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A Study of Intra-Operative and Clinicopathological Correlation in Neurogenic Appendicopathy

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Background : This prospective study is to know the clinical presentation histological features and management of neurogenic appendicopathy.

Methods: It is a prospective clinical study on patients with acute appendicitis presenting to department of general surgery, Bhaskar general hospital, yenkapally.

Results: A total of 60 cases with clinical diagnosis of acute appendicitis, were included in the study , 23 were females and 37 are males , Maximum incidence of appendicitis was noted in the age group of 20-30yrs , pain in the right lower abdomen is the most common complaint .

Conclusion: Neurogenic appendicopathy is one of the cause for pain in the right iliac fossa which mimics as acute appendicitis.

I. INTRODUCTION

Acute Appendicitis is the most common intra-abdominal disorder causing pain that requires emergency surgery, with a 6% lifetime risk.[1] Neurogenic appendicopathy represents an almost unknown pathology which clinically cannot be differentiated from acute appendicitis. Neurogenic appendicopathy or neurogenous hyperplasia of appendix was first discovered by Masson in 1928.[2]

It involves hyperplasia of enterochromaffin-like endocrine cells and non myelinated nerve fibres.[3] It has been reffered to as neuroma, neurogenous hyperplasia, neurogenic appendicitis, neuromatosis, traumatic neuroma, fibrous obliteration, neuroimmune appendicitis, appendiceal fibrosis[4] .

The spectrum of disease for this lesion ranges from intramucosal hyperplasia with intact appendiceal lumen that frequently coexists with nerve growth in submucosal and muscularis area to transverse obliteration of appendix formed by variable proportion of fibrous tissue are considered as a final stage of this disease. It is thought that repeated subclinical episodes of minimal inflammation could cause this lesion.[5]

The emphasis is primarily directed at the neurogenous component, visualized by immunohistochemistry for s-100 protein which play a key role in pathogenesis of appendiceal obliteration. These neurofibers can be decorated with antibodies against S-100, a calcium-binding protein commonly expressed by cells of neural origin[6].

Neurogenic appendicopathy is characterised by the presence of pale spindle cells and proliferation of schwann cells in the mucosal lamina [3] propria or in distal segments of appendix. The role of inflammatory reactions involving the local endocrine cells and neuroproliferation in causing repeated attacks of pain has been described in the literature.[7] 20-25% appendices patients with suspected appendicitis appear normal intraoperatively[8].

In most of these patients appendicectomy relieves pain. Since appendicectomy relives pain, an unkown pathology is likely to exist. The findings are compared with those found in normal appendix. The objective of this study is to assess its clinical presentation, its histological features including immunohistochemistry the treatment carried out and its clinical involution and compare the study with other existing studies.

II. AIMS AND OBJECTIVES

To study the clinical presentation histological features and management of neurogenic appendicopathy.

III. MATERIALS AND METHODS:

It is a prospective clinical study on patients with acute appendicitis presenting to department of general surgery, Bhaskar general hospital, yenkapally.

All patients who presented to the surgical emergency with clinical diagnosis of acute appendicitis who subsequently underwent appendicectomy were included in the study.

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In all these cases intraoperative features of the appendix were recorded
Based on the intraoperative findings these cases are classified into two groups.

- Those who have normal looking appendix intraoperatively.
- Those who have inflamed appendix

All these appendix specimens are followed postoperatively by histopathological examination by H&E staining and IHC by S100 staining.

INCLUSION CRITERIA:

All patients presenting with RIF pain (s/o acute appendicitis clinically) to Bhaskar General Hospital. Those who give consent to participate in this study.

EXCLUSION CRITERIA:

Patients with appendicular lump.

IV. OBSERVATION AND RESULTS

The present study was conducted in Bhaskar medical College & Hospital, moinabad, yenkapally during period of one and a half year from January 2019 to June 2020.

A total of 60 cases with clinical diagnosis of acute appendicitis, were included in the study. The study has been analyzed using ANOVA statistical analysis SPSS software 2010.

In our study among 60 patients, 23 were females accounting to 38.3% and 37 were males accounting to 61.6% , with male to female ratio 1.78:1.

Table.1 : Distribution of cases according to sex.

Sex	Male	Female	Total
No. Of cases	37	23	60

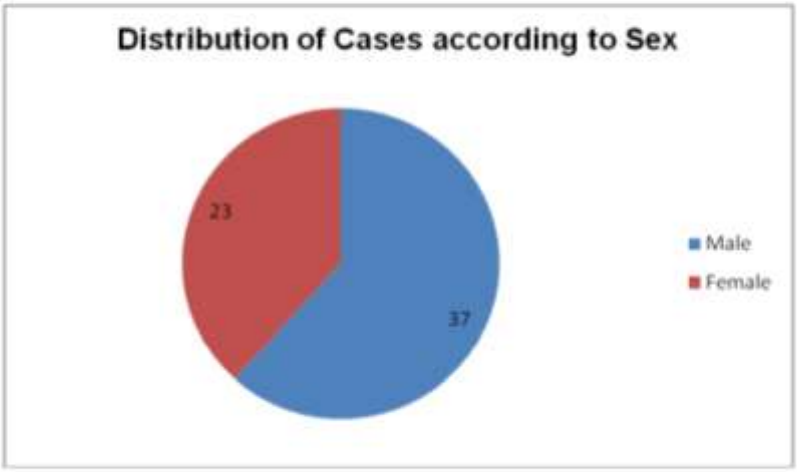

Chart. 1: PIE chart - Distribution of cases according to sex

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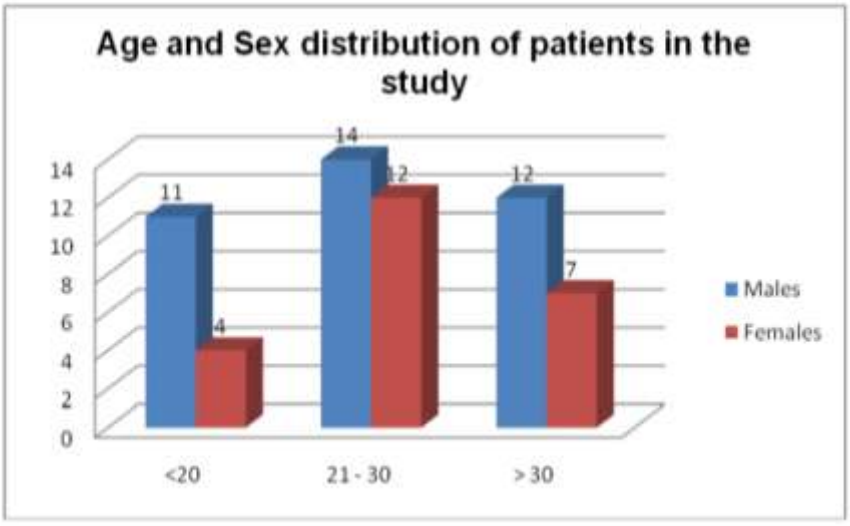
Table.2 : Age and Sex distribution of patients in the study.

AGE	MALES	FEMALES	TOTAL
<20	11	04	15
21-30	14	12	26
>30	12	07	19



Cases were predominantly in the second and third decade of life.

The Maximum incidence of appendicitis was noted in the age group of 20-30yrs followed by 31- 39 years of age with preponderance in males than females.

The youngest patient was 10 years and eldest patient age was 55 years in this study. Clinical presentation:

Most of the patients in study group were presented with pain in the right lower abdomen followed by vomitings and fever .

Table. 3 : Clinical symptomatology

SYMPTOMS	% OF PATIENTS WITH SYMPTOMS
Typical pain	90%
Anorexia	85%
Nausea/vomiting	75%
Fever	70%
Other(burning or frequency of micturation, tenesmus, diarrhoea, constipation)	8%
Atypical	10%

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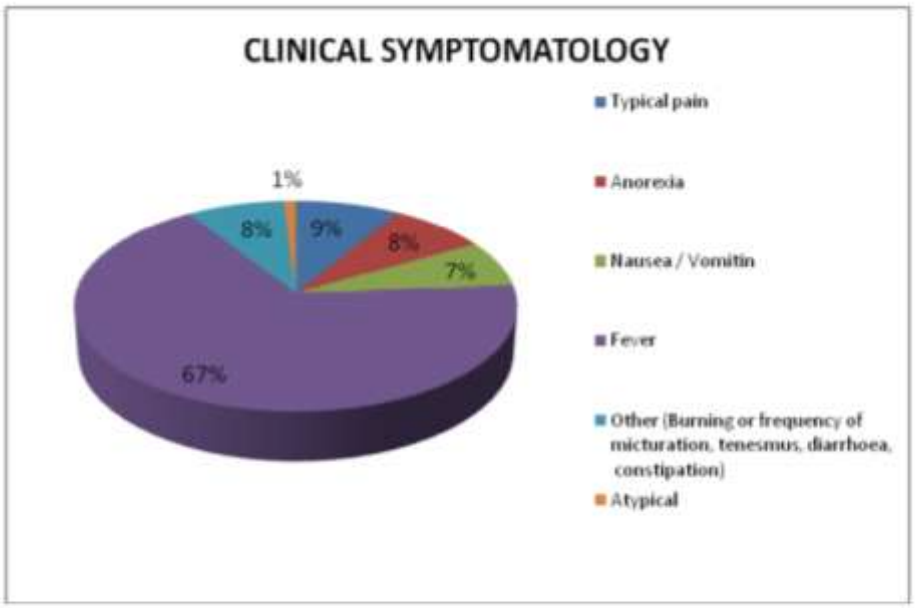

Chart . 3 : Pie chart- Clinical symptomatology.

Figure. 6 : Gross specimen of appendix showing congested serosa.

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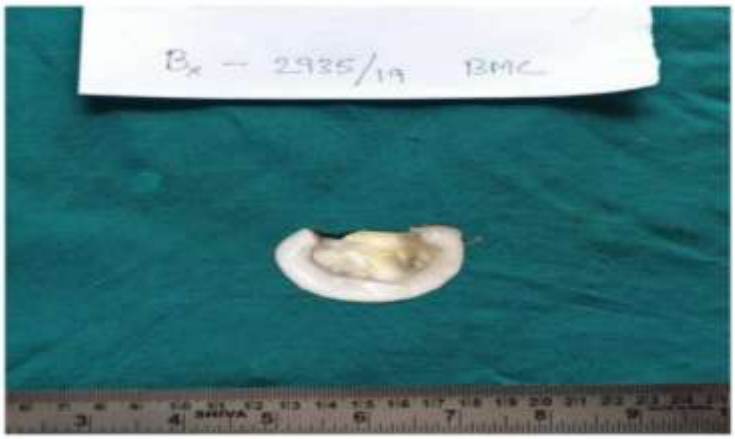

Figure. 7 : Gross specimen of appendix without inflammation and congested serosa.

Cases of HNAA were categorized 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, edema and fibrinous exudates over the serosa.

Scattered inflammatory cells within the lumen and/or in the serosa was not considered sufficient for a diagnosis.

Among HNAA and HPAA cases, relatively more number of neutrophils were found in HPAA.

Group A- HNAA- Histologically negative acute appendicitis (03 cases-05%).

Group B- HPAA- Histologically positive acute appendicitis (57 cases – 95%).

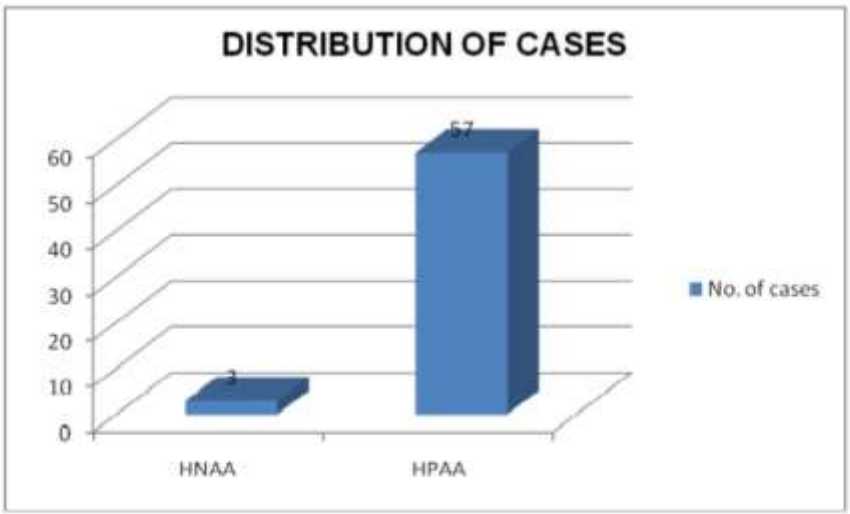

Chart. 4 : Distribution of HNAA & HPAA cases.

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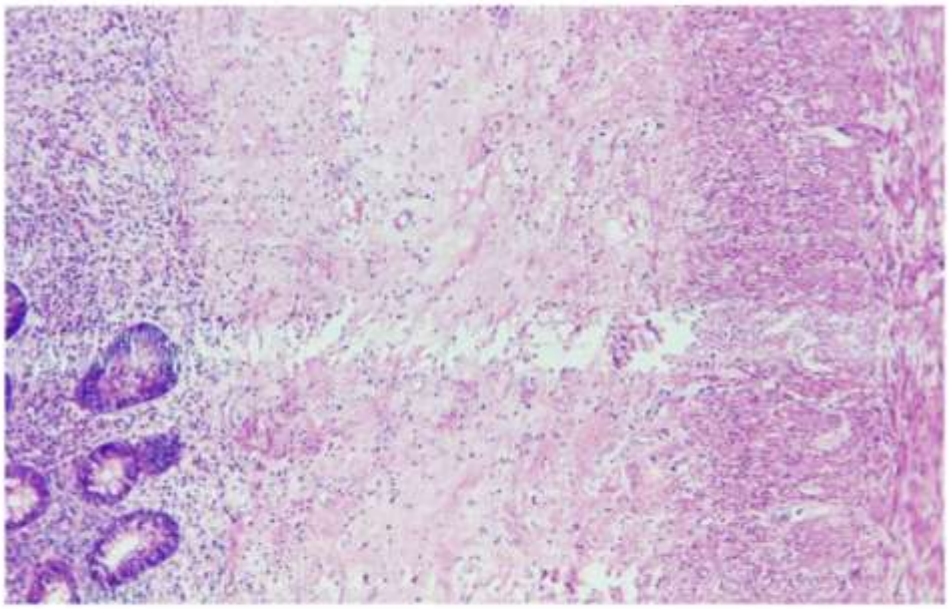

Figure. 8 : H&E , 10X HNAA Absence of neutrophils in muscularis propria.

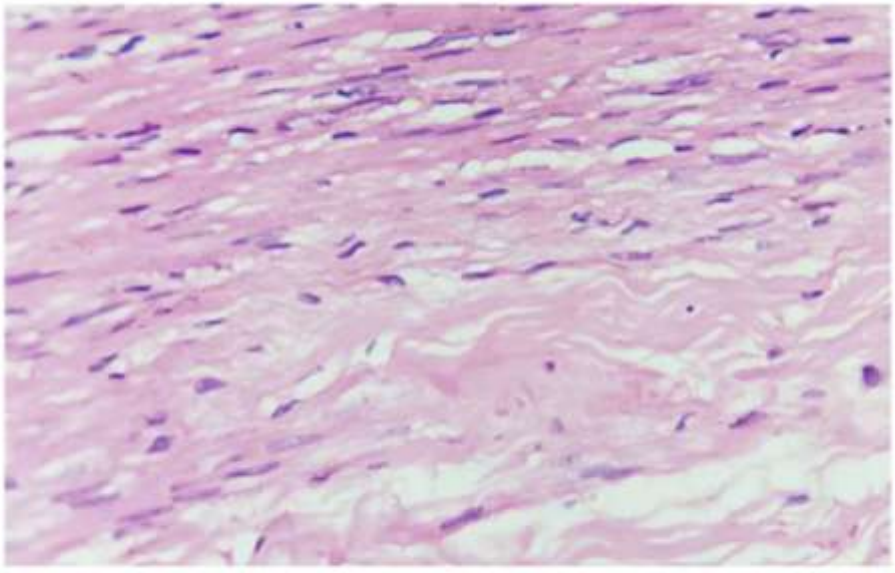

Figure. 9 : H&E , 40X HNAA Absence of neutrophils in muscularis propria.

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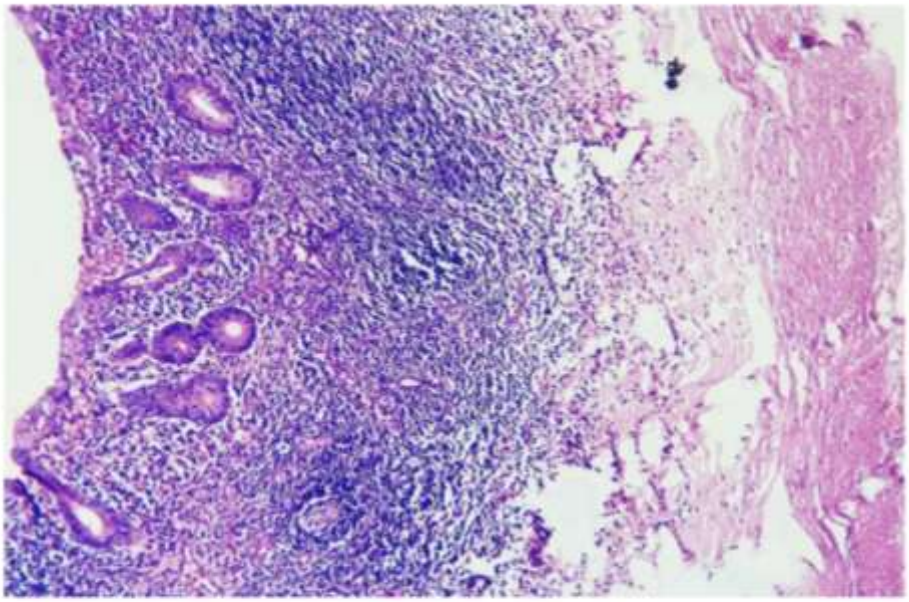

Figure. 10 : H&E, 10X Presence of eosinophils & neutrophils in muscularis propria.

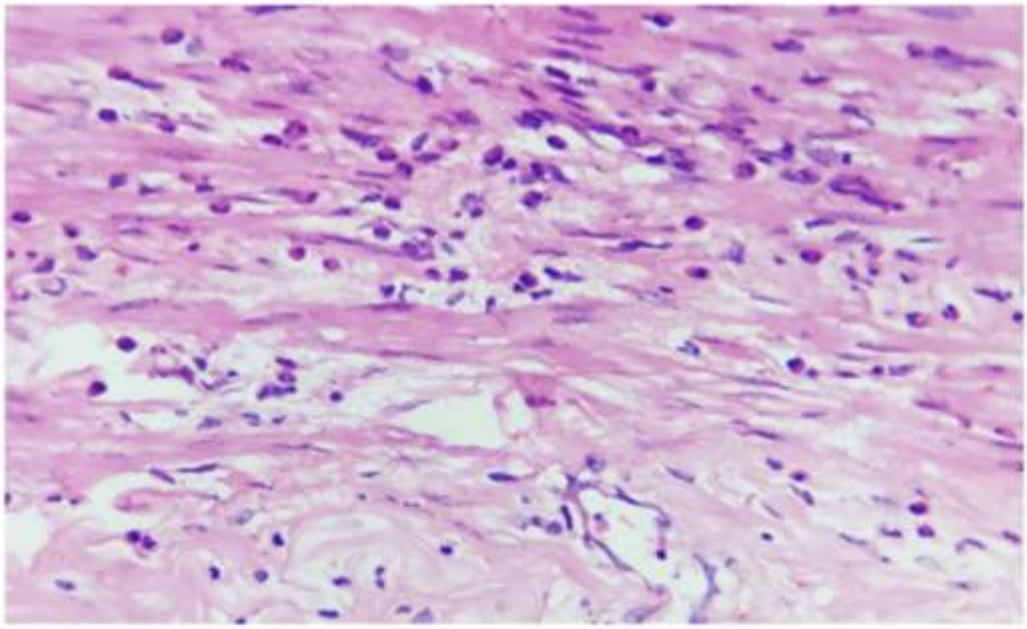

Figure. 11 : H&E, 40X Presence of eosinophils & neutrophils in muscularis propria.

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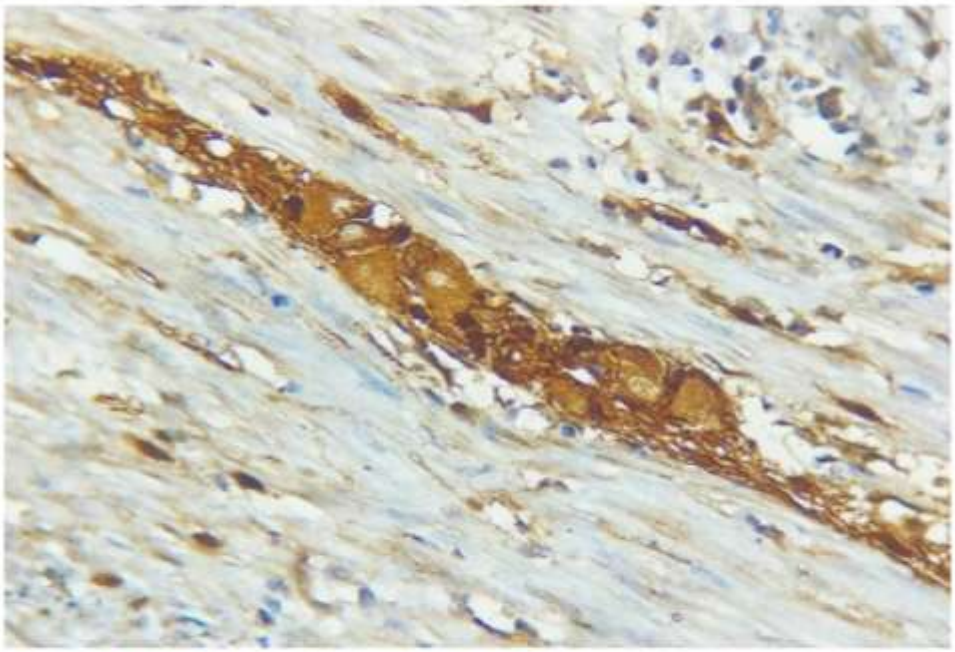

Figure. 12: IHC with S-100 Increased number and size of ganglia in HNAA.

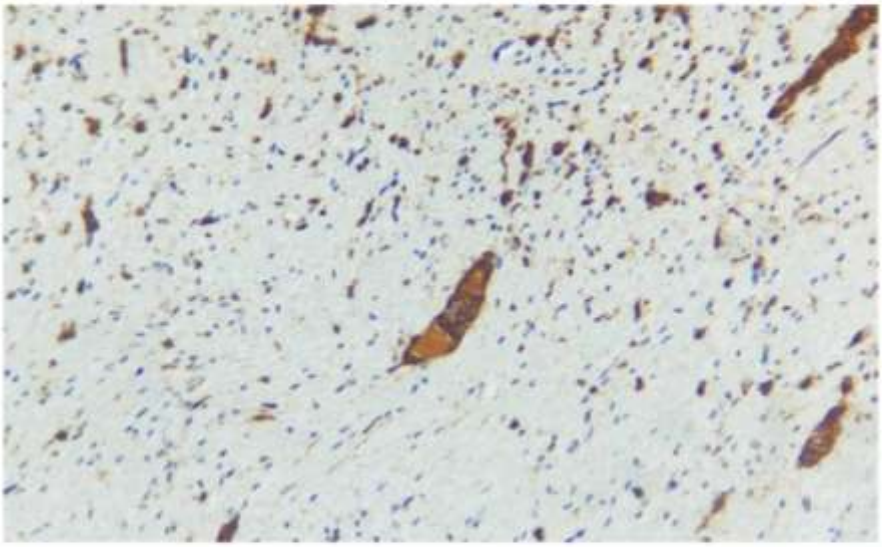

Figure. 13 : IHC with S-100, 40X: inflamed appendix with mild increase in Number and size of ganglia in HPAA

V. DISCUSSION

The present study has emphasized the uncommon occurence of neuromatous hyperplasia in acute appendicitis. Primary neuroma of the appendix is a rare tumour that was first described by the Work et al[35-37]. It was described as

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proliferation of neural tissue that obstructs the appendiceal lumen causing fibrosis and obliteration of its lumen. The diagnosis of AA, the most common condition requiring emergent abdominal surgery in childhood, still represents a challenge[43,44,45]. Delayed diagnosis of AA, especially in young children, may result in perforation of the appendix, peritonitis and a longer hospital stay[46]. NA may cause AA-like symptoms in children.

Neurogenous hyperplasia of appendix is described as a proliferative lesion and not be considered as tumour is proposed by Sunil V. Jagtap. It is not familial, and should not be confused with other intestinal nerve lesions such as the mucosal neuromas of MEN 2B syndrome, ganglioneuromatosis and neurofibromas of type I neurofibromatosis (Von Recklinghausen's disease), and schwannomas, perineuriomas and ganglioneuromatosis of Cowden syndrome [38,39]. Therefore, this is not a tumor lesion but rather a hyperplasia.

The continuing pursuit of surgeons to improve the accurate pre-surgical diagnosis of acute appendicitis [40-41] has not diminished the intraoperative dilemma of deciding whether or not to remove a macroscopically unaffected appendix [42]. Despite special investigations (ultrasound, laboratory, scores), the diagnosis of AA in patients with equivocal signs of an acute inflammation is still a challenge[47,48,49].

It was further demonstrated that nerve elements were a consistent component of obliterated appendices. 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 aging phenomenon according to B.S OSLEN et. al[50]. This theory, however, does not explain the observed neuronal hypertrophy in their study, because most of their patients were young with no evidence of appendiceal fibrosis. Furthermore, it does not explain why there is no appendiceal obliteration in up to 50% of patients older than 60 years[51]).Several studies have shown that inflammation can effect or affect nerve remodeling. Extensive sprouting of neurofilament-immunoreactive nerve fibers has been observed 1 to 2 weeks after a superficial skin wound in patients[53]. Dental periapical inflammation, pulpectomy, and pulpal neurosis may result in the formation of a disorganized sprouting and branching of axons[52]

There are 3 microscopic histological patterns of neurogenous hyperplasia of appendix

1. lumen obliteration.
2. mucosal hyperplasia.
3. submucosal hyperplasia

The aim of present study is to analyse the expression of S-100 in acute appendicitis cases and corelate it with clinicopathological variables. This is prospective study for a period of 18 months. [60] cases of appendectomy specimens which were clinically diagnosed as acute appendicitis were included in the study. All the parameters and variables are compared to the recently published articles available on different national and international journals .

Table. 4 : Comparision of literature with present study. Sample size and duration.

	Sample size	Study duration
Present study	60	18 months
Jose RUIZ	4,969	3yrs
Disebastiano	96	1yr
franke	282	1yr
Serigo sesia	385	2yrs
partike	121	1yr
B.S.Olsen&S.Holck	237	1yr

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Table. 5 : Comparision of mean age and M:F ratio with other studies

	Mean age	M:F sex ratio
Present study	25.63	1.6:1
Jose Ruiz	27.8	1:1
partike	28.5	2:1
Serigo sesia	26.2	1:1.4
Franke	27	2:1
DiSebastiano	24	1:2

Table . 6 : Description of the clinical symptomatology in neurogenic appendicopathy in the present study.

Case	Age	Sex	Pain	Temp	Leuco-cytosis	Abdominal ultrasound	Intra-operative findings	H&D, immune-histochemistry (S100)
1	25 yrs	male	6hrs	36.8	10,439	Not carried out	Normal appendix	Submucosal hyperplasia
2	30 yrs	female	24hrs	37.1	8,300	Mesentric lymphadeno-pathy	Normal appendix	Submucosal hyperplasia
3	24 yrs	male	8hrs	38.5	9,140	Not carried out	Normal appendix	Submucosal hyperplasia

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Table. 7 : Comparision of present study of S100 marker positive with literature.

	STUDY OF YEAR	POSITIVE PERCENTAGE
Present study	2020	5%
YILMAZ	2013	3.8%
Sesia SB	2013	7.5%
Franke C et al.	2002	18.4%

In the study of Di Sebastiano et al the finding of nerve fibres in contact with the marginal layer of lymphatic follicles that were commonly detected in non-acute appendicitis suggests an important role for neuroimmune interaction in the pathogenesis of pain in non-acute appendicitis.

Recently, much attention has been paid to the ways in which the immune and enteric nervous systems can interact to regulate the physiological functions of the intestine, including the epithelium and smooth muscle. Similarly, there has been interest in the effect that substances released from enteric nerves have on immune cells, and vice versa. The close spatial relation between nerve fibres and lymphoid cells detected in the outer zone of lymph follicles suggests that upregulated neuroimmune interaction is a sustaining basic mechanism in non-acute appendicitis.

In study of shigang xiang et al, signifificantly increased numbers of synaptophysin stained nerve fibers and S100- Schwann cells in the acutely inflamed appendix specimens—challenges our understanding of the pathophysiological processes that give rise to acute appendicitis.

In study of B.S.OLSEN & S.HOLCK the S-100 protein-positive material could be demonstrated in all cases, sometimes confined to a narrow sleeve encasing small aggregates of lymphocytes or as a centrally placed, barely discernible slit-like spaces. More often one or more sizeable aggregates of nerve tissue occupy these regions.

In study of Lars Ivo Partecke the localization of S-100-positive neurofibers was described as (a) mucosa type (m), (b) submucosa type (sm), (c) mixed mucosa–submucosa type (m/sm), and (d) the presence of neuromas . Normal unaffected appendices also contained neurofibers. Therefore, immunostained sections were scanned at low magnification (×10) and dense proliferations of S100–Schwann cells were identified.This includes changes for which terms like chronic appendicitis, neurogenic appendicitis, or more recently neurogenic appendicopathy have been suggested. The latter two were diagnosed if histopathologically S-100-positive neurofibers were found and recurrent episodes of right lower abdominal pain had been present. This combination has justified the removal of a macroscopically unaffected appendix. Originally, Maresch and Masson introduced the term neurogenic appendicitis for macroscopically unaffected appendices if the absence of histopathological signs of acute inflammation was accompanied by an increase of neurofibers.[54]

5 % positively stained for S-100, but also “normal appendices” from patients displaying no symptoms were significantly positive for S-100 in almost 50 % of cases. Hence, S-100 itself is not a useful marker for the identification of an appendicopathy of neurogenic origin.

In the study of Oneil Machado incidence of S-100 positive myelinated fibers has been reported to be found in a significantly higher proportion of patients with NA compared to patients with acute appendicitis The pathogenesis of the process remain unknown. Various study stated it is secondary to inflammation giving rise to hyperplasia of neuroendocrine cells. Many times neuroma shows endocrine cells within hypertrophied nerve bundles. On clinical presentation patient present with pain abdomen, vomiting or repeated attacks of acute appendicitis. It is reported more in male than female, with in adolescents and adults. On gross, lesions are mostly of obliterative type with fibrosis of appendix. Others are intramucosal lesions with patent appendiceal lumen. Repeated subclinical attacks of inflammation are thought to trigger this lesion. On microscopic examination, submucosal hyperplasia consists of proliferation of spindle cells, elongated cells arranged in fusiform or nodular pattern. The background may contain myxoid material, adipose tissue, fibrosis, mononuclear cell infiltrate or predominant eosinophilic infiltrate. The fibrous obliteration is predominant in advance stage of disease.

Aravindan et al suggested that infiltration of eosinophils was an early consequence of mediators released by mast cells in acute appendicitis. Eosinophils reach the site due to eosinophilic chemotactic factors present in the mast cells granules or due to histamine released by them. 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. Although the physiopathology is not yet fully understood, it is believed that a persistent state of inflammation or repeated minimal subclinical attacks of inflammation within the appendix promotes the growth of neuroendocrine cells, non-myelinated nerves and Schwann cells that occlude the appendiceal lumen. Appendiceal neuromas show positivity for S-100 protein.

Ganglia were found between the circular and longitudinal layers and also deep within the muscle layers. Only mild neural hyperplasia was seen in all cases of AA on H&E staining and may be explained by the mucosal destruction and dense inflammatory exudates that obscure the morphology of the tiny nerve twigs. Immunostaining by S 100 is mandatory in such cases to make an accurate estimation like in the present study and was in agreement with that of Xiong et al.

In this study, we also noticed mild to moderate infiltration by esinophils and neutrophils in the muscularis propria in almost all cases of acute appendicitis. In appendices with acute clinical presentation but not diagnostic of acute appendicitis(HNAA), transmural eosinophil and neutrophil counts were not in the same range as seen in acute appendicitis(HPAA)

Peripheral nerves may physiologically be in a constant state of modelling under different situations and that a variety of stimuli, such as inflammation or injury, can effect or affect nerve remodelling. Neural proliferation may therefore represent a form of inflammatory response. Well-developed neuronal changes of the extent seen in this study are unlikely to develop during a single episode of acute inflammation (frequently bouts of only hours or days in duration) and suggest a pre-existing stimulus such as repeated episodes of subclinical inflammation or a response to an obstruction. In the present study S100 positive fine nerve fibres were seen in the muscularis propria where as, 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.

Franke et al observed ‘neurogenic appendectomy’ (NA) in 3.8% of patients with HPAA and in 47% of those which are HNAA and found neither history nor clinical examination will enable preoperatively to differentiate HPAA and HNAA.

On comparing H&E staining with S100 staining, 100% accuracy for H & E staining was found for the presence of ganglion plexus as compared to 93% accuracy observed by Franke et al in the diagnosis of ‘neurogenic appendicopathy’.

VI. CONCLUSION

1. Neurogenic appendicopathy is one of the cause for pain in the right iliac fossa which mimics as acute appendicitis.
2. In the present study 5% patients specimens were intraoperatively normal, but they presented with symptoms of acute appendicitis i.e; recurrent right iliac fossa pain.
3. They were diagnosed as HNAA and confirmed as neuronal hyperplasia on IHC.
4. 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.

5. As S100 is non specific marker for neuronal hyperplasia .Further, studies are required to have a rational preoperative identification of neurogenic appendicopathy.

Scope of further study: In these cases of neurogenic appendicopathy further studies are required to identify the neuronal hyperplasia in other parts intestine and symptoms of pain abdomen caused by such neuronal hyperplasia. Such studies will help in specific diagnosis and management of pain abdomen cases.

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