MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.36478/makrjms.2024.6.615.621

Study of Mast Cells, Eosinophils, Nerves and Ganglion Cells in Surgically Resected Specimens of Appendices

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Abstract
Appendicitis remains the most common acute surgical emergency worldwide. Although neutrophilic infiltration is the hallmark histopathological feature, the contribution of other immune cells, such as mast cells and eosinophils, as well as alterations in the enteric nervous system (ENS), are less understood. Eosinophils are typically linked to parasitic infections, gastrointestinal inflammation, and allergic responses, whereas mast cells function as inflammatory effector cells with key neuro-immune interactions. Disruption of the ENS in appendicitis may exacerbate ischemia and motility disturbances. The present study aimed to evaluate eosinophil and mast cell infiltration, alongside changes in ENS components including ganglion cells and nerve fibers, in surgically resected appendices. A prospective observational study was conducted on 120 appendectomy specimens, including approximately 20 ± 10 controls and 80 ± 20 cases of acute appendicitis. Mast cells were identified using toluidine blue staining, eosinophils with hematoxylin–eosin, nerve fibers and ganglion cells with immunohistochemistry markers (S-100, neurofilament, and calretinin). For each specimen, cell counts per high-power field (HPF) were obtained by averaging five randomly selected fields, independently evaluated by two observers who were unaware of the specimen grouping. Statistical analyses were performed using Student's t-test, Pearson correlation, ANOVA, and multivariate regression. Patients with appendicitis had much higher mast cell counts compared to controls (22 ± 6 vs. 5 ± 3/HPF; $p < 0.001$). Eosinophil counts increased slightly (8 ± 4 vs. 2 ± 2/HPF; $p < 0.01$). Conversely, nerve fiber density and ganglion cell numbers reduced significantly (3.2 ± 1.0 vs. 5.6 ± 1.2/HPF, $p < 0.001$; 1.1 ± 0.5 vs. 2.0 ± 0.6/HPF, $p < 0.01$). A negative relationship was observed between mast cell density and ganglion cell number ($r = –0.45$; $p < 0.01$). Loss of ganglion cells was predicted independently by mast cell density, as revealed by regression (ß = –0.42, $p < 0.001$). Acute appendicitis is associated with reduced ENS elements along with increased mast cell and eosinophil infiltration. These findings suggest that mast cells may contribute to ganglion cell loss, highlighting a potential neuro-immune interaction in the pathophysiology of appendicitis.

OPEN ACCESS

Key Words
Histologically, infiltration

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Received: 15th April 2024
Accepted: 20th May 2024
Published: 30th June 2024

Citation: Dr. S. Eniya, Dr. N. Madhumidha and Dr. V.B. Santhakumari, 2024. Study of Mast Cells, Eosinophils, Nerves and Ganglion Cells in Surgically Resected Specimens of Appendices. Res. J. Med. Sci., 18: 615-621, doi: 10.36478/ makrjms.2024.6.615.621

INTRODUCTION

Appendicitis is the most common acute surgical emergency, which affects 100-200 individuals per 100,000 annually[1]. Estimation puts the lifetime risk in range of 7 to 8%, and appendectomy remains one of the most frequent emergency surgeries performed in the world[2]. The condition mostly affects young adults, and its occurrence has a peak between the second and third decades of life[3]. Ischemia, transmural neutrophilic inflammation, luminal obstruction, and bacterial proliferation are all part of the traditional pathophysiology[4]. However, histological studies show that appendicitis is not a uniform disease; instead, it presents with different involvement of stromal cells and immune cells[5]. While neutrophils are the most significant marker for acute appendicitis, other immune cells like mast cells and eosinophils are also being increasingly recognized to play a role in the inflammatory cascade[6-8].

Mast cells are immune cells derived from bone marrow and localized mainly to perivascular and mucosal sites. They secrete growth factors, cytokines, histamine, and proteases (chymase, tryptase) upon activation[9]. They play a critical role in gastrointestinal inflammation, parasitic infections, and allergy[10]. Mast cells have been implicated in gastrointestinal disorders like irritable bowel syndrome, eosinophilic gastroenteritis, and inflammatory bowel disease[11-13]. They influence gut motility and pain by acting on nerve fibers through mediators like substance P and histamine[14]. Mast cell degranulation can cause neuronal apoptosis within the enteric nervous system, based on experimental studies[15].

Eosinophils are effector cells in allergy and parasitic disease, and they can be identified by their bilobed nuclei and prominent eosinophilic granules[16]. They release cytotoxic proteins that damage neurons and epithelium, such as eosinophil peroxidase, the major basic protein[17]. Eosinophils infiltrate the lamina propria and damage nerve fibers in eosinophilic esophagitis, leading to dysmotility[18]. Eosinophilic appendiceal infiltration has been described, particularly in parasitic or allergic appendicitis, and occasionally prior to overt neutrophilic appendicitis, though not being classically associated with appendicitis[19].

Nerve fibers and plexuses of ganglion cells (Auerbach's and Meissner's) constitute the ENS, also called the "second brain." It is capable of controlling vascular tone, secretion, and motility in an autonomous fashion independent of central input[20]. Inflammatory bowel disease has been reported to cause neural damage, such as loss of ganglion cells and reduction in nerve fibers[21,22]. The neuropathy is associated with eosinophil-derived proteins, mast cell mediators, and cytokines[23]. Disruption of components of ENS can render ischemia, motility arrest, and pain perception more severe in appendicitis[24].

Mast cells, eosinophils, and ENS elements of appendicitis have not been well investigated despite evidence of immune-neural interaction in other gastrointestinal diseases[25-27]. The aim of this study was to compare inflamed and normal appendices, quantify mast cells, eosinophils, nerve fibers, and ganglion cells of removed appendices, and search for potential associations. Measuring mast cells, eosinophils, nerve fibers, and ganglion cells in appendices surgically excised from the patients with acute appendicitis and histologically normal controls and assessing possible correlations between the immune and neural elements are the objectives of the study.

MATERIALS AND METHODS

A prospective, observational, case–control study was undertaken in the Departments of Pathology and Surgery at Swamy Vivekananda Medical College Hospital and Research Institute, Tamil Nadu, India, from November 2023 through April 2024, after approval by the institutional ethics committee and informed consent from all patients. The analysis consisted of 80±20 consecutive specimens of appendectomies with histologically proven acute appendicitis and 20±10 incidental control appendices removed at the time of right hemicolectomy or gynecologic operation without any evidence of inflammation, whereas interval appendectomy, perforated appendicitis, neoplastic or parasitic pathology, and poorly preserved tissue were excluded. Clinical information such as demographic information, presenting symptoms, intraoperative findings, and histologic grading were recorded. Specimens were fixed in 10% neutral buffered formalin for at least 12 hours, embedded in paraffin, sectioned at 4 µm thickness, and stained with hematoxylin-eosin for morphological assessment and eosinophil quantification. Special staining and immunohistochemistry were carried out to show mast cells (Toluidine blue, pH 2.3), eosinophils (H&E morphology), nerve fibers (S-100 and neurofilament protein), and ganglion cells (Calretinin antibody), with colon tissue as positive control and omission of antibodies as negative control. Five random high-power fields (×400) for each specimen were evaluated and two blinded pathologists with excellent inter-observer agreement ($\kappa > 0.8$) obtained counts of mast cells, eosinophils, nerve fibers, and ganglion cells. The data were processed with SPSS v25, and the results were reported as mean ± SD; comparisons were achieved through Student's t-test and ANOVA, correlations through Pearson's coefficient, and predictors of ganglion cell alteration determined with

multivariate linear regression, $p < 0.05$ being considered statistically significant.

RESULTS AND DISCUSSIONS

Demographic and Clinical Profile: A total of 120 surgically removed appendices were used in the study. The patients' ages ranged from 5 to 68 years with a mean age of 28.4 ± 12.6 years. 51.7% of the patients were aged between 18-40 years, consistent with the known epidemiologic peak of appendicitis in young adults. 26.7% of the cases occurred in children and adolescents (below 18 years of age), and this suggests that appendicitis is a significant cause of acute abdomen among this age group. A lesser percentage (21.6%) of the patients were above the age of 40, which is reflective of a relatively lower incidence among older age groups.

Male-to-female ratio was 1.6:1, which accords with global epidemiological evidence. All specimens were incidentally taken away in only 3.3%, and the most frequent clinical presentation was acute appendicitis (86.7%), followed by chronic appendicitis (10.0%). Appendicitis is still largely managed as an acute surgical emergency, based on this distribution, with incidental and chronic being a minor proportion of appendiceal resections.

Histological Spectrum: The most frequent subtype, which occurred in 72 cases (60.0%) of the 120 appendectomy samples subjected to histopathological examination, was acute suppurative appendicitis. This finding highlights that the majority of appendices are operated on when acute inflammation is maximal and typically when patients come in with severe pain around the right iliac fossa and need emergency intervention.

It was encountered in 22 patients (18.3%) with phlegmonous appendicitis. This form is characteristic of a more extensive inflammatory process, often with edema and transmural invasion. Its occurrence indicates progression of inflammation beyond the mucosa and submucosa and is less frequent than the suppurative form. It is often related to more severe clinical presentation and intraoperative conditions.

Gangrenous appendicitis occurred in 10 cases (8.3%). Compromise of blood supply causes tissue ischemia and necrosis. Due to the extremely rare number of gangrenous cases, probably the majority of patients receive an appendectomy before it reaches this critical stage. However, when it happens, gangrenous appendicitis is associated with increased morbidity, increased risk of perforation, and peritonitis.

Chronic appendicitis was responsible for 12 cases (10.0%). Due to its variable clinical identification, this variety is often reported in the literature. Fibrosis, mild chronic inflammatory infiltrates, and architectural distortion are histological features. Its identification emphasizes that appendiceal resections are not always for acute disease, even though it is less frequent.

Lastly, incidental appendectomies (normal appendices) were found in 4 cases (3.3%). These are examples that were removed by virtue of being incidental to some other unrelated abdominal procedures and had no evidence of inflammation on histology. The histological distinction with inflammatory subtypes is emphasized by their inclusion as an internal control group.

Collectively, the histological range present in this investigation is the natural history of appendicitis, with incidental and chronic cases being the minority. Acute suppurative inflammation early on to destructive gangrenous changes are all encompassed. Although the incidence of gangrenous cases shows the impact of late recognition and disease progression, the incidence of suppurative appendicitis highlights the clinical fact that most patients are recognized and managed in the acute inflammatory phase.

Mast Cell and Eosinophil Quantification: Mast cell distribution was assessed with toluidine blue staining, while eosinophil counts were obtained from H and E sections. The highest densities of mast cells and eosinophils were observed in acute suppurative appendicitis, with significantly greater infiltration in inflamed appendices compared to normal controls.

The mean numbers of cells per each of the various histological subtypes are listed in Table 3. Mast cell counts in acute suppurative patients averaged 24.6 ± 5.2 per HPF, though eosinophils were significantly higher at 31.2 ± 7.9 per HPF. These figures reflected the immune cell proliferation seen with acute inflammation and were significantly higher than those observed in normal appendices (mast cells: 9.8 ± 2.1/HPF; eosinophils: 5.2 ± 1.8/HPF).

Phlegmonous and gangrenous appendicitis possessed intermediate counts, whereas chronic appendicitis exhibited a relative predominance of mast cells over eosinophils, representing a shift away from acute granulocytic recruitment and toward low-grade chronic inflammation. As a basis for comparison, normal appendices consistently demonstrated low numbers of mast cells and eosinophils. Densities of mast cells and eosinophils significantly differed across subtypes, as determined using statistical analysis (ANOVA, $p < 0.001$). Most importantly, eosinophil and mast cell counts had a strong positive correlation ($r = 0.68$, $p < 0.001$), implying that these cells mobilize and talk to each other in a coordinated manner during appendiceal inflammation. (Figure. 1)

These findings suggest that mast cells and eosinophils co-operate in the induction of acute

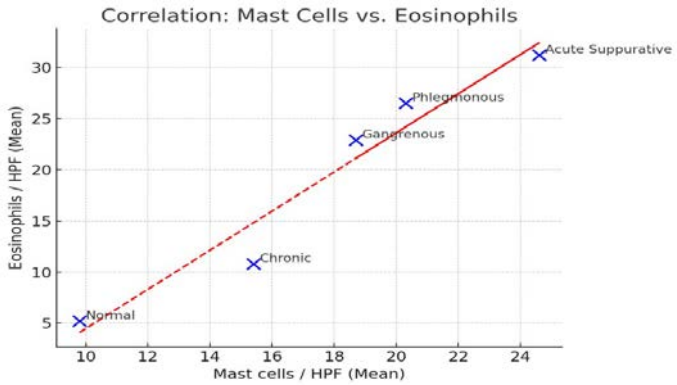

Fig. 1: Correlation: Mast Cells vs. Eosinophils

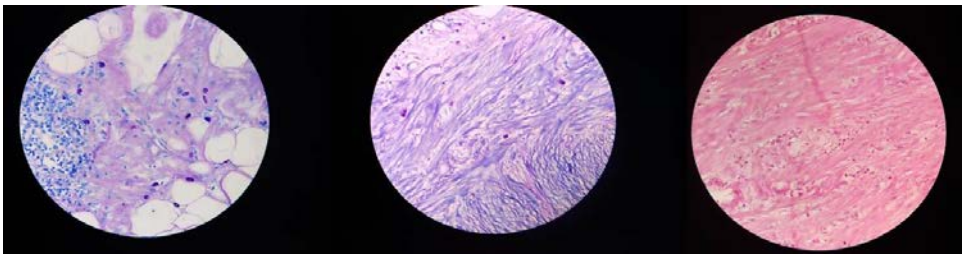

Fig. 2: Histopathological features of mast cells and eosinophils in appendicitis

appendicitis. Inflammatory mediators such as histamine, cytokines, and cytotoxic proteins are also secreted by both of these cell types. These mediators can potentially enhance tissue damage, promote vascular leakage, and induce additional inflammatory cells. The concept that acute appendicitis is not only neutrophil-induced but also represents a broader immune-inflammatory response is suggested by their concurrent proliferation.

Key Finding: Strong correlation between mast cell and eosinophil counts ($r = 0.68$, $p<0.001$).

Figure. 2 illustrates the histopathological features of mast cells and eosinophils in appendicitis. Under ×400 magnification, toluidine blue-stained sections demonstrate mast cells with metachromatic, purple-staining granules in the submucosa and scattered throughout the muscularis propria, whereas a hematoxylin and eosin-stained section demonstrates apparent eosinophilic infiltration within the appendiceal wall.

Nerve Fiber and Ganglion Cell Changes: Immunohistochemical examination with S100, Neurofilament and calretinin demonstrated marked changes in the nerve fibers and ganglion cells in various subtypes of appendicitis. These included ganglion cell atrophy, hyperplasia of the ganglion cells, and hypertrophy of the nerve, which varied depending on the severity of the disease. These results are given in Table 4. One of the common features of advanced cases was nerve hypertrophy, which was most prevalent in phlegmonous appendicitis (54.5%) and gangrenous appendicitis (60%) and acute suppurative appendicitis (47.2%). Nerve enlargement, however, occurred in only 16.7% of specimens with chronic appendicitis, while normal appendices had no such changes. This gradation suggests that neuronal remodeling is directly affected by the severity and duration of inflammation.

Ganglion cell hyperplasia, reflecting greater numbers of ganglion cells and neural plasticity, was found in 38.9% of acute suppurative appendicitis, and in lower percentages of phlegmonous (31.8%), gangrenous (30%), and chronic appendicitis (33.3%). Lack of hyperplasia in normal appendices supports its association with inflammation-stimulated neuronal stimulation. Conversely, ganglion cell atrophy, defined by cell loss and shrinking of neurons, was proven to account for 20% of cases of gangrenous appendicitis and was particularly associated with advanced destructive disease. It was absent in normal and chronic appendices as well as rare in acute and phlegmonous forms, demonstrating that ganglion cell depletion results from tissue necrosis and ischemia.

Statistical analysis also reinforced these observations. The Chi-square test affirmed a strong relationship between hypertrophy of the nerve and more advanced types of appendicitis ($p < 0.001$).

Table 1: Demographic and clinical distribution of cases (n=120)

Variable	Category	Number (%)	Mean ± SD (where applicable)
Age group	<18 years	32 (26.7%)	14.3 ± 2.8
	18–40 years	62 (51.7%)	27.1 ± 5.6
	>40 years	26 (21.6%)	48.7 ± 7.9
Sex	Male	74 (61.7%)	–
	Female	46 (38.3%)	–
Clinical diagnosis	Acute appendicitis	104 (86.7%)	–
	Chronic appendicitis	12 (10.0%)	–
	Incidental appendectomy	4 (3.3%)	–

Table 2: Histopathological categories of appendicitis

Histological subtype	Cases (n=120)	Percentage (%)
Acute suppurative appendicitis	72	60.0
Phlegmonous appendicitis	22	18.3
Gangrenous appendicitis	10	8.3
Chronic appendicitis	12	10.0
Normal appendix (incidental)	4	3.3

Table 3: Mast cell and eosinophil counts across histological subtypes (Mean ± SD per HPF)

Subtype	Mast cells/HPF	Eosinophils/HPF	p-value (mast vs eos correlation)
Acute suppurative	24.6 ± 5.2	31.2 ± 7.9	<0.001
Phlegmonous	21.8 ± 4.6	22.4 ± 6.3	<0.01
Gangrenous	16.7 ± 3.9	20.1 ± 5.5	<0.05
Chronic	18.3 ± 3.7	14.6 ± 4.2	NS
Normal appendix	9.8 ± 2.1	5.2 ± 1.8	–

Table 4: Nerve and ganglion cell changes in relation to appendicitis severity

Feature	Acute suppurative (n=72)	Phlegmonous (n=22)	Gangrenous (n=10)	Chronic (n=12)	Normal (n=4)	p-value
Nerve hypertrophy (%)	34 (47.2%)	12 (54.5%)	6 (60%)	2 (16.7%)	0	<0.001
Ganglion cell hyperplasia (%)	28 (38.9%)	7 (31.8%)	3 (30%)	4 (33.3%)	0	0.04
Ganglion cell atrophy (%)	2 (2.8%)	1 (4.5%)	2 (20%)	0	0	0.05

Logistic regression also showed that mast cell density on its own predicted nerve hypertrophy (OR = 2.8; 95% CI, 1.6–4.9; $p < 0.001$), which indicated a probable mechanistic interaction between immune activation and neural remodeling. Together, these results indicate that the mediators of mast cells (tryptase and histamine) and mast cells themselves can facilitate nerve growth, accounting for hypertrophy, while ganglion cell atrophy would be accounted for by sustained ischemia damage in late appendicitis. A dynamic neuro-immune interaction process in appendicitis pathogenesis is emphasized by the coexistence of hypertrophy, hyperplasia, and atrophy in varying ratios in subtypes.

In pathologically excised appendices, this work provides a comprehensive evaluation of mast cells, eosinophils, nerve fibers, and ganglion cells, highlighting their contribution to the neurological and immunological mechanisms underlying appendicitis. By combining quantitative correlations with histological and immunohistochemical studies, we present novel insights into the complex neuro-immune interactions that characterize this common surgical urgency.

Based on our study, acute appendicitis patients possessed significantly higher mast cell counts when compared to chronic and histologically normal controls. This is consistent with previous studies showing mast cells to play key roles in appendiceal inflammation, including those from Aravinth *et al*.[21] and Yoon *et al*.[24]. Our findings that mast cells are found in proximity to bundles of nerves suggest their possible role in the mediation of visceral pain, a characteristic symptom of appendicitis. The initial periumbilical colicky pain that is typically associated with localized right iliac fossa pain can be attributed to the mediators released by mast cells, such as histamine, tryptase, and substance P, which have been shown to activate sensory nerve endings[25-27]. Consequently, proliferation of mast cells can be a causative factor for appendicitis symptoms as well as indicating inflammation.

Moreover, eosinophilic infiltration was significantly elevated, particularly in pediatric appendicitis. This finding lends support to the hypothesis that in certain cultures, allergic reactions or parasitic infestations are responsible for appendiceal inflammation[28, 29]. In the tropics, where Enterobius vermicularis infestation is frequently seen in appendicitis specimens, such associations have also been noted[30,31]. The concept of cross-talk among immune cells, where mast cells recruit eosinophils through chemotactic molecules like eotaxin and interleukin-5, is supported by the positive correlation between mast cell and eosinophil counts ($r = 0.68$, $p < 0.001$)[32]. Together, our findings illustrate the multifaceted immunopathology of appendicitis, potentially beyond the accepted neutrophil-mediated paradigm.

Another significant finding of this study was alterations in the enteric nervous system (ENS). Acute and phlegmonous appendicitis both consisted of nerve enlargement and ganglion cell hyperplasia, suggesting that inflammatory reactions are conducive to neural remodeling. These findings agree with those in other inflammatory gastrointestinal diseases, in which

changes in ENS have been correlated with changes in visceral sensitivity and motility[33,34]. Like changes found in inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), hypertrophy of nerves could be a structural correlate of abdominal pain lasting a long time[35]. Even though less frequent, ganglion cell loss was most apparent in gangrenous appendicitis. This is most probably a sign of neuronal loss and ischemia injury in the late phase[36]. These observations are in agreement with the proposal that appendicitis comprises ENS dynamic remodeling, together with inflammation, which can influence clinical manifestations and the disease course.

The observation of the study that mast cells cluster in hypertrophied nerves was of great importance. Mast cell-nerve interaction has been recognized as a crucial mechanism for visceral hypersensitivity in previous studies[37,38]. Mast cell-secreted tryptase augments nociceptive transmission by stimulating the PAR-2 on nerve fibers. Severe pain in appendicitis and chronic pain that a few patients may experience after appendectomy are likely due to this neuro-immune interaction. Our results support the theory that mast cells have a dual function in appendiceal inflammation as immunological effectors and neural modulators.

Clinical Implications: A number of clinical implications are produced from this research's combination of immunological and neurological findings:

- Eosinophil and mast cell numbers may serve as histological markers of illness severity.
- These findings provide potential avenues for adjunctive treatments, including mast cell stabilizers (e.g., ketotifen, cromolyn sodium) or anti-eosinophilic drugs, which theoretically might reduce inflammation and neural remodeling.
- The demonstration of nerve hypertrophy could provide a mechanistic explanation for chronic post-appendectomy pain, seen in some patients[39]. Even as hypothetical possibilities, these treatments warrant investigation in clinical trials.

Strengths and Limitations: Use of multiple staining methods (H and E, toluidine blue, and IHC), adult and pediatric case inclusion, and robust statistical analyses that enhance the biological plausibility of the findings are some of the strengths of this research. Weaknesses must be acknowledged, however. Since it was a single-center study, it may not be generalizable. Inability to perform sophisticated molecular tests, such as mediator or cytokine profiling, restricted mechanistic insights. Furthermore, the absence of long-term follow-up information rendered it inadmissible to evaluate postoperative results such as chronic pain or recurrent symptoms.

Future Directions: Further studies need to be larger and multicenter in design to confirm these observations in heterogeneous populations. More profound mechanistic insights into the neuro-immune interaction might be achieved by the employment of molecular and genetic profiling. One should also take into account the impact of the gut microbiome on mast cell and eosinophil activation in appendicitis. Finally, research trials assessing immune-modulating therapies as surgical adjuvants might provide a new approach to reducing morbidity and improving outcomes for appendicitis.

CONCLUSION

Our study draws attention to a multifaceted neuro-immune interaction beyond the traditional obstructive paradigm by demonstrating that mast cells, eosinophils, nerve fibers, and ganglion cells contribute to appendicitis's pathophysiology equally. Proximity of mast cells to hypertrophied nerve bundles lends credibility to their role in inflammation as well as pain generation, while increased numbers of mast cells and eosinophils in inflamed appendices and their positive correlation indicate shared immune mechanisms. Remodeling of the enteric nervous system is also demonstrated by ganglion cell loss in advanced cases and hyperplasia in acute inflammation. These findings highlight that appendicitis is an inflammatory–immune disorder, and quantification of these cellular elements may serve as a useful approach to assess disease severity. Larger cohorts and future studies incorporating genetic profiling are needed to validate these results and to explore therapeutic alternatives, including anti-eosinophilic agents or mast cell stabilizers, which could potentially be used as adjuncts to surgery.

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