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Research Article

EVALUATION OF MAST CELLS IN SURGICALLY RESECTED APPENDIX

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A R T I C L E I N F O

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A B S T R A C T

Introduction: Appendicitis is the most common surgical emergency. Mast cells release biologically active substances which on activation alter intestinal functioning therefore their change in the number and distribution may explain the cause of pain.
Aims and objectives: To compare the number and distribution of mast cells in various layers in cases of acute appendicitis, chronic appendicitis and appendix with no significant change on histology.
Materials and methods: Total 100 specimen of appendix were studied prospectively during the time period of 1 year from May 2017 to June 2018 in the Department of Pathology, Faculty of Medicine & Health Sciences, SGT University, Gurugram. Hematoxylin and Eosin staining was done on paraffin sections, and Special staining for mast cells was done with 1% toluidine blue. Results: Maximum number of cases belonged to the age group of 21 -30 years with males predominance (75%). Acute appendicitis was seen in 56% cases. Highest mast cell count was observed in cases of chronic appendicitis Maximum grades of mast cells were seen in the submucosa layer associated with fibrosis.
Conclusion: Increase in mast cell count was observed in cases of appendicitis as compare to those appendices with normal histology. Thus it was concluded that the growth interaction between the mast cells, nerves and fibrosis may be due to release of chemical mediators leading to type I hypersensitivity reaction that might be a predisposing factor for the sequence of events leading to appendicitis.

INTRODUCTION

Appendix is the normal true diverticulum of the caecum that is prone to acute and chronic inflammations.[1] It was first described by the physician, anatomist, Energario Da Capri in 1521[2] The lifetime risk for appendicitis is 7%; males are affected slightly more often than females.[3] Appendicectomy is the most frequent intra abdominal surgery and it is the most common surgical emergency in children[4] with 6% to 20% of the general population developing appendicitis during their lifetime.[5]Untreated, mortality is high, mainly because of peritonitis and shock.[5] Most cases of acute appendicitis (50% to 60%) develop as a result of obstruction mostlybyfaecolith[6] ,other causes could be lymphoid hyperplasia, intestinal worms, foreign body, true calculus, gallstone, tumor of the caecum or a primary tumor of the appendix.[7]The usual presentation of acute appendicitis is with periumbilical colicky pain and vomiting, with the pain later localizing in the right lower abdominal quadrant.[8] Chronic appendicitis represent recurrent appendicitis, the finding of a significant increase in neural fibers, schwann cells, and enlarged ganglia in cases of clinically acute appendicitis may be indicative of repeated bouts of inflammation.

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Mast cells are derived from the multipotent cells in the bone marrow. They have basophilic granules, which when stained, appear red or violet with the blue basic aniline dyes.[2]They are widely distributed in the gastrointestinal tract. They release large number of biologically active substances (heparin, tryptase, arylsulfatase, beta glucourinidase, the eosinophilic chemotactic factor, the neutrophilic chemotactic factor, prostaglandin-D2, the platelet activating factor and the leukotrienes, LTC and LT B4)[2] which on 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, systemic mastocytosis, graft versus host disease etc.[9] Evidence shows 20% to 30 % appendices removed from patients with suspected appendicitis appear normal on histology. The cause of acute abdominal pain in these patients remains unexplained. The association of the changes in the number of mast cells in appendicitis though unclear, could be responsible for the nerve proliferation and the hypertrophy in the cases with clinically and histopathologically diagnosed appendicitis. So, in present study by using 1% toluidine blue stain, an attempt was made to study the mast cell count, their pattern of distribution and to correlate these findings in variety of appendicular pathologies thereby to understand the role of mast cells in cases coming to SGT hospital.

MATERIAL AND METHODS

The present study was conducted on a total of 100 cases of appendicitis in the Department of Pathology, SGT Medical college and Hospital, Gurugram, Haryana. Cases were categorised on the bases of clinical diagnosis into:-

- Acute appendicitis
- Chronic appendicitis
- Normal appendix obtained on ileocaecal resection.

Detailed gross examination was done, and all the paraffin sections were subjected to Hematoxylin & eosin staining for histopathological examination. Further categorisation of the cases were done on the basis of histological findings under 4 Groups: -

- Group A (Acute appendicitis with neutrophilic infiltration only)
- Group B (Acute Appendicitis with both eosinophilic and neutrophilic infiltration)
- Group C (Chronic appendicitis with lympho-mononuclear infiltrate and fibrosis)
- Group D (Appendix found normal on histology).

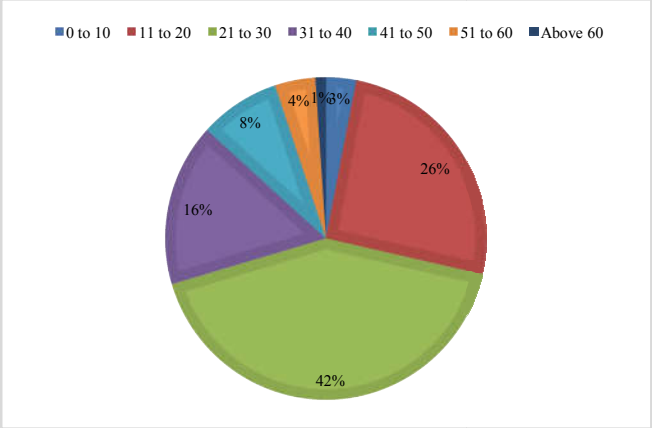
Special staining with 1% toluidine blue was done on all the sections for evaluation of mast cells. Mast cells appeared violet/red purple with blue background. The total number of mast cells were counted in all the layer (mucosa, submucosa, muscularis and serosa) in 10 consecutive high power fields in all sections and were categorised under four Grades:-

- Grade 0 (No mast cells cells)
- Grade 1 (Less than 10 cells seen in 10 high power fields)
- grade 2 (More than 10 cells seen in 10 high power fields)
- Grade 3 (Several such clusters of more than 10 cells seen in more than 10 high power fields).

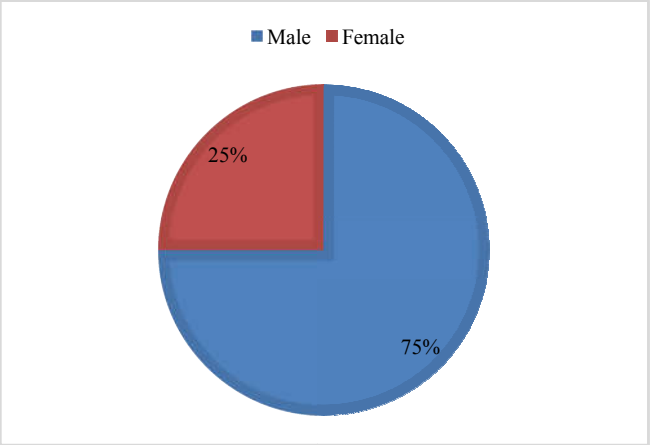
Statistical Analysis

Statistical analysis was done using Microsoft excel .Descriptive analysis of the data was done and categorical variables were analysed using chi square test. For all statistical tests, p value less than 0.05 was taken to indicate a significant difference.

OBSERVATIONS AND RESULTS



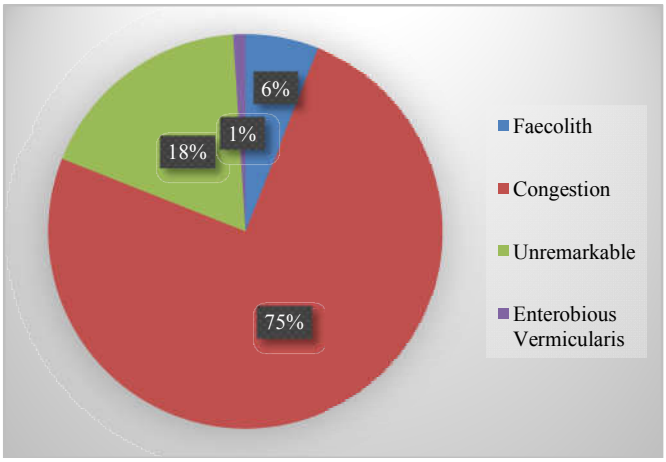
Age Distribution of the Patients



Sex distribution of the Patients

Distribution of cases under various types of clinical diagnosis

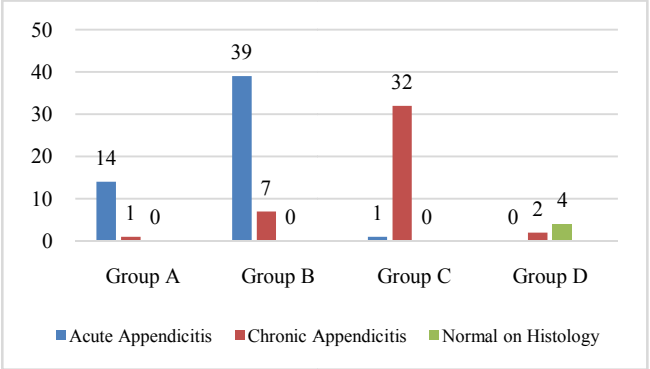
Clinical diagnosis	frequency	percentage
Acute Appendicitis	56	56%
Chronic Appendicitis	38	38%
Normal appendix	6	6%
Total	100	100%



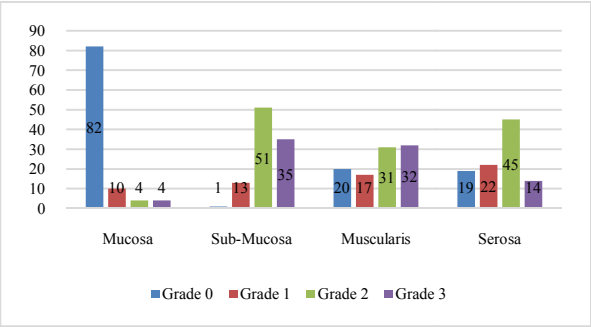
Distribution of findings on cut sections

Distribution of cases according to histopathological Criterions

Groups	Frequency	Percentage
Group A	15	15 %
Group B	46	46 %
Group C	33	33%
Group D	6	6%
Total	100	100 %



Correlation of clinical diagnosis with histopathological criteria



Distribution of mast cells in mucosa

Grading of mast cells in different layers of appendix

Grades	Group A (n=15)	%	Group B (n=46)	%	Group C (n=33)	%	Group D (n=6)	%
Grade 0	8	53.3%	40	87%	28	85%	6	100%
Grade 1	5	33.3%	1	2.2%	4	12%	0	0%
Grade 2	1	6.7%	2	4.3%	1	3%	0	0%
Grade 3	1	6.7%	3	6.5%	0	0%	0	0%

Distribution of mast cells in sub-mucosa

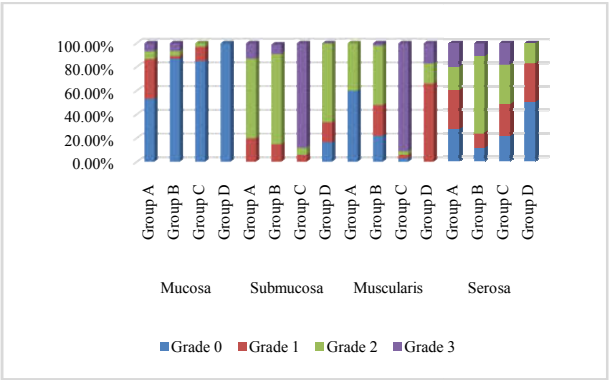
Groups	Group A (n=15)	%	Group B (n=46)	%	Group C (n=33)	%	Group D (n=6)	%
Grade 0	0	0%	0	0%	0	0%	1	16.7%
Grade 1	3	20%	7	15%	2	6%	1	16.7%
Grade 2	10	67%	35	76%	2	6%	4	66.6%
Grade 3	2	13%	4	8%	29	88%	0	0%

Distribution of mast cells inmuscularis

Groups	Group A (n=15)	%	Group B (n=46)	%	Group C (n=33)	%	Group D (n=6)	%
Grade 0	9	60%	10	22%	1	3%	0	0%
Grade 1	0	0%	12	26%	1	3%	4	66%
Grade 2	6	40%	23	50%	1	3%	1	17%
Grade 3	0	0%	1	2%	30	91%	1	17%

Distribution of mast cells in serosa

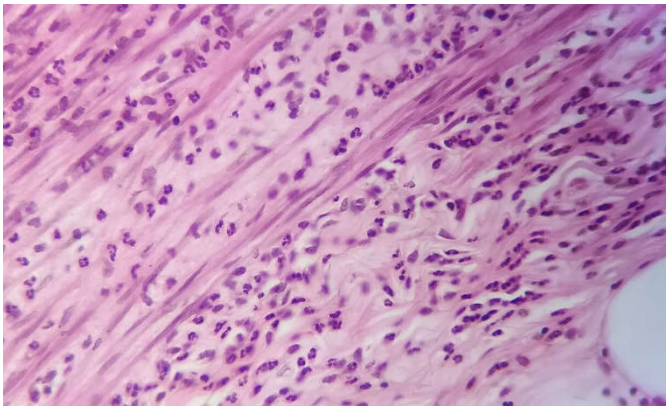
Groups	Group A (n=15)	%	Group B (n=46)	%	Group C (n=33)	%	Group D (n=6)	%
Grade 0	4	27%	5	10.8%	7	21.2%	3	50%
Grade 1	5	33%	6	13%	9	27.3%	2	33%
Grade 2	3	20%	30	65.2%	11	33.3%	1	17%
Grade 3	3	20%	5	10.8%	6	18.2%	0	0%



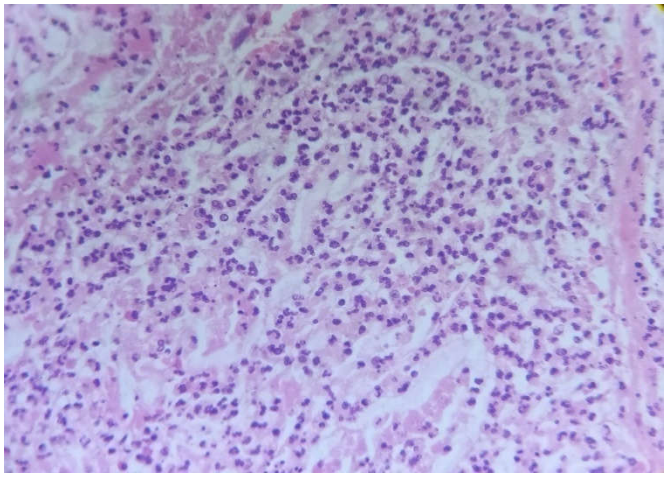
Distribution of cases according to presence of mast cells in all the four layers of appendices in all the histological groups.

There was a significant difference found between the mast cell grades for all the four layers of mucosa, sub-mucosa, muscularis and serosa in all the groups under histopathological criteria, as the p-values for all the Groups was found to be less than 0.05 in every case.

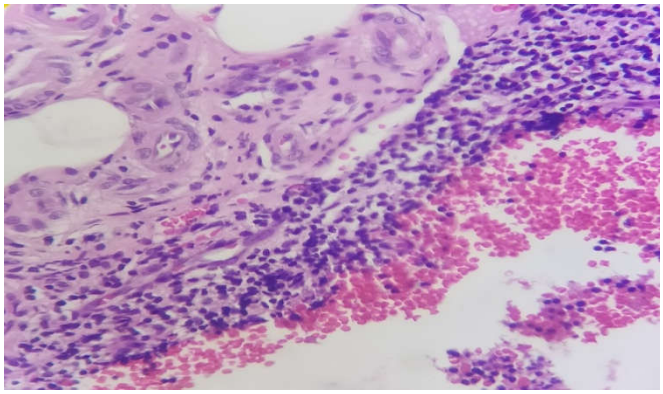
Figures



Group A Acute appendicitis with neutrophilic infiltrate only (H&E stain, 400 X)

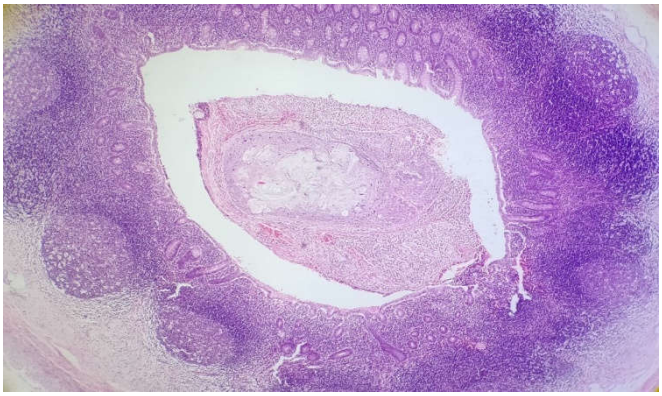


Group B Acute appendicitis with both eosinophilic and neutrophilic infiltrate (H&E, 400X)

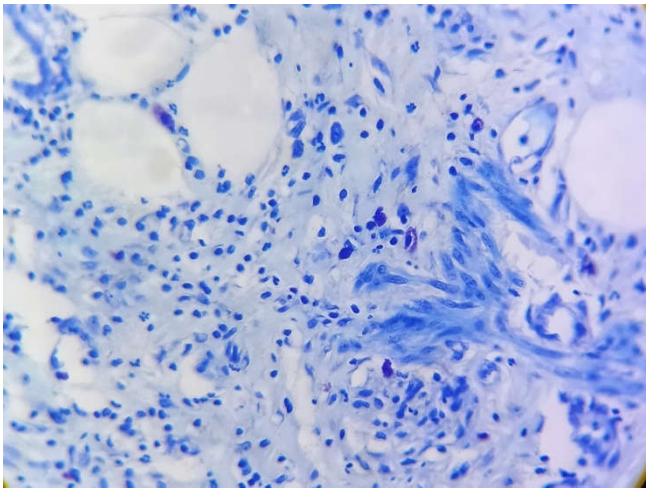


Group C Chronic Appendicitis showing lymphoplasmacytic infiltrate (H&E, 400X)

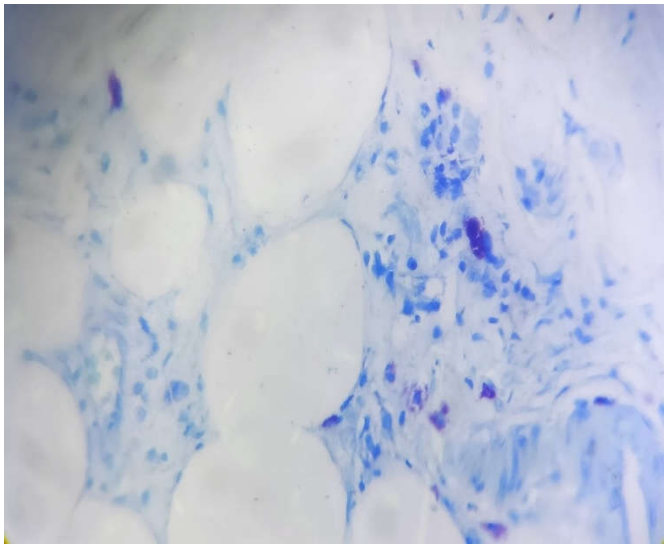
Grade	Mucosa				Submucosa				Muscularis				Serosa			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D
Grade 0 Grade 1 Grade 2 Grade 3		p<0.05 (p=0.00032)				p<0.05 (p=0.00002)				p<0.05 (p=0.0045)				p<0.05 (p=0.0310)		



EnterobiousVermicularis in the lumen of appendix (H&E, 100 X)



Mast cells seen in submucosa(Grade 2) (Toluidine blue, 400X)



Mast cells seen in submucosa (Grade 3) (Toluidine blue, 400X)

DISCUSSION

In our study maximum number of cases were seen between age group of first and second decades of life with maximum cases (42%) in the age group 21 - 30 years. Mean age was 27.3±11.6.This is comparable to the study by Chittawadgi *et al* [5], Banerjee *et al*[10], Singh *et al*[11]

Maximum number of cases were that of acute appendicitis (56%) followed by chronic appendicitis (38%) and appendix found normal on histology (6%). These results are consistant with the study done by Kumaran *et al*,[3] Singh *et al*,[10]Sonti *et al*,[2]Kolur *et al*,[12]Anuradha *et al*.[13]

Acute appendicitis seems to be the end result of a primary obstruction of the appendix lumen according to Wangensteen OH and Pieper R *et al*.[14] The prevalence of faecoliths in patients with appendicitis is significantly higher in developed than in developing societies[15]and is commonly associated with complications.[16] However in the present study, majority of cases (75%) had luminal congestion due to inflammation and only (6%) of cases had obstruction of the lumen due to faecolith. These findings are similar to the study of Kolur *et al*[12] who found that obstruction due to faecolith could not be demonstrated in many cases of appendicitis.

Gastrointestinal infection due to Enterobius Vermicularis occurs worldwide and is considered to be the most common helminthic infection found in the appendix.[17]The recent literature revealed the incidence of E. Vermicularis in surgically removed appendices from patients with clinical appendicitis, varying from 2.7% to 4.1% .[18] In the study done by Eleftherios *et al*,[19]E. Vermicularis was identified only in 0.65% of patients with clinical appendicitis and overall 0.60% for all appendices removed during the same period. Similarly our study showed only one case having luminal obstruction by E. vermicularis on histological examination. This finding is also supported by the studies of Mysorekar *et al*[20], Banerjee *et al*[10] , who found no significant causal relationship between parasitic infestation and acute appendicitis. This lower prevalence could be justified by two factors. The first one is the geographic location and social circumstances, leading to prominent variations in the prevalence of the parasite. The other factor could possibly be the existence of minimal differentiations in the techniques followed by the pathologists.

In the present study it was found that out of 100 cases the maximum number of cases of acute appendicitis were of Group B (46%) i.e acute appendicitis showing neutrophilic and eosinophilicinfiltration. These findings are similar to the study of Kumaran *et al*[3] in which out of the 108 cases, 60 cases (55.5%) of acute appendicitis had neutrophilic and eosinophilic infiltrate. The second highest number of cases were found in Group C (33%)i.e chronic appendicitis. These findings are similar to the study of Kumaran *et al*,[3]and Anuradha *et al*.[13]Appendix in cases of repeated episodes shows fibrosis indicative of previous inflammation. However, existence of recurrent appendicitis has always been controversial and only readdressed.[10]Thackray *et al* found progressive fibrosis with infiltration by lymphocytes and plasma cells together with hyperplasia of lymphoid tissue is normally present.[21]

Mast cell count was highest in chronic appendicitis followed by acute appendicitis, which correlated with the studies done by Shah *et al*[9], Kolur *et al*[12], Singh *et al*[11], Coskun *et al*[22], Nagaraj *et al*[23], Kumaran *et al*[3], Yang *et al*[24], Sonti *et al*[2] and Naik *et al*[25] . Significant difference in mean mast cell count was observed in all the histopathological groups ($P<0.05$). The least mast cell count was observed in Group D, higher in Group B and Highest in Group C. These findings are comparable with the study of Sonti *et al*,[2]Naik *et al*,[25] Banerjee *et al*.[10]

Of the 100 cases studied, 6 cases (6%) were of normal appendix that was incidentally removed during other abdominal surgeries. Histologically, the appendix that was normal showed the lowest mast cell count. These findings were supported by the study carried out by Naik *et al*.[25] and Nagraj

et al.[23]However, in contrast to present study Singh *et al*[11] 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 their degranulation.[11]

We found that mucosal and submucosal mast cell counts in group A and B and C were significantly higher than the group D. This may explains the symptoms of nausea, vomiting and pain in patients with acute appendicitis as well as those who were clinically diagnosed as acute appendicitis but had apparently normal histology .[11]These findings are similar to the study of Sonti *et al*,[2]Mysorekar *et al*,[26] Banerjee *et al*.[10] The mast cells serve a function of host defence against invading pathogens and have a central role in the mediation of allergic responses.[27] Although they synthesise a wide variety of mediators that can induce both acute and chronic inflammation,[28] they also generate and release several mediators that may act on nerve development and neural functions, and contribute to neuroimmune reactions.[29],[30] They synthesise, store and release several mediators that may influence neural functions such as leukaemia-inhibitory factor, IL-6 and TNF. Mast cells exert their biological effects by releasing preformed mediators stored in granules such as leukotrienes, prostaglandins, and cytokines.[4]There is considerable evidence that mast cells are micro anatomically and functionally opposed to the peripheral nerves,[31]resulting in a homeostatic unit in the regulation of gut physiology and host defence. The increase in mast cells in cases of appendicitis together with the broad spectrum of activities of biological mediators, suggests that this cell type could play a role in the pathogenesis of appendicitis. [4]

CONCLUSION

Toluidine staining technique is easy, simple, long lasting and gives good result for identification of mast cells in appendix. Mast cell counts were lowest in appendices removed in the course of surgeries done for some other disease, while they were significantly higher in, acute appendicitis, thus indicating an immunological or a non immunological injury and highest count was observed in cases of chronic appendicitis indicating the growth interaction between the mast cells, nerves and the fibrosis. Also obstruction due to faecolith could not be demonstrated in many cases thus supporting the theory of inflammation rather than obstruction in the pathogenesis of appendicitis. Increased eosinophil count with an increase in mast cell count observed in the present study in appendicitis may be due to the consequence of the type I hypersensitivity reaction with release of mediators by mast cells that might be a predisposing factor for the sequence of events leading to appendicitis. Definitive increase in mast cells count as the disease progresses, thus substantiate their contributing role in pathogenesis of appendicitis.

Bibliography

1. Kumar V, Abbas A., Aster J. robbins & cotrans pathologic basis of diseases. 9th ed. philadelphia: Elsevier Inc; 2015. 816 p.
2. SONTI S. A Study on the Mast Cells in Appendicitis. *J Clin Diagnostic Res*. 2012;6(7).
3. C K, L DL. Mast Cells in Appendicitis: a Study. *J Evid Based Med Healthc*. 2015;2(34):5165-70.
4. Bhramaramba K, Srujana S, Devi PR, Kumar OS. Role of mast cells and neuronal hypertrophy in acute appendicitis. *Int Arch Int Med*. 2016; 3(11): 94-102.
5. Chittawadgi BB, Mhetre SC, Chittawadgi BB. A morphometric analysis of diameter of blood vessels in relation to degree of eosinophils and basophils (mast cells) infiltration for analysis of severity and prognosis of acute appendicitis. *Ind J PatholOncol*. 2016; 3(3):463-9.
6. Nitecki S, Karmeli R, Sarr MG. Appendiceal calculi and fecaliths as indications for appendectomy. Surgery, gynecology& obstetrics. 1990 Sep; 171(3):185-8.
7. Edino ST, Mohammed AZ, Ochicha O, Anumah M. Appendicitis in Kano, Nigeria: a 5-Year Review of Pattern, Morbidity and Mortality. Ann Afr Med. 2004;3(1): 38-41 .
8. Rosai J. Gastrointestinal tract: Appendix. Rosai and Ackerman's Surgical Pathology. 10th ed. Edinburgh: Mosby Elsevier; 2011. p.714-730.
9. Shah AA, Patel M, Jitendra R, Joshi D. Mast cells in Surgically Resected Appendices. *Int J Sci Res*. 2013 Jul;2(7):372-6
10. Banerjee IA, Datta S. Comparative study of mast cells in different pathological types of surgically resected appendix specimens . 2018;17(4):52-7.
11. Singh UR, Malhotra A, Bhatia A. Eosinophils, mast cells, nerves and ganglion cells in appendicitis. *Indian J Surg*. 2008;70(5):231-4.
12. Kolur A, Patil AM, Agarwal V, Yendigiri S, Sajjanar BB. The Significance of mast cells and eosinophils counts in surgically resected appendix. Journal of interdisciplinary Histopathology. 2014 Jan 1;2(3):150-3.
13. Anuradha GP, Anita AM, Seemant SK, Pratima S. Mast cell profile in appendicitis. *Indian J Pathol Oncol*. 2017;4(4):555-9.
14. Andersson RE, Hugander A, Thulin AJ. Diagnostic accuracy and perforation rate in appendicitis: association with age and sex of the patient and with appendicectomy rate. *Eur J surgery Acta Chir*. 1992;158(1):37-41.
15. Jones BA, Demetriades D, Segal I, Burkitt DP. The prevalence of appendicealfecaliths in patients with and without appendicitis. A comparative study from Canada and South Africa. Ann Surgery. 1985 Jul; 202(1):80.
16. Barcia JJ, Reissenweber N. Neutrophil count in the normal appendix and early appendicitis: Diagnostic index of real acute inflammation. Ann Diagn Pathol. 2002;6(6):352-6.
17. Aydin Ö. Incidental parasitic infestations in surgically removed appendices: a retrospective analysis. Diagn Pathol. 2007; 2(1):16.
18. Sah SP, Bhadani PP. Enterobius vermicularis causing symptoms of appendicitis in Nepal. Trop Doct. 2006;36(3):160-2.
19. Gialamas E, Papavramidis T, Michalopoulos NA, Karayannopoulou G. Enterobius vermicularis : A rare Cause of Appendicitis Enterobius vermicularis : A rare Cause of Appendicitis Enterobius vermicularis : Apandisitin Nadir Bir Nedeni. 2012;(May 2014).
20. Mysorekar VV, Chanda S, Dandeka CP. Mast cells in surgically resected appendices. *Indian journal of pathology & microbiology*. 2006 Apr;49(2):229-33.

21. Thackray AC. "Chronic Appendicits"-Some Pathological Observations. *Br J Radiol*. 1959; 32(375): 180-2.
22. Coşkun N, Indel SM, Elpek GO. Mast cell density and neuronal hypertrophy in patients with acute appendicitis. *Turk J Gastroenterol*. 2003 Mar; 14(1):54-8.
23. Nagaraj G, Das S, Venkatesha M, Lingiah HK. A comparative study of mast cells in appendix. *International Journal of Medical Science and Public Health*. 2015 Nov 1; 4(11):1611-4.
24. Yang Z, Esebua M, Layfield L. Increased Mast Cells in Histologically" Normal" Appendices Following Emergency Appendectomy in Pediatric Patients. *American Journal of Clinical Pathology*. 2015 Oct 1; 144(2):A370.
25. Naik R, Pai MR, Bansal R, Pai R. Relation of mast cell, nerve and fibrosis in appendix. *Ind J patholmicrobiol*. 1998 Jan;41(1):27-30
26. Mysorekar VV, Chanda S, Dandeka CP. Mast cells in surgically resected appendices. Indian journal of pathology & microbiology. 2006 Apr; 49(2):229-33.
27. Galli SJ. New concepts about the mast cell. *N Engl J Med*. 1993;328(4):257-65.
28. Gordon JR, Burd PR, Galli SJ. Mast cells as a source of multifunctional cytokines. Immunol Today. 1990; 11:458-64.
29. Nakafuku M, Satoh T, Kaziro Y. Differentiation factors, including nerve growth factor, fibroblast growth factor, and interleukin-6, induce an accumulation of an active Ras. GTP complex in rat pheochromocytoma PC12 cells. *J Biol Chem*. 1992;267(27):19448-54.
30. Selmaj KW, Raine CS. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann Neurol. 1988;23(4):339-46.
31. Dvorak AM, McLeod RS, Onderdonk AB, Monahan-Earley RA, Cullen JB, Antonioli DA, *et al*. Human gut mucosal mast cells: ultrastructural observations and anatomic variation in mast cell-nerve associations in vivo. Int Arch Allergy Immunol. 1992;98(2):158-68.

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