

Mast cell profile in appendicitis

G. Patil Anuradha¹, AM Anita², Saini Kr. Seemant^{3,*}, S. Pratima⁴

¹HOD, ²Associate Professor, ³PG Student, ⁴Professor, Dept. of Pathology, MR Medical College, Kalaburgi, Karnataka

***Corresponding Author:**

Email: seemanthsaini@gmail.com

Abstract

Introduction: Today appendicitis remains the most common indication for emergency laparotomy, especially in the young. Mast cell is one of the most important cells of inflammation and acute appendicitis is triggered by Type -1 hypersensitivity reaction and infection is later consequence.

Materials and Method

- In the present study mast cell number & distribution in surgically resected appendix were studied to see variation in normal & inflamed appendix, in Department of pathology Mahadevappa Rampure Medical College, Kalaburagi.
- 180 appendectomy specimen received from Oct 2015 to Oct 2016. Routine Hematoxylin & Eosin section for histopathology and 1% aqueous toluidine blue was used to study mast cells.
- These cases were divided into two groups, Group A: Acute appendicitis showing neutrophilic infiltration- Group A1, with eosinophilic infiltrate- Group A2
Group B: Chronic appendicitis showing lympho mononuclear infiltrate and fibrosis.
- Mast cells were counted in 10 consecutive high power fields and number were assessed and recorded semi-quantitatively.

Results: There is variation in mast cell counts in appendicitis as observed in present study. In acute appendicitis, mucosal and sub mucosal mast cell counts were decreased. Eosinophilic appendicitis showed increased number of eosinophils in mucosal and sub mucosal layer but mean mast cell counts were decreased. In chronic appendicitis was excessive lympho mononuclear infiltrate in mucosal and sub mucosal layer & mean mast cell counts were highest.

Conclusion: Our study concluded that acute appendicitis is IgE mediated type 1 hypersensitivity reaction and mast cells count showed variations in different form of appendicitis.

Keywords: Acute appendicitis, Mast cell

Introduction

The function of the human vermiform appendix is obscure. Two hundred years after its anatomical description, the appendix was observed to be the site of inflammatory disease.⁽¹⁾

Today appendicitis remains the most common indication for emergency laparotomy, especially in the young. It is difficult to diagnose in a certain proportion of patients and in some, perforation occurs with prolonged morbidity and occasional mortality.⁽²⁾ Mast cell is one of the most important cells of inflammation. It's granules contain inflammatory mediators those having role in acute inflammation, healing and fibroblastic proliferation. Acute appendicitis is triggered by Type-1 hypersensitivity reaction and infection is later consequence.⁽³⁾

In the present study mast cell numbers and distribution in surgically resected appendix were studied to investigate their variation in normal and inflamed appendix. An attempt is made in the present study to investigate the mast cell profile in the appendicitis in surgically resected appendix. A particular emphasis is made to compare and evaluate the mast cell in appendicitis as follows:

- Mast cell distribution in inflammatory disorders of appendix.
- Comparative evaluation of mast cell alteration in various inflammatory lesions.

- To observe any variation and try to explain possible mechanism for the same.

Materials and Method

The study of 180 cases was carried out in Department of Pathology Mahadevappa Rampure Medical College, Gulbarga after institutional ethical clearance.

Appendix removed from suspected appendicitis cases.

The sections were cut at 5 microns thickness and staining was done with hematoxylin and Eosin as routinely and with 1% aqueous toluidine blue for mast cells. Hematoxylin and Eosin was observed for the presence or absence of Inflammation and 1% toluidine blue stain was used to study mast cells.

Mast Cell Staining and Counting

- To identify the mast cells with the typical metachromatic granules, special stain 1% aqueous toluidine blue (at PH 4) was used.
- **Results:** The mast cell granules stain purple and the surrounding tissue blue

Mast Cell Counting and Observation: Toluidine blue stained sections are examined under high power magnification. The number of mast cell present in 10 consecutive high power fields were counted in all the sections and tabulated.

- These 180 cases were divided into two groups based on clinical and histopathological criteria.

Group A: Acute appendicitis presenting with acute abdomen and histopathologically showing

- **A-1** -Neutrophilic infiltration
- **A-2** - Eosinophilic infiltration

Group B: Chronic Appendicitis clinically presenting with pain abdomen recurrently and histopathologically showing lympho mononuclear infiltrate and fibrosis.

- The following histological features were looked for specifically.
- **Luminal exudate**-if present, whether predominantly neutrophils, lymphocytes or eosinophils.
- **Ulceration of the mucosal lining.**
- **Crypt abscesses.**
- **Presence of neutrophils, eosinophils and mast cells in the mucosa.**

The number of mast cells were semi-quantitatively assessed and recorded in the following manner:

Grade 0 -No cells.

Grade 1+ -10 cells seen per 10 high power fields.

Grade 2+ -Clusters of more than 10 cells seen in per 10 high power fields.

Grade 3+ -> 10 clusters seen in 10 per high power fields.

- Widening of sub mucosa due to edema.
- Sub mucosal fibrosis.
- Neutrophils, eosinophils, mast cells in submucosa.
- Muscularis propria -Edematous or not. Neutrophils present or not.
- Inflammatory cells in serosa- Eosinophilic present or not.
- In addition to these observations, the actual count of mast cells in mucosa and count of mast cells in submucosa was done.

Results

- **Group A:** Total of 180 cases, 90 cases showed neutrophilic infiltration of the muscularis propria grouped as Group A1 while Eosinophilic infiltration of the muscularis propria categorized as Group A2 had 50 cases.

Group A1

- This group showed a slight male preponderance with 28 males and 22 females giving M: F ratio of 2: 1. The group had an age range from 2 to 50 years with maximum age range is 10 to 30 year. 66 out of 90 cases were below 30 years of age.

Gross

- Fecolith was seen only in two cases. Luminal dilation which has been considered a sign of obstruction in previous studies by Sisson and Cooley Butler was also recorded when present, Eleven cases were described with Luminal dilatation. An arbitrary upper limit of 1 cm luminal

diameter was used to assess dilatation. Fibrinous surface exudate was noted in five cases.

Histology

- All cases in Group-A1 showed neutrophilic infiltration of the muscularis propria. Crypt abscess was seen in 8 cases. In almost all cases marked or prominent eosinophilic infiltration was seen in the lamina propria in addition to the neutrophilic infiltration.

Group A2

- A total of 50 out of 180 cases showed eosinophilic infiltration in the muscularis propria. This group showed a slight male preponderance with 28 males and 22 Females giving M: F ratio of 1.3: 1.
- This group had an age range from 5 to 50 years with maximum number of cases seen in the 10 to 30 year age group. 28 out of 46 cases were below 30 years of age.

Gross

- Fecolith was seen only in two cases. Ten cases were described with luminal dilation. An arbitrary upper limit of 1 cm Luminal diameter was used to assess dilatation. Fibrinous surface exudates was noted only in one case. Thickened wall was noted in eight cases. Rest of the specimens did not show any notable gross findings.

Histology

- Prominent eosinophilic infiltration was seen in muscularis propria in 46 cases. Mast cells were found to be reduced or hypo granular in areas of marked eosinophilic concentration. In the submucosa, the mast cells were less as compared to the mucosal mast cell count. All the cases that showed marked increase in eosinophils in Group-A2 also showed marked edema.
- In the mucosa the mast cell count ranged from 3-84/10 HPF. Mean mast cell count was 44/10 HPF. In the sub mucosa, the mast cell count ranged from 6-90/10 HPF. Mean mast cell count was 38/10 HPF.

Group B

- Total of 40 cases were included in this group Out of 180 cases accounting to about 22% of cases of whole series. Cases included those with chronic appendicitis clinically presenting with pain abdomen recurrently.
- The sex ratio showed an equal male: female ratio. This group had an age range from 5 to 53 years with maximum cases seen in 10 to 30 year age group. 28 cases out of 40 were between 10 to 30 years.

Gross

- Fecolith was seen only in one case. Eleven cases were described with luminal dilatation. Surface exudate was noted in one case. Thickened wall was seen in eight cases. Rest did not show any notable gross findings.

Histology

- A total of 36 cases out of 40 showed submucosal fibrosis and 6 cases showed marked submucosal fibrosis extending into the lamina propria with more marked fibrosis at the tip.
- It was noted that mast cells were seen in foci of fibrosis, particularly in the vicinity of dilated vessels within the fibrotic foci
- In the mucosa the mast cell count ranged from 12-115/10 HPF. Mean mast cell count was 48.5/10 HPF. In the sub mucosa the mast cell count ranged from 8-100/10 HPF. Mean mast cell count was 46.3/10 HPF

		Total no of cases (180 cases)		Acute appendicitis (140 cases)		Chronic appendicitis (40 cases)	
Group		A1		A2		B	
No of cases		90		50		40	
Sex	M:F	2 : 1		1.3 : 1		1 : 1	
Age (years)		2 to 50		5 to 50		5 to 53	
Gross	No gross changes	72		29		30	
	Fecolith	02		02		01	
	Luminal dilation	11				10	
	FibrinousExudate	05				01	
	Thickened wall	-				08	
Microscopy	Mucosa	Mast cells 5-64 / 10 HPF Mean - 38 /10 HPF		Mast cells 6-90 / 10 HPF Mean - 38 /10 HPF			
	Lamina propria	All cases showed Eosinophilic infiltration		-		Fibrosis - 06 cases	
	Sub mucosa	-		Mast cells 3-84 / 10 HPF Mean - 44 /10 HPF		Fibrosis	Mast cells
						36 cases	8-100/10 HPF Mean - 46.3/10 HPF
	Muscularis propria	All cases showed Neutrophilic Infiltration		All cases showed Eosinophilic Infiltrate		-	
Crypt abscess in 08 cases		-		-			

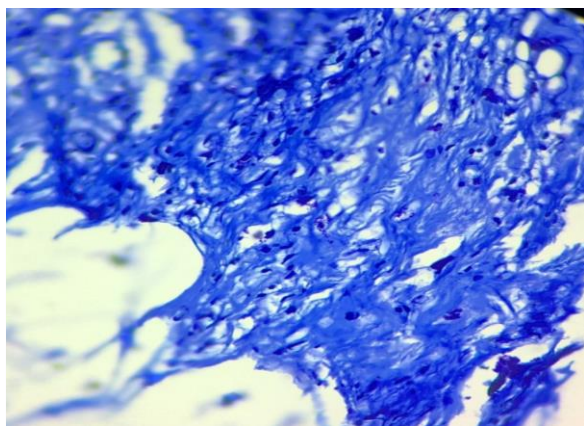


Fig. 1: Demonstration of mast cells (40 x) toluidine blue in chronic appendicitis

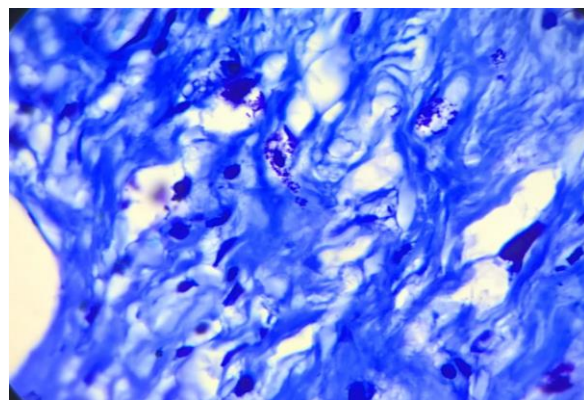


Fig. 2: Demonstration of mast CELLS (100 X) toluidine blue in chronic appendicitis

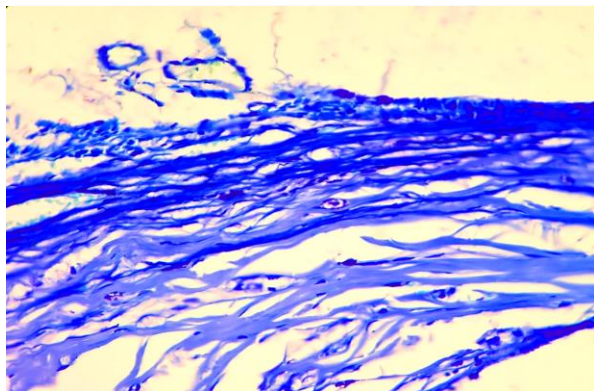


Fig. 3: Demonstration of Mast Cell in Neutrophilic Appendicitis (40 X)

Discussion

Mast cells are Present through Out the digestive tract especially in the mucosa and sub mucosa. The present study showed significant mast cells variation in normal appendices but there was only little variation in the average mucosal and submucosal mast cells along the length of the appendix. There was not much variation of mast cells with Sex and age of the Patient. TWO theories were put forward to explain Patho physiology of appendicitis:

- a. **Theory of obstruction:** Bowers⁽⁴⁾ held obstruction and enterogenous infection to be of major importance. The belief that luminal obstruction is an important agent in the pathogenesis of acute appendicitis was based on the frequent finding of fecoliths in the most advanced cases of acute appendicitis.
- b. **Theory of infection:** So Doraiswamy⁽⁵⁾ concluded that inflammation proceed infection in pathogenesis of acute appendicitis

In 1971, Richard G. Sisson concluded that superficial mucosal ulceration is the earlier lesion and occurs often before the dilatation of the organ is demonstrable. Doraiswamy found the ESR and total leukocyte count to be elevated only after 24 hours and swab for pus cells to be positive only after 24-36 hours.⁽⁵⁾

Mast Cell and Fibrosis in Appendicitis

These fibrous foci showed a clear increase in mast cell clustering particularly in the vicinity of blood vessels.⁽⁶⁾ An increase in mast cell count in region of fibrosis in the appendix has been previously reported. It has been observe that foci of fibrosis in lamina propria show a rise in mast cell density during initial phase of fibrosis i.e. an approximately 3 fold increase.⁽⁷⁾

This Increase in density declines until the mucosa is obliterated by fibrous tissue. In same study, compared to lamina propria the foci of submucosal fibrosis did not show a significant increase in mast cell density. It suggested that initiating stimulus for fibrosis

is luminal and the lamina propria mast cells are therefore activated.⁽⁷⁾

The mast cells are frequency apposed to nerves. In the gastrointestinal mucosa and there is microanatomic basis for potential communication between nerves and mast cells,⁽⁸⁾ supporting the concept that mast cells and nerves might interact as a part of normal homeostasis and repair process.

Group-B showed evidence of mast cell degranulation. The close association of mast cells and nerves⁽⁸⁾ makes it, an attractive and possible hypothesis that every episode of mastcell degranulation occurring in response to a luminal antigenic stimulus is reflected as appendicular colic.

Following up Wangenstein⁽¹⁰⁾ observation that separate inflammatory episodes that may or may not resolve. Butsch in 1973 studied 8 cases from a series of 75 appendectomies which showed a typical history of multiple attacks of right lower quadrant pain (as many as three attacks over a period of three months to one year). Three of the eight cases showed inflammation with fibrosis, rest of the five cases showed dilatation and eosinophilic infiltration of the wall and four cases showed acute appendicitis. This Butsch and his associates inferred that recurrent bouts of right lower quadrant pain of acute appendicitis, which may resolve leaving the telltale marks of inflammation in the form of fibrosis.⁽¹¹⁾

Sub Mucosal Fibrosis in Recurrent Appendicitis

Another observation that benefits discussion is that sub mucosal fibrosis was seen more frequency in Group B; patients with a recurrent appendicitis. In these cases, the sub mucosal fibrosis was often focal and seen extending to the lamina propria suggesting a local event in that region which may have preceded the fibrosis and possibility of growth factors released reading to fibroblastic proliferation

Role of Immunological Mechanisms

Butter has observed the fact, as confirmed in this study also, that acute appendicitis is most common among older childhood, adolescence and young adulthood, when the lymphoid tissue is most prominent. This apparent association between inflammation and lymphoid tissue is consistent with mechanism such as immune complex injury.

An interesting observation was made on young male patients with acute presentation (the epidemiological most prone group). This group gave history of change of diet sources over past 3-6 months.⁽⁹⁾ It is possible that young males in the group studied have in general, a greater variety of antigenic exposure which in turn increases the chances that hypersensitivity may develop during episodes of antigenic exposure. The range of mast cell values in non-inflamed appendices were found to show considerable variation from patient to patient. This

variability may be because of variation between individuals or granule depletion that in turn leads to reduced ability to detect their presence.

In eosinophilic appendicitis the wall of the appendix shows eosinophilic infiltration in the muscularis propria. It is possible that eosinophils are attracted due to eosinophilic chemotactic factor present in mast cell granules or due to histamine released from mast cells. Eosinophils contain an enzyme histaminase which destroys histamine.

It is interesting that the nerve growth factor has been shown to cause hyperplasia both mucosal and submucosal mast cells. Since certain fibroblasts have the ability to make nerve growth factor, it appears that there is abundant potential for growth interaction between mast cells, nerves and fibroblast.⁽⁷⁾

Conclusion

Mast cell count did not show significant variation in non-inflamed appendices between mucosal and submucosal coats. In acute appendicitis mucosal and submucosal mast cell counts were decrease. Variation in count, relation to age and Sex was not noted. More the inflammation severe is the decrease in the number of mast cells indicating that mast cell count were directly proportional to the extent of inflammation

There is marked decrease in mast cell counts in group A. This is explained as the mast cell recruitment occurs response to luminal stimulus which can be either immunological or non-immunological. The decrease of mast cells in acute appendicitis is temporary. The reactions of mast cell with injury is eliminated through mucosa or there progressive degranulation making it difficult for the mast cell detection.

In chronic appendicitis there was excessive lymphomononuclear infiltrate in mucosal and submucosal layer. Maximum number of mast cell was observed in chronic appendicitis. Nerve growth factor causes hyperplasia of mast cells and there is growth interaction between mast cells, nerve and fibrosis.

Summary

Mast cell is one of the most important cells of inflammation. Its granules contain mediators of inflammation and its role in acute inflammation and healing is known. Acute appendicitis is triggered by type 1 hypersensitivity reaction and infection is a later consequence.

There is variation in mast cell counts in appendicitis as observed in the study:

- In acute appendicitis, mucosal and submucosal mast cell counts were decreased.
- Eosinophilic appendicitis showed increased number of eosinophils. Both mucosal and submucosal mean mast cell counts were decreased and were intermediate between those of acute appendicitis and normal appendices.

- In chronic appendicitis, excessive lymphomononuclear infiltrate in mucosal and submucosal layer seen. The mean mast cell counts were high

References

1. Waldron R and Jonson JG (1983) "Appendicitis 357 consecutive cases" *Ir. Med J* 76(11);446 – 448.
2. Butler Cooley (1981), "Surgical Pathology of acute appendicitis", *Hum Pathol*, 12 (10) ;870-878.
3. Arvindan KP 'Eosinophilis in acute appendicitis; possible significance, *Indian J Pathol Microbiology*,40(4);491-498.
4. Bower WF (1939), "Appendicitis with special reference to pathogenesis, bacteriology and healing", *Arch surg*,39;362-370.
5. Doraiswamy NV (1979), "Leukocyte count in the diagnosis & prognosis of acute appendicitis in children". *Br J Surg*,66;782-784.
6. Stead and Franks (1992). "Mast cells, nerve and fibrosis in the appendix", *J Pathol*, 161;209-219.
7. Ramdas Naik, Muktha R Pai, Rani Bansal (1998). "Relation of mast cell, nerve and fibrosis in appendix". *Indian journal Of Pathology and Microbiology*,41(1);27-30.
8. Rajendar Bhatnagar, Gupta AS (1978), "Acute appendicitis "A clinico Patholoical Study of 100 cases " *Indian Journal of Surgery*, Vol. 51;13-18.
9. Barker DJP (1985), "Acute appendicitis and dietary fiber; an alternative hypothesis, *Br Med J*,290;1125-1127.
10. Wangsteen OH and Dennis C (1939), "Experimental proof of the obstructive origin in man", *Ann Surg*,110; 629-633.
11. Butsch W. David and Jothi Rishyur (1973), "Recurrent appendicitis – fact no Fallacy", *Postgrad Med*,54;132-136.