IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 18, Issue 2 Ser. 5 (February. 2019), PP 01-07
www.iosrjournals.org

Mast Cells in Surgically Resected Appendices

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Abstract
Background
The vermiform appendix is the most common intra-abdominal organ subjected to frequent surgical intervention and resection. Appendicitis is the inflammation of appendix. It is usually a disease of the young, with 40% of cases occurring between the ages of 10 and 29 years. Appendicitis is the most common intra-abdominal condition requiring many times emergency surgery. The pathogenesis of appendicitis remains poorly understood. Mast cells are known to be effector cells in various inflammatory reactions but their role in appendicitis is unclear.
Objectives
1. To find out the correlation between the histological findings and number of mast cells in appendicitis.
2. To evaluate the role of mast cells and their number in the pathogenesis of appendicitis.
3. To find out whether there is any association between specific age group and number of mast cells.

Material and Methods
The study was conducted on 200 surgically resected specimens of appendices received in the department of pathology, Govt. Medical College, Patiala. The appendix specimens were kept in 10% formalin overnight for fixation. After processing, two sections of 5-micron thickness were cut from the paraffin block. One of the sections was stained with 1% aqueous toluidine blue (Stain for the mast cell) and the other with routine haematoxylin and eosin (H&E) and examined under light microscope.
Keywords: *Mast cell, appendicitis, appendix.*

Date of Submission: 26-01-2019 Date of acceptance: 09-02-2019

I. Introduction

Appendicitis is the inflammation of the appendix. It is a disease of the young, with 40% of cases occurring between the ages of 10 and 29 years. In 1886, Fitz reported the associated mortality rate of appendicitis to be at least 67% without surgical therapy.[1]

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.[2]

Mast cells are being found in varying numbers in practically all tissues, primarily in respiratory, digestive, urogenital systems and skin[3] 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 constantly subjected to surgical intervention and removal.[4]

II. Material and Methods

The study was conducted on 200 surgically resected specimens of appendices received in the department of pathology, Govt. Medical College, Patiala. These included all appendices surgically resected as a therapeutic measure for clinically diagnosed appendicitis and normal appendix specimens if any which were removed as part of some other illness or injury.

The appendix specimens were kept in 10% formalin overnight for fixation. Each specimen was grossly examined for any abnormalities. Pieces were taken from different part of appendix proximal, middle and distal i.e. the tip of appendix. After processing, two sections of 5 micron thickness were cut from the paraffin block. One of the sections was stained with 1% aqueous toluidine blue[5] (Stain for the mast cell) and the other with routine haematoxylin and eosin (H&E) and examined under light microscope. The toluidine blue (that stains the background pale blue and mast cells purple in colour) stained section were examined for the counting of mast cells in various layers of appendix specimens microscopically. The number of mast cells in the mucosa, submucosa and muscle layers were counted at 400X magnification (High Power).Mast cell granules stained dark blue or purplish against a faintly satined blue background.The average count obtained in 20 non overlapping

high power fields was taken in each specimen and expressed as the number of cells per mm^2. The haematoxylin and eosin section were studied for inflammatory changes in the layers of appendix under light microscope to definehistopathological diagnosis.The relationship between the mast cell numbers and histological findings were assessed statistically

III. Results

TABLE 1: AGE AND SEX WISE DISTRIBUTION

Age Group (in years)	Male	Female	Total	Percentage
0-10	14	9	23	11.50
11-20	33	25	58	29.00
21-30	32	23	55	27.50
31-40	17	16	33	16.50
41-50	6	10	16	8.00
51-60	3	6	9	4.50
61-70	2	1	3	1.50
71-80	2	1	3	1.50
Total	**109**	**91**	**200**	**100**

In the present study 200 cases of appendicitis were evaluated. Maximum number (29%) of patients were in the 2nd decade followed closely by 3rd decade (27.5%) with slight male preponderance in both the age groups. Number of cases in the 4thdecade(16.5%) was followed closely by number of case in the 1st decade(11.5%). 5th decade presented with 8% cases, 6th with 4.5% cases and 7th and 8th decade with 1.5% cases each. Number of male patients were slightly more than number of female patients. No definite sex predilection was seen.

TABLE 2: HISTOLOGICAL DIAGNOSIS

Diagnosis	No of Cases	Percentage
Acute Appendicitis	95	47.50
Resolving Appendicitis	24	12.00
Chronic Appendicitis	77	38.50
Normal	4	2.00
Total	200	100

In the present study of 200 surgically resected appendices the histological categories are as follows: Acute appendicitis, Resolving Appendicitis, Chronic Appendicitis and Normal non-inflamed appendices.

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.

Grossly some of the appendices showed rough surface because of fibrous tissue surrounding the appendices with thickening of the wall, in case of chronically inflamed appendices.

In resolving appendicitis and acute appendicitis grossly the specimens had similar appearance, few exhibiting congested blood vessels and others showing mild fibroses along with peri appendicular fat.

Grossly the normal appendices showed normal frozen surface with or without attached peri appendicular fat.

In our study, on cut section, appendices showed faecoliths and faecal matter. Many of the appendices showed almost complete obliteration of lumen. Some appendices grossly did not have recognizable lumen because of perforation.

Histological changes in the appendices mostly co-related with the duration of symptoms.

TABLE-3: CATEGORIZATION OF HISTOLOGICAL DIAGNOSIS IN EACH AGE GROUP

Age Group (in years)	Histological Diagnosis in Each Category			
	Acute Appendicitis	Chronic Appendicitis	Resolving Appendicitis	Normal
0-10	19	4	0	0
11-20	31	19	8	0
21-30	23	23	8	1
31-40	11	20	2	0
41-50	6	6	2	2
51-60	2	2	4	1
61-70	1	2	0	0
71-80	2	1	0	0
Total	95	77	24	4

Maximum number of patients of appendicitis were of acute appendicitis followed by chronic and resolving resp. It was observed that acute cases of appendicitis were seen-be highest in age group of 10-20 years. Highest number of cases in chronic appendicitis were observed in the age group of 21-30 years. In resolving appendicitis, it is observed that age groups of 10-20 and 20-30 were showing 8 cases each.

The diagnosis of acute appendicitis was made when neutrophilic infiltration was seen in different layers of the appendix. In few cases slight lymphocytic of eosinophilic infiltration was also present. The layers of appendix showed congestion in some cases mucosal ulceration was also evident. Chronic appendicitis was diagnosed when the major infiltrating population was of lymphocytic cells or lymphoid follicles. Cases of chronic appendicitis presented with varying degree of fibrosis. In our study few cases of perforated appendices were observed and diagnosed histologically as chronic appendicitis. In resolving appendicitis the histological hallmark is eosinophilic infiltration of different layers of appendices with or without accompanying edema and majorly absence of neutrophils. Four cases of normal appendix showed no disruption of mucosal lining with no inflammatory infiltrate in submucosa and muscularis propria.

TABLE-4: MEAN MAST CELL COUNT PER mm^2±SD FOR ALL AGE GROUPS

Histopathological Category	**Mucosa (Mean±SD)**	**Submucosa (Mean±SD)**	**Muscularis Propria (Mean±SD)**
Acute Appendicitis	6.30±0.93	17.10±1.13	10.44±0.97
Resolving Appendicitis	6.72±0.79	15.79±0.82	11.26±0.78
Chronic Appendicitis	10.22±0.89	22.37±0.93	15.63±0.98
Normal Appendix	4.69±1.25	6.70±1.65	5.69±1.42

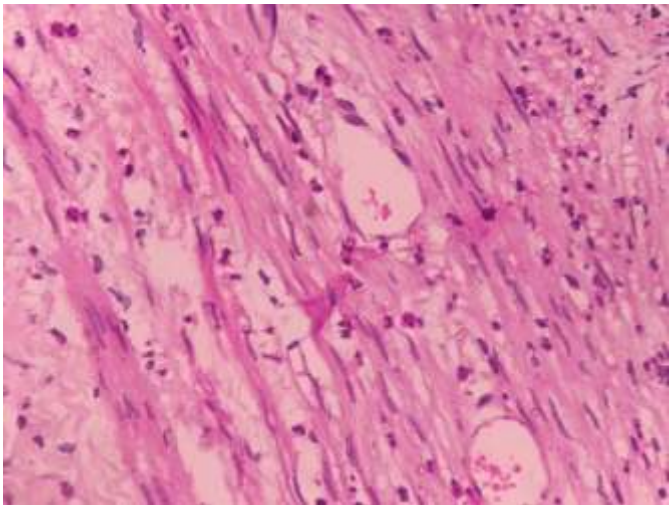
Statistical analysis of Variance test (ANOVA) – significant differences were found between mast cell numbers in all categories (p value < 0.01, highly significant).

Statistical analysis of Variance test (ANOVA) – significant differences were seen between mast cell numbers in different layers of all appendicitis cases (p value<0.01, highly significant)
Of all the appendicitis cases minimum number of mast cells were seen in the normal appendices in all the layers. Minimal number of mast cell were seen in the mucosal layer and maximum in the submucosa. Cases of chronic appendicitis exhibited maximum numbers of mast cell followed by acute appendicitis

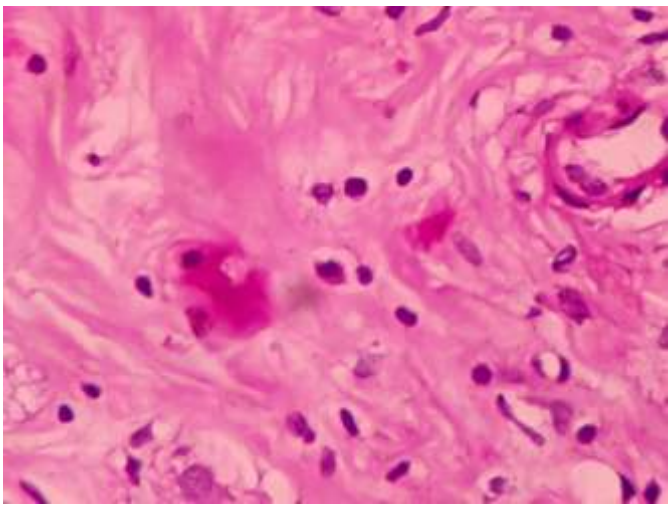
TABLE-5: MEAN MAST CELL COUNT PER mm^2±SD FOR AGE GROUP LESS THAN AND MORE THAN 20 YRS.

Histopatho-logical Category	**Mucosa**		**Submucosa**		**Muscularis Propria**	
	0-20 years	**>20 years**	**0-20 years**	**>20 years**	**0-20 years**	**>20 years**
Acute Appendicitis	6.28±0.95	6.33±0.91	17.11±1.12	17.09±1.15	10.43±0.92	10.44±1.03
Resolving Appendicitis	6.55±0.85	6.795±0.77	15.69±0.98	15.84±0.75	11.12±0.90	11.31±0.73
Chronic Appendicitis	10.23±0.75	10.20±0.95	22.43±0.78	22.33±0.99	15.67±0.87	15.6±1.03
Normal	0±0	4.68±1.24	0±0	6.70±1.64	0±0	5.69±1.42

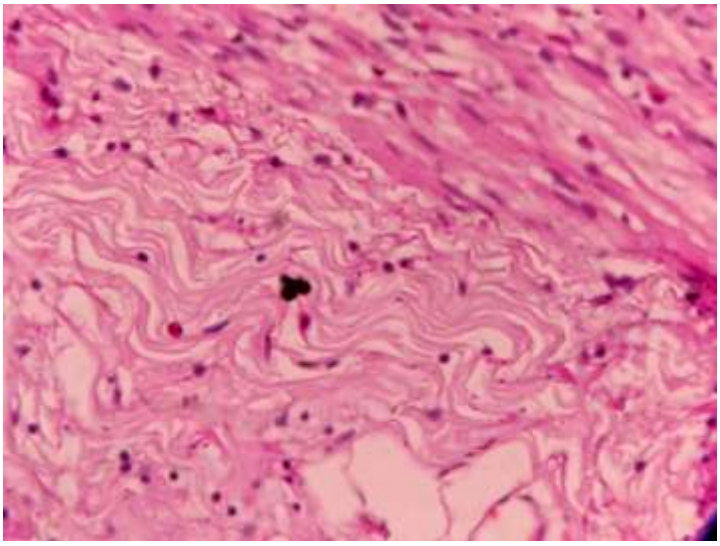
Analysis of the results were done by dividing the patient in two broad age groups 0-20 years (children and adolescents) and above 20 years (Adults). 81 patients were from the age group of zero-twenty and 119 patients were above twenty years of age.
Statistical analysis by z test: there was no significant difference in mast cell count in younger and older age groups (p>0.05), not significant.



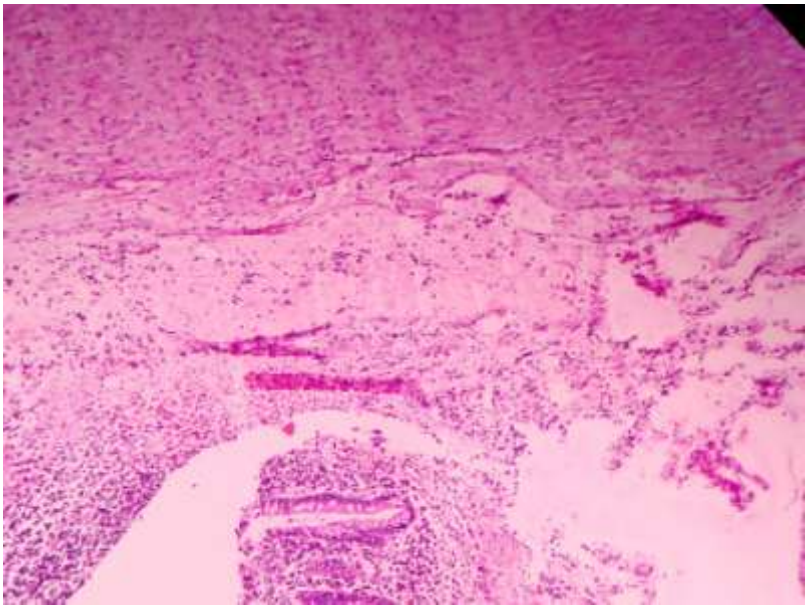
Micrograph : Acute appendicitis - Section showing submucosa along with multiple congested blood vessels, slight edema and numerous neutrophils (H & E x 400)



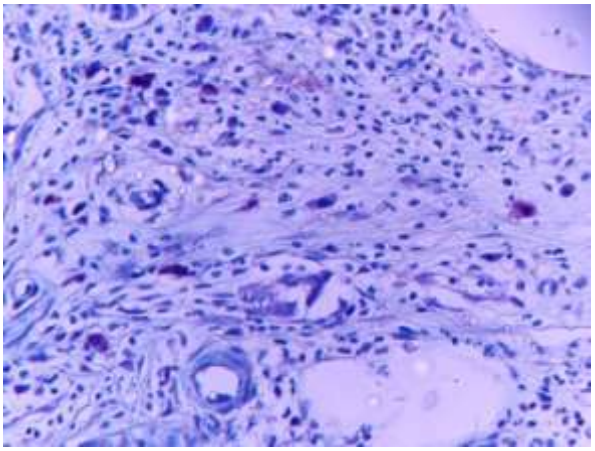
Micrograph : Chronic appendicitis - Sections showing submucosal layer along with multiple congested blood vessels (H & E x 100) and chronic inflammatory infiltrate comprising of lymphocytes (H & E x 400)



Micrograph : Resolving appendicitis - Sections showing submucosal layer and a bit of muscularis propria (H & E x 100) majorly eosinophilic infiltrate in submucosal layer (H & E x 400)



Micrograph : Normal appendix - Sections showing mucosa, submucosa and muscularis propria (H & E x 40). Only slight edema was seen in the layers with no specific inflammatory infiltrate (H & E x 100)



Micrograph : Appendicitis – Sections showing submucosa and muscularis propria along with numerous mast cells (Toluidine Blue x 400)

IV. Discussion

Mysorekar et al in 2006 studied 150 cases, of which 46 were that of acute appendicitis. The pathogenesis of this disease is poorly understood and cause for inflammation remains unknown in significant number of cases. Obstruction by faecolith and infection followed by inflammation were some of the common causes. The mast cell count was found to be decreased in the areas of mucosal ulceration. This study stated that mast cell count were lowest in normal appendices, significantly higher in acute appendicitis and highest in chronic appendicitis. Hence a type 1 hypersensitivity reaction with release of mediators by mast cells might be another triggering factor for the sequence of events leading to appendicitis.[6]

Singh et al in 2008 conducted studies that found a significant increase in eosinophil count in acute appendicitis compared to normal appendices and has stated that increase in eosinophil and mast cells may be the cause of pain in histologically normal but clinically suspected acute appendicitis. A significant increase in mast cell counts, nerves along with prominence of ganglion cells in sub mucosa and muscularis propria in acute appendicitis establishes an association between increase mast cell count and nerve proliferation in acute appendicitis.[7]

Sonti in 2012 studied 290 cases of surgically resected appendices and concluded that mast cell count was higher in acute appendicitis indicating immunological and non immunological injury causing acute appendicitis. The mast cell count was highest in chronic appendicitis, indicating growth interaction between mast cells, nerves and fibrosis.[8]

Singh et al in 2014 studied 200 appendices to access for histological changes and mast cell infiltration. Maximum number of mast cells were seen in recurrent acute appendicitis cases followed by chronic appendicitis

and least were in acute appendicitis. Maximum number of mast cells were observed in the submucosa. There was no significant difference in the mast cell count in the younger and older age groups. Obstructions due to faecoliths were seen in only 6 percent of the appendicitis.[9]

Kolur in 2014 studied 777 cases of surgically resected appendices and concluded that eosinophil counts in all the layers were very high in acute eosinophilic appendicitis compared to normal appendices. A higher mast cell count was seen in acute eosinophilic appendicitis and recurrent appendicitis. No correlation was found in mast cell and eosinophilic density.[10]

Kumaran C and Divya LL in 2015 concluded that the mast cell count was higher in chronic appendicitis thus indicating immunological and non-immunological injury caused appendicitis. They suggested that there is an association between the mast cells, nerves and the fibroblast. [11]

Nagraj et al in 2015 correlated with a study done by Xiong, the mast cells were counted, which showed that neuroproliferation in appendix was associated with increased mast cell density. This might occur in patients with clinical and histopathological diagnosis of acute appendicitis. These findings were significant in the GI tract, because changes in the neuronal component and the mast cell density have been reported in chronic inflammatory conditions such as Crohn's disease and ulcerative colitis. They concluded that the mast cell count was highest in chronic appendicitis, thus indicating the growth interaction between the mast cells and fibroblast in areas of fibrosis.[12]

Verma et al in 2016 found that mast cells count was lowest in the appendices removed in the course surgeries done for some other disease/control, while they were significantly higher in histology negative acute appendicitis (HNAA) group, acute appendicitis and highest in chronic appendicitis. Increased eosinophil count with an increase in mast cell count observed in the study in appendicitis could be due to consequence of mediators released by mast cells such as eotaxin. They concluded that Type I hypersensitivity reaction with release of mediators by mast cells might be a pre disposing factor for sequence of events leading to appendicitis. [13]

Sharma et al in 2017 conducted a study with 150 patients with appendicitis and concluded that the mast cell count was higher in acute appendicitis, thus indicating an immunological or non-immunological injury and the highest in chronic appendicitis, which indicates the growth interaction between the mast cells, nerves and fibrosis. It showed that there was definite increase in mast cell count as the disease progresses thus substantiating their contributing role in pathogenesis.[14]

Banerjee et al in 2018 his study observed that females dominated (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. A positive correlation with mean eosinophilic and mast cell counts in appendicitis noted with increasing mast cell count with fibrosis.[15]

V. Conclusion

Toluidine staining technique is easy, simple, long lasting and gives very good result for identification of mast cell in appendix. In the present study, mast cell count was lowest in histologically normal appendices, resected in course of surgeries done for some other disease. They were higher in acute and resolving appendicitis and highest in chronic appendicitis. This shows that there is definite increase in mast cell count as disease progresses thus substantiating their contributing role in pathogenesis. It was also observed that faecolith or obstruction was seen in only a few cases. Therefore, mast cell activation could be a factor responsible for appendicitis. Mast cell count variation was not seen in different age groups ruling out association between specific age groups and mast cell number.

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