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Research Paper

Medical Science

KEYWORDS : Mast cell, Appendicitis

Mast cells in Surgically Resected Appendices

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ABSTRACT

In the present study total 100 appendices were studied by using simple histochemical techniques, an attempt was made to study the mast cell in variety of appendicular pathology to find out the mast cell variation in the normal and inflamed appendix.

Appendices were received from 97 patients operated for the signs and symptoms of appendicitis. 3 normal appendices were collected. After morphological assessment, appendices were grouped in to acute appendicitis (64), recurrent (28), worm infestation (1), tuberculosis (1), appendix from tuberculosis affecting ileocaecal region (1) , appendix from typhoid perforation of intestine (1), appendix from mucin secreting adenocarcinoma of colon (1).

Recurrent or subacute appendicitis, mast cell count is intermediate between normal appendices and acute appendicitis. This indicates that mild inflammation causes degranulation of less number of mast cells or due to simultaneous reparative process in recurrent appendicitis, mast cell number are increasing from low count in acute appendicitis towards normal count in normal appendices.

As mucosal mast cell count is more liable to changes with inflammation or fibrotic process as demonstrated by lower mucosal mast cell count as compared to submucosal mast cell count in acute inflammation and higher mucosal mast cell count than submucosal in different grades of fibrosis, it can be concluded that initial stimulus may be luminal.

INTRODUCTION

Mast cells are widely distributed in the gastrointestinal tracts [2.5.6], and as they release a large number of biologically active substances, they are potentially important regulator of the gut functions in both health and disease. The mast cell activation alters intestinal function including epithelial ion transport, mucus secretion and motility. They also have been implicated in the pathogenesis of a number of gastrointestinal conditions including inflammatory bowel diseases, celiac disease, food allergy and systemic mastocytosis [6], graft versus host disease [7] and so on. This suggest that mast cells are likely in an ongoing fluctuating balance between physiological functions and pathological effects in normal individuals and poorly known factors can crate an imbalance and lead to pathological reaction [8].

Role of mast cells in gastrointestinal pathophysiology can be studied by searching morphological, biochemical, immunological or pharmacological evidences [9]. Appendices are most common part of intestinal tract received in pathology department and mast cells of other part of intestinal tract are similar to that of appendix in distribution, morphology and biological response to variety of stimuli, so study of appendicular mast cells can help to understand biological functions of mast cells in gastrointestinal tract.

So, in present study by using simple histochemical techniques, an attempt was made to study the mast cell in variety of appendicular pathology to find out the mast cell variation in the normal and inflamed appendix and there by to understand the role of mast cells in gastrointestinal conditions as whole.

MATERIAL AND METHODS

The present study comprises evaluation of 100 appendices received from the department of surgery, L. G. Hospital, Ahmedabad. The appendices were processed and assessed at the Department of Pathology, N.H.L Medical College, Ahmedabad. The study was done during 3 years.

In the Department of Surgery, all patients were assessed clinically for signs and symptoms of appendicitis and in cases of acute, severe appendicitis; appendectomy was carried out within 48 hrs. In less severe cases, appendectomy was done one and half month after subsiding an acute attack. For recurrent appendicitis, same strategy was applied and thus received appendices were classified as for the clinical presentation (vide table No. 2).

Appendices were removed during surgical laparotomy done for some other purposes like for diverticulitis, resection anastomosis and were considered normal.

On receiving of appendices, further processing and evaluation of appendices was carried out.

Fixation:

After gross examination, all the appendices were fixed in 10% formaline for 24 hrs. After fixation, blocks were taken from the tip, base and one intermediate site along the length of the appendix and processed by routine paraffine embedding method in graded alcohol and Xyline and then embedded in paraffine wax. Two serial sections of 5 micron thickness were obtained from each and taken up on properly prelabeled slides. These sections were deparaffinized in Xyline and brought to water through descending grade of alcohol.

Staining:

The hydrated sections were then subjected to haematoxyline and eosine stain and 1% toluidine blue stain (vide appendix – 2).

Histological assessment:

The haematoxyline and eosine stained sections were assesses for the following:

1) Presence or absence of inflammation
2) Type of inflammation
3) Degree of fibrosis
4) Presence of parasites
5) Presence of other pathology.

Criteria for the grading of fibrosis in appendix proposed by stead et al (1990)[43] were used here.

Mast cell counting:

In toluidine stained sections, mast cells were counted in 50 oil immersion field and mast cell number per 100 such field were then counted and expressed as per sq.mm. Using micrometry.

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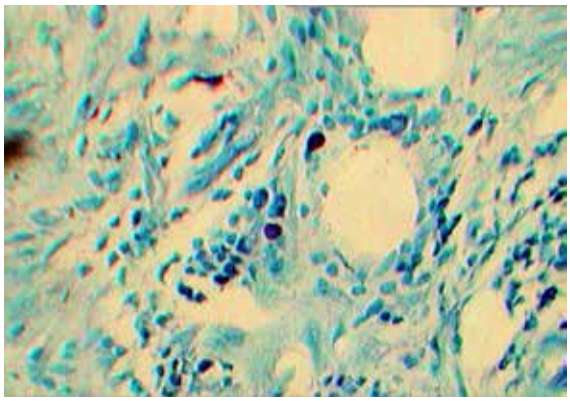
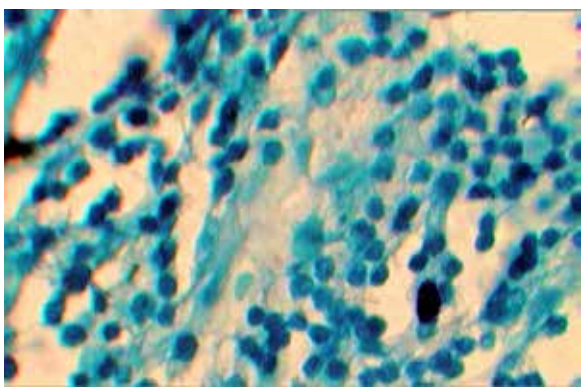


RECURRENT APPENDICITIS – MAST CELLS IN SUBMUCOSA TOLUIDINE BLUE STAIN (400X)



ACUTE GANGRENOUS APPENDICTIS – MAST CELLS IN SUB-MUCOSA
TOLUIDINE BLUE STAIN (1000X)

Mast cell counting was done both in mucosa and submucosa when ever possible. When mucosa was absent due to ulcerative necrosis, counting was done in submucosa and portion of appendices inside the submucosa separately.

According to the number of mast cells per 100 microscopic fields, cases in study could be classified in to three broad groups as follows:

GROUP	MAST CELL COUNT / mm2
GROUP -1	Mast cell count less than 25 / mm2
GROUP -2	Mast cell count between 25 -50 / mm2
GROUP -3	Mast cell count above 50 / mm2

RESULTS AND OBSERVATION
CLINICAL AND MORPHOLOGICAL DIAGNOSIS OF 100 CASES

SR. NO	Clinical presentation	Morphological diagnosis	No. of cases
1.	Appendicitis: either acute or recurrent (subacute). With morphological changes according to duration between clinical symptoms and appendicectomy	Acute appendicitis: a. acute catarrhal appendicitis b. acute suppurative appendicitis c. acute gangrenous appendicitis	64 38 14 12
		Recurrent (subacute) appendicitis	28
		Worm infestation	01
		Tuberculosis of appendix	01
2.	Appendix removed for other reason	a. tuberculosis of ileocaecal region b. typhoid perforation c. mucin secreting adenocarcinoma of colon	01 01 01
3.	Other autopsy specimen	Normal appendix	03
	Total		100

CLINICAL ASSESSMENT:
Clinical presentation:
Age and Sex:

In the present study, age of patients was ranging from 10 yrs to 70 yrs with maximum number of patients in second and third decades (Vide Table No. IV)

In present study, there were 64 male and 36 females. So the male-female ratio was 1.4:1 with little male preponderance. (Vide table no. V)

AGE INCIDENCE IN 100 CASES

Diagnosis	Total no. of cases	Incidence of cases in different age groups						
		1-10 yr	11-20 yr	21-30 yr	31-40 yr	41-50 yr	51-60 yr	>61 yr
Acute appendicitis	64	03	24	28	04	03	01	01
Recurrent appendicitis	28	00	15	04	05	04	00	00
Worm infestation	01	00	01	00	00	00	00	00
In tuberculosis	01	00	00	01	00	00	00	00
Tuberculosis of ileocaecal region	01	00	00	00	01	00	00	00
Typhoid perforation	01	00	01	00	00	00	00	00
Mucin secreting adenocarcinoma of colon	01	00	00	00	00	00	01	00
Autopsy specimen	03	00	01	00	00	00	02	00
Total	100	03	42	33	10	07	04	01

SEX INCIDENCE IN 100 CASES

Sex	Acute appendicitis	Recurrent appendicitis	Tuberculosis of ileocaecal region	Typhoid perforation	Mucin secreting adenocarcinoma of colon	Normal appendices	Total
Male	48	12	01	01	00	02	64
Female	16	18	00	00	01	01	36
Total	64	30	01	01	01	03	100

Morphological assessment of appendices and mast cell count:

The morphological assessment of 100 appendices was done using H & E stained sections and were classified accordingly (vide table no: III and X) and mast cell study was done using toluidine stained sections.

Mast cell morphology:

In toluidine stained sections, mast cells were round, spindle or pyriform cells, 9 -12 microns in diameter, cytoplasm of which was stuffed with numerous granules (Fig no: 11). Nuclei were unsegmented oval or round, appreciated as lightly stained areas in centre or just obscured by granules (Fig no: 2,3). Many mast cells also showed degranulation with metachromatic granules

dispersed in surrounding tissue. (Fig no: 2,3)

They were distributed from basement lamina to serosa with preponderance around the blood vessels. In mucosa, mast cells were smaller round to oval and having fine granules. In submucosa, they were larger more pleomorphic and having larger granules.

Mast cell count in normal appendices:
In present study 3 normal appendices were selected. The mast cell count in mucosa and submucosa was done in formaline fixed paraffine embedded sections (vide table no: VI). Mean mucosal mast cell count was 31 ± 10 / sq.mm. and mean submucosal mast cell count was 26 ± 8 sq.mm. These mast cells counts were taken as control in the present study.

Effect of fixation on mast cell count:
3 normal appendices were also used to see effect of fixation on mast cell count. Each normal appendix was fixed in the Carnoy's fluid and mast cell count was done. The count obtained from tissue fixed in Carnoy's fluid was higher than that of formaline fixed tissue.

EFFECT OF FIXATION ON MAST CELL COUNTS IN 3 NORMAL APPENDICES

Type of fixation	Mean mast cell count/ sq. mm	
	mucosal	Submucosal
Formaline (10%)	32± 10	27± 8
Carnoy's fluid	127± 28	106± 23

Correlation of age and sex with mast cell count in normal appendices:
The mast cell count in 3 normal appendices was studied in relation to different age groups (vide table no: VII). Below age of 15yrs mean mucosal mast cell count was 36 ± 8.5 / sq.mm. and above the 45 yrs of age 22 sq.mm. In submucosa, mean mast cell count was 29 ± 6.5 / sq.mm. 26 ± 8 / sq.mm. and 18 / sq.mm. in respective age groups. An attempt was made to correlate mast cell count in relation to sex in 3 normal appendices obtained from 2 male and 1 female (vide table no: VII).

MEAN MAST CELL COUNTS IN 3 NORMAL APPENDICES IN VARIUOS AGE GROUPS

Age	No. of cases	Mean mast cell count/ sq.mm	
		Mucosal	Submucosal
15-45 year	01	31	26
>45 year	02	22	18
total	03		

MEAN MAST CELL COUNT IN RELATION TO SEX IN 3 NORMAL APPENDICES

Sex	No. of cases	Mean mast cell count/ sq. mm	
		mucosal	submucosal
Male	02	30±5.2	26±8
Female	01	31	24
Total	03		

Mast cell COUNT in various pathological cONDITIONS:
In the present study of 100 appendices, 97 appendices showed various pathological lesions during morphological assessment (vide table no: III, IX).

Acute appendicitis:
In 64 appendices various morphological changes of acute appendicitis had been observed as follow (vide table no: III). Acute catarrhal appendicitis (in 38 appendices) showing edema, congestion and minimum acute inflammatory infiltrate in wall of appendix (fig. 7). Acute suppurative appendix (in 14 appendices) showing abundant pus cells in lumen and infiltration of neutrophils in all layers of appendix with reactive follicle (fig.10) and gangrenous appendicitis (in 12 appendices) showing black discoloration and marked ischemic necrosis with thrombosed and congested vessels.(fig 13).

Mast cell count in acute appendicitis: In various forms of acute appendicitis, mucosal mast cell count ranged from 10 to 22 / sq.mm. and submucosal mast cell count ranged from 6 to 26 / sq.mm. Because of mucosal ulceration, mucosal mast cell counting was possible in only 49 appendices and submucosal mast cell counting was possible in all 57 appendices. (Vide table no: IX) (Fig. 8,9,11,12,14)

MAST CELL COUNT IN VARIOUS DISEASE OF APPENDICES (STUDY OF 100 CASES)

Morphological diagnosis	No. of cases	Mast cell count/ sq. mm			
		Mucosal mast cell count		Submucosal mast cell count	
		range	mean	range	mean
Normal	03		31±10		26±8
Acute catarrhal appendicitis	38	12-22	11.58±5.2	15-26	18.8±6
Acute suppurative appendicitis	14	11-14	13±3.6	6-17	14.6±4.4
Acute gangrenous appendicitis	12	-	-	11-15	13±2
Recurrent (sub acute) appendicitis	28	20-39	29±9	15-32	22±8
Worm infestation	01	-	35	-	29
Tuberculosis	01	-	* 40	-	32
Tuberculosis of ileocaecal region	01	-	36		29
Typhoid perforation	01	-	35		29
Mucin secreting adenocarcinoma of colon	01	-	34		28
total	100				

* At the periphery of granuloma

Recurrent (subacute) appendicitis:
28 appendices showing lymphocytes, particularly in perivascular region, plasma cells and eosinophils in all layer and subserosa were diagnosed to have changes of recurrent (subacute) appendices

Mast cell count in recurrent (subacute) appendicitis: Mast cell count ranged 20-39 / sq.mm. in mucosa and 15-32 / sq.mm. in submucosa

In one appendix removed from patients with recurrent attacks, **E. Vermicularis** was seen in lumen. Mean mast cell count in this case was 35 / sq.mm and 29 / sq.mm. in mucosa and submucosa respectively. (Vide table IX) and was similar to recurrent appendicitis

In four cases, there was a chance to study appendices being involved as a part of bowel pathology. Among these, one cases each of **tuberculosis affecting appendix, ileocaecal tuberculosis, and typhoid perforation of intestine and mucinous carcinoma of colon.**

After histological diagnosis of **tuberculosis affecting appendix**, mean mast cell count was 40 / sq.mm. This is little higher than normal and found around and in between tubercle.

In tuberculosis of ileocaecal region, appendix showed mean mucosal mast cell count 36 / sq.mm. and submucosal mast cell count 29 / sq.mm. As compared to normal appendices mast cell count was at higher value

In typhoid perforation of intestine, appendix showed mean mucosal mast cell count 34 / sq.mm. and submucosal mast cell count 28 / sq.mm. As compared to normal appendices mast cell count was at higher value

In mucin secreting adenocarcinoma of colon, appendix showed mean mucosal mast cell count 30 / sq.mm. and submucosal mast cell count 22 / sq.mm. As compared to normal appendices mast cell count was at lower value

COMPARISON OF EFFECT OF FIXATION ON MAST CELL COUNT

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Reference	Mast cell count in		% reduction of mast cell count in the Formaline
	Carnoy's fluid	Formaline (10%)	
Strobel et al (1981)	268 sq mm	47/sq mm	83%
Shanahan et al (1987)	1603/ mm3	453/ mm3	71%
Gomez et al (1987)	43.75/10HPF (400x)	11.25/10 HPF (400x)	73.3%
Ghanem et al (1988)	1.00%	0.37%	63%
Walla et al (1990)	4%	2.4%	40%
Present study	120±28.2/sq mm	31.0±10/sq.mm	74%

Mast cell counting:
In the present study, mast cell count was done by using micrometry and was expressed as count / sq.mm.

Various authors stead et al (1990)[43], Naik et al (1997)[40], Naik et al (1998)[42], Crowe and Howe (1988)[44] expressed mast cell count per square millimeter while study of mast cells in appendices. Authors also used various units for the expression of mast cell count in study of bowel and other organs

Morphology Heterogenicity and distribution of mast cells in appendices:
According to the various authors[10,18], mast cells usually 9 – 12 micron in diameter, round, spindle or pyriform cells, cytoplasm of which was stuffed with numerous metachromatic round or oval granules. Nuclei usually unsegmented oval shaped. Mast cells were distributed from the basement lamina to serosa with preponderance around the blood vessels[22]. Submucosal mast cells in appendices where larger, polygonal and contained coarse and more numerous granules than that of mucosal mast cells. Many mast cells were degranulated with dispersion of metachromatic granules in surrounding tissues[22].

So in present study findings were consistent with other workers in respect to morphology of mast cells.

Mast cells count in normal appendices:
Age: Mast cell count in normal appendices in relation to various age groups was done. The age groups were classified as < 15 yrs, 15 – 45 yrs and > 45 yrs. In normal appendices, mast cells count observed in mucosa was higher than that of submucosa. Findings were correlated and with other workers[40,44] as mentioned

COMPARISON OF MAST CELL COUNT IN RELATION TO AGE IN NORMAL APPENDICES IN VARIOUS STUDIES

Reference	Age group	Mean MMC	Mean SMMC
Crowe and Howe (1988)	< 15 years > 15 years	224+/-88.6 170+/-73.3	130+/-38.4 114+/-21.3
Naik et al (1997)	< 15 year 15-45 years >45 years	69.28 47.63 69.28	44.27 44.27 58.42
Present study	< 15 year 15-45 years >45 years	36±8.5 31±10 22±5	29±6.5 26±8 18±4

MMC – Mucosal Mast Cell Count SMMC – Submucosal Mast Cell Count

Mast cell count observed in age group < 15 yrs was higher as compared to age group 15 -45 yrs and > 45 yrs. So findings in present study were correlated to that of Crowe and Howe (1988)[44]. Naik et al (1997)[40] observed high mast cell count in mucosa in age <15 yr and > 45 yrs and higher submucosal count only in age > 45 yrs.

Sex: No significant findings related with sex were observed in present in normal appendices which were correlated with findings of other workers.

COMPARISON OF MAST CELL COUNT IN RELATION TO SEX IN NORMAL APPENDICES

Reference	Sex	Mean MMC	Mean SMMC
Crowe and Howe (1988)	Male Female	201+/-47.8 205+/-103	125+/-30.4 122+/32.5
Present study	Male Female	30.0±5.2 31.0±14	26±8 8.025

MMC: Mucosal mast cell count SMMC: Submucosal mast cell count

MAST CELL COUNTS IN DISEASED APPENDICES:
Morphological assessments of 100 appendices were done using H & E stain and were classified in different groups. Mast cells count was done in appendices of each group and was compared to that of normal appendices.

Acute appendicitis:
In present study, mean mast cell count in various forms of acute appendicitis was significantly lower as compared to normal appendices. In gangrenous appendicitis, it was lowest and in catarrhal appendicitis, it was highest in these groups. Mean mucosal mast cell count was lower as compared to mean submucosal mast cell count. Findings are correlated with Naik et al (1997)[40] who observed decreased mucosal and submucosal mast cell count in acute appendicitis as compared to normal.

COMPARISON OF MAST CELL COUNT IN ACUTE APPENDICITIS

Reference	No. of cases	Mean Mast cell count/ sq. mm			
		Normal appendix		Acute Appendicitis	
		Mucosal	Submucosal	Mucosal	Submucosal
Naik et al (1997)	50	47.63	44.37	29	34.48
Present study	64	31±10	26±8	14±4.3	15±6

Recurrent appendicitis (Subacute appendicitis):
In present study mean mast cell count was in between that of normal and acute appendicitis findings observed were correlated with that of Naik et al (1997)[40]. (Vide table no: XIV)

SUMMARY
1. In toluidine stained sections, mast cells were easily identified with better differention and stain did not fade.
2. Mean mast cell count in mucosa and submucosa of normal appendices were 31 ± 10 / sq.mm. and 26 ± 8 / sq.mm. respectively in formaline fixed tissues. In Carnoy's fluid, higher mast cell count (75%) was observed as compared to formaline fixation.
3. Mast cell count in normal appendices was higher in age group <15 yrs than the age group >15 yrs in mucosa and submucosa.
4. In acute appendicitis, mast cell count (14 ± 4.3 / sq.mm.) was markedly reduced in mucosa, while in submucosa mast cell count (15 ± 6 / sq.mm.) was relatively less reduced.
5. In recurrent appendicitis, mast cell count was less reduced and was in between normal and acute appendicitis with higher mucosal mast cell count. (29 ± 9 / sq.mm.)
6. Mast cell count in appendix with enterobious Vermicularis was similar to recurrent appendicitis.
7. Number of mast cell was depending on the degree of fibrosis in non-inflamed appendices. In initial stage of fibrosis (grade – II mast cell 60 ± 5.8 / sq.mm. in mucosa and 51 ± 6.0 / sq.mm. submucosa) count was higher but as fibrosis increased, mast cell count was reduced. (So, in grade –IV fibrosis in mucosa 52 ± 4.8 / sq.mm. and in submucosa 47 ± 4.9 / sq.mm. mast cell count observed). Markedly reduced count was obtained in acellular fibrotic area of grade –V fibrosis. Mucosal mast cell count had good correlation with degree of fibrosis as compared to submucosal mast cell count.
8. In tuberculosis, mast cell was abundant in periphery of caseating granuloma where fibroblastic proliferation was going on. Here mast cell count (40 / sq.mm.) was relatively

higher than normal.

9. In tuberculosis affecting ileocaecal region, typhoid perforation of intestine and mucin secreting adenocarcinoma of colon, mast cell count was increased in mucosa and submucosa of appendices.

CONCLUSION

Comparison to the aim of the study, the following conclusion are made:

1. Toluidine stain is easy, simple and gives good result, it can be used as routine staining method for the studying the mast cells where fixative does not matter much and stain does not fade away, and it is long lasting and should always be done after routine histopathological assessment.
2. Although, Carnoy's fluid fixation gives higher mast cell count than formaline fixation, as later is routinely used and effect of it remains same on mast cell count irrespective of diseased process and results obtained are reproducible so formaline fixation is preferred.
3. There is some influence of age on mast cell, with increase in age mast cell count decreases. Sex does not have any such effect.
4. Mast cell count decrease in acute inflammatory condition as demonstrated by mast cell count in acute appendicitis.
5. As healing process starts, progressive fibroblastic proliferation occur and mast cell numbers increase. Later on young granulation tissues is replaced by dense fibrous tissue and is accompanied with decrease in number of mast cells.
6. Recurrent or subacute appendicitis, mast cell count is intermediate between normal appendices and acute appendicitis. This indicates that mild inflammation causes degranulation of less number of mast cells or due to simultaneous reparative process in recurrent appendicitis, mast cell number are increasing from low count in acute appendicitis towards normal count in normal appendices.
7. As mucosal mast cell count is more liable to changes with inflammation or fibrotic process as demonstrated by lower mucosal mast cell count as compared to submucosal mast cell count in acute inflammation and higher mucosal mast cell count than submucosal in different grades of fibrosis, it can be concluded that initial stimulus may be luminal.
8. In any chronic inflammatory conditions with fibroblastic proliferation, mast cell count is higher as compared to normal.

Hence, it is concluded that mast cells play an important role in pathogenesis of appendicular disease and by understanding it, one unravel pathophysiology of many non-immunological inflammatory and fibrotic lesions.

So, it is still mysterious to understand the key role of "Master Player" mast cells in the drama of diseases, special appearance of which have been clicked by keen observers.

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