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Original Research Article.

A Histological Study of Surgically Resected Appendices in Clinically Suspected Appendicitis with Special Reference to Eosinophils and Mast Cells

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ABSTRACT

Background: The appendix is a normal true diverticulum of the caecum that is prone to acute and chronic inflammation. Acute appendicitis is most common in adolescents and young adults, but may occur in any age group. The life time risk for appendicitis is 7%.

Methods: The present study is being undertaken to study the role of eosinophils and mast cells in the pathogenesis of appendicitis. Total 50 cases of clinically suspected acute appendicitis and 10 cases of normal appendices were taken for the study. The tissues were sectioned and stained. The haematoxylin and eosin sections were studied for inflammatory changes in various layers of appendix and 1% toludine blue stained sections were examined for mast cells. Average cell counts of neutrophils, eosinophils and mast cells in all the layers were obtained.

Results: In our study, histological features of acute appendicitis were found as increased neutrophils in submucosa, muscularis and serosa. The average number of eosinophils is higher in cases of acute appendicitis than control cases.

Conclusion: It had been suggested that eosinophilic infiltrate in the appendix might be a reflection of a resolving or regression phase of acute appendicitis.

Key Words: Acute Appendicitis, Histology, Eosinophils, Mast Cells.

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INTRODUCTION

The appendix is a normal true diverticulum of the caecum that is prone to acute and chronic inflammation. Currently, the most accepted explanation for the development of acute appendicitis is obstruction and secondary infections. But it has been suggested that obstruction is not an important causative factor in acute appendicitis, it mostly develops as a result of inflammatory process.[1]

Eosinophilic infiltration of muscularis has been seen in acute appendicitis and it is postulated that acute appendicitis is triggered by Type I Hypersensitivity.[1] Mast cells have a central role in Type I Hpersensitivity. Mast cells have been implicated in the pathogenesis of pain.[2-4] Role of mast cells in the pathogenesis of appendicitis has been studied by very few authors. Hence, the present study on surgically removed appendices with clinically suspected appendicitis with special reference to eosinophils and mast cells was being done.

MATERIALS AND METHODS

Fifty cases of surgically resected appendices for clinically suspected acute appendicitis and ten cases of normal appendices (appendices removed in other abdominal surgeries) were taken for the study. Appendices removed in ileocecal surgeries were included while those of acute gangrenous appendicitis were excluded. After formalin fixation, sections were taken from the tip, base and one intermediate site along the length of the appendix. Sections were routinely processed and stained with haematoxylin and eosin (H &E) for inflammatory changes and 1% toludine blue for mast cells in all the layers of appendix. 50 cases were divided into histologically positive and negative depending upon the presence and absence of neutrophils in the muscle layer. Mean cell counts/ hpf were calculated and compared in different categories. Significance was calculated based on standard deviations.

Table.1a: Maximum number of neutrophils in various layers: Layers of Appendix

	HPAA	HNAA	Controls
Mucosa	80/hpf	03/hpf	03/hpf
Submucosa	100/hpf	02/hpf	01/hpf
Muscularis	175/hpf	Nil/hpf	Nil/hpf
Serosa	200/hpf	03/hpf	04/hpf

Table.2b: Average number of eosinophils in various layers Layers of appendix

	HPAA	HNAA	Total HPAA+HNAA	Controls
Mucosa	28.32±24.8	20.8±18.9	25.4±22.8	12.8±14.2
Submucosa	7.5±6.6	13.4±26.2	9.74±16.9	3.9±6.2
Muscularis	17.6±23.4	9.2±16.1	14.4±21.14	4.2±7.1
Serosa	6.6±12.6	2.8±6.7	5.14±10.8	2.0±2.3

Table.3a: Maximum number of mast cells in various layers Layers of appendix

	HPAA	HNAA	Controls
Mucosa	10/HPF	10/HPF	10/HPF
Submucosa	30/HPF	30/HPF	30/HPF
Muscularis	30/HPF	15/HPF	15/HPF
Serosa	10/HPF	10/HPF	08/HPF

Table.3b: Average number of mast cells in various layers Layers of appendix

	HPAA	HNAA	Total HPAA+HNAA	Controls
Mucosa	3.1±2.5	2.5±2.4	2.9±2.5	3.2±3.3
Submucosa	9.7±5.6	12.3±7.4	10.8±6.4	10.0±7.8
Muscularis	7.2±5.17	6.9±3.9	7.1±4.7	4.0±4.4
Serosa	4.9±1.8	4.4±2.8	4.7±2.2	3.3±2.4

RESULTS

A total of 60 cases of surgically resected appendices were studied. Out of these, 50 were removed in patients presenting with signs and symptoms of acute appendicitis and 10 cases of appendices were removed as complementary to other abdominal surgeries; these were taken as controls. Fifty cases were divided into histologically positive acute appendicitis (HPAA) and histologically negative acute appendicitis (HNAA) on the basis of neutrophils found in the muscle layer. The cases in which neutrophils were found in the muscle layer, were taken as HPAA and were 31 cases out of 50 (62%), while no neutrophils were found in the muscle layer in HNAA in 19 cases out of 50 (38%). Out of 50 cases studied, 50% were male and 50% were female. Thus the incidence of acute appendicitis was equal in both the sexes. The age range for acute appendicitis was found to be 1 to 62 years and it was most common in third decade.

Luminal inflammation was seen in 74% of HPAA, 68% cases of HNAA and in 30% of normal controls. Mucosal ulceration was seen in 55% of HPAA , 63% of HNAA and in 30% of normal controls.[Fig.1a] Thus it was concluded that mucosal ulceration and luminal inflammation were not specific features of acute appendicitis and not much helpful to differentiate from normal appendix.

Mucosal neutrophils >10/5 hpf can be considered a sign of early appendicitis because 74% cases of HPAA were found to have this feature, while 95% of HNAA cases showed mucosal neutrophils </=10/5 hpf. The number of neutrophils found in submucosa, in cases of HPAA varied from 10/hpf to 100/hpf, while in HNAA and in control cases, the number was not more than 15/hpf. The number of neutrophils found in muscularis, in cases of HPAA varied from 10/hpf to 175/hpf, while in HNAA and in control cases, there were no neutrophils The number of neutrophils found in serosa, in cases of HPAA varied from 10/hpf to 200/hpf, while in HNAA and in control cases, the number was not more than 20/hpf. The maximum number of neutrophils/hpf was found in serosa, but the maximum average count/hpf was found in muscularis.[Table. 1a and b] [Fig.1b].

The maximum number of mast cells were found in submucosa and muscularis of HPAA cases.[Table.3a][Fig.3a and b]. In muscularis layer of appendix, the number of mast cells/hpf in cases of acute appendicitis were significantly higher than the control cases; but there was no significant difference in mast cell count/hpf in rest of the layers. Mast cell count/hpf in HPAA is significantly higher in mucosa and muscularis layer than HNAA. The maximum average count/hpf was found in submucosa.[Table.3b].

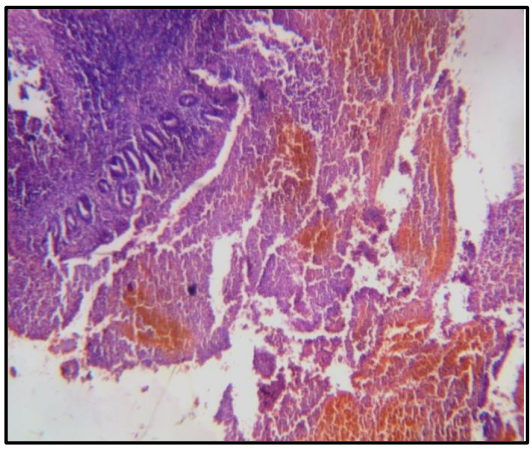

Fig.1a: Luminal inflammation and mucosal ulceration (H & E, X100)

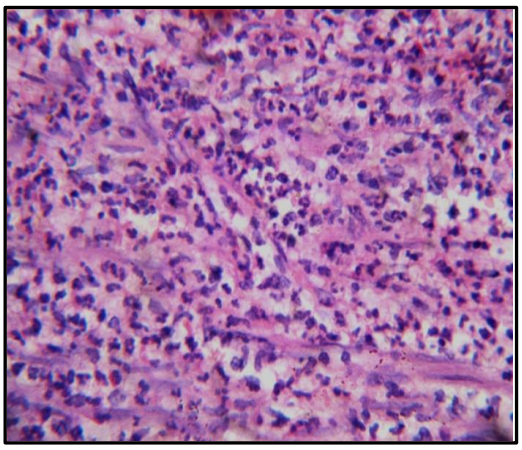

Fig.1b: Neutrophils in muscularis propria of HPAA cases (H & E,X400)

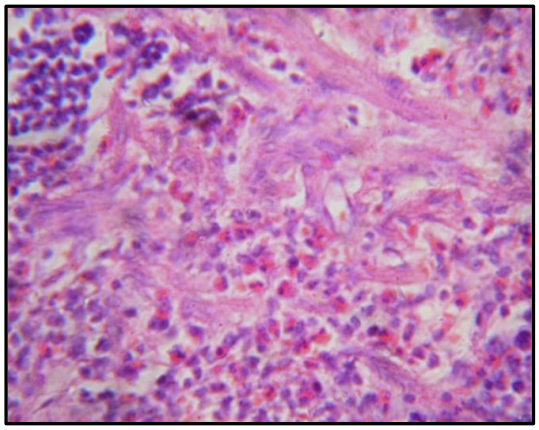

Fig.2a: Eosinophils in submucosa of HNAA cases (H & E,X400)

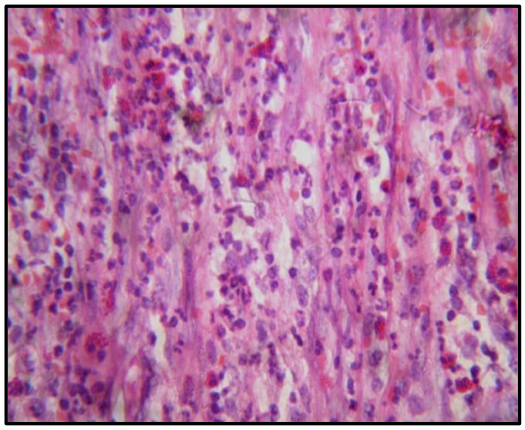

Fig.2b: Neutrophils and eosinophils in muscularis propria of HPAA cases (H & E,X400)

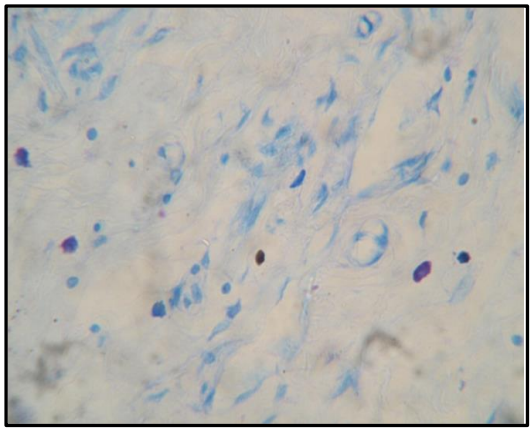

Fig.3a: Mast cells in submucosa (Toludine blue,X 400)

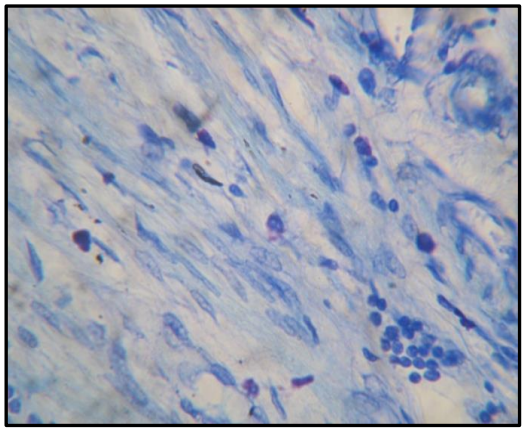

Fig.3b: Mast cells in muscularis propria (Toludine blue, x400)

DISCUSSION

In our study, total of 50 cases of clinically suspected cases of acute appendicitis and 10 cases of normal appendices were taken for the study. Fifty cases were divided into HPAA and HNAA on the basis of neutrophils found in the muscle layer, as it was concluded by Petras RE et al5. Aravindan KP et al1 also defined suppuration or the presence of neutrophils as the feature of acute appendicitis.

In our study, 62% cases were HPAA, while 38% were HNAA. Our findings were consistent with the study by Jones EA et al[6], they reported acute inflammation in 77% of cases and 23% appendices were within normal limits. Singhal V et al[7] found 60% cases to be positive histologically. In our study, the incidence of acute appendicitis was equal in both the sexes. HPAA was found to be more common in males, while histologically normal appendices were found more commonly in females. These findings were similar to the study by Mysorekar VV et al.[8]

In our study, acute appendicitis was most common in third decade (40% of cases). We found that 68% of cases occurred in patients less than 30 years of age. Chang AR[9] found 80% of cases occurring in the same. In our study, exudation of pus in the lumen of the viscus, ulceration and loss of mucosal epithelium were

found in many cases of HPAA (74% had luminal inflammation, 55% had mucosal ulceration) but the diagnosis of classic suppurative appendicitis should not be made only in their presence, because luminal inflammation was seen in 68% cases of HNAA and in 30% of normal controls. Ashley JB David[10] recognized in his study that in acute inflammation, there was often exudation of pus in the lumen. In many instances, there was also ulceration and loss of the mucosal epithelium.

In our study, 74% cases of HPAA had mucosal neutrophils >10/5HPF, while 95% of HNAA cases showed mucosal neutrophils </=10/5HPF. These results were similar to the study of Barcia JJ et al[11], who considered more than 10 neutrophils/5HPF found in the mucosa to be evidence of early appendicitis. In our study, the increased number of neutrophils/ HPF in submucosa >15/HPF and in serosa >20/HPF, were also diagnostic of acute appendicitis. The number of neutrophils/HPF was significantly increased in all the layers in cases of HPAA as compared to HNAA and in control cases. Our findings were similar to the study by Herd ME et al[12], who suggested that serosal inflammatory response was diagnostic of acute appendicitis. The average number of neutrophils /HPF were found to be maximum in muscularis propria. The average number of eosinophils/HPF in our study were significantly higher in cases of acute appendicitis than the control cases in all the layers of appendix. Findings in the present study were in accordance with the study of Aravindan KP1 and Singh UR et al[13], who also found eosinophil count to be significantly higher in acute appendicitis compared to normal appendices from other abdominal surgeries. In our study, the eosinophils were found to be maximum in mucosa, whereas, in the study of Singh UR et al[13], the maximum number of eosinophils were seen in muscularis propria. The average number of mast cells/HPF, in our study were significantly higher in muscularis propria of acute appendicitis cases than the controls. Our findings were similar to the study of Aravindan KP1 and Xiong et al[14], who also found the mast cells number to be significantly higher in acute appendicitis compared to normal appendices from other abdominal surgeries. In our study, mast cells were detected in all layers of the appendiceal wall, but were found to be significantly increased in the submucosa and muscularis propria in cases of acute appendicitis. Xiong et al[14] found significantly increased numbers of mast cells in the submucosa, muscularis and especially in the lamina propria. We have found the average number of mast cells/HPF to be significantly higher in HPAA than HNAA in mucosa and muscularis propria. Singh UR et al[13] also found mean mast cell count to be higher in mucosa and submucosa in HNAA cases, but in muscularis layer the count was higher in HPAA, similar to our study. Amber et al[15] found that the mean mast cell count was highest in HNAA cases in all the four layers as compared to the HPAA.

CONCLUSION

To conclude, histological features of acute appendicitis were found as: increased neutrophil counts in submucosa, muscularis and serosa. Mucosal neutrophils >10/5HPF could be considered a sign of early appendicitis. The average number of eosinophils/ HPF were significantly higher in cases of acute appendicitis than the control cases in all the layers of appendix, while the average number of mast cells/HPF was significantly higher in the muscularis layer.

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