STUDY OF INTRAOPERATIVELY AND HISTOLOGICALLY NEGATIVE CASES IN CLINICALLY DIAGNOSED CASES OF ACUTE APPENDICITIS

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ABSTRACT

BACKGROUND

Acute appendicitis remains the most common cause of acute abdomen requiring surgical intervention. Diagnosis of appendicitis is done on clinical grounds. The aetiology and pathogenesis of appendicitis are poorly understood. The cause of pain in the cases with clinical diagnosis of acute appendicitis but intraoperatively normal looking appendix remains controversial.

The present study aims at determining the cause of pain in these cases by combining routine histopathology, IHC to investigate the status of the enteric nervous system in normal and inflamed appendices.

MATERIALS AND METHODS

In the present study, 694 patients who underwent appendicectomy with diagnosis of acute appendicitis were included. In 89 cases, there were no signs of inflammation intraoperatively. These appendix specimens were followed by histopathological examination by H&E staining. 69 out of 89 specimens did not have signs of inflammation in histological examination by H&E staining.

Cases which are diagnosed as acute appendicitis but histologically negative in H&E staining are taken as study cases.

49 acute appendicitis cases- histologically positive acute appendicitis (HPAA) and 20 appendices from right hemicolectomy specimens were taken as controls.

All these specimens were subjected to;

1) Routine Haematoxylin & Eosin staining.
2) Toluidine blue stain for mast cell evaluation.
3) Immunohistochemistry by S-100 stain for evaluating size and number of the ganglia.

OBSERVATION

The mean mast cell count was more in the histologically negative acute appendicitis group when compared to histologically positive acute appendicitis and control group.

The mean size and number of the ganglia were more in the histologically negative acute appendicitis group when compared to histologically positive acute appendicitis and control groups.

CONCLUSION

Neuronal hypertrophy and mast cells may play a role in the pathogenesis of "appendicitis like pain" in patients with intraoperatively and histologically normal appendices.

KEYWORDS

Acute appendicitis, Histologically positive acute appendicitis (HPAA), Histologically negative acute appendicitis (HNAA), Mast cells, Immunohistochemistry, S-100 stain, Haematoxylin and eosin stain, Toluidine blue stain, Neuronal hypertrophy, Neurogenic appendicitis.

ABBREVIATIONS USED

H & E, IHC, HNAA, HPAA.

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INTRODUCTION: Acute appendicitis remains the most common cause of acute abdomen requiring surgical intervention.[1] Diagnosis of appendicitis is done on clinical grounds. The aetiology and pathogenesis of appendicitis are poorly understood. Although obstruction of the lumen, which results in distension and interference with circulation has been suggested to be a major factor, invasion of the

appendix wall by microorganisms is considered to be the last event in the pathogenesis of acute appendicitis.

Since typical presentations are only encountered in 60% of patients, accurate preoperative diagnosis has been a great challenge even to experienced surgeons. Various imaging modalities, biochemical markers, scoring systems are introduced with a view to lower the negative appendicectomy rate.

Recently, much attention has been paid to the ways in which the immune and enteric nervous systems interact to regulate the physiological functions of the intestine, including the epithelium and the smooth muscle.

The role of inflammatory reactions involving the local endocrine cells and neuroproliferation in causing repeated attacks of pain has been described.[2]

20-25% appendices in patients with suspected appendicitis appear normal intraoperatively.[3] In most of these patients appendicectomy relieves pain. Since appendicectomy relieves pain, an unknown pathology is likely to exist.

AIM: To study the cause of pain and outcome in clinically diagnosed cases of acute appendicitis, but intraoperatively and histologically negative cases.

To study the various pathological changes in surgically removed appendices with the clinical diagnosis of acute appendicitis, but no evidence of acute appendicitis intra operatively.

MATERIALS AND METHODS: The present study was conducted in department of general surgery in a tertiary care hospital. The study was prospective and conducted for a period of one and half year from Jan 2014 to October 2015. All patients who presented to the surgical emergency with clinical diagnosis of acute appendicitis who subsequently underwent appendicectomy were included in the study.

In all these cases, intraoperative features of the appendix were recorded.

Based on the intraoperative findings, these cases are classified into two groups.

1) Those who have normal looking appendix intraoperatively.
2) Those who have inflamed appendix.

All these appendix specimens were followed post operatively by histopathological examination by H&E staining of the removed appendix specimen.

Postoperative pain relief was also recorded in these cases.

In our study, 694 cases underwent appendicectomy with clinical diagnosis of acute appendicitis.

Out of these in 89 patients, the gross findings of appendicitis were lacking intraoperatively. These cases were followed in the postoperative period by histopathology by H & E stain.

In 69 of 89 cases, the histological features of acute appendicitis were lacking.

These 69 cases were taken as study cases. (Histologically negative acute appendicitis).

49 Patients with both clinical and intraoperative diagnosis of acute appendicitis (Histologically positive acute appendicitis-HPAA) and 20 Appendix specimens resected from right hemicolectomy specimens were taken as controls. Total sample size therefore is 138.

All these specimens were subjected to Toluidine blue stain for evaluation of mast cells and S100 staining for evaluation of size and number of the ganglia.

OBSERVATION AND RESULTS: The present study was conducted in a tertiary care hospital during period of one and a half year from January 2014 to October 2015.

A total of 138 cases with clinical diagnosis of acute appendicitis, 20 controls from right hemicolectomy specimen were included in this study.

In our study among 138 patients, 81 were females accounting up to 60% and 57 were males accounting up to 40%, with female to male ratio 1.5:1. Age was ranging from 9-59 yrs., predominantly in the second and third decade of life. The maximum incidence of appendicitis was noted in the age group of 10 to 20 and 20 to 30 years followed by 30 to 40 years of age with preponderance in females than males. The youngest patient was 9 years and eldest patient age was 59 years in this study.

Cases of HNAA were categorised based on absence of inflammation and intact epithelium with or without lymphoid hyperplasia.

HPAA was diagnosed on the basis of signs of inflammation that included neutrophils infiltrating throughout the muscular layer, epithelial erosion, vasodilatation, oedema, abscesses and fibrinous exudates over the serosa. Scattered inflammatory cells within the lumen and/or in the serosa was not considered sufficient for a diagnosis.

In both HNAA and HPAA cases, there were relatively more number of eosinophils observed in this study.

Group A: HNAA- Histologically negative acute appendicitis (69 cases-58.4%).

Group B: HPAA- Histologically positive acute appendicitis (49 cases – 41.5%).

Group C: Controls-from normal appendix specimens.

The mast cells were counted in 10 contiguous fields (All layers) using 40× objective lens and 10× eye piece. The average number of mast cells per hpf was calculated and expressed as:

0-2/hpf (Grade 1+),
2.1 -4/hpf (Grade 2+),
4.1-6/hpf (Grade 3+)
More than 6/hpf (Grade 4+).

Of these 138 appendices, mucosal mast cells <4/hpf was seen in 122 cases and >4/hpf in 16 cases. In submucosa, mast cells <4/hpf were seen in 89 cases and >4/hpf in 49 cases. In muscularis, mast cells <4/hpf were seen in 138 cases and >4 in submucosa and muscularis propria. Mast cell counts in group A and group B were

significantly higher than those in group C. Mast cells were highest in submucosa in all Groups. In submucosa, mast cells were larger, ranging from spindle to polygonal in shape and they contained coarse and numerous granules.

Quantitative Estimation of Neuronal Hypertrophy: Greatest dimensions of ganglia in 10 high power fields were measured by calibrated ocular micrometre for the number of divisions it spans and the value is multiplied by conversion factor (For 10x 1 division = 9.8 µm; 40x 1 division= 2.5 µm). Ganglion cells were counted in 10 high power fields per specimen/hpf in 20 cases. There were no cases with mast cells >4/hpf in the serosa.

Number and size of ganglia in different groups mean size of ganglia (Greatest dimension – muscularis propria) and median ganglion cell count/10 hpf were as follows:

1. A- HNAA-198.82±71µm, 50.
2. B-HPAA-166.44±56µm, 44.5.
3. C-CONTROLS-70.08±28µm, 21.5.

DISCUSSION: Appendicitis still continues to challenge the diagnostic skills of a surgeon. The exact aetiopathogenesis is poorly understood in those appendices resected for suspected appendicitis and subsequently classified as normal by histopathology on conventional staining.[3,4]

In present study, all patients who underwent appendicectomy with clinical diagnosis of acute appendicitis were included. These patients were divided into two groups.

A) Those who were found to have normal appendix intraoperatively.
B) Those with inflamed appendix intraoperatively.

Appendices from right hemicolectomy done for some other reason were taken as controls.

These cases are followed by histopathological examination of the appendix.

Based on the histopathological findings, these patients were divided into histologically negative (HNAA) and histologically positive appendicitis (HPAA).

All cases were subjected to Toluidine blue for evaluating mast cell density.

All specimens were subjected to immunohistochemistry by S100 stain to evaluate to neuronal status.

Mast cells,[4] typically located in close association with tissue that form barriers such as the skin, mucosa, and submucosa of the gastrointestinal and respiratory tract, exert their biological effects by releasing preformed mediators stored in granules such as leukotrienes, prostaglandins, and cytokines. There is considerable evidence that mast cells are micro-anatomically and functionally apposed to the peripheral nerves, resulting in a homoeostatic unit in the regulation of gut physiology and host defence.

The increase in mast cells in acute appendicitis (HNAA, HPAA) together with the broad spectrum of activities of mast cell–derived mediators, suggests that this cell type could play a role in the pathogenesis of acute appendicitis. It has always been of clinical concern that up to one third of appendices removed at surgery for suspected appendicitis are subsequently classified as normal by conventional histological staining. Though neutrophils in the muscularis propria of the appendix are the hallmark of acute appendicitis, the presence of eosinophils and mast cells in HNAA may suggest that these cells represent the beginning of an inflammatory process. As mast cells are widely distributed in the mucosa and submucosa of the digestive tract, these cells are closely apposed to nerves in the human gastrointestinal mucosa and get stimulated by major basic protein derived from eosinophils, substance P, Vasoactive Intestinal Peptide, Neurokinin A (NKA) and Neurokinin B (NKB). On stimulation, these cells release mediators, which cause clinical symptoms of nausea, vomiting, abdominal pain and diarrhoea.

The surprising finding in this study—of significantly increased numbers of S-100 stained nerve fibres in the acutely inflamed appendix specimens—challenges our understanding of the pathophysiological processes that give rise to acute appendicitis.

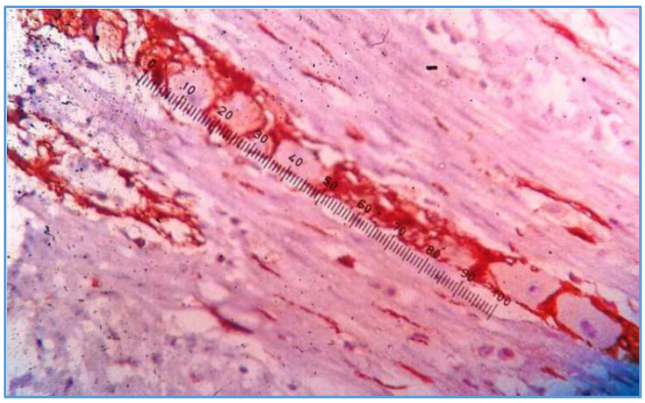
The pathophysiological basis for this neuronal hypertrophy in acute appendicitis is not known. It has previously been suggested that neuronal proliferation in association with appendiceal fibrosis represents a physiological ageing phenomenon.[5] However, it does not explain why there is no appendiceal obliteration in up to 50% of patients older than 70 years.[6]

Since enteric nervous system is in close association with immunocompetent cells like mast cells, Xiong[7] et al studied neuronal hypertrophy[8,4] and mast cells in acute appendicitis and histologically normal appendices which were clinically diagnosed as acute appendicitis. They found an increased number & enlarged ganglia and increased number of mast cells in the submucosa and muscularis externa in all cases of acute appendicitis and in 40% of apparently normal appendices having symptoms of appendicitis, as compared to controls. Our study also showed similar finding. The ganglion cells were significantly higher in muscularis propria in group 'A' when compared to group 'B' and 'C'.

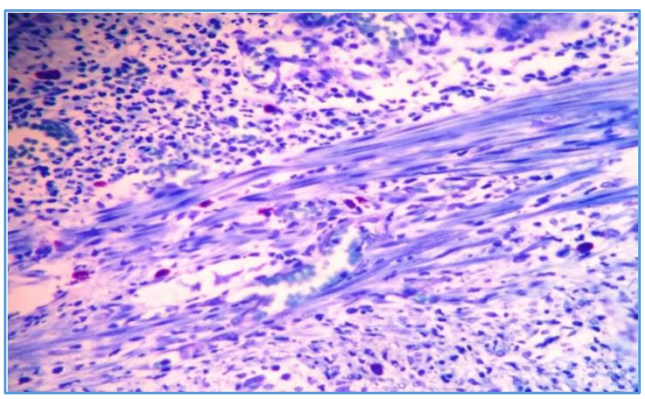
The mast cell density was more in the HNAA group when compared to HPAA and control group.

The mean size and number of the ganglia were more in the HNAA group when compared to HPAA and control groups.

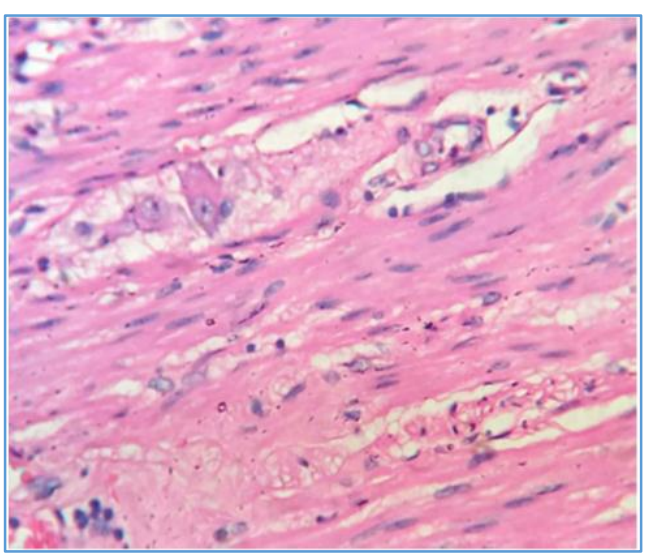
In the present study, S100 positive fine nerve fibres were seen in the muscularis propria whereas, Xiong et al observed fine nerve fibres near the epithelial surface and large nerve fibres near the bottom and in between the crypts in 40% of HNAA cases. Neural components seen in all four layers of appendices in HNAA cases were increased or comparable with cases of HPAA suggesting the possibility of right iliac fossa pain in the absence of inflammation.



IHC Picture showing Increase in Number and size of the Ganglia.



Toluidine Blue Staining showing Grade 4 mast Cells



H & E staining of HNAA showing Absence of Neutrophils.

CONCLUSIONS

1. Quantitative immunohistochemical analysis provides a supplementary technique to the conventional staining method to observe the changes in the pattern of innervation and morphology of nerve fibres in the appendiceal wall.
2. Neurogenic appendicopathy is a distinct histopathological entity clinically presenting in the same way as acute appendicitis and confirmed by S100 immunostaining.
3. Neuronal hypertrophy and mast cells may play a role in the pathogenesis of "appendicitis like pain" in patients with intraoperatively and histologically normal appendices.
4. Neurogenic appendicopathy must be considered in patients who have clinical signs of acute appendicitis even if intraoperative findings show normal looking appendix.
5. These patients of neurogenic appendicopathy are relieved of pain following appendicectomy. Hence appendicectomy is advocated in cases with clinical diagnosis of acute appendicitis and having normal appendix intraoperatively.
6. Further studies are required to have a rationale preoperative identification of neurogenic appendicopathy.

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