

Study of mast cells in appendectomy specimen

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ABSTRACT

Background: Mast cells play an important role in various inflammatory and immune reaction. Mast cells are constantly present in the appendices, and appendix remains one of the common surgical specimens received at pathology laboratory. Mast cells could be one of the important cell population responsible for nerve proliferation and hypertrophy in cases with clinically and histopathologically diagnosed appendicitis.

Material and Methods: The study was done on 100 surgically resected specimens of appendix received for routine histopathological evaluation at the Department of Pathology, Government Medical College, Bhavnagar, between June 2018 and May 2019. All appendices surgically resected as a therapeutic measure for clinically suspected appendicitis included. Cases of normal appendix and acute gangrenous appendicitis were excluded from the study. The appendices were assessed for their histological changes and density of mast cell infiltration. The mast cells were counted in 1% toluidine blue stained sections.

Results: Out of 100 cases of surgically resected appendices, chronic appendicitis was found in 65% of cases and occurred frequently during the second and third decades of life. Highest mast cell count was seen in acute eosinophilic appendicitis and chronic appendicitis. No significant difference was observed in the mean mast cell counts among the different layers of the appendices studied.

Conclusion: Mast cell counts were found to be highest in acute eosinophilic appendicitis and chronic appendicitis. Intermediate mast cell counts were seen in acute appendicitis and acute suppurative appendicitis. Mast cells appear to play roles in accentuation of inflammatory process and fibrosis.

Keywords: Appendix, Mast Cells, Toluidine Blue

Introduction

For well over a century the biomedical literature has recognized two populations of basophilic leukocytes, namely mast cells and basophils.¹ Mast cells are ubiquitous cells found at the interface of environment and the organisms.² Mast cells arise from hematopoietic progenitor cells and mature mast cells ordinarily do not circulate in the blood instead, mast cells acquire their mature phenotype locally in the tissues where they ultimately reside.³

Mast cells have many similarities to basophils. They are small cells, round to ovoid in shape with a diameter of 12 – 15 microns and are packed with numerous cytoplasmic granules of 0.2-0.5 microns in size. The precise nature of their relationship is poorly understood. Mast cells and basophils are bone marrow derived cells. Both contain electron dense granules staining metachromatically with selected basic dyes and produce numerous inflammatory mediators such as histamine. For the demonstration of mast cells, metachromatic staining with basic aniline dye, especially toluidine blue has been widely employed. They are also visualized with a number of Alcian blue methods, Azure A, Bismarck Brown and Thionin. **4**

Both express high affinity receptor for IgE and upon activation by anti IgE antibodies, mediator synthesis and

secretions are induced which play prominent roles in allergic inflammation and immune responses.⁵ The type-1 hypersensitive reaction with a release of the mediators by the mast cells may be a triggering factor for the sequence of events which lead to appendicitis.

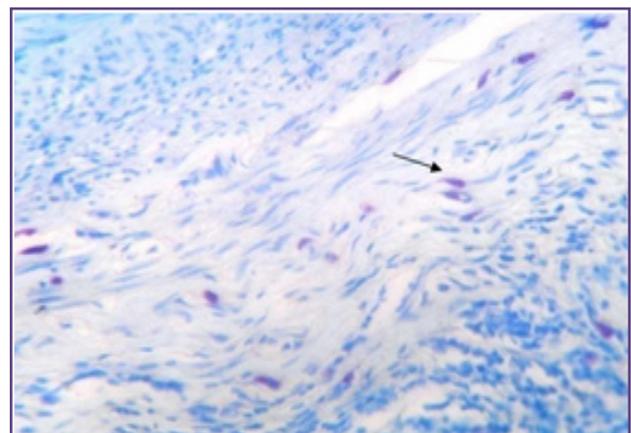


Fig. 1: Photomicrograph of mast cells (arrow) in appendicitis [Toluidine blue 40x].

Mast cells mature outside the bone marrow, generally in connective tissue or the serous cavities, while basophils circulate in blood and are recruited during the allergic

responses.⁶ Mast cells are thought to be critical for the maintenance of tissue integrity and function. They produce several growth factors and mediators such as nerve growth factor (NGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), histamine and tryptase, which induce proliferation of epithelial cells and fibroblasts. Mast cells are known to participate in and influence wound healing at every step, including the initial inflammatory response followed by re-epithelialization and revascularization of the damaged tissue, and finally the deposition of collagen and remodeling of the matrix.⁷

Mast cells are constantly present in appendix, the organ most commonly subjected for surgical intervention and removal. Although, the clinical and operative protocols are well known, the pathogenesis of acute appendicitis is poorly understood.⁸ Currently, luminal obstruction due to faecoliths or less commonly submucosal lymphoid hyperplasia especially in children appears to initiate acute inflammation in appendix.⁹

In about 20–25% of appendectomies performed for clinically suspected acute appendicitis, definite morphological changes are lacking on histopathological examination.¹⁰

Since appendectomy relieves pain, an unknown pathology is likely to exist. The role of inflammatory reactions involving local endocrine cells and neuroproliferation in causing repeated attacks of pain has been described and several staining techniques including immunohistochemistry have been tried in detecting the pro-inflammatory mediators. Histopathological examination still remains the gold standard method for the confirmation of the appendicitis.¹¹ Acute appendicitis is thought to be initiated by progressive increases in intraluminal pressure that compromise venous outflow. Currently, luminal obstruction due to faecoliths or less commonly submucosal lymphoid hyperplasia especially in children appears to initiate acute inflammation in appendix.¹²

The present study aims at finding mast cell variation in inflamed appendix and also to suggest the possible role of mast cells in the pathogenesis.

Material and Method

Total 100 cases of suspected appendicitis were included in this study over period of one year from June 2018 to May 2019 received for histopathological examination in the Department of Pathology, Govt. Medical College Bhavnagar.

Clinical details were noted in the proforma enclosed, including age, sex and clinical diagnosis.

Inclusion Criteria

- Case with clinical features suggestive of appendicitis will be included in this study.

Exclusion Criteria

- Cases of acute gangrenous appendicitis due to associated necrosis of muscle fibres
- Autolysed sample
- Normal appendix

Appendices were received in 10% formalin. Minimum of 24 hours was allowed for proper tissue fixation. After fixation, one section from each of tip, base and intermediate site along the length of appendix was taken and sent for routine paraffin processing. After the processing and embedding of tissue sections in paraffin blocks, 2 sections of 5 micron thickness were cut from each block. One of the sections was stained by haematoxylin & eosin and the other by 1% toluidine blue for the identification of mast cells. Result obtained was mast cells-violet and background showed shades of blue Toluidine blue stain (1%) was used for the identification of mast cells. The number of mast cells in mucosa, submucosa and muscular layer were counted at 40X magnification (high power).

Results

The present study was conducted on 100 surgically resected appendices received for histopathological evaluation at the Department of Pathology, Government Medical College, Bhavnagar.

Out of 100 cases (n=100) of appendices studied, 52 cases (52%) were males (n=52) and 48 cases (48%) were females (n=48) with a male to female ratio of 1:0.92.

The age of the patients in the present study ranged from 0-65 years (Table-4 and Graph-1) with a mean age of 26.5 years.

Maximum number, that is thirty three cases (males=14; females=19) were seen in the age group of third decade (n=33), followed by thirty cases (males=17; females=13) in second decade (n=30).

(Fig-2) External surface showed hyperaemia and congested vessels in eighty eight cases (n=88). Six appendices were oedematous on gross examination while three were fibrosed externally. Two appendices were perforated whilst appendectomy while a single one was markedly inflamed.

Out of 100 appendices, lumen showed normal content in sixty one cases (n=61). Luminal dilatation was seen by stool in thirty five cases (n=35) and by fecolith in three cases (n=3). One case showed pus in the lumen (n=1).

Acute appendicitis was diagnosed when the patient presented with acute onset of fever, severe umbilical or right lower abdominal quadrant pain with tenderness and vomiting and muscularispropria showed infiltration by neutrophils. Thirty two cases of acute appendicitis were seen (n=32) (32%).

Acute suppurative appendicitis with clinical presentation of acute symptoms and showing dense infiltration of muscle wall with neutrophils, eosinophils, plasma cells and lymphocytes throughout all layers and frequently into serosa were seen in one cases (n=1) (1%).

Acute eosinophilic appendicitis presenting clinically like classic acute appendicitis with inflamed and oedematous

appendix showing marked eosinophilic infiltration into the muscularis propria without neutrophils were seen in two cases (n=2) (2%).

Maximum number of cases seen in this study were of chronic appendicitis (n=65) (65%) presenting with history of repeated attacks of right lower abdominal quadrant pain greater than two weeks and pathologic finding of chronic inflammation with or without fibrosis.

Table-5 show mast cell density in various layers of appendix. The concentration of mast cell is relatively more in submucosa compared to other layers. Also, submucosal density of mast cell is more pronounced in chronic appendicitis compared to acute appendicitis

Table 1: Age and gender distribution of patients studied.

AGE(IN YEAR)	MALE	FEMALE	TOTAL
0-10	07	02	09
11-20	17	13	30
21-30	14	19	33
31-40	10	04	14
41-50	02	05	7
51-60	01	04	5
>60	01	01	2
TOTAL	52	48	100

Table 2: Histological findings in ,mucosa,submucosa and muscularis of the appendices studied.

Histopathology Diagnosis	Mean mast Cell Count per mm ²		
	Mucosa	Submucosa	Muscularis
Acute Suppurative Appendicitis	13	10	11
Eosinophilic Appendicitis	11.5 ± 0.5	21 ± 1	13 ± 1
Acute Appendicitis	11.56 ± 2.84	11.41 ± 2.26	12.25 ± 2.14
Chronic Appendicitis	12.02 ± 2.71	16.26 ± 3.14	12.17 ± 2.94
TOTAL	11.87 ± 2.73	14.74 ± 3.76	12.2 ± 2.67

Table: 3 Comparison Of Incidence Of Male and Female Of Appendices In Various Studies.

Authors	Total No of case	Male(%)	Females(%)
Verma A ¹²	150	56.66 %	43.33 %
Dr.Alpa ¹⁵	100	64 %	36 %
Present study	100	52 %	48 %

Table: 4 comparative study of mast cells in mucosa, submucosa and muscular layer.

Author	Acute appendicitis	Chronic appendicitis	Acute suppurative	Eosinophilic Appendicitis
Verma A ¹²	10/12.5/14.2	18.2/16.7/17.1	11.7/14/16.2	14.6/18/18.6
Sharma J et al ¹⁷	13/11	25/24	-	22/18
Mysorekar VV etal ¹⁹	36.3/27.4/27.6	55.6/42.8/32.4	-	-
Dr.SomaDatta ¹⁶	7.5/22.1/12.3	10.5/27.4/15.7	8/19/11.5	12.5/28.7/20.4
Present study	11.5/11.4/12.2	12.0/16.2/12.1	13/10/11	11.5/21/13

Table: 5 Comparison Of Incidence Age Of Appendices In Various Studies.

Age(In Year)	Verma A ¹²	Dr.Alpa ¹⁵	Present study
0-10	4.66 %	3 %	9 %
11-20	32 %	42 %	30 %
21-30	41.33 %	33 %	33 %
31-40	10 %	10 %	14 %
41-50	6 %	7 %	7 %
51-60	4 %	4 %	5 %
>60	2 %	01 %	2 %

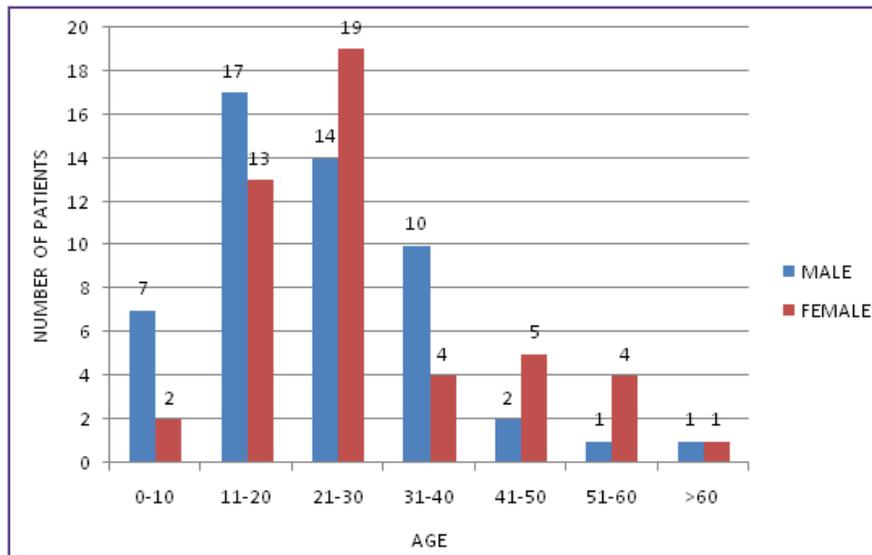


Chart 1: Age (in Year) and Gender Distribution of Patients Studied.

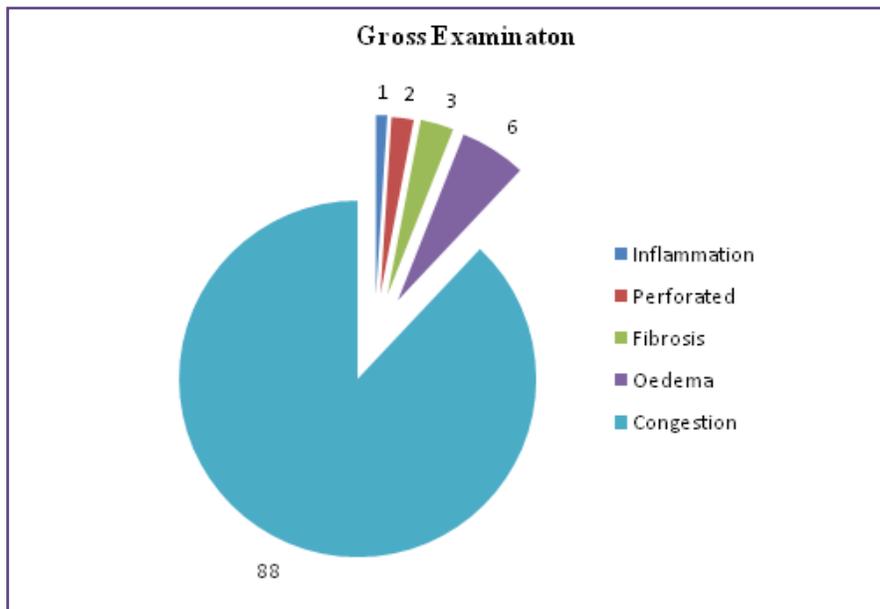


Fig. 2: Gross features of the various appendices studied.

Discussion

In the present study, surgically resected appendices of 100 patients were grossly and histopathologically evaluated along with mast cell .

Out of 100 resected appendices, 52 cases were male and 48 cases were female with slight male preponderance with male to female ratio of 1:0.92. This correlate with Verma A¹² and Alpa¹⁵ study in table 1.

A study done by Sulochana, 2012²⁰ Who concluded that the mast cell count was more in chronic appendicitis when compared to acute appendicitis. This study correlates with the present study.

Immunologic and non immunologic stimuli cause degranulation of the mast cells. So there is a highest frequency of the mast cell and the nerve apposition in the appendix. The nerve growth factor causes hyperplasia of both the mucosal and the sub mucosal mast cells. Even the fibroblasts have an ability to make the nerve growth factor; hence, there is an abundant potential for growth interactions between the mast cells, nerves and the fibroblast

Alpa¹⁵ study also showed highest incidence in second decade(42%) followed by third decade(33%).

The age of the patients in the present study ranged from 0-65 year with a mean age of 26.5 year. Maximum number, that is 33 cases were seen in the age group of third decade, followed by 30 cases in second decade. Verma A¹² study had also slight male predominance with highest incidence of appendicectomy was seen in third decade (41.33%) followed by second decade (32%).

Maximum number of cases seen in present study were of chronic appendicitis (65%) followed by acute appendicitis (32%); other cases were of acute suppurative appendicitis and eosinophilic appendicitis though comparatively rare. This compares well with study carried by Dr. Soma Datta¹⁶ which also showed majority of cases with chronic appendicitis.

Qualitative data were represented in the form of frequencies and percentages. Quantitative data were represented in the form of mean and standard deviation (SD). ANOVA was the test of significance to compare the mean between the three groups. Post hoc Bonferroni test was done to compare the individual values between the three groups. $P < 0.05$ was considered as statistically significant.

There was no correlation between the sex and mast cell count in the present study as has also been described by Naik et al¹⁸ and Mysorekar et al.¹⁹

Conclusion

A higher mast cell count was seen in eosinophilic appendicitis and chronic appendicitis followed by acute appendicitis. Mast cell seen in all the layers of the appendix ,maximum number of mast cell count seen in submucous and mucous layer of appendix.

Mast cell count is the highest in chronic appendicitis, thus indicating the growth interaction between the mast cells and fibroblasts in areas of fibrosis. The fibroblasts have an ability to make NGF; hence there is an abundant potential for growth interaction among the mast cells, nerve and fibroblasts.

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Conflicts of Interest

The authors declare no conflict of interests.

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