinal dysbacteriosis, food intolerance, brain-gut axis dysregulation, and psychological stress, which are important biomarkers of IBS.8 However, the generation and association of these biomarkers are incompletely understood. It is now clear that these mechanisms, whether considered alone or in combination, are insufficient to explain symptom generation. In recent years, irritable bowel syndrome is increasingly viewed as a low-grade inflammatory disorder, nine and mucosal mast cells are considered to be a key component of the intestinal mucosal inflammation, accounting for the close relations of mast cells with major intestinal functions, such as epithelial secretion, epithelial permeability, blood flow, neuroimmune interactions, visceral sensation, and peristalsis.10 Intestinal mucosal mast cells in healthy individuals are preferentially located in close proximity to enteric nerve terminals in the lamina propria[11], with an estimated 70% of mast cells are in direct contact with enteric nerves, and another 20% are within 2 μm[12] and release a wide array of inflammatory mediators such as histamine, tryptase, chymase, serotonin that are capable of affecting enteric nerve function[13] and muscle contractility.14 The intestinal mucosal mast cells are involved in gut inflammation through their interplay with the enteric nerve.15 This interaction between the mast cell and the enteric nerve is bidirectional; each secretes mediators or neuropeptides that act on the other. Activated mast cells release mediators such as histamine, tryptase, serotonin, etc., acting on the afferent nerve, while neuropeptides such as substance P, vasoactive intestinal peptide, and calcitonin-gene related peptides are secreted from nerve endings and affect mast cells activity.16 A number of observations have been cited as evidence for a relationship between IBS and mucosal mast cell counts. The first evidence of possible involvement of an inflammatory component in the intestinal wall of patients with IBS was reported in 1962 by Hiatt and Katz, who detected an increased number of mast cells in the colonic muscular layer in four patients.[17] O'Sullivan et al. carried out a study in Ireland, and biopsy specimens were taken from the caecum, ascending colon, descending colon, and rectum. Mast cells were significantly increased in the caecum in IBS patients compared to controls in that study. Mast cells were also elevated in the ascending and descending colon in IBS than controls, but this was not statistically significant[18]. In Korea, Choi et al. obtained biopsy specimens from the caecum, ascending colon, descending colon, and rectum. They found mast cell counts in IBS patients at the descending colon were significantly higher than normal controls. In a group of patients with IBS, mast cell counts at the caecum were significantly higher in constipation-predominant IBS than diarrhea-predominant IBS [19]. However, Irvine et al. carried out a study in Canada and failed to show differences in mucosal mast cell counts in the colon in patients with IBS compared to controls [20]. Though many studies were performed in relating mucosal mast cell counts in the colon in patients with IBS in different countries with somewhat different results, there were no studies in Bangladesh related to this topic. In these circumstances, we

evaluate mucosal mast cell counts in the colon in patients with IBS in our population.

Materials and Methods

The hospital-based case-control study was conducted in the Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University, Dhaka, during one year period from April 2016 to March 2017 on cases of IBS and comparison group. We recruited 25 IBS patients and 25 healthy controls. IBS was diagnosed on ROME III criteria; none of them had alarm symptoms or concurrent allergic disease. Individuals with no symptoms of IBS who had undergone colonoscopy for screening purposes (e.g., evaluation of anemia, family history of colon cancer) and found normal findings were taken as controls. A purposive nonprobability method of sampling was employed. After a full explanation of the study procedure, informed written consent was taken, and then they were subjected to a colonoscopy procedure. Biopsy was taken from the caecum, ascending colon, descending colon, and rectum. Tissue was stained with Giemsa, and then quantitative evaluation of mast cells was performed by a single expert pathologist. Mast cell counts were compared between the two groups of patients. Statistical analysis was performed using SPSS (Statistical Package for Social Science). P values <0.05 were considered significant.

Results

The age range of both IBS patients and control was 18 to 55years. The mean age of IBS patients was 34.44±9.92 years, and controls were 37.40±9.22 years. 64% (16) were male among the IBS patients, and 36% (9) were female. On the other hand, 60% (15) were male, and 40% (10) were female in the control group. Male patients were predominant in both groups. Out of 25 IBS patients, IBS-D was 56% (14), IBS-M was 24% (6), and IBS-C was 20% (5). Most of the patients were IBS-D. Among the control group, the reasons for colonoscopy were evaluation of anemia 76.0% (19) and for surveillance of colonic neoplasm in subjects with a family history of colon cancer 24.0% (9). Mast cell counts were significantly higher in the caecum, ascending colon, descending colon, and rectum in IBS patients compared to control, as shown in table-1

Table-1: Comparison of mucosal mast cell counts between two study groups (n=50)

Site	Case (n=25) Mean±SD	Control (n=25) Mean±SD	p value
Caecum	10.40±2.10	4.20±1.01	<0.001[s]
Ascending colon	6.76±1.83	3.32±0.69	<0.001[s]
Descending colon	8.08±2.19	3.04±0.84	<0.001[s]
Rectum	9.16±2.46	3.84±1.07	<0.001[s]

Mucosal mast cell counts in the caecum, ascending colon, descending colon, and rectum was significantly higher in all subtypes of IBS patients (IBS-D, IBS-C, and IBS-M) compared to control and mast cell counts were relatively elevated in IBS-D patients compared to IBS-C and IBS-M patients but this was not statistically significant as shown in table-2.

Table-2: Comparison of mucosal mast cell counts among subgroups of IBS patients (n=25)

Site	Subgroups of IBS			
	IBS-D (n=14) Mean±SD	**IBS-C (n=5) Mean±SD**	**IBS-M (n=6) Mean±SD**	**p-value**
Caecum	11.0±2.04	8.60±1.82	10.5±1.87	0.085^{ns}
Ascending colon	7.0±1.66	6.4±2.61	6.5±1.76	0.773^{ns}
Descending colon	8.64±1.98	6.80±2.39	7.83±2.40	0.270^{ns}
Rectum	9.71±2.27	7.80±3.11	9.0±2.28	0.337^{ns}

Among four sites, mast cell counts were significantly higher in the caecum, as shown in table-3.

Table - 3: Comparison of mucosal mast cell counts among different sites of the colon of IBS patients (n=25)

	Site				
	Caecum (n=25) Mean±SD	**Ascending colon (n=25) Mean±SD**	**Descending colon (n=25) Mean±SD**	**Rectum (n=25) Mean±SD**	**p-value**
Mast cell counts	10.4±2.1	6.8±1.8	8.1±2.2	9.2±2.5	<0.001^{s}

No associations were seen between mucosal mast cells and other variables, including different age groups, gender, and duration of symptoms of IBS patients.

Discussion

In the present study, the mean age of the case group was 34.44±9.92 years, and the control group was 37.40±9.22 years. The difference in mean age was not statistically significant between the two groups. O'Sullivan et al. conducted a similar study and found the mean age of the IBS group was 42±11 years, and the mean age of the control group was 44±8 years.[18] Considering gender distribution, 16(64.0%) were male among the IBS patients group, and 9(36.0%) were female. On the other hand, 15(60.0%) were male, and 10(40.0%) were female in the control group. Male patients were predominant in both groups. O'Sullivan et al. demonstrated that most IBS patients (86%; 12/14) and normal controls (71%; 5/7) were female [18]. Choi et al. reported 50% of IBS patients and 25% of control were female.[19] In our study, female patients were lower in number in both groups. Female patients still get less medical privilege in our country. It may be the cause of the lower number of female patients. Our study focused on mucosal mast cell counts in the caecum, ascending colon, descending colon, and rectum. We found that the mucosal mast cell counts with Giemsa stain in the caecum, ascending colon, descending colon, and rectum were 10.40±2.10, 6.76±1.83, 8.08±2.19, and 9.16±2.46 per HPF in IBS patients and 4.20±1.01, 3.32±0.69, 3.04±0.84, and 3.84±1.07 per HPF respectively in the control group. Mast cell counts were significantly higher in the caecum, ascending colon, descending colon, and rectum in IBS patients compared to control. Among four sites, mast cell counts were significantly higher in the caecum. We also

demonstrated that mucosal mast cell counts in the caecum, ascending colon, descending colon, and rectum were substantially higher in all subtypes of IBS patients (IBS-D, IBS-C, and IBS-M) compared to control. De Silva et al. from Srilanka found that the mucosal mast cell counts with Giemsa stain in the caecum, ascending colon, descending colon, and rectum were 8.71 (2-14), 5.54 (3-8), 8.67 (4-20), and 10.08 (7-16) per HPF among 39 diarrhea-predominant IBS patients and 4.00 (2-6), 3.20 (1-5), 3.35 (3-4) and 4.13 (2-7) per HPF respectively among 13 controls[21] Mast cell counts were significantly higher in the caecum, ascending colon, descending colon, and rectum in diarrhea-predominant IBS patients compared to control. This result was consistent with our study result. O'Sullivan et al. carried out a similar study in Ireland and included a total of 14 IBS patients and seven normal controls, and biopsy specimens were taken from the caecum, ascending colon, descending colon, and rectum. They demonstrated tryptase positive mast cells (as represented by mean volume density of mast cells) were significantly higher in the caecum of IBS patients (0.91±0.18; CI 0.79-1.0) compared to controls (0.55±0.14; CI 0.40-0.69). Mast cells were also elevated in the ascending and descending colon in IBS patients than controls, but this was not statistically significant [18]. Another similar study conducted in Korea by Choi et al. included a total of 16 IBS patients, and eight normal controls and biopsy specimens were obtained from the caecum, ascending colon, descending colon, and rectum. They found tryptase-positive mast cell counts in IBS patients at the descending colon were significantly higher than normal controls (15.2±4.0 vs. 10.9±2.7/400X magnification) [19]. In Korea, Park et al. obtained biopsy specimens from the caecum and rectum of 14 IBS-D patients and 14 normal controls and demonstrated an increased number of mucosal mast cells with toluidine blue stain (as represented by a mean number of mast cells per mm^2) in IBS-D patients in comparison with the control group in both caecum (262.7±35.5 vs. 165.1±25.3/mm^2) and rectum (184.7±27.0 vs. 124.6±10.7/mm^2) [22]. In Korea, Sohn et al. included a total of 22 IBS-D patients and 21 normal controls, and biopsy specimens were taken from the rectum. They demonstrated the number of tryptase-positive mast cells was substantially higher in the IBS-D patients than the normal controls (9.6±3.3 vs. 5.7± 2.5/ HPF) [23]. We observed that the results of mucosal mast cell count in IBS patients of different studies were varied. These variations may be due to methodological differences such as patient selection, sample size, biopsy site, and the method of mast cell identification. Different ethnic groups of people in different geographic areas may be another cause of these varying results. Our study was based on a sample from the caecum, ascending colon, descending colon, and rectum of both IBS patients and controls, and microscopic examination allowed precise identification of mast cell counts with Giemsa stain.

Conclusion

Mast cell counts were significantly increased in the caecum, ascending colon, descending colon, and rectum in patients with IBS compared to controls in our study. Among four sites of the colon, mast cell counts were significantly higher in the caecum. An increase in mast cell numbers in the colon may be significant in the pathogenesis of IBS by releasing potent mediators such as histamine, serotonin, tryptase that alter the enteric nervous system and smooth muscle function. More work is clearly needed to further investigate the role of mast cells in the pathogenesis of IBS.

Conflict of Interest – None

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