Original Article

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Diagnostic Significance of Ganglion Cells and Eosinophils in Appendicitis: An Adjunct to Histopathological Diagnosis

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ABSTRACT

Background: Appendicectomy is most commonly performed intra-abdominal surgery. The exact etiopathogenesis of appendicitis is not exactly understood. Though eosinophils and ganglion cells are been observed routinely in appendix by many pathologists, little is being highlighted in literature in this regard. Present study was done to access the significance of mean cell count and topography of ganglion cells and eosinophils in association with histological type of appendicitis.

Methods: It's a retrospective study which included 93 appendicectomy specimens, out of which 65 were included. Remaining 28 cases with gangrenous change, perforation and neoplastic lesions were excluded. Mean cell count of eosinophils and ganglion cells was calculated. Topographical distribution and mean cell count of eosinophils and ganglion cells were correlated with histopathological type of appendicitis. The significance of association was calculated by chi-square test.

Result: The mean eosinophil count was significantly higher in acute appendicitis compared to recurrent appendicitis with a p-value of <0.001. The ganglion cells were found to be more in acute appendicitis than recurrent appendicitis and was statistically significant (p=0.007). Topographic distribution of ganglion cells was predominantly noted within the circular layer only and between longitudinal and circular muscle layer along with circular layer of the muscularis propria in both acute and recurrent appendicitis.

Conclusion: Mean count of eosinophils and ganglion cells were significantly increased in acute appendicitis. Hence, it may be considered as additional diagnostic criteria for acute appendicitis. Atypical localization of ganglion cells in circular layer was noted in both acute and recurrent appendicitis.

Keywords: *ndicitis, Eosinophils, Ganglion cells, Topography.*

Introduction

Appendicectomy is one of the most commonly performed intra-abdominal surgery.[1] There is a chance of 6-20 % of general population experiencing appendicitis during their lifetime out of which only 1 in every 6 people undergo appendicectomy. [2] The exact function of normal appendix and the etiopathogenesis of appendicitis is not completely understood. However, the chain of events are thought to be triggered by progressive increase in the intraluminal pressure which compromises the venous outflow. Luminal obstruction by fecalith, lymphoid hyperplasia, and less common causes such as gall stone, tumor or a mass of worms are observed in 50 to 80% percent of cases. Ischemic injury accompanied by luminal stasis promote bacterial growth and initiate inflammatory response. [3]

Some theories suggest that acute appendicitis is precipitated by type I hypersensitivity reaction. IgE released is thought to produce abundant swelling which further compromises the blood supply and leads to mucosal damage. Subsequent influx of bacteria from the lumen into the mucosa of appendix leads to development of acute appendicitis. The mast cells play central role in type I hypersensitivity reaction. The eosinophils reach the site as a sequel of mediators released from mast cells such as eosinophilic chemotactic factors. Such response also occur in other segments of intestine, but in contrary to the rest of the bowel, appendix is commonly involved because of its size and poor capacity to harbour edema fluid without much compromise in the blood supply to mucosa. [4]

Eosinophils are normal constituents in mucosa and submucosa, but are not found in muscularis propria. Presence of eosinophils and associated inflammatory edema in the muscularis propria points towards the acute onset of disease. On the other hand, eosinophilic infiltrate admixed with lymphocytes and without evidence of inflammatory edema in muscularis propria indicate a resolving lesion. [4]

The neural crest cells originating from neuroepithelium differentiate into neurons and glia, which coalesce to form ganglion plexuses. These ganglion plexuses form myenteric and meissener's plexus in muscle coat and submucosa respectively. The ganglion cells of the muscle coat is situated in between inner circular and outer longitudinal layer. [5,6] These cells under normal

physiological conditions show considerable stability with respect to their regeneration and proliferation. [2]

It requires high motor activity in the gut to produce increased and sustained peristaltic contractions to expel the contents distally. This is brought out by hypertrophy of myenteric plexus. Hence it has been postulated that neuronal hypertrophy may represents a part of reparative phase in an inflammatory process, antecedent to a chronic or acute injury. [1,5,7]

Even though the histological diagnosis of acute appendicitis is made based on the evidence of neutrophilic infiltrate, considerable infiltration of eosinophils and mast cells are seen in all cases of it. This is even correlated with the severity of inflammation. [1] In spite of the fact that eosinophils and ganglion cells are been observed routinely in appendix by many pathologists, little is being highlighted in literature in this regard. Hence the present study was undertaken to access the significance of mean cell count and topography of ganglion cells and eosinophils in association with the histological type of appendicitis.

Materials and Methods

It is a retrospective study. All the appendicectomy specimens received in histopathological laboratory which were reviewed for a period of one year from January 2018 to December 2018. There were a total of 93 appendicectomy specimens out of which 65 were included in the present study. The remaining 28 specimens were excluded. The specimens with evidence of gangrenous change (8 cases) and perforation (13 cases) on gross examination were excluded. Large areas of mucosal ulceration (7 cases) and appendix having neoplastic lesions (0 cases) on microscopic examination were excluded. This was done because of major architectural damage to mucosa and submucosal layers. The hematoxylin and eosin stained slides having minimum three sections of appendix (including base, middle and tip) were only included.

The demographic details of the patients and clinical diagnosis were noted. The hematoxylin and eosin stained slides were retrieved and re-examined by single pathologist to make the histopathological diagnosis. Presence of neutrophilic infiltrate in muscularis propria was considered for diagnosing acute appendicitis. [3] Whereas, evidence of fibrosis and infiltration of chronic inflammatory cells were considered for diagnosis of recurrent appendicitis. The microscopic examination was also carried out to evaluate the population and topographic distribution of ganglion cells and eosinophils in the wall of appendix.

Eosinophils were counted in ten non overlapping high power fields (400x) of mucosa, submucosa and muscularis propria. Mean eosinophil count was calculated for each layer in both acute and recurrent appendicitis.

The ganglion cells were counted in the entire cross section of muscularis propria and submucosa preferably in the longitudinal section of appendix. Mean ganglion cell count was calculated. The ganglion cells of muscularis propria were also graded as mild when the ganglion cells are 1-5 in number, moderate when 6-10 in number and severe when more than 10 in the entire cross section. Further on the basis of topographic distribution of ganglion cells in myenteric plexus, they were divided into seven patterns and was correlated with the histopathological type of appendicitis.

Statistical analysis: Data was analysed using SPSS software v.23.0. and Microsoft office 2007. Mean± standard deviation (SD) was calculated and Chi-square (χ^2) test was used to check the significance of association between two groups of appendicitis with respect to mean cell count, topography of ganglion cells and eosinophils. If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. For other data, numbers and percentage was calculated.

Result

Of the 93 cases of appendicectomies received, 65 were included in the study remaining 28 cases were excluded. Out of 65 cases, 40 were histologically diagnosed cases of acute appendicitis (group A) and 25 were histologically diagnosed cases of recurrent appendicitis (group B). The mean age of presentation among patients of group A and group B was 25.1±7.9 and 24.6±10.1 years respectively. Among the cases of group A, males constituted 80% and females were 20%. Whereas slight female predominance (56%) was noted in group B cases.

Eosinophils were counted in 10 non-overlapping fields of mucosa, submucosa and muscularis propria separately and mean eosinophil count was calculated (Table 1). The mean eosinophil count in the mucosa, submucosa and muscularis propria of the group A is significantly higher compared to that of group B respectively with a p-value of <0.001. Further among the cases of group A, it is also observed that the mean eosinophil count is more in the muscularis propria than in the mucosa and submucosa (Figure 1). Whereas in group B, the eosinophils were more in the mucosa compared to muscularis propria and submucosa.

The ganglion cells were counted in the submucosa and muscularis propria of the entire cross section of appendix (Table 2). The mean ganglion cell count in muscularis propria of group A was 8.6±3.3 and of group B was 6.2±3.2 which was found to be statistically significant (p value 0.007). The mean ganglion cell count of

submucosal layer of group A is 2.0±1.1 and of group B is 1.0±0.6 which was also found to be statistically significant (p value of < 0.001).

All the cases of both the groups were also graded into mild, moderate and severe categories based on the number of ganglion cells in the muscularis propria (Table 3). Majority (50%) of the cases belonging to group A were included in moderate category whereas 56% of the cases belonging to group B were included in mild category which was found to be statistically significant (p-value of 0.01).

The topographic distribution of ganglion cells present in the myenteric plexus was also analysed and were divided into seven patterns among cases of both acute and recurrent appendicitis (Table 4) such as,

Pattern I (Normal): Ganglion cells present at the junction between the circular and longitudinal muscle layer.

Pattern II: Ganglion cells present within the circular muscle layer only.

Pattern III: Ganglion cells present within the longitudinal muscle layer only.

Pattern IV: Ganglion cells present at the junction between the longitudinal and circular muscle layer and also within the circular muscle layer.

Pattern V: Ganglion cells present at the junction between the longitudinal and circular muscle layer and also within the longitudinal muscle layer.

Pattern VI: Ganglion cells seen in both longitudinal and circular muscle layer.

Pattern VII: Ganglion cells present in all three planes.

The commonest topographic distribution of ganglion cells observed was pattern II in both group A (42.5%) and group B (40%) wherein the ganglion cells were found in the circular layer of the muscularis propria only. This was followed by pattern IV, constituting 37.5% cases of group A and 32% of group B cases.

Table 1: Mean eosinophil counts observed in various layers of wall among acute and recurrent appendicitis.

PARAMETER	GROUP A (ACUTE APPENDICITIS)		GROUP B (RECURRENT APPENDICITIS)		p Value
	MEAN	SD	MEAN	SD	
Eosinophils located in Mucosa	36.3	16.9	10.9	7.7	<0.001*
Eosinophils located in Submucosa	16.5	13.5	3.4	3.0	<0.001*
Eosinophils located in Muscularis propria	49.3	21.6	6.0	6.0	<0.001*

*Note: * significant at 5% level of significance (p<0.05)*

Table 2: Mean ganglion cell count in submucosa and muscularis propria of acute and recurrent appendicitis.

PARAMETERS	GROUP A (ACUTE APPENDICITIS)		GROUP B (RECURRENT APPENDICITIS)		p value
	Mean	SD	Mean	SD	
Ganglion cells located in Submucosa	2.0	1.1	1.0	0.6	<0.001*
Ganglion cells located in muscularis propria	8.6	3.3	6.2	3.2	0.007*

*Note: * significant at 5% level of significance (p<0.05)*

Table 3: Grading the ganglion cells belonging to myenteric plexus in acute and recurrent appendicitis.

GRADING OF GANGLION CELLS	ACUTE APPENDICITIS		RECURRENT APPENDICITIS		p value
	N	%	N	%	
MILD	8	20.0%	14	56.0%	0.01*
MODERATE	20	50.0%	8	32.0%	
SEVERE	12	30.0%	3	12.0%	
TOTAL	**40**	**100.0%**	**25**	**100.0%**	

*Note: * significant at 5% level of significance (p<0.05)*

Table 4: Ganglion cells of Myenteric plexus categorised into seven pattern based on the topographical distribution in acute and recurrent appendicitis.

PATTERN OF ARRANGEMENT	GROUP A (ACUTE APPENDICITIS)		GROUP B (RECURRENT APPENDICITIS)		p value
	N	%	N	%	
I	0	0.0%	1	4.0%	0.596
II	17	42.5%	10	40.0%	
III	0	0.0%	0	0.0%	
IV	15	37.5%	8	32.0%	
V	1	2.5%	1	4.0%	
VI	5	12.5%	5	20.0%	
VII	2	5.0%	0	0.0%	
TOTAL	**40**	**100.0%**	**25**	**100.0%**	

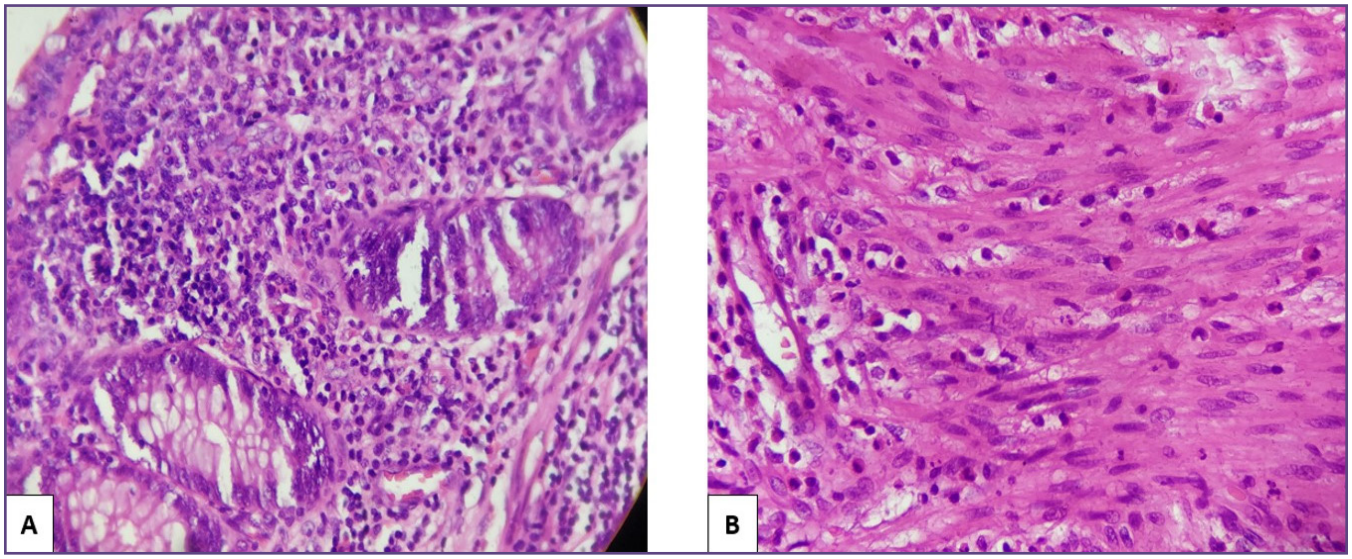

Fig. 1: A) Eosinophilic infiltrate noted in the mucosa of recurrent appendicitis (400x, H&E), B) Eosinophilic infiltrate noted in the muscularis propria of acute appendicitis (400x, H&E).

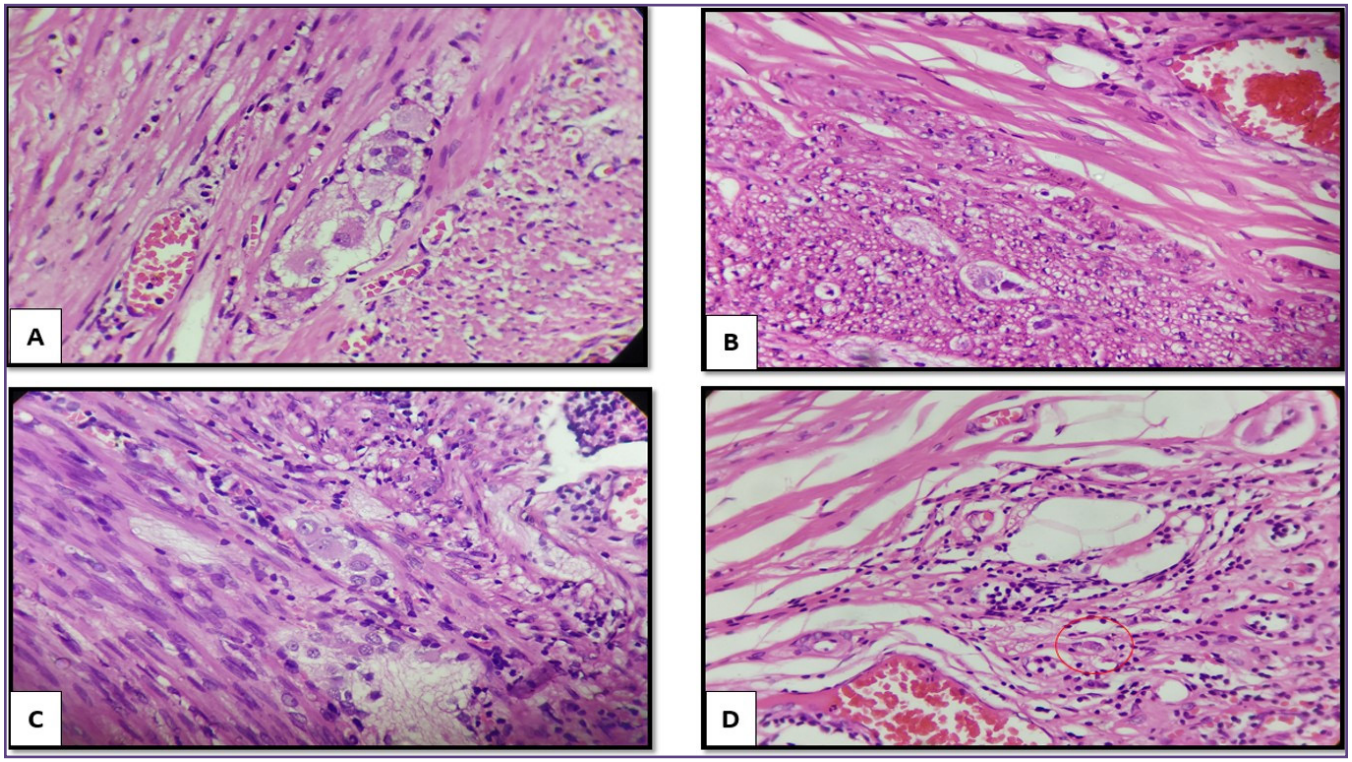

Fig. 2: A) Ganglion cells noted in between circular and longitudinal muscle layer of recurrent appendicitis (100x, H&E), B) Ganglion cells noted longitudinal muscle layer of recurrent appendicitis (100x, H&E), C) Ganglion cells noted in circular muscle layer of recurrent appendicitis (100x, H&E), D) Ganglion cell noted in submucosa of recurrent appendicitis, insight showing ganglion cell (100x, H&E).

Discussion

Gastrointestinal tract is formed from close interaction of the endoderm and splanchnic mesoderm. The latter gives rise to muscularis propria, submucosa and muscularis mucosa. This differentiation occurs from the serosa towards the luminal side of the wall. Enteric nervous system is derived from neural crest cells (NCC). They initially differentiate into neurons and glia which later coalesce to form ganglion plexus. These NCC migrate in rostro-caudal manner to colonise the entire gut by 7 weeks of gestation. This coincides with development of circular and longitudinal muscle layer between 7 to 14 weeks of gestation. The submucosal plexus develops subsequent to this and before differentiation of muscularis mucosa. [5]

The vermiform appendix is the most common intra-abdominal organ which is frequently subjected to surgical intervention followed by resection. [8] The narrow lumen of appendix in humans and apes shows increased susceptibility of obstruction. This luminal obstruction plays a crucial role and triggers the vicious cycle of appendicitis. Whereas, primates and mammals with large and open appendix are less vulnerable to obstruction and appendicitis. [9,10]

Recently many meta-analysis and randomised control trials are done to compare the efficacy of conservative management (CM) of acute appendicitis followed by interval appendicectomy (IA) versus immediate surgery at the time of presentation. CM comprised of two days of intravenous antibiotics and intravenous fluids followed by eight days of oral antibiotics. It has decreased morbidity with reduced overall wound infection rate and post-operative complications. Following CM, the recurrence risk of appendicitis is reduced to 8-14%. It is also hypothesised that the recurrence rate of appendicitis or perforation is more in patients with appendicolith formation after CM. [11,12]

Currently many authors have put insights regarding CM in case of acute appendicitis followed by IA, usually 2-3 months after the disappearance of symptoms. This is done because, adhesions are reduced between the appendix and surrounding tissue, the histological findings does not vary from 1 to 6 months after a CM, further longer waiting period could increase the risk of recurrence. [11]

Singh *et al* [1] in their analysis of 329 cases of appendicectomies found that there was slight male

predominance with many of the cases observed to be within thirty years of age. The mean age of presentation in their study was found to be 25.44±12.14 years which was similar to our study.

Aravindan [4] in his study of 120 appendicectomies has found mural eosinophilic infiltrate as a consistent histological finding in all the cases of acute appendicitis. Eosinophilic infiltrate in the muscularis propria along with concomitant edema of the wall had led them to propose the theory of eosinophil-edema lesion which is seen in patients presenting with acute symptoms and inflamed appendix on gross examination. In present study, we found that the mean eosinophil count was significantly high in group A compared to group B in all the three layers of appendix which was similar to studies done by Singh *et al* [1] and Aravindan. [4]

Since mast cells are usually detected in close proximity to tissues forming barrier such as skin, gastrointestinal and respiratory tract, they are found to maintain homeostasis and host defence. There exists a close relationship between nerves and immunocompetent cells like mast cells and lymphocytes. [2,7]

Xiong *et al* [2] studied neuronal hypertrophy in acute appendicitis and had proposed new insights regarding close association between enteric nervous system and mast cells towards the pathogenesis of acute appendicitis. They found an increase number of nerve fibres, schwann cells and enlarged ganglia in the Meissener's and Myenteric plexus in all the cases of acute appendicitis and also in 40% of cases histologically normal appendices with symptoms of appendicitis. Authors suggested that the neuronal hypertrophy represent a reparative process of inflammatory lesion to previous chronic or repeated acute injury. [1,2]

They also found that the size and the number of ganglion cells were considerably increased in the muscularis propria and submucosa of acute appendicitis than the other groups. This was in concordance with our study results wherein there was a significant increase of mean ganglion cell count in muscularis propria and submucosa among the cases in group A as compared to group B cases (Figure 2). The number of ganglion cells remain constant during the course of life. The significant increase in the ganglion cell count observed in acute appendicitis cases is because of the inflammatory edema of the wall. This inflammatory exudate separates the smooth muscle bundles thus making the ganglion cells to appear more prominent in acute appendicitis. [2]

Singh *et al* [1] had also graded ganglion cells into mild, moderate and severe, wherein they found that majority of cases diagnosed as acute appendicitis were belonged to moderate category followed by severe and mild which was similar to cases of group A in our study. Also, among the other groups, many of the cases were classified as mild followed by moderate and few severe grade, which correlated with our findings.

Rao *et al* [6] in their evaluation about the topographical arrangement of ganglion cells in inflammatory lesions of appendix described seven patterns based on various location of ganglion cells in the muscularis propria. The authors had found that many of the cases were included in pattern II and IV which was similar to our study. Other authors like Hanani *et al* [13] has described arrangement of myenteric plexus in three concentric networks placed both between circular and longitudinal muscle bundles and also within them. Thus it appears to be unique when compared with small and large intestine. Whereas Kubikova *et al* [9] has observed three different patterns under atypical localisation ganglion cells of myenteric plexus. Such as, ganglion cells situated within circular layer, within the circular layer and rarely in subserosa, and within both muscle layers. Most commonly they were situated in the circular layer.

Conclusion

Appendicectomies are the most frequently encountered specimens by pathologists. Mean counts of eosinophils and ganglion cells were significantly increased in acute appendicitis when compared to recurrent appendicitis. And many of the acute appendicitis cases showed a moderate increase in ganglion cell count. Hence, can be considered as an adjunct in histological diagnosis of acute appendicitis. Atypical localisation of ganglion cells in the circular layer, in between two muscle bundles along with circular layer were predominantly noted in both types of appendicitis.

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