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STUDY OF MAST CELL IN SURGICALLY RESECTED APPENDICES

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ABSTRACT

Mast cells, first discovered by Paul Ehrlich over a century ago, continue to intrigue researchers due to their complex roles in both health and disease. These cells, which are abundantly found in connective tissues and near blood vessels, are involved in various physiological and pathological conditions. They contain a variety of biologically active mediators within their metachromatic granules, and their activation is primarily triggered by the cross-linking of IgE Fc receptors, although they can also be activated by complement components such as C5a and C3a. The role of mast cells in the pathogenesis of acute appendicitis remains poorly understood, despite their ubiquitous presence in tissues, including the appendix. This study aims to investigate the variation of mast cells in inflamed versus non-inflamed appendices and to explore their potential role in the development and outcome of acute appendicitis. Histopathological analysis was performed on 100 appendix specimens, using Toluidine blue staining to identify mast cells and Hematoxylin & Eosin for assessing inflammatory changes. Mast cell counts were compared across different histopathological groups, including acute appendicitis, acute eosinophilic appendicitis, and recurrent appendicitis, in various age groups. The results may provide insights into the involvement of mast cells in acute appendicitis and contribute to a better understanding of its pathogenesis.

Keywords: Mast cells, acute appendicitis, Toluidine blue, Histopathology, Inflammation, Eosinophilic appendicitis, Recurrent appendicitis, IgE Fc receptors, Tissue granules, Appendectomy

INTRODUCTION

Mast cell remains an enigmatic, fascinating and highly engineered cell more than 100 years after its discovery by Paul Ehrlich at the turn of the 18th century. It is a cell that is found widely distributed in the body particularly associated with connective tissues.1 The mast cells with a battery of crucial chemical mediators and substances in their typical metachromatic granules are known to play a role in health and various disease states in man.2 Mast cells are similar to Basophils in many respects, including the presence of cell surface IgE Fc receptors as well as cytoplasmic granules. Mast cells have cytoplasmic membrane-bound granules that contain a variety of biologically active mediators they are activated by the cross-linking of high- affinity IgE Fc receptors; in addition, mast cells may also be triggered by several other stimuli, such as complement components C5a and C3a.3Although, the clinical and operative protocols are well known, the pathogenesis of acute appendicitis is poorly understood. Currently, luminal obstruction due to faecolith or less commonly submucosal lymphoid hyperplasia especially in children appears to initiate acute inflammation in appendix.4 The present study aims at finding mast cell variation in inflamed appendix compared to uninflamed appendix and also to suggest the possible role of mast cells in the pathogenesis and outcome of acute appendicitis.Mast cells are ubiquitous though they are being found in varying numbers in practically all tissues primarily in respiratory, digestive, urogenital systems, skin and are abundant near blood vessels and nerves and in sub epithelial tissues, which explains why local immediate hypersensitivity reactions often occur at these sites . Mast cells are constantly present in appendix, the organ most commonly subjected for surgical intervention and removal.3 They arise from hematopoietic tissue in the bone

marrow, the progenitors differentiate from primitive cells under the influence of cytokines (IL3), migrate to other body sites and then undergo differentiation and maturation, they settle in connective tissue and usually do not circulate in the blood stream, and are functionally heterogeneous, possibly site specific and have the ability to adapt to their environment, producing secretions commensurate with the needs of any situation.1Mast cells are similar to Basophils in many respects, including the presence of cell surface IgE Fc receptors as well as cytoplasmic granules. Mast cells have cytoplasmic membrane-bound granules that contain a variety of biologically active mediators they are activated by the cross-linking of high- affinity IgE Fc receptors; in addition, mast cells may also be triggered by several other stimuli, such as complement components C5a and C3a.3Although, the clinical and operative protocols are well known, the pathogenesis of acute appendicitis is poorly understood. Currently, luminal obstruction due to faecolith or less commonly submucosal lymphoid hyperplasia especially in children appears to initiate acute inflammation in appendix.4The present study aims at finding mast cell variation in inflamed appendix compared to uninflamed appendix and also to suggest the possible role of mast cells in the pathogenesis and outcome of acute appendicitis.

AIMS AND OBJECTIVES

To investigate the possible role of mast cells in pathogenesis of appendicitis. To compare the mast cell counts in the mucosa, sub mucosa and the muscular layers of the appendix in various histopathological groups. To compare the mast cell counts in the normal appendix and in the acute appendicitis, acute eosinophilic appendicitis and recurrent appendicitis in various age

MATERIAL AND METHODS

The study was conducted on 100 consecutive appendix specimens received for histopathological examination in the Department of Pathology, in our tertiary care centre. The appendices were removed either for suspected appendicitis or as normal course of laparotomy done for other diseases. This study was conducted from November 2017 to August 2019. Clinical details were noted in the proforma enclosed, including age, sex, symptoms and clinical diagnosis.

COLLECTION OF SPECIMENAppendices of patients who underwent surgical intervention in the form of emergency appendectomy, interval appendectomy and ileocaecal resection procedures at the Department of Surgery in our tertiary care Centre were procured with relevant history, clinical details and laboratory investigations. Appendices were received in 10% formalin. Minimum of 24 hours was allowed for proper tissue fixation. After fixation, one section from 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 4 micron thickness were cut from each block. One of the sections was stained by haematoxylin& eosin and the other by 1% toluidine blue. Haematoxylin&Eosin stained sections were studied for various findings of appendicitis including the presence of eosinophil, fibrosis and inflammatory changes. 1% toluidine blue stained sections were studied for identification of mast cells and an average mast cell count in all the layers are obtained.

INCLUSION CRITERIA
All appendices surgically removed as a therapeutic measure for clinically suspected cases of acute appendicitis. Appendices removed in the course of ileo-caecal resection or laparotomy done for some other disease

EXCLUSION CRITERIA
Cases of acute gangrenous appendicitis due to associated necrosis of muscle fibres were excluded from the study. Cases of confirmed malignant and benign lesion of appendix are excluded from study. Toluidine blue stain (1%) was used for the identification of mast cells. The stained sections were studied immediately and photographs were taken. The number of mast cells in mucosa, submucosa and muscular layer were counted at 400X magnification (high power). The average counts obtained in 10 nonoverlapping high power fields were considered in each case. The field of view of the eyepiece of the microscope used was 20 mm and area of the field covered by high power objective (40X) of the microscope was calculated to be 0.50 sq mm. considering this data, the cell counts were expressed as the number of cells per sq mm. The Mean and Standard Deviation were calculated using Microsoft Office Excel 2007 software. The mean mast cell counts in various histopathological categories were compared using ANOVA Test (Analysis Of Variance Test) by using Open Epi software version 3.01. Method of staining(100)

Toluidine blue
Deparaffinise the slides by melting wax and rinsing by xylene followed by isopropyl alcohol Add 8-10 drops of toluidine stain on the slide and wait for 1 minute.Blot the slides quickly with blotting paper.Rinse the slide once with CO2 free water.Rinse the slide again with alcohol reagent in two changes.Rinse the slide with xylene in two changes.Mount and examine under microscope.

RESULT:- Mast cells - violet/ Purple Background – Blue

Haematoxylin and Eosin staining
Deparaffinise the slides by melting wax and rinsing by xylene for 5-10 minutes followed by Absolute alcohol for 5 minutes and 95% ethyl alcohol for 5 minutes.Wash with tap water.Stain with Harris haematoxylin for 5 minutes. Rinse with tap water.Differentiate with 1% hydrochloric acid. Wash in tap water.Blue with running tap water Stain with watery eosin. Rinse in water.Two changes in 95% ethyl alcohol, Absolute alcohol and xylene. Mount with D.P.

RESULT :- Nuclei- Blue Cytoplasm – Pink

OBSERVATIONS AND RESULTS

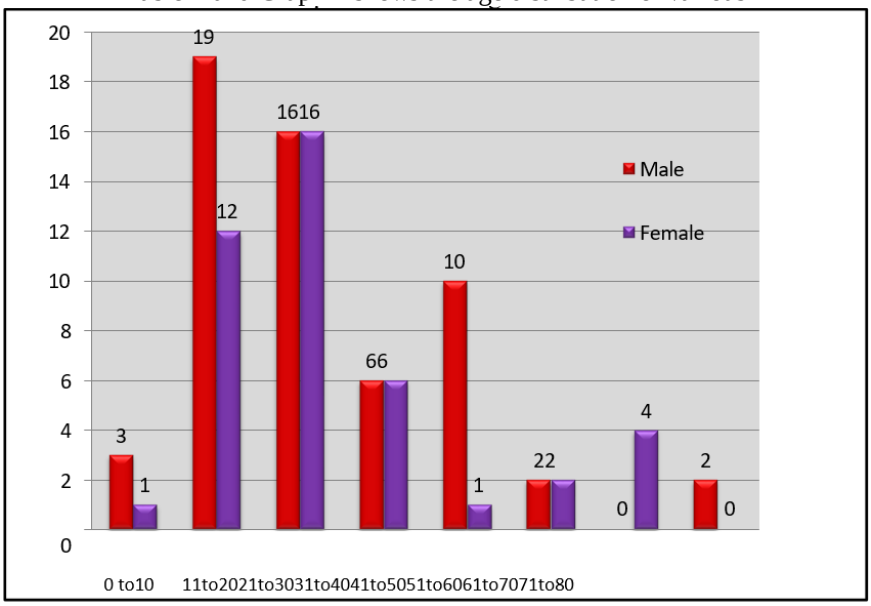
A two year prospective study was done on surgically resected appendices sent for histopathological examination in the Department of pathology in tertiary care centre.

Table 1 & Graph 1 shows age and sex distribution of study

Age group	Male		Female		Total
	No.ofcases	Percentage(%)	No.ofcases	Percentage(%)	
0-10	03	03	01	01	04
11-20	19	19	12	12	31
21-30	16	16	16	16	32
31-40	06	06	06	06	12
41-50	10	10	01	01	11
51-60	02	02	02	02	04
61-70	00	00	04	04	04
71-80	02	02	00	00	02
Total	58	58	42	42	100

Graph 1 :- Age and sex distribution of study subjects.

Table-2 and Graph-2 shows the age distribution of various



appendicitis with inflamed and oedematous appendix showing marked eosinophilic infiltration into the muscularis propria without neutrophils(fig 10) were seen in five cases (n=5) (5%). Recurrent appendicitis presents with history of repeated attacks of right lower abdominal quadrant pain and pathologic finding of chronic inflammation with or without fibrosis (fig 12 & 13). Recurrent appendicitis cases seen in this study were of (n=32) (32%) The peak incidence of occurrence of recurrent appendicitis out of thirty two cases was seen in the age group above 20 years. Among the fifty six cases of acute appendicitis studied, the peak incidence of occurrence was seen in age group above 20 years.

Table No. 2:- Age distribution of various histopathological groups of appendices studied.

Histopathological group	Ageinyears		Total
	0-20	>20	
Normal	00	07	07
Acute appendicitis	21	35	56
Acute eosinophillicappendicitis	02	03	05
Recurrent appendicitis	12	20	32
Total	35	65	100

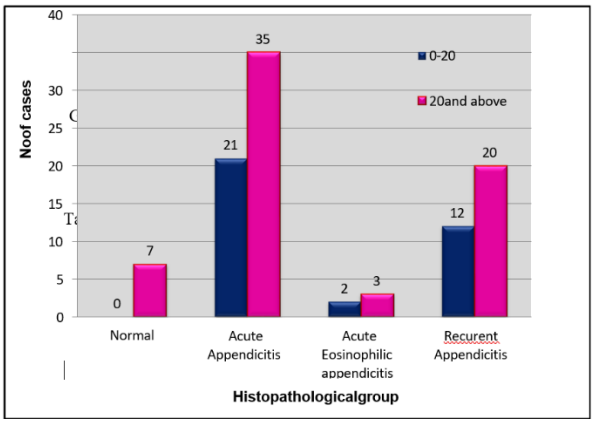


Table 3 and Graph 3 show the distribution of various histopathological groups of appendices among males and females. Out of 58 male patients, the incidence of acute appendicitis n=38(65.51%) was higher when compared to other groups, followed by recurrent appendicitis n=14(24.13%). Out of 42 female patients the incidence of recurrent appendicitis n=18(42.85%) was equal when compared to the incidence of acute appendicitis n=18(42.85%).

Table No. 3 :- Sex distribution of various histopathological groups of appendices studied.

Histopathological group	Sex		Total
	Male	Female	
Normal	03	04	07
Acute appendicitis	38	18	56
Acute eosinophillic appendicitis	03	02	05
Recurrent appendicitis	14	18	32
Total	58	42	100

Graph 3:- Sex distribution of various histopathological groups of appendices studied.

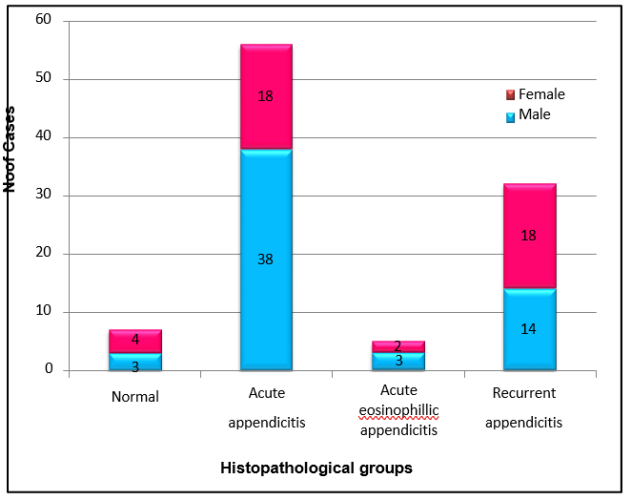


Table 4 shows the shows the gross features of the100 appendices studied.

The length of the appendices studied ranged from 3 cm to 8 cm with a mean length of 5.25cm. All the appendices received were of entire length and eighty five (n=85) appendices had attached mesoappendix (85%). External surface showed hyperaemia and congested vessels in seventy eight cases (n=78) and five cases showed perforation(n=05) while seventeen cases showed normal external surface (n=17). Wall of the appendices were thickened in 44 (n=44) cases and thinned out in 14(n=14) cases.

Table No. 4 : Gross appearance of the appendices in the cases studied.

Gross finding		No. of cases	Percentage(%)
Meso-appendix	Present	85	85
	Absent	15	15
External surface	Normal	17	17
	Hyperemia	78	78
	Perforation	05	05
Wall of appendix	Normal	42	42
	Thickening	44	44
	Thinning	14	14

Table 5 shows the Luminal and Histopathological findings in the mucosa of the appendices studied. Mucosal ulceration was seen in thirty three (n=33) cases and hyperaemia in fifty two (n=52) cases while fifteen cases showed normal mucosal lining(n=15). Out of 100 appendices, lumen showed faecolith in fourteen cases (n=14); luminal dilatation was seen in thirty cases (n=30) and narrowing in fifty four cases (n=54). Two cases showed pus in the lumen (n=2). There were no parasites found in the studied cases.

Table No. 5 :- Luminal and Histopathological findings in the mucosa of the appendices studied.

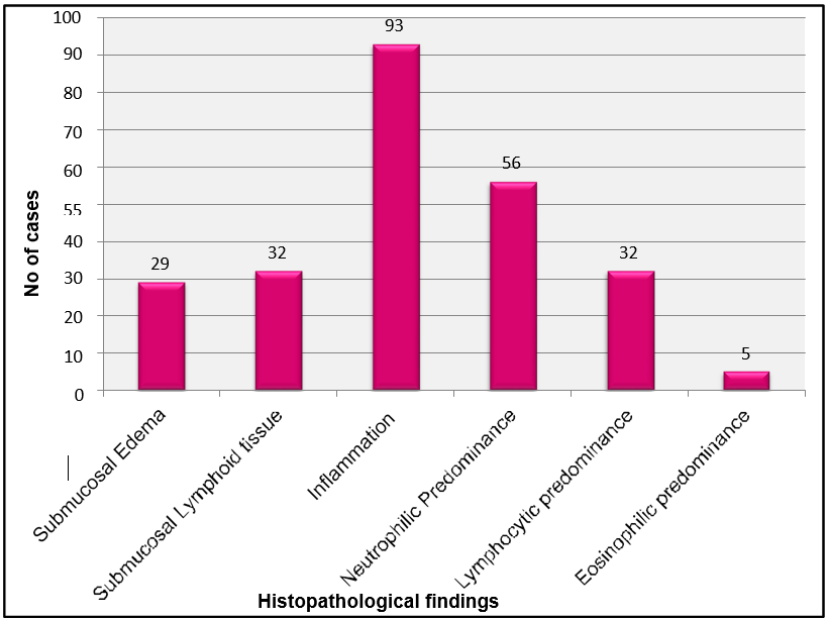
Histopathological findings		No. of cases	Percentage(%)
Mucosa	Normal	15	15
	Ulceration	33	33
	Hyperemia	52	52
Lumen	Faecolith	14	14
	Pus	02	02
	Narrowing	54	54
	Dilatation	30	30
	Parasite	00	00

Table- 6 and Graph- 4 show various Histopathological findings in the submucosa. Submucosal oedema was seen in twenty nine cases (n=29) and inflammation seen in ninety three (n=93) cases. Majority of the cases showed neutrophilic predominance in the submucosa (n=56) followed by lymphocytic predominance in thirty two cases (n=32) (fig 12) while five cases showed a predominant eosinophilic infiltration (n=5) (fig 9). Submucosal Lymphoid tissue was present in thirty two cases (n=32) (fig 3). Table- 6 and Graph- 4 show various Histopathological findings in the submucosa. Submucosal oedema was seen in twenty nine cases (n=29) and inflammation seen in ninety three (n=93) cases. Majority of the cases showed neutrophilic predominance in the submucosa (n=56) followed by lymphocytic predominance in thirty two cases (n=32) (fig 12) while five cases showed a predominant eosinophilic infiltration (n=5) (fig 9). Submucosal Lymphoid tissue was present in thirty two cases (n=32) (fig 3).

Table No. 6 :- Histopathological findings in the submucosa of the appendices studied.

Histopathological findings		No. of cases	Percentage(%)
Submucosal edema	Present	29	29
	Absent	71	71

Inflammation	Present	93	93
	Absent	07	07
Predominant inflammatory cells	Neutrophils	56	56
	Lymphocytes	32	32
	Eosinophils	05	05
Submucosal Lymphoid tissue	Present	32	32
	Absent	68	68



Graph 4:- Histopathological findings in the submucosa of the appendices studied.

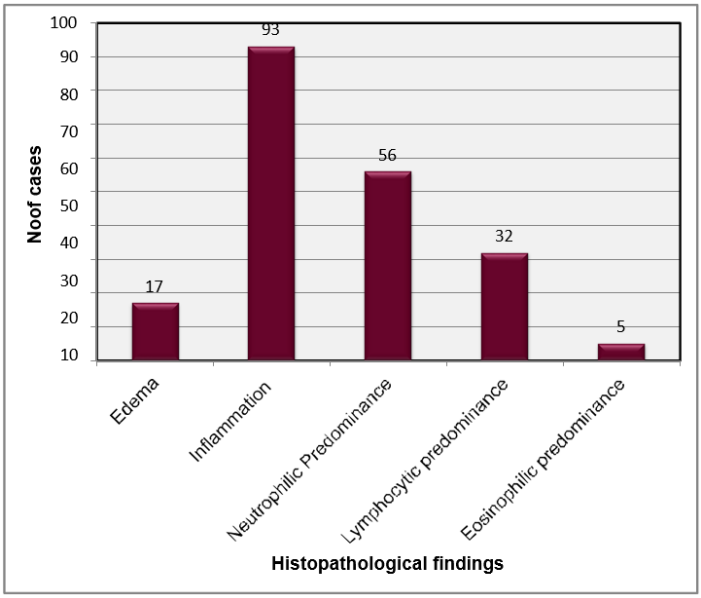
Table-7 and Graph-5 shows histopathological findings in the muscularis propria. Seventeen cases showed oedema (n=17) in the muscularis, ninty three cases showed inflammation (n=93). Majority of the cases showed neutrophilic predominance in the (n=56) followed by lymphocytic predominance in thirty two cases (n=32) while five cases showed a predominant eosinophilic infiltration(n=5).

Table No. 7 :- Histopathological findings in the muscularis propria of the appendices studied.

Histopathological findings		No. of cases	Percentage(%)
Edema	Present	17	17
	Absent	83	83
Inflammation	Present	93	93
	Absent	07	07
Predominant inflammatory cells	Neutrophills	56	56
	Lymphocytes	32	32

	Eosinophils	05	05

Graph 5 :- Histopathological findings in the muscularis propria of the appendices studied.



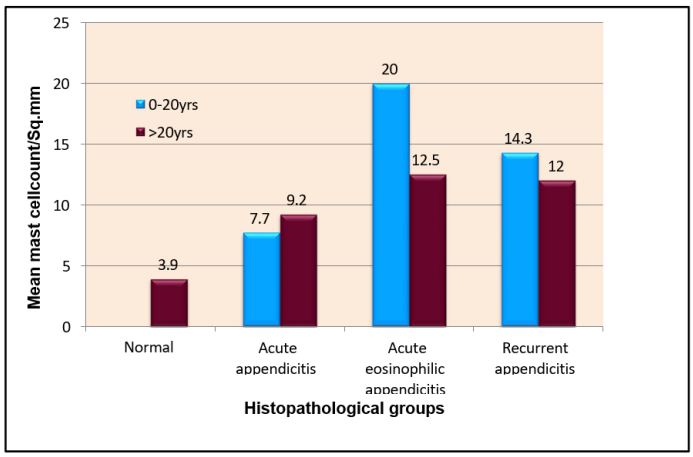
Patients were classified into two broad age groups and the results were analysed.

1. 0-20 Years (Children and Adolescents)
2. >20 years (Adults)

Table 8 and graph 6 shows the mucosal mast cell count in various histopathological groups of appendices in each age group. Among the mucosa highest mean mast cell count was observed in the mucosa of acute eosinophilic appendicitis (mast cell count = 15.5 /mm^2) followed by recurrent appendicitis (mean mast cell count = 12.8/mm^2) (fig 17 & 20) while least mean mast cell count was observed in normal appendices. (mean mast cell count = 3.9/mm^2)

Table No. 8 :- Comparison of mast cell counts in the mucosa of the appendices in various histopathological groups in each age groups.

Histopathological groups	No. of cases	Age group		Mean mast cell count /Sq.mm
		(0-20yrs)	>20yrs	
		Mean mast cell count /Sq.mm	Mean mast cell count /Sq.mm	
Normal	07	--	3.9±1.3	3.9±1.3
Acute appendicitis	56	7.7±3.7	9.2±5.4	8.7±4.9
Acute eosinophilic appendicitis	05	20.0±10.6	12.5±5	15.5±7.5
Recurrent appendicitis	32	14.3±6.4	12.0±5.3	12.8±5.8



Graph 6 :- Comparison of mast cell counts in the mucosa of the appendices in various histopathological groups in each age groups.

Table 9 and graph 7 show the submucosal mast cell count in various histopathological groups of appendices in each age group. Among the submucosa highest mean mast cell count was observed in the submucosa of recurrent appendicitis (mean mast cell count = 21.4 /mm^2) followed by acute eosinophilic appendicitis (mean mast cell count=17.5/mm^2) while least mean mast cell count was observed in normal appendices. (mean mast cell count=4.6/mm^2)

Table No. 9 :- Comparison of mast cell counts in the submucosa of the appendices in various histopathological groups in each age groups.

Histological groups	No. of cases	Age group		Mean mast cell count /Sq.mm
		(0-20 yrs)	>20 yrs	
		Mean mast cell count /Sq.mm	Mean mast cell count /Sq.mm	
Normal	07	--	4.6 ± 1.7	4.6 ± 1.7
Acute appendicitis	56	11.7 ± 6.4	13.3 ± 5.9	12.7 ± 6.1
Acute eosinophillic appendicitis	05	15.0 ± 3.5	19.1 ± 7.6	17.5 ± 6.1
Recurrent appendicitis	32	20.0 ± 7.3	22.2 ± 8.3	21.4 ± 7.9

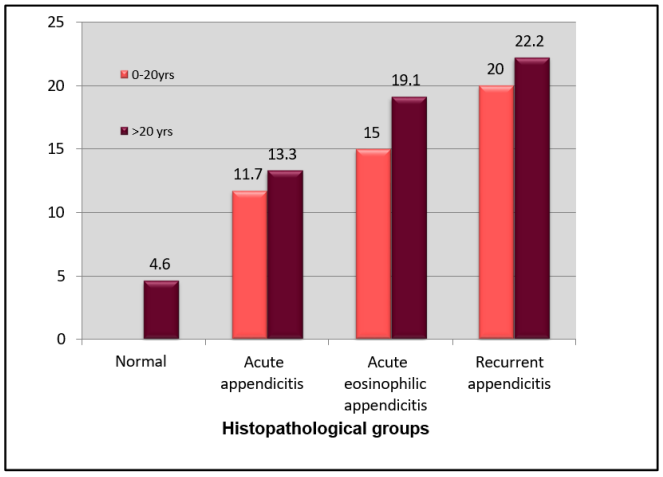


Table 10 and graph 8 show the comparison of mast cell counts in the muscularis propria of the appendices in the various histopathological groups. Among the muscularis propria highest mast cell count was seen in Recurrent appendicitis

(13.9/mm^2) followed by cases of Acute eosinophilic appendicitis (12.5/mm^2) while least mean mast cell count was observed in normal appendices. (mean mast cell count=6.4/mm^2)

Table No. 10 :- Comparison of mast cell counts in the muscularis propria of the appendices in various histopathological groups in each age groups.

Histological groups	No. of cases	Age group		Mean mast cell count /Sq.mm
		(0-20 yrs)	>20 yrs	
		Mean mast cell count /Sq.mm	Mean mast cell count /Sq.mm	
Normal	07	--	6.4 ± 1.9	6.4 ± 1.9
Acute appendicitis	56	12.5 ± 4.8	11.8 ± 3.8	12.0 ± 4.2
Acute eosinophillic appendicitis	05	13.7 ± 5.3	11.6 ± 5.2	12.5 ± 4.6
Recurrent appendicitis	32	14.5 ± 5.9	13.6 ± 5.9	13.9 ± 6.2

Graph 8 :- Comparision of mast cell counts in the muscularis propria of the appendices in various histopathological groups in each age groups.

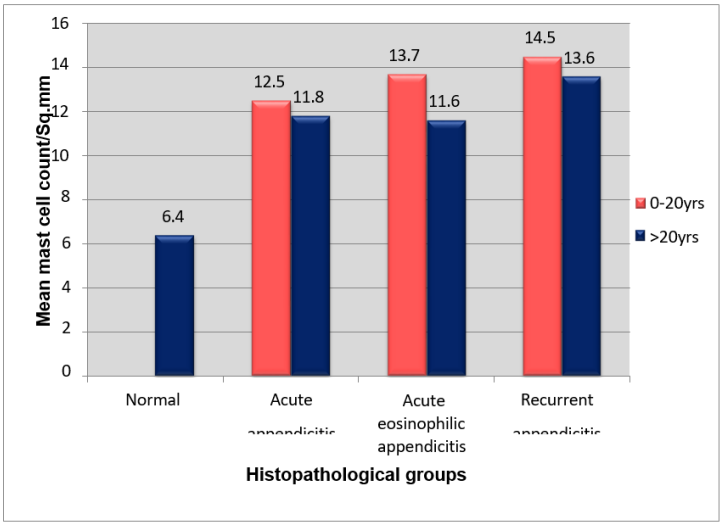


Table No. 11 shows the comparison of mean mast cell counts in the mucosa, submucosa and muscularis propria of the appendices in various histopathological groups. Among the various histopathological groups highest total mean mast cell count was seen in recurrent appendicitis and lowest was found in normal appendices. Among the various layers of appendix highest mean mast cell count was seen in submucosa of recurrent appendicitis while lowest was seen in mucosa of normal appendix.

Table No. 11 :- The comparison of mean mast cell counts in the mucosa, submucosa and muscularis propria of the appendices in various histopathological groups

Histopathological groups	No.ofcases	Mean mast cell count			Total mean mast cell count
		Mucosa	Submucosa	Muscularis propria	
Normal	07	3.9±1.3	4.6±1.7	6.4±1.9	5±1.9
Acute appendicitis	56	8.7±4.9	12.7±6.1	12.0±4.2	11.1±5.4

Acute eosinophillic appendicitis	05	15.5±7.5	17.5±6.1	12.5±4.6	15.1±6.1
Recurrent appendicitis	32	12.8±5.8	21.4±7.9	13.9±6.2	16.1±7.7
Total	100	10.5±5.8	15.2±8.1	12.3±5.1	12.5±6.8

The difference of totalmeanmast cell counts between normalappendix and cases of appendicitis was found statistically significant($p<0.05$).

DISCUSSION

For many years appendix was erroneously viewed as a vestigial organ with no known function. It is now well recognized that appendix is an immunological organ that actively participates in secretion of immunoglobulin, particularly immunoglobulin A.[45]

In the present study, surgically resected appendices of 100 patients were grossly and histopathologically evaluated along with mast cell counts in mucosa, submucosa and muscularis propria.

Out of 100 resected appendices, 58 cases were males and 42 cases were females with a male to female ratio of 1.38:1. Highest incidence of appendectomy was seen in the age group of third decade with a mean age of 28.3 years.

Chang AR in 1981 in his analysis of 3003 appendices found that there was a slight preponderance of males. He found that 82% of appendices were removed from the patients less than thirty years of age, similar to our study. [46]

Peak incidences of appendicectomies were observed in teens and early 20's. After middle age, risk of developing appendicitis is quite small. Incidence is equal among males and females before puberty. In teenagers and young adults, male to female ratio increases to 3:2 at 25 years of age. Thereafter, greater incidence in males decreases.[48]

Histopathological groups of appendicitis

In the present study, the incidence of acute appendicitis was found to be 56 %. A higher incidence of acute appendicitis was seen in the age group above 20 years. A male to female ratio of 1.38:1 was observed (Table 2 & 3).

TABLE 12:- COMPARISON OF INCIDENCE OF DIFFERENT HISTOPATHOLOGICAL GROUPS OF APPENDICES IN VARIOUS STUDIES

Authors	Normal Appendix	Acute Appendicitis	Acute Suppurative/ Resolving Appendicitis	Acute eosinophilic appendicitis	Recurrent/ chronic Appendicitis
NaikRetal (1997)[17]	54.5%	25%	-	-	-
Aravindan KP(1997)[47]	32.9%	44.3%	-	-	22.8%
Mysorekar VV etal (2006)[93]	4%	59.3%	-	-	34.7%
Aravindan KP et al ((2010)[86]	32.8%	60.9%	-	6%	-
KolurA et al (2014)[94]	5.15%	43.75%	2.31%	2.96%	45.82%
Sandhu A et al (2019)[97]	2%	47.50%	12%	-	38.50%
Present study (2019)	7%	56%	-	5%	32%

Male predominance of acute appendicitis was also seen by Elangovan S et al in 1997 and Singh UR et al in 2007 which was consistent with the present study. [55,27] . Acute appendicitis was common in 2nd and 3rd decade with incidence falling gradually after 40 years of age. According, to them males and females are equally affected and females are more affected in the age group of 15-25years with a female to male ratio of 2:1. [15]. Kolur A et al in 2014 conducted study on 777 surgically resected appendices observed that the incidence of acute appendicitis was found to be 43.5%. A higher incidence of acute appendicitis was seen in the age group below 20 years and in females.[94] Sandhu A et al in 2019 conducted study on 200 surgically resected appendices observed that maximum number of patients regardless of the age group were of acute appendicitis that is 95 cases (47.5%) followed by chronic appendicitis i.e. 77 cases (38.5%). Resolving appendicitis cases were 24 (12%) and only 4 (2%) cases showed Normal non-inflamed histology.[97] Acute appendicitis could be seen in either sex at any age but seen most frequently in young men. He also noted that a false positive diagnosis was twice more common in females than in males.[76] Appendicitis is notoriously recurrent. It is not uncommon for patients to attribute such attacks to biliousness or dyspepsia. The attacks vary in intensity and may occur every few months and majority of cases ultimately culminate in severe acute appendicitis. If a careful history is taken from patients with acute appendicitis, many remember having had milder but similar attacks of pain. The appendix in these cases shows fibrosis indicative of previous inflammation. However, the existence of recurrent appendicitis has always been controversial and only recently re-addressed.[48] In the current study, acute appendicitis was seen more frequently than recurrent appendicitis (Table 2 & 3). The incidence of acute appendicitis was higher in age group above 20 years. A significantly high incidence of acute appendicitis was seen in males in the present study. Thackray AC in 1959 found that histological features of progressive fibrosis with infiltration by lymphocytes and plasma cells together with hyperplasia of the lymphoid tissue is normally present.[78] Stephenson J and Snoddy WT in 1961 classified appendices having polymorphonuclear leukocytes infiltration of the muscle wall, with or without other inflammatory cell infiltration as acute suppurative appendicitis. Most of these appendices showed dense infiltration of neutrophils in the muscular layer and constituted 28.6% of inflammatory lesions of appendix in their study.[84] Eosinophils as a sole infiltrate in the muscle layer in appendicitis have been described previously. Stephenson J and Snoddy WT in their study chose to call it 'Subacute appendicitis' when there was an infiltration of muscle wall by at least 5 eosinophils per high power field but failed to mention the clinical correlation to justify this term. [84] Barber MD et al in 1997 concluded that recurrent appendicitis exists and affects at least 6.5 percent of those who ultimately have an inflamed appendix removed.[83] Chang SK and Chan P in 2004 noticed in their study that a 11% incidence of recurrent appendicitis out of 290 patients with appendicitis. Females comprised of 21% of the group. The diagnosis was retrospective, as the patient had to be symptom free after surgical removal of the appendix. 15% of patients had more than three previous episodes of right iliac fossa pain, which is attributed to recurrent inflammation of appendix.[81] 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.77 In the present study of 100 cases of appendices, no case of subacute appendicitis or acute suppurative appendicitis was seen. Jona JZ et al in 1976 in their study observed cases which presented as acute appendicitis but containing only transmural eosinophilic infiltration in the appendix and included them in the spectrum of eosinophilic gastroenteritis rather than a primary appendicitis.[85] Aravindan KP in 1997 observed mural eosinophilic infiltration as a consistent finding in acute appendicitis and also described cases in which 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.[47] In a further study, Aravindan KP et al in 2010 described cases which presented clinically like classic acute appendicitis, in which an inflamed and oedematous appendix does not show neutrophils in the muscle layer, but marked eosinophil infiltration instead. He chose to name this entity 'Acute eosinophilic appendicitis'.[84]

TABLE 13:- COMPARISON OF MEAN MAST CELL COUNTS IN THE MUCOSA OF THE APPENDIX IN DIFFERENT HISTOPATHOLOGICAL GROUPS IN VARIOUS STUDIES.

Authors	Normal	Acute appendicitis	Acute eosinophilic appendicitis	Subacute appendicitis	Recurrent/ chronic appendicitis	Acute suppurative appendicitis
Crow J and Howe S (1988)[80]	66.0	-	-	-	-	-
Naik R et al (1997)[17]	47.63	29.0	-	43.1	-	-
Mysorekar VV et al (2006)[93]	4.7	36.3	-	-	55.6	-
Singh UR et al ((2008)[27]	3.13	5.42	-	-	-	-
Kolur A et al (2014)[94]	7.2	8.8	19.8	-	11.3	8.3
Present Study (2019)	3.9	8.7	15.5	-	12	-

In the present study higher mean mucosal mast cell count was seen in acute eosinophilic appendicitis and recurrent appendicitis. Intermediate counts were seen in mucosa of acute appendicitis whereas a very low mean mast cell counts in mucosa of normal appendix.(Table 7 & 11). Crowe J and Howe S in 1992 showed a high mucosal mast cell count in normal appendix. This was in contrast to the present study which showed a very low mast cell count in normal appendix.[80] Naik R et al in 1997 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 the mucosa. [17]Mysorekar VV et al in 2006 studied 150 appendices, out of which 6 showed normal histology and these appendices showed very low mast cell count in all the layers similar to that seen in the present study. They found a higher mast cell count in acute appendicitis compared to normal appendices but chronic appendicitis showed the highest mast cell count. [93]Mysorekar VV et al in 2006 studied 150 appendices, out of which 6 showed normal histology and these appendices showed very low mast cell count in all the layers similar to that seen in the present study. They found a higher mast cell count in acute appendicitis compared to normal appendices but chronic appendicitis showed the highest mast cell count. [93] Singh UR et al in 2008 found highest mean mast cell count in clinically acute but histologically normal appendices when compared to controls and also acute appendicitis. They remarked that a statistically significant increase in mast cells in appendices which appear normal on histology but clinically have symptoms of acute appendicitis may explain the clinical presentation and opined that the pain in these patients may be caused by degranulated mast cells. [27] Aravindan KP et al in 2010 found wide range of mean mast cell count in normal appendices. He suggested that normal appendices with high initial mural mast cell count are prone to develop acute appendicitis. 86 Kolur et al in 2014 studied 777 cases of surgically resected appendices and concluded that Highest mean mast cell count was seen in cases of acute eosinophilic appendicitis followed by recurrent appendicitis.[94]

TABLE 14 :- COMPARISON OF MEAN MAST CELL COUNTS IN THE SUBMUCOSA OF THE APPENDIX IN DIFFERENT HISTOPATHOLOGICAL GROUPS IN VARIOUS STUDIES

Authors	Normal Appendix	Acute Appendicitis	Subacute appendicitis	Acute Suppurative Appendicitis	Acute eosinophilic appendicitis	Recurrent/ chronic Appendicitis
Naik et al (1997)[17]	38.3	34.48	41.7	-	-	-
Mysorekar VV et al (2006)[93]	3.3	27.4	-	-	-	42.8
Singh UR et al (2008)[27]	7.04	18.58	-	-	-	-
Kolur A et al (2014)[94]	8.15	8.48	-	8.27	16.6	10.2
Present Study (2019)	4.6	12.7	-	-	17.5	21.4

In the present study, submucosal mean mast cell count was lowest in the normal appendix which was in concordance with studies of Mysorekar VV et al and Singh UR et al (Table 14). [93, 27] Naik et al in 1997 found high mean mast cell count in normal appendices and in subacute appendicitis.[17] In the present study, highest mean mast cell count was seen in Recurrent appendicitis and very low in normal appendices similar to the studies by Mysorekar VV et al and Singh UR et al. [93, 27]

TABLE 15 :- COMPARISON OF MEAN MAST CELL COUNTS IN THE MUSCULAR LAYER OF THE APPENDIX IN DIFFERENT HISTOPATHOLOGICAL GROUPS IN VARIOUS STUDIES

Authors	Normal Appendix	Acute Appendicitis	Acute Suppurative Appendicitis	Acute eosinophilic appendicitis	Recurrent/ chronic Appendicitis
AravindanKP (1997)[47]	19.6	26.5	-	-	32.1
MysorekarVVet al (2006)[93]	1.8	27.6	-	-	32.4
SinghURetal (2008)[27]	4.36	8.79	-	-	-
KolurAetal (2014)[94]	7.4	8.16	3.05	16.3	9.99
Presentstudy (2019)	6.4	12	-	12.5	13.9

In the present study, mast cell count was observed in the muscular layer of Acute eosinophilic appendicitis was nearly equal as compared to acute appendicitis Lowest mean mast cell count was seen in normal appendices which was in concordance with other authors. Highest mean mast cell count was observed in cases of recurrent appendicitis. Aravindan KP in 1997 found high mean mast cell count in muscular layer of recurrent appendicitis, which was also found in the present study(Table 15). [47] Mysorekar VV et al in 2006 found highest mean mast cell count in the muscular layer of appendices diagnosed as chronic appendicitis which they defined as transmural infiltration by 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 was used for the cases showing similar histological features along with a clinical history of repeated bouts of abdominal pain in the past. [93] Crow J and Howe S and Naik et al found a higher mucosal mean mast cell count in children as compared to older patients. [90], 17 Our study did not show any such difference which was similar to the study by Mysorekar VV et al.[93]There

was no correlation between the sex and mast cell count in the present study as has also been described by Naik et al and Mysorekar et al.[93,17] Singh UR et al in 2008 also observed an increase in mean mast cell count in different layers with a corresponding increase in mean eosinophil count and attributed it to the chemotactic factor and histamine release from mast cells. 27 Aravindan KP in 2010 found that mast cells tended to be few and degranulated in areas which showed clustering of eosinophils.[86] Naik R et al suggested that eosinophil are attracted to eosinophilic chemotactic factor or histamine release from the mast cell granules. [17] In contrast, Mysorekar VV et al and Kolur A et al et al in their studied did not find any correlation between mast cell and eosinophil density or distribution. [93,94]

Pathogenesis of appendicitis :

Acute appendicitis is a multifactorial disease. The universally accepted theory is that obstruction of the appendiceal lumen increases the risk of acute appendicitis. Obstruction with continued mucinous secretions into the lumen results in increased intraluminal pressure. This pressure in turn leads to the collapse of venous drainage and results in ischaemic injury to the appendiceal mucosa. Mucosal injury predisposes the appendix to bacterial invasion, which also contributes to the inflammatory reaction, further compromising the circulation.[15] Many authors have proved that appendicitis may develop from causes unrelated to obstruction of the appendiceal lumen. It is also well recognized that faecoliths may be present in the appendiceal lumen without causing obstruction or without accompanying evidence of inflammation.[55]

Table No. 16 :- Comparison of presence of faecolith in normal appendix and in cases of appendicitis in various studies

Authors	No. of Cases studied	Cases with faecolith	Cases with faecolith	
			Normal	Appendicitis
Chang AR(1981)[46]	3003	167	67	107
Singh JP et al (2013)[98]	1014	186	08	178
Kolur A et al (2014)[94]	777	200	-	-
Ramdas M et al (2015)[99]	1357	186	65	121
Present study(2019)	100	14	0	14

In the present study, out of 100 appendices studied, 14 cases show faecoliths, out of which 9 are acute appendicitis and 5 cases are recurrent appendicitis. No normal appendix shows presence of feacolith. Burkitt DP in 1971 suggested that the main cause of appendicitis was removal of much of the cellulose content of our food. 62 Gill B and Cudmore RE in 1975 showed majority of the infants and children developed appendicitis due to faecolith obstruction. [79] Butler C in 1981 found 33% of patients with acute appendicitis with perforation had faecolith in the appendices. 53 Aravindan KP in 1997 opined that though obstruction can set into motion the chain of events which lead to mucosal damage and infection, obstruction as a primary cause is overrated. [47]Mysorekar VV et al in 2006 found only 20.1% of the cases of appendicitis showed faecolith. [93] Elangovan et al in 1997 also found no association between the presence of faecolith and acute appendicitis. 55 Singh JP et al in 2013 conducted a retrospective chart review of 1,014 emergency appendicectomy patients . Overall, faecoliths were found in 18.1% (178/986) of appendicitis specimens and 28.6% (8/28) of negative appendicectomies. [98] Ramdas M et al in 2015 performed a study to determine the association between the presence of a faecolith and acute/nonperforated appendicitis, gangrenous/perforated appendicitis and the healthy appendix. During the study period, 1357 appendectomies were performed. Fecaliths were present in 186 patients (13.7%). The main groups with fecaliths were those with acute appendicitis (n = 121) and those with a healthy appendix (n = 65). [99] In the present study, out of total 100 appendices none showed evidence of parasitic infestation. Dorfman S et al in 2003 and Addiss DG and Juranek DD in 1991 have found no significant causal relationship between parasitic infestation and acute appendicitis. [65,66] Mysorekar et al in 2006 found a mere 2.1% of cases showing evidence of parasitic infestation. [93] Dahlen SE and Kumlin M in 2004 stated that although many mast cell mediators or products serve as useful markers of mast cell activation in vitro, it has been notoriously difficult conclusively to establish mast cell activation in human studies. [36] Aravindan KP in 1997 postulated that the pre-infective stage is a Type I hypersensitivity reaction involving IgE mediated responses as a part of defence mechanisms of bowel and when this involves submucosa and muscle and is of massive nature , it may produce enough swelling to compromise the blood supply and thereby damage the mucosa. Influx of organisms into the mucosa and acute inflammation ensues.[47] Mysorekar VV et al in 2006 also favour this theory in preference to obstructive theory for the causation of appendicitis.[93] Thus, the findings in the present study favours the theory of hypersensitivity, which plays an important role in the causation of appendicitis.

SUMMARY AND CONCLUSIONS

In the present study 100 appendices received in the department of pathology from Nov 2017 to Aug 2019 were studied. The appendices were studied for gross findings and stained with hematoxylin & eosin stain and 1% toluidine blue stain. The stained sections were evaluated for histopathological changes and number of mast cells in each layer

1. Out of the 100 cases of appendicectomies studied, male predominance was seen (58%). A male to female ratio of 1.38:1 was observed.
2. Appendicectomies were seen more commonly in 2nd and 3rd decades.
3. Incidence of acute appendicitis (56%) was the highest compared to all the other histopathological groups in the present study.
4. Male predominance was also seen among the cases of acute appendicitis
5. Obstruction due to faecolith could be demonstrated in 14 cases only out of 100 cases of appendicitis
6. A highest mean mast cell count was seen in recurrent appendicitis followed by acute eosinophilic appendicitis.
7. Among the various layers of appendix highest mean mast cell count was seen in submucosa of recurrent appendicitis.
8. Mean mast cell count was significantly high in appendicitis as compared to normal appendix.

Hence considering the results and observations found in the present study, we conclude that the mast cell activation may be one of the important factors in causation of appendicitis and supports allergic theory of appendicitis rather than obstructive theory

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