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Study to compare the mass cell counts in mucosa and submucosa and muscular layer of the appendix in various histopathological groups

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Abstract

Background: Mast cells are immune cells distributed throughout connective tissues, particularly in the gastrointestinal tract, where they play a role in immune regulation and inflammation. Their involvement in appendicitis is not well understood. This study compares mast cell counts in different histopathological groups of appendicitis by evaluating their distribution in the mucosa, submucosa, and muscularis propria.**Objectives :**To compare mast cell counts in the mucosa, submucosa, and muscular layer of the appendix across various histopathological groups.**Methods:** This study analyzed100 appendix specimens from patients undergoing appendectomy or laparotomy for suspected appendicitis. Specimens were stained with hematoxylin& eosin (H&E) for histopathological evaluation and Toluidine Blue for mast cell identification. Mast cells were counted in 10 high-power fields (HPF) at 400X magnification, and the mean mast cell count per mm² was calculated. ANOVA was used to assess statistical significance.**Results:** Among the 100 cases, acute appendicitis was the most common (56%), followed by recurrent appendicitis (32%), acute eosinophilic appendicitis (5%), and normal appendices (7%). Mast cell counts were significantly higher in inflamed appendices compared to normal appendices ($p<0.05$). The lowest mast cell count was in normal appendices (5 ± 1.9 per mm²), while recurrent appendicitis showed the highest count (16.1 ± 7.7 per mm²). The highest mast cell density was observed in the mucosa of eosinophilic appendicitis (15.5 ± 7.5 per mm²) and in the submucosa (21.4 ± 7.9 per mm²) and muscularis propria (13.9 ± 6.2 per mm²) of recurrent appendicitis**. Conclusion**: Mast cells are significantly involved in recurrent and eosinophilic appendicitis, particularly in chronic inflammation and fibrosis. Their statistically significant increase in inflamed appendices suggests their potential as diagnostic markers and possible therapeutic targets in recurrent appendicitis.

Keywords: Mast cells,Appendicitis,Histopathology,Mucosa,Submucosa.

INTRODUCTION

Mast cells are specialized immune cells derived from hematopoietic stem cells in the bone marrow. They are distributed throughout various tissues in the body, particularly within connective tissues, where they play a crucial role in immune regulation and disease processes [1,2]. These cells are abundantly present in the skin, respiratory tract, and gastrointestinal tract, serving as key components of the body's defense system against invading pathogens and parasites [3].

In acute inflammation, the number of mast cells tends to decrease, whereas chronic inflammatory conditions and fibrosis are often associated with an increased mast cell count [4]. These cells contribute significantly to the progression of acute inflammation, particularly through their role in neutrophil recruitment and immune complex-mediated responses [5]. Within the gastrointestinal tract, mast cells are essential for mucosal immune defense, suggesting their involvement in immune system activation and inflammatory processes [6].

Appendicitis is one of the most frequent causes of acute abdominal pain requiring surgical intervention, particularly among adolescents and young adults [7]. The incidence of appendicitis has been rising in developing regions, possibly due to dietary and lifestyle changes. Despite extensive research, the exact pathogenesis of appendicitis remains unclear, though it is commonly linked to luminal obstruction. Factors such as fecaliths, hypertrophic lymphoid follicles, parasitic

infections, trauma, and tumors may contribute to the obstruction, leading to inflammation and infection [8]. Histopathological examination remains the gold standard for confirming appendicitis.
Mast cells have been implicated in appendicitis pathophysiology, with studies suggesting their role in nerve proliferation and tissue hypertrophy [9]. Their count varies across different histopathological forms of appendicitis, being lowest in normal appendices, higher in acute appendicitis, and reaching its peak in recurrent appendicitis.
This study aims to compare mast cell counts across different layers of the appendix—mucosa, submucosa, and muscularis—in various histopathological groups.

OBJECTIVES

To compare the mass cell counts in mucosa and submucosa and muscular layer of the appendix in various histopathological groups

MATERIAL AND METHODS

1. Study Design and Sample Collection

This study was conducted on 100 consecutive appendix specimens received for histopathological examination at the Department of Pathology in a tertiary care center. The appendices were removed for suspected appendicitis or as part of a laparotomy performed for other diseases. The study duration was from November 2017 to August 2019. Clinical details, including age, sex, symptoms, and clinical diagnosis, were recorded in a structured proforma.

2. Specimen Collection and Processing

- **Source:** Appendices were obtained from patients undergoing:
  - Emergency appendectomy
  - Interval appendectomy
  - Ileocecal resection
- **Fixation and Processing:**
  - Appendices were received in 10% formalin and fixed for a minimum of 24 hours.
  - A section from the length of the appendix was taken and processed for routine paraffin embedding.
  - From each paraffin block, two sections (4-micron thickness) were cut:
    - One section was stained with Hematoxylin& Eosin (H&E)
    - The other was stained with 1% Toluidine Blue

3. Histopathological Examination and Mast Cell Counting

- **H&E-stained sections** were analyzed for:
  - Presence of eosinophils
  - Fibrosis
  - Inflammatory changes
- **Toluidine Blue-stained sections** were used for:
  - Mast cell identification
  - Counting mast cells in mucosa, submucosa, and muscularis propria

Mast cells were counted in 10 non-overlapping high-power fields (HPF) at 400X magnification. The mean mast cell count per square millimeter was calculated.

4. Inclusion Criteria

- Appendices surgically removed as a therapeutic measure for suspected acute appendicitis.
- Appendices excised during ileo-caecal resection or laparotomy for other diseases.

Exclusion Criteria

- Acute gangrenous appendicitis cases were excluded due to muscle fiber necrosis.
- Appendices with confirmed malignant or benign tumors were excluded.

5. Staining Protocols

1. **Toluidine Blue Staining for Mast Cells**
   - Deparaffinization with xylene and isopropyl alcohol.
   - Toluidine blue (1%) applied for 1 minute.
   - Rinsed with CO_2-free water, alcohol, and xylene.
   - Mast cells appeared violet/purple, with a blue background.
2. **Hematoxylin& Eosin (H&E) Staining for General Histopathology**
   - Deparaffinization with xylene, alcohol, and water.
   - Staining with Harris Hematoxylin.
   - Differentiation with 1% hydrochloric acid.

- Counterstaining with Eosin.
- Final mounting with DPX.
- Nuclei appeared blue, while cytoplasm appeared pink.

6. Statistical Analysis

- Microsoft Excel was used for calculating mean and standard deviation.
- ANOVA test (Analysis of Variance) was performed using Open Epi Software v3.01 to compare mean mast cell counts across different histopathological groups.

RESULTS

This study analyzed100 cases of appendicitis, comparing mast cell counts across different histopathological groups and appendix layers. The results revealed a significant increase in mast cell density in inflamed appendices compared to normal appendices, with the highest counts observed in recurrent appendicitis (16.1 ± 7.7 per mm² total mean count) and acute eosinophilic appendicitis (15.1 ± 6.1 per mm² total mean count). Normal appendices had the lowest mast cell count (5 ± 1.9 per mm²), confirming that mast cells play a minimal role in non-inflamed tissues.

Among the different layers of the appendix, submucosa consistently exhibited the highest mast cell density, particularly in recurrent appendicitis (21.4 ± 7.9 per mm²), followed by acute eosinophilic appendicitis (17.5 ± 6.1 per mm²) and acute appendicitis (12.7 ± 6.1 per mm²). The mucosa showed the highest mast cell count in acute eosinophilic appendicitis (15.5 ± 7.5 per mm²), suggesting a potential immune-mediated inflammatory response. In the muscularis propria, recurrent appendicitis again demonstrated the highest mast cell count (13.9 ± 6.2 per mm²), reinforcing its role in chronic inflammation, fibrosis, and possible nerve proliferation.

The statistical analysis confirmed that the difference in total mean mast cell counts between normal and inflamed appendices was statistically significant ($p<0.05$). This suggests that mast cells contribute to the inflammatory process in appendicitis and may play a role in disease progression and recurrence. The highest mast cell counts were observed in recurrent appendicitis, indicating that mast cells may be involved in fibrosis, nerve hypertrophy, and prolonged inflammation. Additionally, the elevated mast cell density in acute eosinophilic appendicitis suggests a potential allergic or immune-mediated mechanism.

In conclusion, mast cells appear to play a crucial role in appendicitis pathogenesis, particularly in recurrent and eosinophilic appendicitis, where their increased presence in the submucosa and muscularis propria suggests involvement in chronic inflammation and tissue remodeling. These findings highlight mast cells as potential markers for appendicitis severity and recurrence, opening avenues for further research on their therapeutic targeting in recurrent appendicitis cases.

Table 1: Age Distribution of Patients

Age Group (Years)	Number of Cases	Percentage (%)
0-10	4	4%
11-20	31	31%
21-30	32	32%
31-40	12	12%
41-50	11	11%
51-60	4	4%
61-70	4	4%
71-80	2	2%
Total	**100**	**100%**

Observation: The highest incidence of appendicitis was in the 21-30 years age group (32%), followed by 11-20 years (31%), indicating that appendicitis is most common in young adults.

Table 2: Age-wise Distribution of Histopathological Groups

Age Group (Years)	Normal Appendix	Acute Appendicitis	Acute Eosinophilic Appendicitis	Recurrent Appendicitis	Total Cases
0-10	1	2	0	1	4
11-20	1	18	1	11	31
21-30	2	19	1	10	32
31-40	1	7	1	3	12
41-50	1	6	1	3	11
51-60	0	2	1	1	4

61-70	0	1	0	3	4
71-80	1	1	0	0	2

Observation:Acute appendicitis was the most common pathology across all age groups, particularly in 11-30 years. Recurrent appendicitis was more frequent in older age groups.

Table 3: Gender Distribution of Patients

Gender	Number of Cases	Percentage (%)
Male	58	58%
Female	42	42%

Observation: The study showed a male predominance (58%), with a male-to-female ratio of 1.38:1.

Table 4: Gender-wise Distribution of Histopathological Groups

Histopathological Group	Male (n=58)	Female (n=42)	Total Cases
Normal Appendix	4	3	7
Acute Appendicitis	32	24	56
Acute Eosinophilic Appendicitis	3	2	5
Recurrent Appendicitis	19	13	32

Observation:Acute appendicitis was more common in males (57%), while recurrent appendicitis was also higher in males compared to females.

Table 5: Histopathological Groups Distribution

Histopathological Group	Number of Cases	Percentage (%)
Normal Appendix	7	7%
Acute Appendicitis	56	56%
Acute Eosinophilic Appendicitis	5	5%
Recurrent Appendicitis	32	32%

Observation:Acute appendicitis (56%) was the most common finding, followed by recurrent appendicitis (32%).

Table 6: Mast Cell Count in Mucosa

Histopathological Group	Mast Cell Count in Mucosa (Mean ± SD per mm^2)
Normal Appendix	3.9 ± 1.3
Acute Appendicitis	8.7 ± 4.9
Acute Eosinophilic Appendicitis	15.5 ± 7.5
Recurrent Appendicitis	12.8 ± 5.8

Observation:Acute eosinophilic appendicitis had the highest mast cell count in the mucosa.

Table 7: Mast Cell Count in Submucosa

Histopathological Group	Mast Cell Count in Submucosa (Mean ± SD per mm^2)
Normal Appendix	4.6 ± 1.7
Acute Appendicitis	12.7 ± 6.1
Acute Eosinophilic Appendicitis	17.5 ± 6.1
Recurrent Appendicitis	21.4 ± 7.9

Observation:Recurrent appendicitis had the highest mast cell count in the submucosa, followed by acute eosinophilic appendicitis.

Table 8: Mast Cell Count in Muscularis Propria

Histopathological Group	Mast Cell Count in Muscularis Propria (Mean ± SD per mm^2)
Normal Appendix	6.4 ± 1.9
Acute Appendicitis	12.0 ± 4.2
Acute Eosinophilic Appendicitis	12.5 ± 4.6
Recurrent Appendicitis	13.9 ± 6.2

Observation:Recurrent appendicitis had the highest mast cell count in the muscularis propria, indicating its role in chronic inflammation.

Table 11: Comparison of Mean Mast Cell Counts in Different Layers of the Appendix Across Histopathological Groups

Histopathological	No. of	Mucosa	Submucosa	Muscularis	Total Mean Mast

Group	Cases	(Mean ± SD)	(Mean ± SD)	Propria (Mean ± SD)	Cell Count (Mean ± SD)
Normal Appendix	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 Eosinophilic 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**

Statistical Significance: The difference in total mean mast cell counts between normal and inflamed appendices was found to be statistically significant ($p<0.05$).

DISCUSSION

The appendix, historically regarded as a vestigial organ, is now understood to play an active role in mucosal immunity. Inflammation of the appendix remains a common cause of acute abdomen, with acute appendicitis being the most frequently diagnosed condition. This study aimed to evaluate the distribution of mast cells across different histopathological groups of appendicitis by assessing their presence in the mucosa, submucosa, and muscularis propria.

Our study found acute appendicitis (56%) as the most common condition, followed by recurrent appendicitis (32%) and acute eosinophilic appendicitis (5%). This aligns with Shrestha et al. [10] and Lee et al. [11], who reported similar appendicitis incidence patterns.

Mast cells were present in all layers of the appendix, with varying densities depending on the histopathological group. Normal appendix had the lowest mast cell count (5 ± 1.9 per mm^2), whereas recurrent appendicitis had the highest count (16.1 ± 7.7 per mm^2), particularly in the submucosa (21.4 ± 7.9 per mm^2). These results correlate with findings from Mysorekar et al. [12], Kolur et al. [13], and Sandhu et al. [14], who reported increased mast cells in chronic and recurrent appendicitis, suggesting their role in fibrosis and tissue remodeling.

Layer-Wise Distribution of Mast Cells

- Mucosa: The highest mast cell density was observed in acute eosinophilic appendicitis (15.5 ± 7.5 per mm^2). Farhadi et al. [15] and Aravindan et al. [16] described a similar mast cell increase in eosinophilic-driven inflammation.
- Submucosa:Recurrent appendicitis had the highest count (21.4 ± 7.9 per mm^2), supporting its role in chronic inflammation and fibrosis, as suggested by Chang et al. [8].
- Muscularis propria:Recurrent appendicitis displayed the highest count (13.9 ± 6.2 per mm^2), in agreement with Chang et al. [8], who proposed mast cells as contributors to chronic inflammation and fibrosis.

The significant increase in mast cell density in inflamed appendices, particularly in the submucosa and muscularis propria of recurrent appendicitis, suggests their role in chronic inflammation, fibrosis, and potential nerve hypertrophy. Similar findings were reported by Mysorekar et al., who identified increased mast cells in recurrent appendicitis cases [12]. The elevated mast cell density in acute eosinophilic appendicitis supports their involvement in allergic and immune-mediated inflammation [8].

Our findings align with earlier studies demonstrating increased mast cell counts in inflamed appendices. Chang et al. [8] and Kolur et al. [13] reported similar distributions of mast cells in recurrent appendicitis, supporting their role in chronic inflammation and fibrosis. Aravindan et al. [16] highlighted mast cell involvement in acute eosinophilic appendicitis, corroborating our study findings.

The elevated mast cell count in recurrent appendicitis suggests potential therapeutic interventions targeting mast cells. Given their contribution to fibrosis and tissue remodeling, Mekori et al. [17] and Maltby et al. [18] previously discussed mast cells' role in fibrosis and immune modulation, supporting the potential for mast cell-targeted therapies in recurrent appendicitis.

CONCLUSION

This study underscores the significant role of mast cells in appendicitis pathogenesis, particularly in recurrent and eosinophilic appendicitis, where their increased presence in the submucosa and muscularis propria suggests involvement in chronic inflammation, fibrosis, and nerve proliferation. The statistically significant differences in mast cell counts between normal and inflamed appendices indicate their potential diagnostic and therapeutic relevance. Further research should explore the mechanistic role of mast cells in appendicitis progression and assess whether mast cell-targeted interventions could improve outcomes in recurrent appendicitis cases.

PHOTOGRAPHS OF STUDY

Fig.1 Gross specimen of normal appendix

Fig 2 Gross specimen of acute appendicitis

Fig 3 Gross specimen of recurrent appendicitis

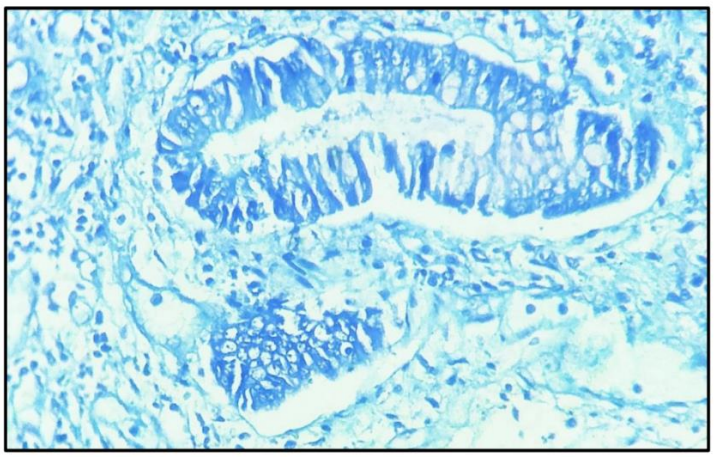

Fig 4 presents a photomicrograph of mast cells surrounding the mucosal crypts of a normal appendix, stained with Toluidine Blue at 100X magnification.

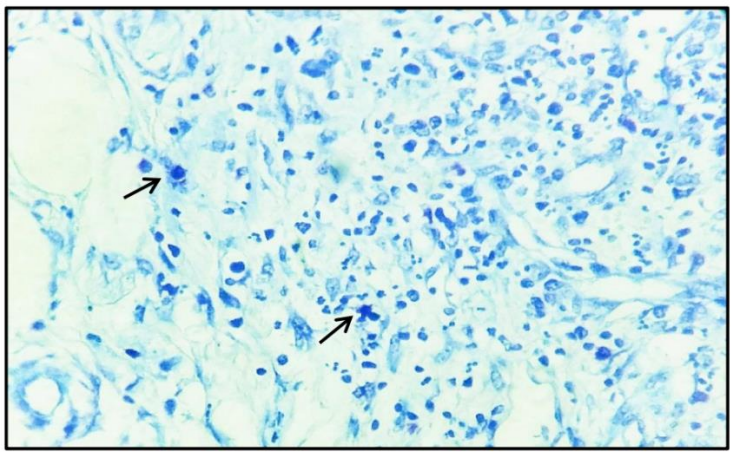

Fig 5 presents a photomicrograph of mast cells in the submucosa of a normal appendix, stained with Toluidine Blue at 100X magnification. An arrow highlights the mast cell in the image.

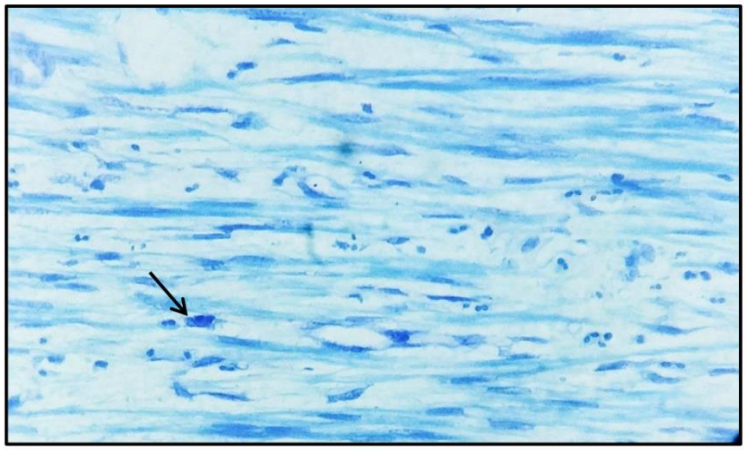

Figure 6 presents a photomicrograph of mast cells located between the muscle fibers in the normal appendix, stained with Toluidine Blue at 400X magnification. Arrows indicate the presence of mast cells.

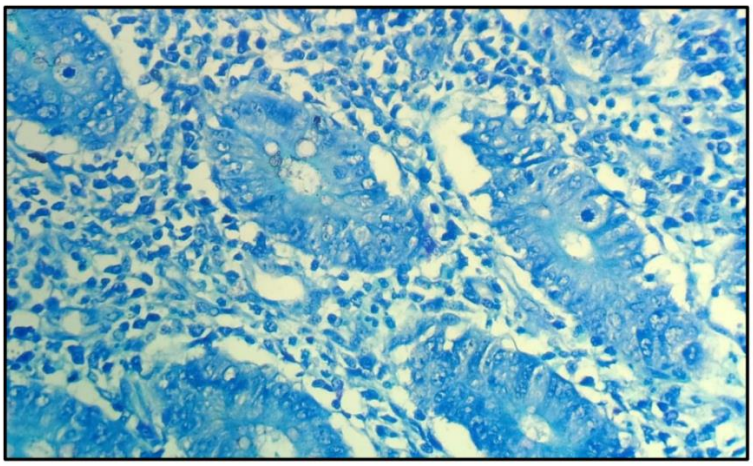

Fig 7 presents a photomicrograph of mast cells around the mucosal crypts in acute appendicitis, stained with Toluidine Blue at 100X magnification.

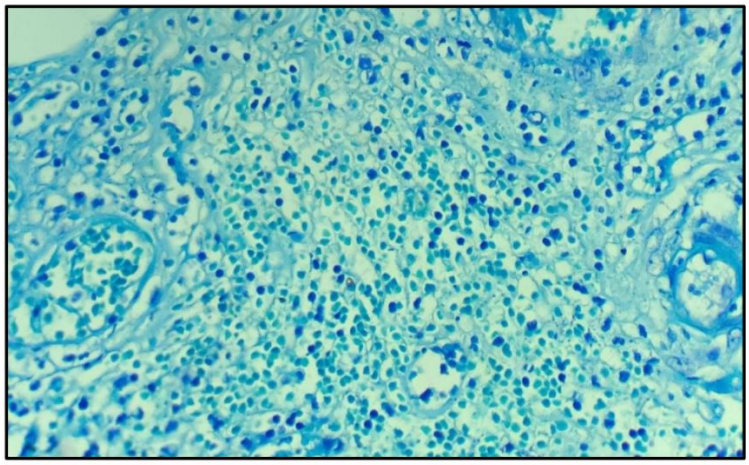

Fig 8 presents a photomicrograph of mast cells in the submucosa of acute appendicitis, stained with Toluidine Blue at 100X magnification

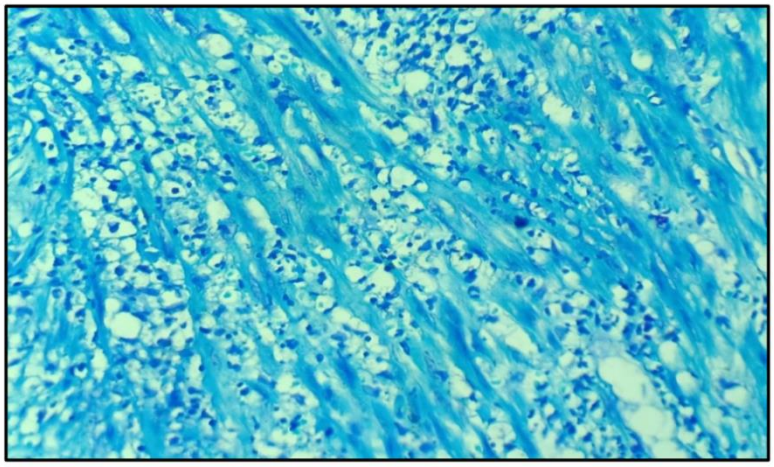

Fig 9: Photomicrograph of mast cells in the muscularis propria of acute appendicitis. Toluidine blue 100X.

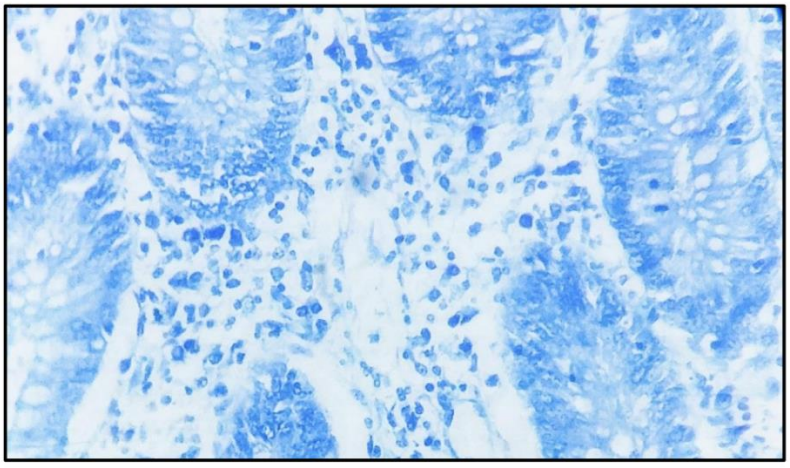

Fig 10: Photomicrograph of mast cells around the mucosal crypts of recurrent appendicitis. Toluidine blue 100X.

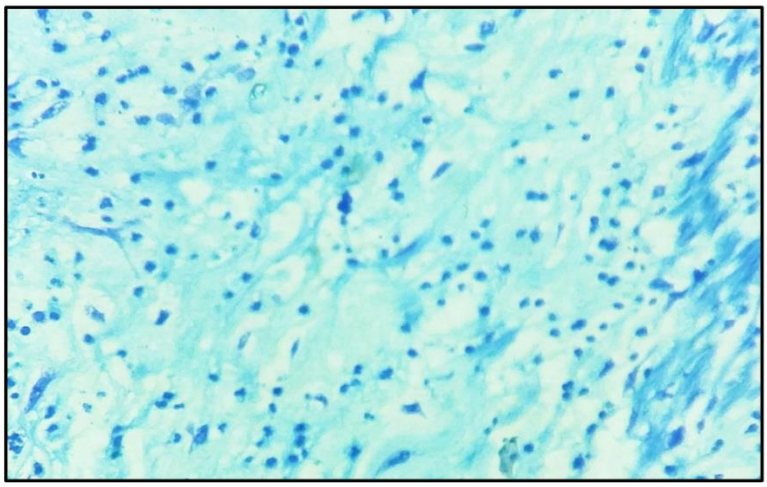

Fig 11: Photomicrograph of mast cells in the submucosa of recurrent appendicitis. Toluidine blue 100X.

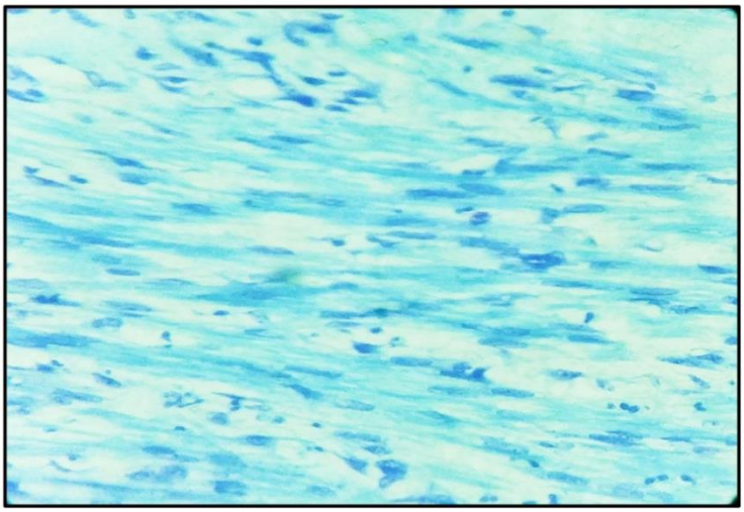

Fig 12: Photomicrograph of mast cells in the muscular layer of the recurrent appendix. Toluidine blue 100X.

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