

Comparative study of mast cells in different pathological types of surgically resected appendix specimens.

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Abstract: Background : Mast cells, constantly present in appendix can vary in numbers in normal and pathological states. **Objectives :** Looking for role of mast cells in pathogenesis of appendicitis, their quantitative variation in normal appendix and appendicitis as well as in different layers of wall of appendix in varied pathological entities and their relationship with eosinophils. **Materials and methods :** One hundred patients selected and mast cells, eosinophils counted and compared in formalin fixed appendectomy specimens by H&E, toluidin blue and Von Gieson stains. **Results :** Females dominated in the study (56%) with maximum cases in the age group of second to third decade. Recurrent appendicitis dominated in the pathological group (69%). High eosinophil count observed in acute eosinophilic appendicitis and high mast cell count in acute eosinophilic and recurrent appendicitis. **Conclusion :** A positive correlation with mean eosinophilic and mast cell counts in appendicitis noted with increasing mast cell count with fibrosis.

Key Words : Mast cell, eosinophil, appendicitis, fibrosis.

Date of Submission: 04-04-2018

Date of acceptance: 17-04-2018

I. Introduction

The biomedical literature included mast cells and basophils in the group of basophilic leucocytes. Paul Ehrlich recognized mast cells for their remarkable staining characteristics of proteoglycan and protease rich cytoplasmic granules(1).

Mast cells arise from hematopoietic progenitor cells and mature ones usually do not circulate in the blood but acquire their mature phenotype locally in the tissues where they ultimately reside(1).

Mast cells resemble basophils and both are bone marrow derived cells, contain electron dense granules staining metachromatically with selected basic dyes and produce numerous inflammatory mediators like histamine. Both express high affinity receptor for IgE which on activation by anti IgE antibodies induce mediator synthesis and secretions playing important roles in allergic inflammation and immune response. Mast cells mature outside bone marrow, generally in connective tissue or serous cavities, while basophils circulate in blood and recruited during allergic responses(1,2,3).

Most mast cells are constantly present in appendix, the organ most commonly subjected for surgical intervention and removal. Although, clinical and operative protocols are well known but pathogenesis of acute appendicitis is poorly understood. Currently, luminal obstruction due to faecoliths or less commonly submucosal lymphoid hyperplasia especially in children appears to initiate acute inflammation in appendix(1,2,3).

The present study aims at finding role of mast cells in pathogenesis of appendicitis; their variation in counts in mucosa, submucosa, and muscle layers in different histopathological groups of inflamed appendix compared to uninflamed one in various age groups. The study also looks for relationship between mast cells and fibrosis in appendix as well as relationship between number of eosinophils and mast cells in inflamed appendix.

II. Materials And Methods

The prospective study was conducted in a tertiary care hospital in West Bengal in a time period of one year after taking clearance from the institutional ethical committee and consent of patient. One hundred patients who attended and got admitted in surgery department with appendicitis included in the cross sectional observational study where appendices got removed for acute appendicitis, in course of laparotomy and those present with pathological ileo-caecal specimens. Exclusion criteria were applicable for cases of acute gangrenous appendicitis with associated necrosis.

Appendix fixed in 10% formalin received in the department of pathology for study. One section from tip, base and body taken after twenty four hours of fixation. Post processing and embedding of tissue sections

into paraffin blocks; two sections of five micron thickness cut from each block. One of the sections stained by hematoxyline & eosin (H&E) and the other by one percent toluidine blue for identification of mast cells.

H&E stained sections studied for histopathological evaluation of acute appendicitis with emphasis on presence of eosinophils and fibrosis. one percent toluidene blue solution staining showed purple coloured mast cell granules in blue background.

Van Gieson stain used to confirm the presence of fibrocollagenous tissue where working solution of Working Weigert's Iron hematoxyline for fifteen to thirty minutes and washed in water until nuclei were dark blue-black in paler blue-black background. Collagen fibres took red colour; nuclei were brown black and yellow colour visualized in muscle and red blood cells.

The number of mast cells in the mucosa, submucosa and muscular layer counted at high power(HP) and average counts obtained in ten overlapping HP fields considered in each case. Average eosinophil count in all layers in H&E stained sections obtained as described for mast cells. Field of view of eyepiece of microscope used was 18mm and area of field covered by HP objective of microscope was calculated to be 0.4sqmm. Considering this data, cell counts expressed as number of cells per sqmm. Further, the relationship of mast cells with eosinophilic infiltrate and fibrosis also assessed.

III. Analysis Of Data

The relationship between mast cell numbers and degree of eosinophilic infiltration evaluated using Spearman's rank correlation test. The eosinophil and mast cell counts in various histopathological categories compared using ANOVA.

IV. Results

The present study conducted on hundred surgically resected specimens of appendix. Of 100 cases; 44 were males (44%) and 56 females (56%) with male to female ratio of 1:1.27. Age of patients ranged from 7-64 years with mean age of 25.7 years. 38cases(16males and 22 females) were in the third decade and 31 cases(14males and 17females) in second decade.

Seven appendices(7%) were removed during other surgical procedures without any clinical symptoms of appendicitis and showed normal gross and histology.

Acute appendicitis presented muscularis propria,submucosa infiltrated by neutrophils in thirteen cases(13%).

Acute suppurative appendicitis showed dense infiltration of muscle wall with neutrophils,eosinophils,plasma cells and lymphocytes throughout all layers ; seen in five cases(5%).

Acute eosinophilic appendicitis showed marked eosinophilic infiltration into the mucosal crypts,submucosa, muscular and serosal layer without neutrophils;found in six cases(6%).

Maximum cases were of recurrent appendicitis(69%) having pathologic findings of chronic inflammation with or without fibrosis.Forty four out of sixty nine cases of recurrent appendicitis were seen above 20 years of age. Among nine cases of acute appendicitis studied, peak incidence of occurrence seen in age group above 20.

Out of 56 females;40(71.4%) presented with recurrent appendicitis compared to all other groups. This was statistically significant.Out of 44 males; 29(65.9%) presented with recurrent appendicitis which was higher compared to other groups, followed by 8 cases(18.2%) of acute appendicitis. Out of 69 cases of recurrent appendicitis, higher incidence seen in females(n=40 ;57.9%) which was statistically significant(p<0.01).

All appendices received were of entire length and seventy five appendices had attached mesoappendix(75%).

External surface showed hyperemia with congested blood vessels in seventy cases. Sixteen appendices were swollen and five showed pus on surface. Mucosal ulceration seen in sixteen cases and hyperemia in twenty four cases.Lumen of appendix showed faecolith in thirty eight out of hundred cases, luminal dilatation seen in twenty cases and narrowing in thirty four cases. Five showed pus in lumen , neutrophilic predominant exudates in eighteen appendices and eosinophilic predominant exudates in six. No parasite found in the studied cases. Appendiceal mucosa was ulcerated in forty one cases and congested in fifty two. Neutrophilic predominance seen in eighteen cases and eosinophilic predominance in six. Maximum cases (n=76) showed lymphocytic predominance.

Inflammation found in eighty two with majority (n=76) showing lymphocytic predominance in submucosa followed by neutrophilic predominance in eighteen and six showed predominant eosinophilic infiltration. Seventy nine cases showed submucosal fibrosis. Fifteen cases showed edema in the muscularis, thirty four showed inflammation, fibrosis.Edema seen in ten cases, inflammation in twenty , congestion in sixty and fibrosis in ten. Out of 100 appendices studied, maximum recurrent appendices showed fibrosis in submucosa(n=62) and muscularis propria(n=24)

Patients divided into two age groups of below and above 20 years and results analysed.

Mean eosinophilic counts in mucosa in various histopathological groups in each age group compared. Highest mean eosinophilic count of 143.75/sq.mm seen in mucosa of acute eosinophilic appendicitis and was statistically significant ($p < 0.01$). It was found that mean eosinophilic count in mucosa were low in acute appendicitis. Mean eosinophilic count in submucosa in various histopathological groups in each age group compared. Highest mean eosinophil count of 43.33/sqmm seen in acute eosinophilic appendicitis followed by 32.5/sqmm in acute suppurative appendicitis. It was found that eosinophil count in mucosa were high in acute eosinophilic appendicitis; low in acute appendicitis and normal appendices. Mean eosinophil count in muscularis propria of appendices in various histopathological groups in each age group compared. Highest mean eosinophil count of 65/sqmm seen in acute eosinophilic appendicitis and that in acute appendicitis was 25/sqmm.

Mucosal mast cell count in various histopathological groups in each group compared. Highest mean mast cell count observed in mucosa of acute eosinophilic appendicitis (12.5/sqmm) followed by recurrent appendicitis (10.5/sqmm). (Table-1)

Table:1 :Comparison of mast cell count in mucosa,submucosa,muscularis propria of appendix

Histopathological groups	No. of cases	Age (0-20 yrs)	Age >20yrs	Total mean mast cell count/sqmm.
		Mean mast cell/sqmm	Mean mast cell /sqmm	
Normal	7	6.25/11.25/6.25	4/7.5/4	4.64/8.57/4.64
Recurrent	69	12.3/25.9/16.9	8.4/28.35/15.68	10.57/27.46/15.76
Acute	13	7.8/18.21/9.28	7/26.67/15.83	7.5/22.11/12.3
Acute suppurative	5	10/24.16/15.83	5/16.25/5	8/19/11.5
Acute eosinophilic	6	0	12.5/28.75/20.41	12.5/28.75/20.41

Highest mean mast cell count of 28.75/sq.mm seen in submucosa of acute eosinophilic appendicitis followed by 27.46/sqmm in recurrent appendicitis (Table-1)

Highest mast cell count of 20.41/sqmm seen in muscularis propria of acute eosinophilic appendicitis followed by 15.76/sqmm in recurrent appendicitis (Table-1)

Appendectomy specimens from both genders did not show any significant differences in mast cell counts in any of the layers. Highest mean mast cell seen in acute eosinophilic appendicitis and lowest found in normal appendices. Recurrent appendicitis showed higher mean mast cell count compared to other inflamed appendices. Acute appendicitis and acute suppurative appendicitis showed intermediate counts

A positive correlation established using Pearson's test between mean eosinophilic counts/sqmm and mean mast cell count/sqmm. In the different layers of appendix in various histopathological groups showing trend towards significance ($p < 0.05$). Mast cell count in mucosa found to be higher in acute eosinophilic appendicitis and recurrent appendicitis with or without fibrosis as compared to other inflamed appendices. Submucosal mean mast cell count was higher in acute eosinophilic appendicitis and recurrent appendicitis when compared to other inflamed appendices and showed a trend towards significance ($p < 0.05$).

No significant difference observed in mean mast cell count in the different layers of appendices in each age group.

V. Discussion :

The surgically resected appendices of 100 patients were grossly and histopathologically evaluated along with mast cell and eosinophil counts. Appendix, previously thought as vestigial organ, now recognized as immunological organ actively participating in secretion of immunoglobulins, particularly immunoglobulin A(3,5,7,8,14).

In the present study, out of 100 resected appendices, 44 were males and 56 females with male to female ratio of 1:1.27. Highest incidence of appendectomy seen in third decade with mean age of 25.7yrs. Chang AR in his analysis of 3003 appendices found a slight preponderance of males with 82% less than thirty years of age, similar to current study(4). In the present study, incidence of acute appendicitis was 13%. A higher incidence of acute appendicitis seen above 20years and in males. Male predominance of acute appendicitis also seen by Elangovan S et al and Singh UR et al which was consistent with present study(5). Petras RE and Goldblum JR showed common occurrence of appendicitis in second and third decade with incidence falling gradually after forty years of age, equally affecting males and females with affection of females more in 15-25 years group with female to male ratio of 2:1. This difference is not noted in other age groups(6). Rosai J observed acute appendicitis seen in either sex at any age but most frequently in young men. He also noted that a false positive diagnosis was twice as common in females and males(7).

Appendix in cases of repeated episodes shows fibrosis indicative of previous inflammation . However, existence of recurrent appendicitis has always been controversial and only readdressed(8).In the present study, recurrent appendicitis seen more frequently than acute appendicitis with higher frequency above 20yrs of age.A significantly high incidence of recurrent appendicitis seen in females in this study.Chang SK and Chan P noticed a 11% incidence of recurrent appendicitis out of 290 patients with appendicitis comprising 21% females. 15% of patients attributed to recurrent inflammation of appendix(9).Thackray AC found progressive fibrosis with infiltration by lymphocytes and plasma cells together with hyperplasia of lymphoid tissue is normally present(10).

Crabbe MM et al found 10% incidence of recurrent appendicitis ,Barber MD et al concluded that recurrent appendicitis exists and affects at least 6.5% of those who ultimately have an inflamed appendix removed (11,12).In the present study, no case of chronic appendicitis was found which collaborated with most of the authors. They were probably represented as recurrent appendicitis as noted by Hertzler AE(13). In the current study of 100cases, no case of subacute appendicitis found but showed 5% of acute suppurative appendicitis.Stephenson J and Snoddy WT classified appendices having polymorphonuclear leucocyte infiltration of muscle wall, with or without other inflammatory cell infiltration as acute suppurative appendicitis where most showed dense infiltration of neutrophils in the muscular layer and constituted 28.6% of inflammatory lesions of appendix in their study(14).

Strphenson J and Snoddy WT chose to call “Subacute appendicitis” when there was an infiltration of muscle wall by at least five eosinophils/hpf but failed to mention the clinical correlation to justify this term(14).Jona JZ et al observed cases which presented as acute appendicitis but containing only transmural eosinophilic infiltration in appendix and included them in the spectrum of eosinophilic gastroenteritis rather than primary appendicitis(15).

Aravindan KP observed mural eosinophilic infiltration as consistent finding in acute appendicitis and described cases where eosinophilic infiltrate was the sole finding. He was the first to suggest that eosinophilic infiltration in acute appendicitis is an early event linked possibly to Type I hypersensitivity reaction(16,17). He chose to name this entity as “Acute eosinophilic appendicitis”. In the current study, six out of hundred appendices showed the histological features fitting into the category of acute eosinophilic appendicitis, the incidence of which was similar to that seen by Aravindan KP.

In the current study, higher mucosal mast cell count seen in acute eosinophilic appendicitis and recurrent appendicitis . Intermediate counts seen in mucosa of acute appendicitis and acute suppurative appendicitis.Crowe J and Howe S showed high mucosal mast cell count in normal appendix. This was in contrast to present study which showed very low mast cell count in normal appendix(18). Naik T et al found that mean mast cell counts were decreased in mucosa and submucosal layers of acute appendicitis. They concluded that the cause of decreased mast cell count is due to inability to detect them because of degranulation or elimination through mucosa(19,22).Aravindan KP suggested that normal appendices with high initial mural mast cell count are prone to develop acute appendicitis(16,17).

Mysorekar VV et al studied 150 appendices, out of which six showed normal histology and these appendices showed very low mast cell counts in all the layers similar to that seen in the current study. They found a higher mast cell count in acute appendicitis compared to normal appendices but chronic appendicitis showed highest mast cell count(20).Singh UR et al found highest mean mast cell count in clinically acute but histological normal appendices when compared to controls and also acute appendicitis. They remarked that a statistically significant increase in mast cells in appendices may explain the clinical presentation and opined that the pain in these patients may be caused by degranulated mast cells (21).(Table-2)

Table-2 : Comparative study of mast cell in mucosa, submucosa and muscle layers

Author	Normal	Recurrent appendicitis	Acute appendicitis	Subacute appendicitis	Acute eosinophilic appendicitis	Acute suppurative appendicitis
Crowe & Howe S	66.0/-/25.4	-	-	-	-	-
Naik R etal	47.63/38.3/-	-	29.0/34.48/-	43.1/41.7/	-	-
Mysorekar VV etal	4.7/3.3/1.8	55.6/42.8/32.4	36.3/27.4/27.6	-	-	-
Sing UR etal	3.13/7.04/4.36	-	5.42/18.58/8.79	-	-	-
Present study	4.64/8.57/4.64	10.57/27.46/15.76	7.5/22.11/12.30	-	12.5/28.75/20.41	8.0/19/11.5

In the present study, submucosal mean mast cell count was lowest in normal appendix which was in concordance with Mysorekar VV et al and Singh UR et al. Naik et al found high mean mast cell count in normal appendices and subacute appendicitis. In present study, highest mean mast cell count seen in acute eosinophilic appendicitis and recurrent appendicitis and very low in normal appendices similar to studies by Mysorekar VV et al and Singh UR et al (19,20,21) (Table-2)

In the current study, a higher mast cell count observed in the muscularis propria in recurrent appendicitis when compared to acute appendicitis. Lowest mean mast cell count seen in normal appendices which were in concordance with other authors. Highest mean mast cell count observed in cases of acute eosinophilic appendicitis. Aravindan KP found high mean mast cell count in muscular layer of recurrent appendicitis which was also found in the present study (16) (Table-2)

Mysorekar VV et al found highest mean mast cell count in the muscular layer, lymphocytes and plasma cells with presence of submucosal lymphoid hyperplasia and varying degree of fibrosis. In the present study, the term chronic appendicitis was avoided since its existence has been disputed. Instead, the term recurrent appendicitis used for the cases showing similar histological features along with a clinical history of repeated bouts of abdominal pain in the past (20).

Crowe J and Howe S and Naik et al found a higher mucosal mean mast cell count in children as compared to older patients. Our study did not show any such difference which was similar to study by Mysorekar VV et al (18,19,20). There was no correlation between sex and mast cell count in the present study as has also been described by Naik et al and Mysorekar et al (19,20).

Aravindan KP found that mast cells tend to be few and degranulated in areas which showed clustering of eosinophils. (17,19,36). Singh UR et al also observed an increase in mean mast cell count in different layers with a corresponding increase in mean eosinophilic count and attributed it to the chemotactic factor and histamine release from mast cells (21). In contrast, Mysorekar VV et al did not find any correlation between mast cell and eosinophil density or distribution. In the present study, a positive correlation found between mast cell and eosinophil count. It was also observed that highest mean mast cell count seen in acute eosinophilic appendicitis which also showed highest mean eosinophil count in all the layers (20).

Stead RH et al found that appendices with fibrosis having higher mast cell count and histamine. (23). Naik R et al observed a rise in mast cell count in early stages of fibrosis and as the fibrosis increased this association was retained only in submucosa (19). The present study showed fibrosis in 62% cases in submucosal and muscularis layers. These showed a concurrent higher mast cell count as compared to other inflammatory appendices.

The mast cell mediators are involved in different acute and chronic inflammatory and repair processes. Increased mast cells are demonstrated in keloid, pulmonary fibrosis, favouring their involvement in repair processes (24,25,26,27,28).

In the present study, none showed evidence of parasitic infestation. Dorfman S et al and Addiss DG and Juranek DD found no significant causal relationship between parasitic infestation and acute appendicitis (32,33). Mysorekar VV et al found a mere 2.1% of cases showing evidence of parasitic infestation (20).

Dahlen SE and lumlin M stated that it has been notoriously difficult conclusively to establish mast cell activation in human studies (34). Tsuji et al suggested that antigen stimulation of effector system could give rise to appendicitis (35).

VI. Conclusion

A higher incidence of recurrent appendicitis found in this study. Eosinophil counts in all the layers were very low in normal appendices and very high in acute eosinophilic appendicitis and acute appendicitis. A higher mast cell count seen in acute eosinophilic appendicitis and recurrent appendicitis. There were no cases of parasitic infestation.

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