

Role of mast cells in surgically resected specimens of appendices

Ravishankar Katkar¹, Shaista Choudhary^{2*}, Neha Singh³

¹Assistant Professor, ²Associate Professor, ³Post Graduate, Dept. of Pathology, Dr. B R Ambedkar Medical College, Bengaluru, Karnataka, India

*Corresponding Author: Shaista Choudhary

Email: drshaista5@rediffmail.com

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Abstract

Introduction: Mast cells are very closely linked to diseases and have a significant role in inflammatory process. The functions of mast cells depends on release of preformed granules. The aims and objectives of present study are to investigate the possible role of mast cells in pathogenesis of appendicitis.

Materials and Methods: This prospective study was conducted on 100 surgically resected appendectomy specimens at our hospital. Study period was from March 2016 to February 2017 for a period of one year. Histopathological diagnosis was made and status of mast cells was studied.

Results: In this study presence of mast cells and its relationship with appendicitis was studied and it was found that increased number of mast cells were noted in inflammatory conditions of appendix. Presence of mast cells has an impact on pathology of appendix.

Keywords: Appendix, Mast cells, Toluidine blue, Appendicitis.

Introduction

Appendectomy specimens are the most common specimens in any histopathology laboratory with most common pathology being acute appendicitis. Besides the usual histopathological features of appendicitis the presence of mast cells was noted and documented by various authors. There is a sense of curiosity regarding the association of mast cells and appendicitis. Mast cells are derived from multipotential stem cells in bone marrow, with precursors also found in blood, mucosal and connective tissue. With appropriate stimulation these precursors proliferate and differentiate into mast cells.¹

Mast cells are round, oval, or fusiform in shape measuring 8 to 15 micrometers in diameter and contains light basophilic granules.² The granules stain metachromatically with toluidine blue, thio bismarck brown, Azure –A and Giemsa. Basophilic granules contain histamine, proteoglycans, carboxypeptidase A, glycosidases and sulphatases. Mast cells and basophils resemble each other in their metachromasia, acid nature and content of histamine and heparin. But mast cells contain hydrolytic enzymes, 5-hydroxy-tryptamine and serotonin which basophils do not have.³ In addition to preformed products mast cells along with basophils produce a variety of lipid and protein mediators with pro inflammatory activities including chemotactins, cell activating and cell growth factor. Enzymes within the granules of mucosal and connective tissue mast cells are different from each other. Thus the range of mast cell activities are specialized for their anatomic locations.³

Mast cells have been regarded as potentially important in the initiation and/or amplification of acute inflammatory response. These cells have also been

found to be important for the induction of neutrophil influx in the immune mediated responses. This in turn has been found to involve IgG Fc receptors. This indicates that mast cells may not only contribute to but also initiate the process.²

The role of mast cells in fibrosis has been studied by Rothe and Kerdel who concluded that mast cell mediators may act as growth factors for fibroblasts and endothelial cells. Also a tumor necrosis factor like molecule is synthesized and released by mast cells hence it is clear that mast cells participate in various biologic events, some of which result in fibrosis.

Materials and Methods

This study comprised of surgically resected appendix specimens which were received at histopathology section of Pathology department. Specimens were fixed in 10% formalin solution in the ratio of 1:10. After fixation appendix with mesoappendix was studied for macroscopic features or grossing was done. Surface changes were noted for e.g. enlarged appendix, congestion of blood vessels, any discoloration and necrosis etc. Any changes were noted down. Serial slicing was done and every bit was observed. Minimum of three bits were given for further processing. In case of suspicion on gross appearance multiple bits were given from representative areas.

Sections were processed by manual processing by following all the steps. Slides were stained with routine haematoxyllin & eosin and one section was stained with Toluidine blue stain for the identification of mast cells. Haematoxylin & eosin stained sections were studied for various findings like presence of inflammatory cells, status of appendiceal glands, muscular layer, fibrosis

etc. In case of predominance of any inflammatory cells was also noted and documented.

The Toluidine blue stained sections were studied for the presence of mast cells and images were taken. Principal of toluidine blue staining: Mast cells should stain red –purple (metachromatic staining). Background stain blue (orthochromatic staining). Toluidine blue stock solution was prepared and 1% sodium chloride was taken. Both the reagents were mixed and at the time of staining working solution was prepared freshly. Working solution consisted of Toluidine blue stock solution-5.0 ml (ph 2.0-2.5)
1% sodium chloride-45.0 ml.

Procedure

1. Deparaffinize and hydrate to distilled water
2. Working Toluidine blue 1 to 2 minutes
3. Rinse in distilled water three changes
4. Dehydrate quickly through 95% and absolute alcohol.
5. Clear in xylene.

Result obtained was mast cells-violet and background showed shades of blue.

The number of mast cells in the mucosa, submucosa and muscular layer were counted under high power i.e. 40X of binocular microscope. Per field mast cells were counted and average of ten fields were taken and count documented. Associated features like edema, inflammatory cell infiltrates, fibrosis and predominant inflammatory cells were noted in each layer of appendix.

Results

The present study was conducted on 100 surgically resected specimens of appendix which were received at histopathology section of Pathology department at our institution over a period of one year.

Out of 100 cases, 49 were males and 51 females. Age of the patients in present study ranged from 10 to 80 years. Maximum number of cases were seen between second and third decade of life. Table 1 shows the age distribution and table 2 shows the histopathological diagnosis in male and female patients.

Acute appendicitis was diagnosed when the presenting symptoms showed acute onset of fever, severe umbilical or right lower abdominal quadrant pain with tenderness and vomiting. Diagnosis was confirmed by histopathology. When the patient presented with repeated episodes of pain in right iliac fossa and fever it was clinically diagnosed as recurrent appendicitis.

Highest mean mast cell count was seen in recurrent appendicitis with fibrosis.

Lowest mast cell count was seen in normal appendices as shown in table 3

Table 1

| Age group(years) | Number of patients | Percentage % |
|------------------|--------------------|--------------|
| 10 -20 | 28 | 28 |
| 21-30 | 42 | 42 |
| 31-40 | 14 | 14 |
| 41-50 | 11 | 11 |
| 51-60 | 2 | 2 |
| 61-70 | 3 | 3 |

Table 2

| Histopathological diagnosis | Males % | Females % | Total % |
|--------------------------------|---------|-----------|---------|
| Normal | 0 | 8 | 8 |
| Acute appendicitis | 29 | 25 | 54 |
| Recurrent appendicitis | 10 | 16 | 26 |
| Acute suppurative appendicitis | 9 | 2 | 11 |
| Eosinophilic appendicitis | 1 | 0 | 1 |
| Total | 49 | 51 | 100 |

Table 3

| Diagnosis | No. of cases | Mast cell count in mucosa | | Mast cell count in submucosa | |
|---------------------------|--------------|---------------------------|------|------------------------------|------|
| | | Range | Mean | Range | Mean |
| Normal | 8 | 0-2 | 1 | 0-2 | 1 |
| Acute appendicitis | 54 | 2-10 | 4 | 4-15 | 5 |
| Acute suppurative | 11 | 3-12 | 5 | 2-10 | 4 |
| Recurrent appendicitis | 26 | 5-16 | 8 | 6-18 | 9 |
| Eosinophilic appendicitis | 1 | 1-6 | 2 | 0-2 | 1 |
| Total | 100 | | | | |



Fig. 1: Gross appearance showing thickened wall in acute appendicitis

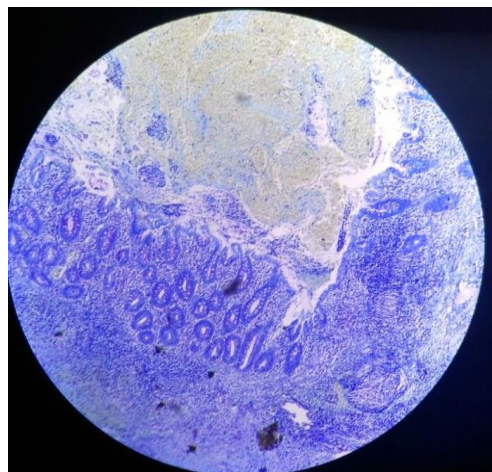


Fig. 4: Sheets of inflammatory cells 10X

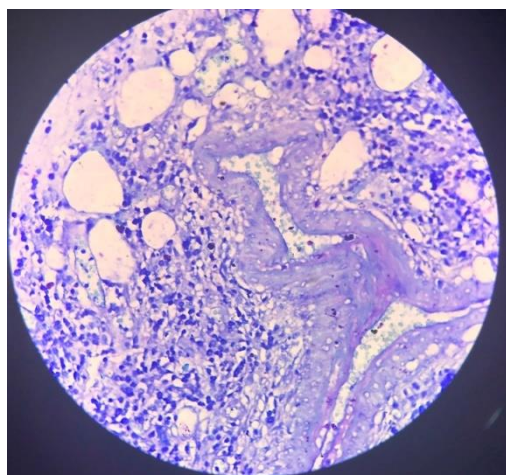


Fig. 2: Inflammatory cells (neutrophils) in acute appendicitis

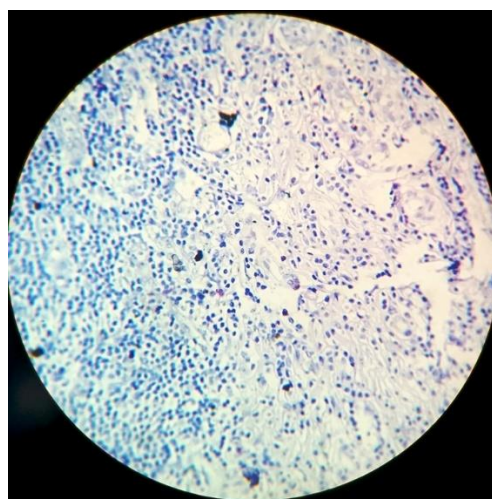


Fig. 5: Toluidine blue stain showing mast cells along with inflammatory cells

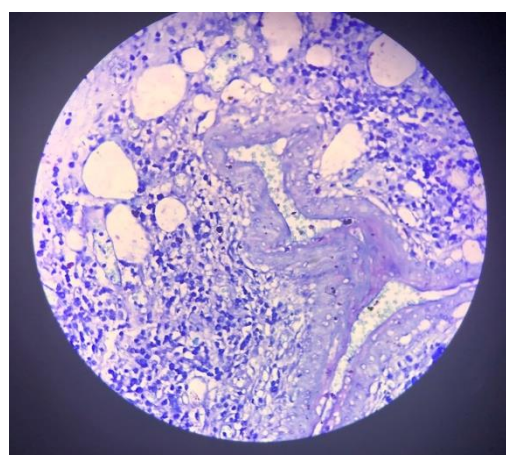


Fig. 3: Toluidine stained sections showing mast cells in submucosal layer

Discussion

In the present study 100 surgically resected specimens of appendix were studied for the mast cell profile. In present study we found the results comparable to various authors. The percentage of recurrent appendicitis was 26% in present study as observed by Dymock et al and Aravindan et.al. Highest number of appendicectomies were seen in 21 to 30 years (42%) age group followed by 11 to 20 years (24%) age group in our study.

In present study mast cell count was highest in recurrent appendicitis especially when it was associated with fibrosis and the mast cell count was low in normal appendix. In a study by Sulochana normal appendices did not show any significant variation in mast cells count. Study concluded that mast cell count was highest in acute appendicitis.⁶ Ashwini et al. in their study concluded that highest mast cell count was associated with acute eosinophilic appendicitis.⁷

Karakus et al. detected that mast cells are increased in appendicitis and their presence correlates with degree

of inflammation. Study involved children and their focus was on mast cells and eosinophils.⁸

Usha Rani Singh et al in their study on eosinophils, mast cells and ganglion cells in appendicitis concluded that there is significant increase in eosinophils and mast cells in acute appendicitis.⁹

In present study also we found mast cells to be increased significantly in acute appendicitis and to a certain extent in recurrent appendicitis. Various other studies also support this conclusion. Muktha et al in their study found paneth cells metaplasia in colonic adenocarcinoma.¹⁰

Hence in conclusion mast cells have a role in long standing injury, i.e. recurrent appendicitis. In present study we found that number of mast cells was significantly high in acute appendicitis followed by recurrent appendicitis. Mucosal ulceration could be one reason for less mast cell count in acute appendicitis in few studies. Each type of cell is significant when present in large numbers. It would be ideal if we treat appendix as a learning experience and not mere a vestigial organ.

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