Eosinophils, mast cells, nerves and ganglion cells in appendicitis

Usha Rani Singh · Anu Malhotra · Arati Bhatia

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Abstract

Introduction Twenty to thirty percent appendices removed from patients with suspected appendicitis appear normal on histology. The cause of pain in these patients is unknown. The presence of eosinophils and mast cells should be looked at skeptically which may explain the cause of pain. The aim was to study the eosinophils, mast cells, nerves and ganglia in normal and inflamed appendices.

Materials and methods A total of 329 appendices including 192 case of acute appendicitis (group A), 94 cases of clinically acute but histologically normal appendices (group B), 13 cases of complementary/elective appendicectomies (group C) and 30 normal controls from medico-legal autopsies (group D), were studied for the presence of eosinophils, mast cells, nerves and ganglia in mucosa, submucosa and muscularis propria. Routine haematoxylin and eosin stained sections were used for eosinophils, nerves and ganglia and Toluidine blue sections for mast cell counts. One way ANOVA and logistic regression was used for statistical analysis

Results The mean eosinophil and mast cell counts were significantly higher in mucosa, submucosa and muscularis

propria in Group A and B, when compared to group C+D. The number of nerves and ganglion cells were significantly higher in group A when compared to groups B and C+D. The correlation between eosinophil and mast cell count was not found to be statistically significant.

Conclusion A significant increase in Eosinophils, mast cells, nerves and ganglion cells was seen in acute appendicitis. Increase in eosinophils and mast cells may explain the pain in histologically normal but clinically suspected acute appendicitis.

Keywords Eosinophils · Mast cells · Nerves · Ganglion · Appendicitis

Introduction

Appendicectomy is the most frequent intra abdominal surgery [1]. The diagnosis of Appendicitis is mainly clinical based on right sided abdominal pain and correlated with a raised white cell count. In about 15–25% of appendices removed at surgery because of suspected acute appendicitis signs of acute appendicitis are absent [2]. The cause of acute abdominal pain in these patients remains unexplained [3]. In most of these patients appendicectomy proves curative [4]. Obstruction and inflammation is implicated in the pathogenesis Acute Appendicitis [5]. Fecolith and lymphoid hyperplasia is seen in only small percentage of cases. Eosinophilic infiltration of muscularis has been seen in Acute Appendicitis and it is postulated that Acute Appendicitis is triggered by Type1 hypersensitivity [6]. Mast cells have a central role in Type1 hypersensitivity leading to recruitment of eosinophils and discharge of primary and secondary

U. R. Singh · A. Malhotra · A. Bhatia
Department of Pathology,
University College of Medical Sciences & G.T.B. Hospital,
Delhi - 110 095, India

U. R. Singh (✉)
e-mail: ursingh@hotmail.com, drursingh@yahoo.co.in

mediators of inflammation. Mast cells have been implicated in the pathogenesis of pain [7, 8, 9, 10]. Mast cells are found primarily in the skin, gastrointestinal and respiratory tract. It has been found that various inflammatory diseases at these sites are associated with increase in mast cells [11]. Mast cells are frequently apposed to peripheral nerves [12]. Proliferation of nerves [13] and ganglion [14] cells is seen in Appendicitis. As there are very few studies related to eosinophils, mast cells, nerves and ganglion cells the aim was to study eosinophils, mast cells, nerves and ganglia in patients presenting as Acute Appendicitis.

Materials and methods

A total of 329 appendices including 192 patients of acute appendicitis (group A), 94 cases of clinically acute but histologically normal appendices (group B), 13 complementary or elective appendicectomies (group C) and 30 normal controls from medico legal autopsies (group D) were studied for the presence of eosinophils and mast cells in mucosa, submucosa and muscularis propria. Routine Hematoxylin and eosin (H and E) stained sections were used for eosinophil, nerve and ganglion cell counts and 1% Toluidine blue stained sections for mast cells counts. Eosinophils, mast cells and nerve counts were done in 20 non-overlapping high power fields (HPF). The diameter of one HPF measured using a slide micrometer was 0.44 mm. The cell counts were done on 20 HPF, the mean cell counts / mm² was calculated to be number of cells in 20 HPF /3. Three sections each of 5 micron each from tip, base and middle of the appendix were examined. Ganglion cells were graded as mild when 1–5, moderate when 6–10, severe when more than 10 in whole cross-section. One way ANOVA was used for comparison of cell counts in mucosa, submucosa,

and muscularis propria. Logistic regression analysis to see the relationship of nerves with mast cells and eosinophils and eosinophils with mast cells. The protocol was approved by the ethical committee of the institution.

Results

The age of the patients ranged from four months to seventy years. Mean ages were 25.44 ± 12.14 years. Group C + D was taken as control. There was a male preponderance among patients in group A.

A total of 14% of patients gave a history of allergy and an equal number a history of worm infestation. There was no statistically significant association of this history with any of the groups,

Eosinophils were present in mucosa, submucosa and muscularis propria in all the four groups. The mean eosinophil counts in mucosa, submucosa and muscularis propria were significantly higher in group A and B in comparison to Group C + D. The mean eosinophil count in, submucosa and muscularis propria of appendices in group A were significantly higher ($p=0.00$) than those of group B. However, the mucosal mean eosinophil count of group A was significantly lower than group B (Table 1). In acute appendicitis (group A) eosinophils in muscularis propria were significantly higher than eosinophils in Mucosa and submucosa ($P<0.05$). Highest eosinophils Counts were seen in the mucosa in group B.

In submucosa and muscularis propria mast cell counts in group A and group B were significantly higher than those in group C+D. In Mucosa, mast cell counts in group B were significantly higher than mast cell count in group C + D (Table 1). Mast cells were highest in submucosa in all Groups.

The nerve density was studied in all the groups in submucosa and muscularis propria (Table 2). The ganglion cells were counted in whole cross section (Table 3). The nerve density and Ganglion cells in group A was significantly higher than in group B and C+D. There was no significant difference between group B and group C+D.

When the logistic regression analysis was applied to find out the relationship of nerve density with mast cells, it was not found to be statistically significant. Similarly the relationship of nerve density with eosinophils and that of eosinophils with mast cells was not statistically significant.

Discussion

Acute appendicitis is the most frequent cause of emergency abdominal surgery and results in postoperative complications in as many as 30% patients [15]. Almost all the patient of acute appendicitis present with pain abdomen. However, 15–20% of appendices removed from the patients with

Table 1 Eosinophil and Mast cell counts in different groups

Groups	No.	Mean Eosinophil count $\pm$ SD	Mean Mast Cell count $\pm$ SD
Mucosa			
Group A	192	10.15 ± 14.01	5.42 ± 6.32
Group B	94	17.04 ± 13.23	7.03 ± 6.47
Group C + D	43	4.53 ± 6.07	3.13 ± 4.64
Submucosa			
Group A	192	10.56 ± 9.10	18.58 ± 11.76
Group B	94	3.12 ± 6.68	22.20 ± 11.70
Group C+D	43	1.53 ± 3.30	7.04 ± 11.93
Muscularis Propria			
Group A	192	21.96 ± 18.79	10.47 ± 8.26
Group B	94	5.76 ± 14.3	8.79 ± 5.99
Group C+D	43	1.32 ± 3.12	4.36 ± 7.89

clinically suspected acute appendicitis appear near normal on histopathological examination and cause of this pain remains unknown [7].

Chang [16] in his analysis of 3003 appendices found that there was slight preponderance of males. He found that acute appendicitis was more often seen in younger age group and 82% of the appendices were removed from the patients less than thirty years of age, similar to our study.

Aravindan [6] observed that eosinophils were found in abundance in many cases of acute appendicitis involving all the layers of the organ. He found moderate to heavy infiltration by eosinophils in the muscularis propria in almost all cases of acute appendicitis. In appendices with acute clinical presentation but not diagnostic of acute appendicitis, transmural eosinophil counts were in the same range as seen in acute appendicitis whereas, normal appendices showed only occasional eosinophils. We also found the same. He [6] pointed out that perhaps the previous studies, which labeled appendices with transmural eosinophilic and lymphocytic infiltration as resolving or chronic appendicitis, had not correlated these findings with the clinical presentation. Jona et al [17] reported two cases presenting clinically as acute appendicitis, which had transmural eosinophilic infiltration in the absence of neutrophils. We had 94 such cases.

Aravindan [6] suggested that infiltration of eosinophils was an early consequence of mediators released by mast cells in acute appendicitis. Eosinophils reach the site due to eosinophilic chemotactic factors [18, 19] present in the mast cells granules or due to histamine released by them. In our study we found that mean eosinophil counts in all the three layers were significantly increased in group A and B as compared to normal appendices in group C+D similar to Mysorekar et al [20]. This could be explained by the presence of the substances like eosinophil chemotactic factors and histamine released by mast cells which were significantly higher in numbers in group A and B as compared to Group C + D Table 2.

We found that mean mast cell counts in submucosa and muscularis propria in group A and B were significantly higher than those of group C+D similar to Mysorekar et al

[20] and at variance with Naik et al 21. Mucosal mean mast cell counts in group B were significantly higher than those of group C +D indicating that both in acute appendicitis and those who only had clinical evidence of disease, the mast cells were significantly raised.

Since enteric nervous system is in close association with immuno-competent cells like mast cells [22]. Xiong et al [14] studied neuronal hypertrophy and mast cells in acute appendicitis and histologically normal appendices which were clinically diagnosed as acute appendicitis. They found an increase number of nerve fibers, schwann cells and enlarged ganglia in the submucosa and muscularis externa in all cases of acute appendicitis and in 40% of apparently normal appendices having symptoms of appendicitis. They also found that the size and number of ganglion cells were significantly increased in muscularis externa and submucosa of acute appendicitis than those of the controls [14]. The mast cells were significantly increased in submucosa and muscularis propria in acute appendicitis as compared to controls. Our study also showed similar finding. The nerve density and ganglion cells were significantly higher in submucosa and muscularis propria in group A when compared to group B and C+D. Though nerve density was higher in group B compared to group C+D and this was not statistically significant.

Though neutrophils in the muscularis propria of the appendix are the hallmark of acute appendicitis, the presence of eosinophils and mast cells in surgically resected appendices with a clinical diagnosis of acute appendicitis that are apparently normal on histology may suggest that these cells represent the beginning of an inflammatory process which has hitherto been labeled as normal [23]. As mast cells are widely distributed [24] in the mucosa and submucosa of the digestive tract [25], these cells are closely apposed to nerves in the human gastrointestinal mucosa [12] and get stimulated by major basic protein derived from eosinophils, substance P, Vasoactive Intestinal Peptide, NeurokininA (NKA) [12] and Neurokinin B (NKB) [26]. On stimulation, these cells release mediators, which cause clinical symptoms of nausea, vomiting, abdominal pain and diarrhoea. We found that mucosal and submucosal mean mast cell counts in group A and B were significantly higher than the group C+D (Table 3). This explains the symptoms of nausea, vomiting and pain in patients with acute appendicitis as well as those who were clinically diagnosed as acute appendicitis but had apparently normal histology (Group A and B)

Table 2 Nerve density in different groups

Groups	No.	Mean Nerve count $\pm$ SD
Submucosa		
Group A	192	18.92 $\pm$ 7.07
Group B	94	12.39 $\pm$ 4.95
Group C + D	43	11.65 $\pm$ 12.61
Muscularis Propria		
Group A	192	6.31 $\pm$ 3.06
Group B	94	3.89 $\pm$ 2.05
Group C+D	43	3.70 $\pm$ 1.79

Table 3 Ganglion cells in various groups

Group	No	Mild	Moderate	Severe
Group A	192	9	95	82
Group B	94	81	11	0
Group C+D	43	36	4	2

Conclusion

Eosinophils and mast cells are increased in the appendices clinically diagnosed as acute appendicitis. Statistically significant increase in mast cells in appendices that appear normal on histology but clinically have symptoms of acute appendicitis may explain the clinical presentation. The pain in these patients may be caused by the mediators released by degranulated mast cells. A significant increase in mast cell counts, nerves along with prominence of ganglion cells in submucosa and muscularis propria in acute appendicitis establishes an association between increased mast cell counts and nerve proliferation in acute appendicitis.

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