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Mast cell density, neuronal hypertrophy and nerve growth factor expression in patients with acute appendicitis

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In acute appendicitis, although the relationship between the enteric nervous system (ENS) and mast cells (MCs) has been described in a few studies, neither the expression of nerve growth factor (NGF) nor its relation to mast cell density (MCD) and ENS has been delineated yet in this disease.

The aim of this study was to immunohistochemically investigate the relationship between MCD, nervous system and NGF expression in the appendices of cases with clinically and histopathologically diagnosed acute appendicitis and of normal controls.

Twenty-five patients with acute appendicitis and twelve normal controls were included in our study. Mast cell tryptase, PGP 9.5 and anti-NGF immunostained tissue sections were subjected to quantitative image analysis.

Our results showed that MCD, the number of Schwann cells, the number and size of ganglia and NGF staining were significantly greater in acute appendicitis than in the control group ($p < 0.01$). A strong correlation between MCD and NGF staining was detected ($r = 0.92$) only in cases with acute appendicitis. Similarly MCD was also related to neuronal proliferation and hypertrophy in this group. We failed to detect any relationship between NGF staining and neural components either in the acute appendicitis or control groups.

Our findings indicate that mast cells could be one of the important cell populations responsible for nerve proliferation and hypertrophy in acute appendicitis. The relationship between NGF staining and MCD and the lack of correlation between NGF staining and changes in neural components suggest that, in acute appendicitis, NGF might be responsible for the increased number of MCs, but not for neuronal proliferation and hypertrophy.

key words: acute appendicitis, mast cell, nerve growth factor, neuronal hypertrophy, image analysis

INTRODUCTION

Mast cells (MCs) are derived from the bone marrow and are frequently located at body sites that interface with the external environment, such as the skin, respiratory tract and gastrointestinal mucosa. They serve a function of host defence against invading pathogens and have a central role in the mediation of allergic responses [4, 12, 13]. Although MCs synthesise a wide variety of mediators that can induce both acute and chronic inflammation [4, 12–14], they

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also generate and release several mediators that may act on nerve development and neural functions, and contribute to neuroimmune reactions [22, 24, 27]. They synthesise, store and release several mediators that may influence neural functions such as leukaemia-inhibitory factor, IL-6 and TNF [5, 36, 38]. A number of neuropeptides have been demonstrated to alter both mast cell development and function, and nerve growth factor (NGF) is amongst them [3, 20, 29, 37]. Despite being a neurotropic factor for the survival and maintenance of peripheral and sensory neurones [19], NGF has a wide range of other effects, especially on MCs and both have been reported to be involved in inflammation [6, 16, 20, 21, 23, 25, 26, 40].

There is increasing evidence for spatial and functional interactions between MCs and peripheral nerves in many tissue sites [2]. In the gastrointestinal system, MCs are closely apposed to nerves, suggesting they are essential for nerve growth and repair [30]. Recent studies have also demonstrated the involvement of the enteric nervous system (ENS) and MCs during intestinal inflammation [1, 7, 18, 28, 31]. In inflammatory bowel diseases an association between neural hypertrophy and increased mast cell density (MCD) has been detected [11, 39]. Moreover, enhanced expression of NGF and its receptor has suggested a functional interaction between ENS and MCs in these diseases [8]. In acute appendicitis, although increased neuronal proliferation, as well as increased levels of neuropeptides such as substance P (SP) and vasoactive intestinal peptide (VIP), have been demonstrated by some researchers [9, 10], the relationship between neuronal hypertrophy and increased mast cell density has been demonstrated in few studies [41]. To our knowledge, neither the expression of nerve growth factor (NGF) nor its relation to MCD and ENS has been delineated yet in this disease.

Therefore this study was undertaken to immunohistochemically investigate the relationship between MCD, the nervous system and NGF expression in cases with clinically and histopathologically diagnosed acute appendicitis and in normal appendices.

MATERIAL AND METHODS

Twenty-five patients with clinical diagnosis of acute appendicitis, admitted to the Department of General Surgery, Akdeniz University Hospital, Antalya, Turkey, were selected for the present study. All cases met the clinical criteria of acute appendicitis and histopathological evidence of acute inflammation. Twelve normal appendices removed from organ donors served as control group.

Three paraffin sections (5 μm) from tissue specimens, originally fixed in buffered formalin and divided into three segments from tip to base of the appendices, were prepared.

Sections were deparaffinised and heated in a microwave oven for 10 minutes to retrieve antigens. Slides were immunostained with PGP 9.5 (dilution 1:200, Dako, Denmark), mast cell tryptase (dilution 1:500, Neomarkers, USA) and NGF (dilution 1:500, Santa Cruz, USA) monoclonal antibodies by the avidin-biotin immunoperoxidase technique. Finally, all slides were treated with DAB reagent to develop colour and counterstained with haematoxylin. Slides were interpreted for number of Schwann cells, number and size of ganglia, MCD and NGF staining by a pathologist who had no knowledge of the clinicopathological data.

Image analysis was performed using a SAMBA 2005 image processor (Alcatel-TITN, Grenoble, France). This system consisted of a Leitz Diaplan Microscope connected to a personal computer through a Sony colour camera and a data translation frame grabber board. In each section, Schwann cells, ganglia and mast cell were counted at X400 magnification and their number was expressed as the number per square millimetre of tissue. Morphometric evaluation was performed to obtain the mean size of each ganglion and expressed as a micronmetre per ganglion.

For detection of the density of NGF immunostaining, the system was calibrated for a 10X objective. In each area antigen immunoreactivity was measured in the whole appendiceal wall. The results obtained were expressed as percentage of the ratio of NGF immunoreactive area to the total scanned area (both in μm^2).

The differences in NGF staining, MCD, number of Schwann cells, size and number of ganglia between two groups were compared by Student's *t* test. Correlations between MCD, NGF expression and other quantitative data were tested by calculating Spearman's correlation coefficient. A significance level of 0.05 was used throughout the analysis.

RESULTS

In two groups mast cells were detected in all layers of the appendiceal wall. In the acute appendicitis group MCs were clustered especially in the lamina propria and MCD was significantly higher than in control tissues ($p < 0.01$) (Table 1) (Fig. 1A, B).

In all cases PGP 9.5 positive Schwann cells were distributed throughout the submucosa and the mus-

Table 1. Schwann cells and ganglia in the submucosa and muscularis, MCD and percentage of NGF positivity in acute appendicitis and normal appendices

	Acute appendicitis Mean ± SD*	**Control group** Mean ± SD
MUCOSA		
Number of Schwann cells/mm^2	19.52 ± 6.83	7.08 ± 2.82
Number of Ganglia/mm^2	2.1 ± 1.04	0.87 ± 0.48
Mean area of Ganglia/μm^2	699.08 ± 103.15	182.83 ± 29.24
MUSCULARIS		
Number of Schwann cells/mm^2	58.62 ± 24.82	7.78 ± 3.32
Number of Ganglia/mm^2	5.07 ± 2.27	1.99 ± 0.72
Mean area of Ganglia/μm^2	684.29 ± 114.70	294.65 ± 44.18
MCD/mm^2	64.99 ± 17.14	25.94 ± 11.22
NGF positivity (%)	45.95 ± 19.44	9.33 ± 4.63

p values < 0.01 is always between two groups in Student's *t* test, *standard deviation, MCD — mast cell density, NGF — nerve growth factor

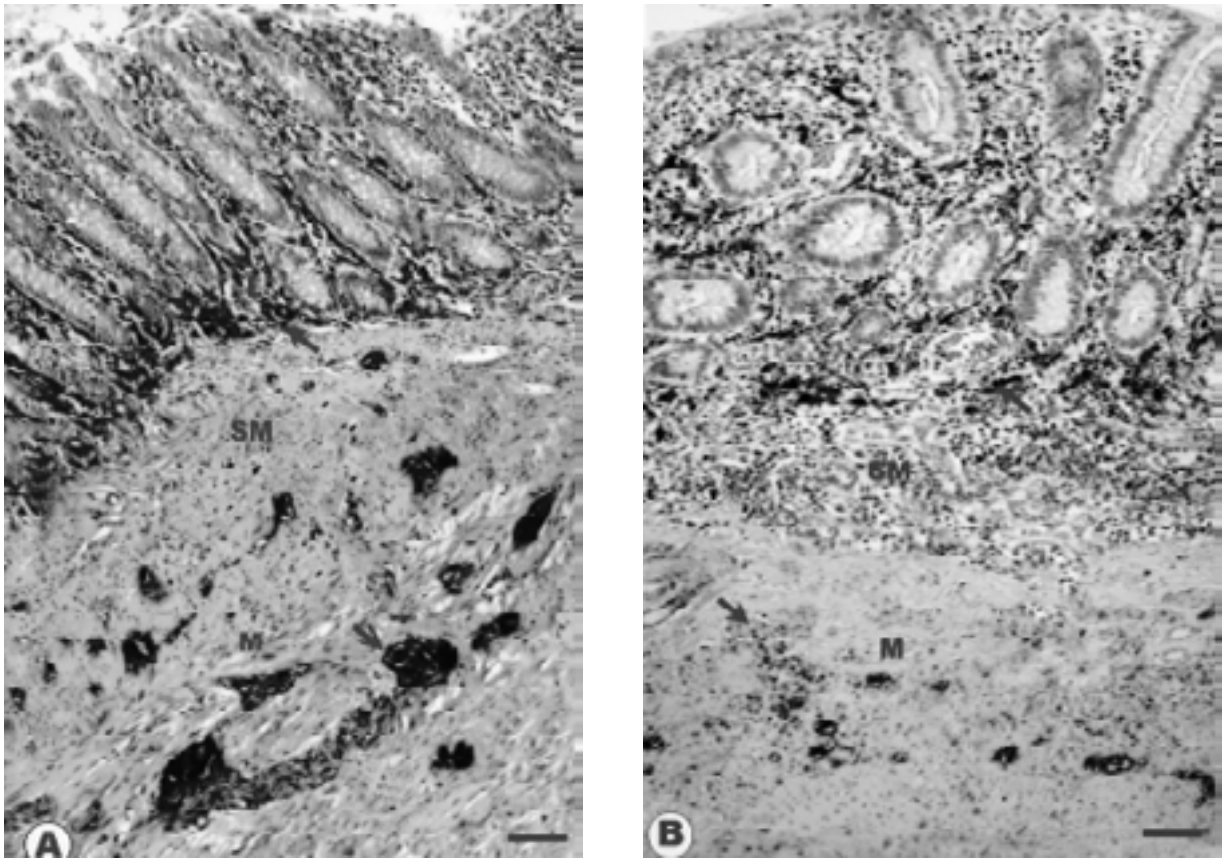

Figure 1. PGP9.5 immunoreactivity of (**A**) acute appendicitis and (**B**) normal appendices (arrows). Significantly increased number of PGP 9.5 positive nerve fibres and ganglia and enlarged ganglia are seen in acute appendicitis; L — indicates lamina propria, SM — submucosa, M — muscularis. Scale bar: 500 μm.

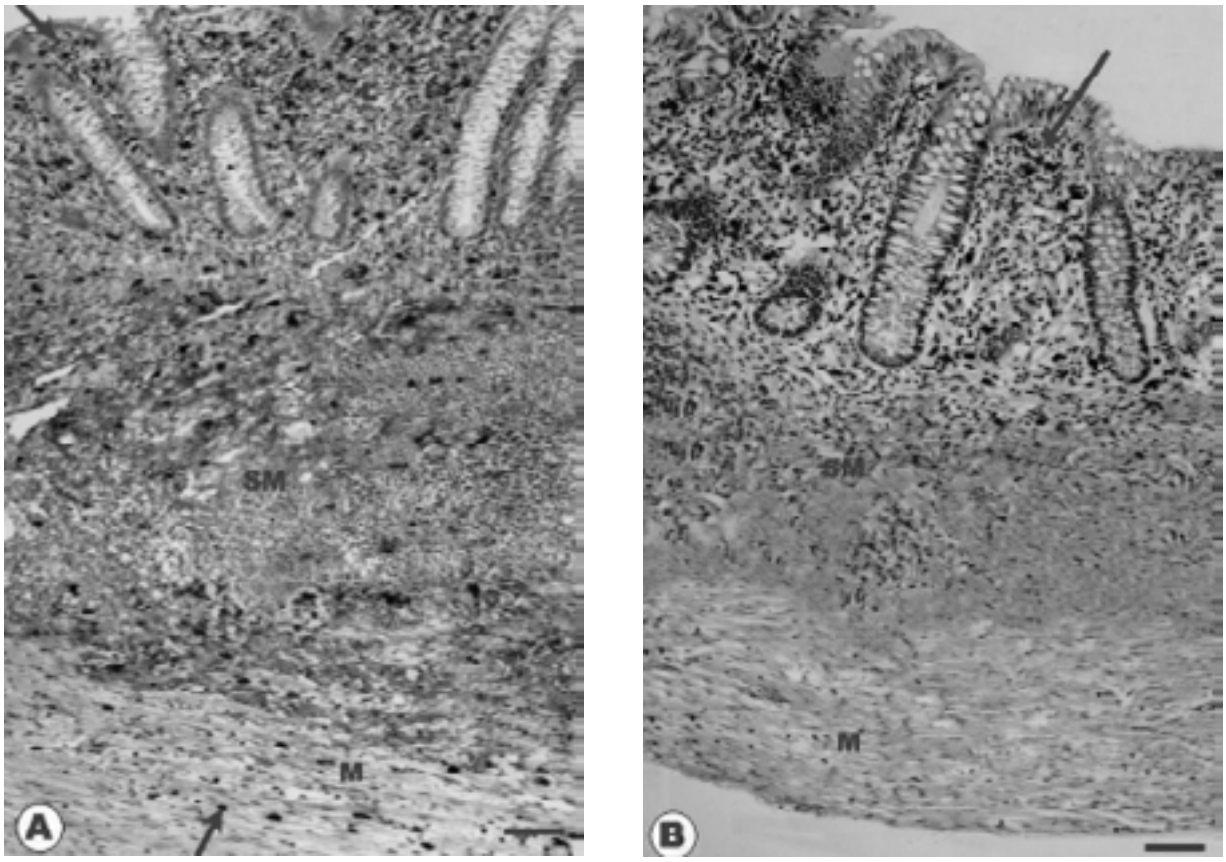

Figure 2. Distribution of tryptase positive mast cells in appendiceal wall of (**A**) acute appendicitis and (**B**) normal appendices (arrows). Significantly increased number of mast cells seen in acute appendicitis; L — indicates lamina propria, SM — submucosa, M — muscularis. Scale bar: 500 μm.

cularis externa. The number of Schwann cells and the number and size of ganglia located in submucosa and muscularis externa were significantly increased when compared with those in the control group ($p < 0.01$) (Table 1) (Fig. 2A, B).

NGF expression was detected in Schwann cells, ganglia and in mast cells. The percentage of staining was significantly greater in acute appendicitis than in control tissues ($p < 0.01$) (Fig. 3A, B).

Spearman correlation test revealed a strong correlation between MCD and NGF staining ($r = 0.92$). MCD was also related to the number of Schwann cells (in submucosa $r = 0.633$, in muscularis $r = = 0.701$) and the number (in submucosa $r = 0.610$ in muscularis $r = 0.870$) and size (in submucosa $r = = 0.891$, in muscularis $r = 0.803$) of ganglia. However, we failed to find a significant correlation between NGF staining and other quantitative data.

DISCUSSION

Although MCs are involved in the local regulation of immune events, there is a growing body of evidence that these cells, by their numbers, distribution and content of chemical mediators, might influence neural functions and nerve remodelling during inflammation [2, 18, 22, 27, 30]. In the gastrointestinal system, bi-directional communication between MCs and ENS has been described [34]. In acute appendicitis, only a few studies have been addressed to investigate the relationship between MCs and ENS. In an elegant study, Xiong et al. [34], by using a similar antibody to define mast cells and a counting procedure similar to our study, observed a significant increase in neural components and MCs in cases with acute appendicitis. Parallel to this finding, our study shows that neuroproliferation in the appendix, in association with increased MCD, may occur in patients with clinical and histopathological diagnosis of acute appendicitis. These findings are interesting because, in the gastrointestinal system, changes either in neural components or in MCD have been reported in chronic inflammatory conditions. For instance, in chronic inflammatory bowel diseases, such as Crohn disease and ulcerative colitis, an

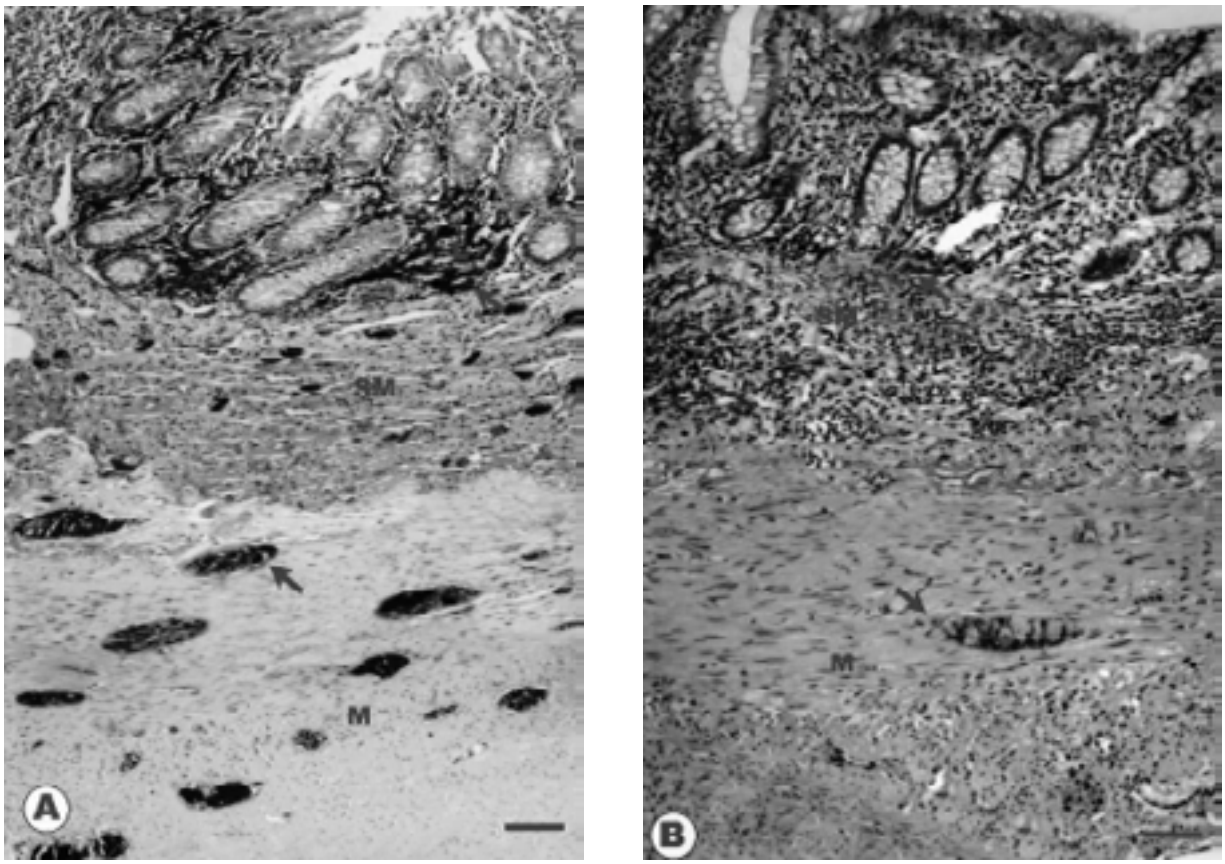

Figure 3. NGF positivity of (**A**) acute appendicitis and (**B**) normal appendices (arrows). NGF expression is more frequent in acute appendicitis, L — indicates lamina propria, SM — submucosa, M — muscularis. Scale bar: 500 μm.

association of MCs and hypertrophic nerve fibres has been detected [11, 39]. In previous studies neuronal hypertrophy and increased levels of neuropeptides have been observed in clinically suspected but histopathologically normal appendices [9, 10, 41]. Moreover, in one study an increase in MCD accompanying neuronal hypertrophy has been observed in some cases with negative appendectomies [41]. It has been suggested that the neuronal cell hypertrophy might represent the reparative phase of an inflammatory process to previous chronic or repeated acute injury and it is more appropriate to suggest that acute appendicitis might represent an exacerbation of an inflammatory process that already exists in the appendix [9, 10, 41]. Because the existence of chronic inflammation of the appendix is not generally accepted as a pathological entity, negative appendectomies were not included in our study. For this reason it is not possible to conclude with our findings that the significant increase in Schwann cells and number and size of ganglia, as well as increased MCD in acute appendicitis, had developed during a single episode of inflammation or not. However, it has been described that well developed neuronal changes necessitate molecular and cellular events over time [32]. For this reason, neuronal hypertrophy and proliferation observed in our study are unlikely to develop during a short and single episode of acute inflammation.

On the other hand, although the exact mechanism remains to be elucidated, the close association of increased MCD and neural components in acute appendicitis suggests a functional link between the MCs and ENS in this disease [41]. There is considerable evidence that MCs are spatially and functionally appeased to nerves in the gastrointestinal system with the highest level in the appendix [31]. It has been suggested that neuronal proliferation or changes in number of MCs might be associated with fibrosis and reflect a physiologic aging phenomenon [35]. However, in our study in all cases with acute appendicitis fibrosis was minimal or not present and histopathological findings of acute inflammation were prominent. Moreover, in control group neural proliferation and MCD were lower than in acute appendicitis and no relationship

was detected between neural components and MCD in the statistical analysis.

MCs' mediators may influence neural components, which further activate MCs by releasing neuropeptides [2]. A number of neuronally derived factors have been demonstrated to alter both mast cell development and function and these include growth factors like NGF [3, 20, 29, 37]. Although NGF is essential for the survival of sensory and sympathetic neurons, it has broader biological activities on non-neuronal cells [19]. In experimental studies NGF induces degranulation of MCs [6, 25]. When injected to neonatal rats NGF induces hyperplasia of MCs in both connective tissue and mucosal sites and prevents their apoptosis [6, 16, 21, 25]. MCs have been demonstrated to possess receptors for NGF and interestingly are a newly recognised source of NGF synthesis, storage and release [15, 16, 33]. In inflammatory bowel diseases, an increase in NGF expression was found in inflamed tissues and infiltrating MCs also expressed enhanced levels of NGF [8]. It has been suggested that NGF and MCs contribute to nerve activation and remodelling during inflammation. In Hirschsprung Disease, it has been demonstrated that MCs might be an important factor in the excessive development of adrenergic and cholinergic nerve fibres by releasing NGF [17]. To our knowledge, the relationship between NGF expression, MCD and neuronal proliferation has not been investigated in cases with clinically and histopathologically diagnosed acute appendicitis. In our study, the percentage of NGF expression in cases with acute appendicitis was significantly higher than in control group and a strong correlation between NGF expression and MCD was detected. Results of the previous studies on NGF and mast cell interactions and the present one lead us to speculate that in acute appendicitis NGF might be responsible for the hyperplasia of MCs. The significant relation of MCD with the number of Schwann cells and number and size of ganglia suggests that MCs might have an important role in neural proliferation and hypertrophy. Although NGF is responsible for nerve growth and development, the lack of correlation between NGF staining and neuronal proliferation and hypertrophy observed in this study suggests that NGF does not directly contribute to neuronal proliferation and hypertrophy in acute appendicitis.

In a more recent study it has been demonstrated that NGF can also induce mast cells to produce significant amounts of PGE_2, which might be responsible for the modulation of MCs IL-6 and TNF production and, under conditions of neuronal damage and repair, the long term mast cell PGE_2 response to NGF could provide a mechanism by which local harmful responses might be limited [23]. In the light of this observation, it will be of interest to investigate the effect of NGF on PGE_2 production on MCs in acute appendicitis.

Although only a limited number of cases have been included in this study, this is the first attempt to compare changes in neural components, MCD and NGF expression in acute appendicitis. The significant association of neural components and MCD indicates that mast cells could be one of the important cell populations responsible for nerve proliferation and hypertrophy in acute appendicitis. The relationship between NGF staining and MCD and the lack of correlation between NGF staining and changes in neural components suggest that, in acute appendicitis, NGF might be responsible for the increased number of MCs, but not for neuronal proliferation and hypertrophy.

However, further studies on a large number of patients, including clinically suspected but histologically normal appendectomies, are necessary to further establish the relationships between MCs, NGF and neural components in the pathogenesis of acute appendicitis.

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