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Mast cell density and neuronal hypertrophy in patients with acute appendicitis

Akut apandisit olgularında mast hücre yoğunluğu ve nöronal hipertrofi

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Background/aims: *In the gastrointestinal system, the relationship between the enteric nervous system and mast cells has been described in normal and pathological conditions. However, a few studies have evaluated the association between the numbers of mast cells and the enteric nervous system in acute appendicitis. The aim of this study was to immunohistochemically investigate the relationship between mast cell density and the enteric nervous system in cases with clinically and histopathologically diagnosed acute appendicitis and in normal appendices.* ***Methods:*** *Twenty-five patients with acute appendicitis and 12 cases with normal appendices were included in our study. Mast cell tryptase and PGP 9.5 immunostained tissue sections were subjected to quantitative image analysis.* ***Results:*** *Our results showed that mast cell density, number of Schwann cells and the number and size ofganglia were significantly greater in acute appendicitis than in the control group ($p<0.05$). A strong correlation between mast cell density and neuronal proliferation and hypertrophy was detected only in cases with acute appendicitis. We failed to detect any relationship between mast cell density and neural components in the control group.* ***Conclusions:*** *Our findings indicate that mast cells could be one of the important cell populations responsible for nerve proliferation and hypertrophy in clinically and histopathologically diagnosed acute appendicitis.*

Key words: Acute appendicitis, mast cell, neuronal hypertro phy, image analysis.

Amaç: *Nöronal ve patolojik şartlarda gastrointestinal sistemde, enterik sinir sistemi ve mast hücreleri arasındaki ilişki tanımlanmıştır. Ayrıca akut apandisitte yapılan bir kaç çalışmada, mast hücre sayısı ile enterik sinir sistemi arasındaki ilişki de gösterilmiştir. Bu çalışmanın amacı klinik ve histopatolojik olarak akut apandisit ve normal appendix tanısı almış olgularda mast hücre yoğunluğu ve enterik sinir sistemi arasındaki ilişkiyi immunuhistokimyasal olarak araştırmaktır.* ***Yöntem:*** *Çalışmamız 25 akut apaandisit ve 12 normal appendix içermektedir. Mast cell tryptase ve PGP 9.5 ile immunohistokimyasal boyanan doku kesitleri kantitatif image analizi ile değerlendirilmiştir.* ***Bulgular:*** *Çalışmamızda Mast hücre yoğunluğu, schwann hücre sayısı, ganglion alan ve sayısınının kontrol grubuna göre arttığı gösterilmiştir. Sadece akut apandisit olgularında mast hücre yoğunluğu ile nöronal proliferasyon ve hipertrofi arasında kuvvetli bir korelasyon belirlendi. Kontrol grubunda mast hücre yoğunluğu ve nöronal komponentler arasında herhangi bir ilişki belirleyemedik.* ***Sonuç:*** *Bulgularımız akut apandisitin klinik ve histopatolojik tanısında mast hücre populasyonunun nöronal proliferasyon ve hipertrofiden sorumlu olduğunu göstermektedir.*

Anahtar kelimeler: Akut apandisit, mast hücresi, nöronal hipertrofi, görüntü analizi.

INTRODUCTION

The interaction between the nervous and immune systems has been demonstrated in various organs (1,2). In the gastrointestinal system there is considerable data supporting the involvement of the enteric nervous system (ENS) in immune regulation (3,4). This is suggested from the anatomic micro and functional relationships that exist between ENS and immunocompetent cells in normal and pathological conditions (2-6). Recent studieshaveindicatedthatamongimmunocompetent cells, mast cells (MCs), in addition their pivotal role in hypersensitivity reactions, are closely apposed to nerves in the human gastrointestinal mucosa. Further more, there may be a functional interaction between MCs and ENS in several disorders (7-10). In Hirschsprung's disease, a relationship between increased MC number and hypertrophic nerves has been detected (11). It has been suggested that through release of several neurotrophic factors, MCS might be involved in the excessive development of cholinergic and adrenergic nerve fibers (11). In inflammatory

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bowel diseases, the association between MCs and ENS has also been noted (12,13). In acute appendicitis, although the relationship between ENS and inflammation has been described (14-15), few studies have evaluated the relationship between ENS and MCs in this disease (16).

This study was set up to investigate the association of MCs and ENS in cases with clinically and histopathologically diagnosed acute appendicitis and in normal appendices by using quantitative immunohistochemistry.

MATERIALS AND METHODS

Twenty-five patients with a clinical diagnosis of acute appendicitis admitted at 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 histopathologic evidence of acute inflammation. Twelve normal appendices removed from organ donors served as the control group.

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

Sections were deparaffinized and heated in a microwave oven for 10 minutes to retrieve antigens. Slides were immunostained with PGP9. 5 (dilution 1:200.Dako, Denmark) and mast cell tryptase (dilution 1:500, Neomarkers, USA) and by the avidin-biotin immunoperoxidase technique. Finally, all slides were treated with DAB reagent to develop color and were counterstained with hematoxylin. Slides were interpreted for number of Schwann cells, number and size of ganglia and mast cell density (MCD) by a pathologist who had no knowledge of the clinicopathologic data.

Image analysis was performed using a SAMBA 2005 image processor (Alcatel-TITN, Grenoble, France). This syste consisted of Leitz Diaplan microscope connected to a personal computer through a Sony color camera and a data translation frame grabber board. In each section, Schwann, ganglia cell and MCs 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 micrometer per ganglion.

The differences in MCD, number of Schwann cells, and size and number of ganglia between the two groups were compared by Student's t test. Correlations between MCD 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 the two groups, MCs 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 ($p<0.05$) (Table 1) (Figure IA and IB).

In all cases, PGP 9.5 positive Schwann cells were distributed throughout the submucosa and the muscularis externa. The number of Schwann cells

Table 1. Glutaminase (mmol/hour/mg protein), maltase (mmol/hour/mg protein), the amount of lipid peroxidation malonyldialdehyde level/gram tissue), and lactase (unit/gram tissue) activities in groups

	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 /urn^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 /[im^2	684.29±114.70	294.65±44.18
MCD/mm^2	64.99±17.14	25.94+11.22

P values <0.01 (Student's *t* test) *: Standartd deviation; MCD: mast cell density.

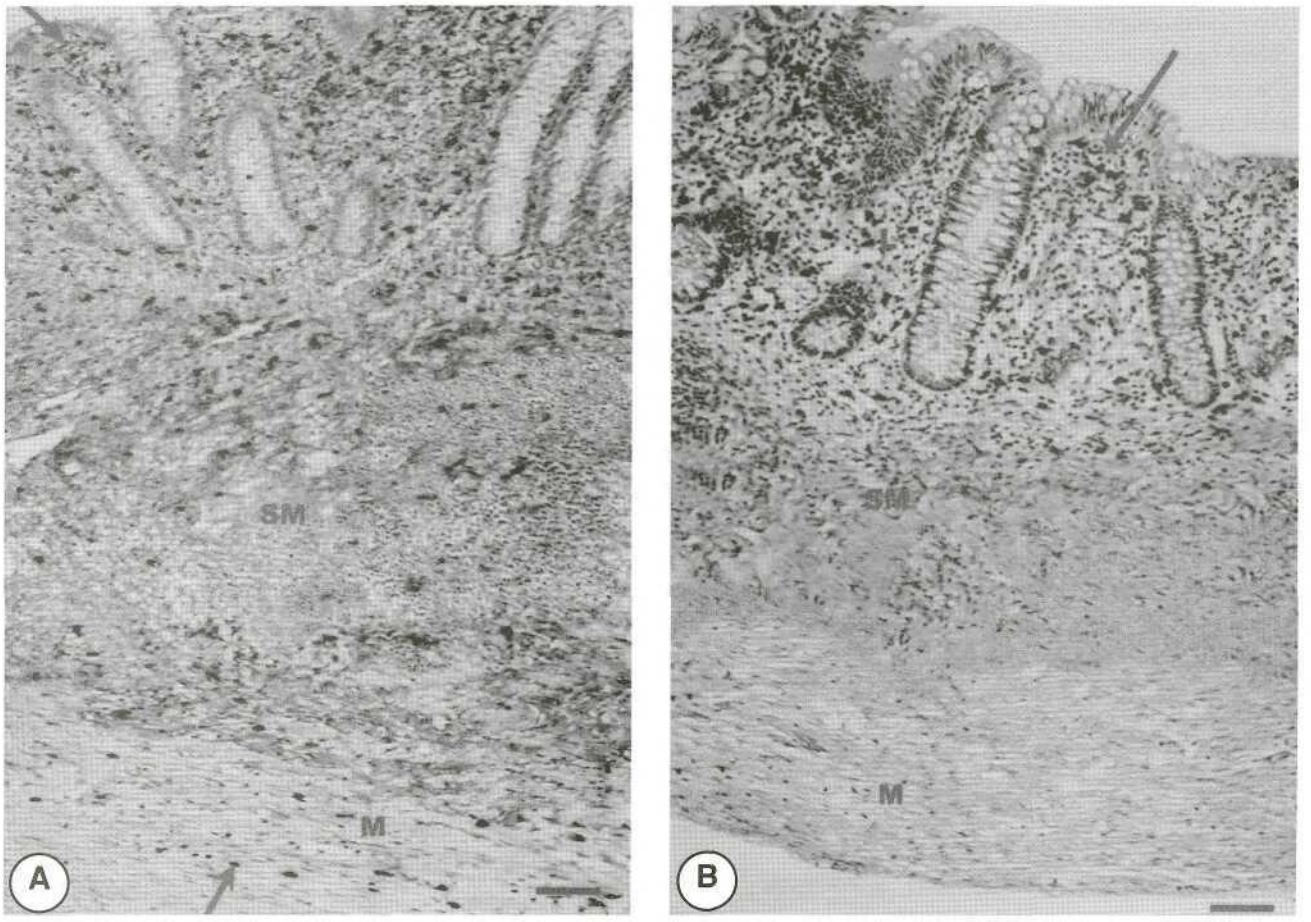

Figure I. Distribution of tryptase-positive mast cells in appendiceal wall of (A) acute appendicitis and (B) normal appendices (arrows). A significantly increased number of mast cells are seen in acute appendicitis.
(L: indicates lamina propria; SM: submucosa and M: muscularis)
(Scale bar -SOOum)

and the number and size of ganglia located in the submucosa and muscularis externa were significantly increased when compared with those in the control group ($p<0.05$) (Table 1) (Figure 2A and 2B).

Spearmans correlation test revealed a strong correlation between MCD and 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 in cases with acute appendicitis.

DISCUSSION

Although MCs are involved in the local regulation of immune events, there is a growing body of evidence that these cells might be essential for nerve growth and repair (7,17-19). MCs, by their numbers, distribution and content of chemical mediators, might influence neural functions and nerve remodeling (17-19). Moreover, coordinated neuroimmune interactions between MCs and nerves are an important aspect of the host response during inflammation (20). MCs contribute to neuroimmune reactions in diseases characterized by inflammation in various organs (1,2). In the gastrointestinal system, bidirectional communication between MCs and ENS has been described in physiologic and pathologic conditions (7-10). In Hirschsprung's disease, MCs have been demonstrated as a possible important factor in the excessive hyperplasia of the nerve network (11). In chronic inflammatory bowel diseases considerable changes in the pattern of innervations and in the morphology of nerve fibers were found to be associated with MCs (12,13). In our study, MCD was significantly increased in cases with acute appendicitis and this increase was highly correlated with an increased number of Schwann cells and number and size of ganglia. Our findings are

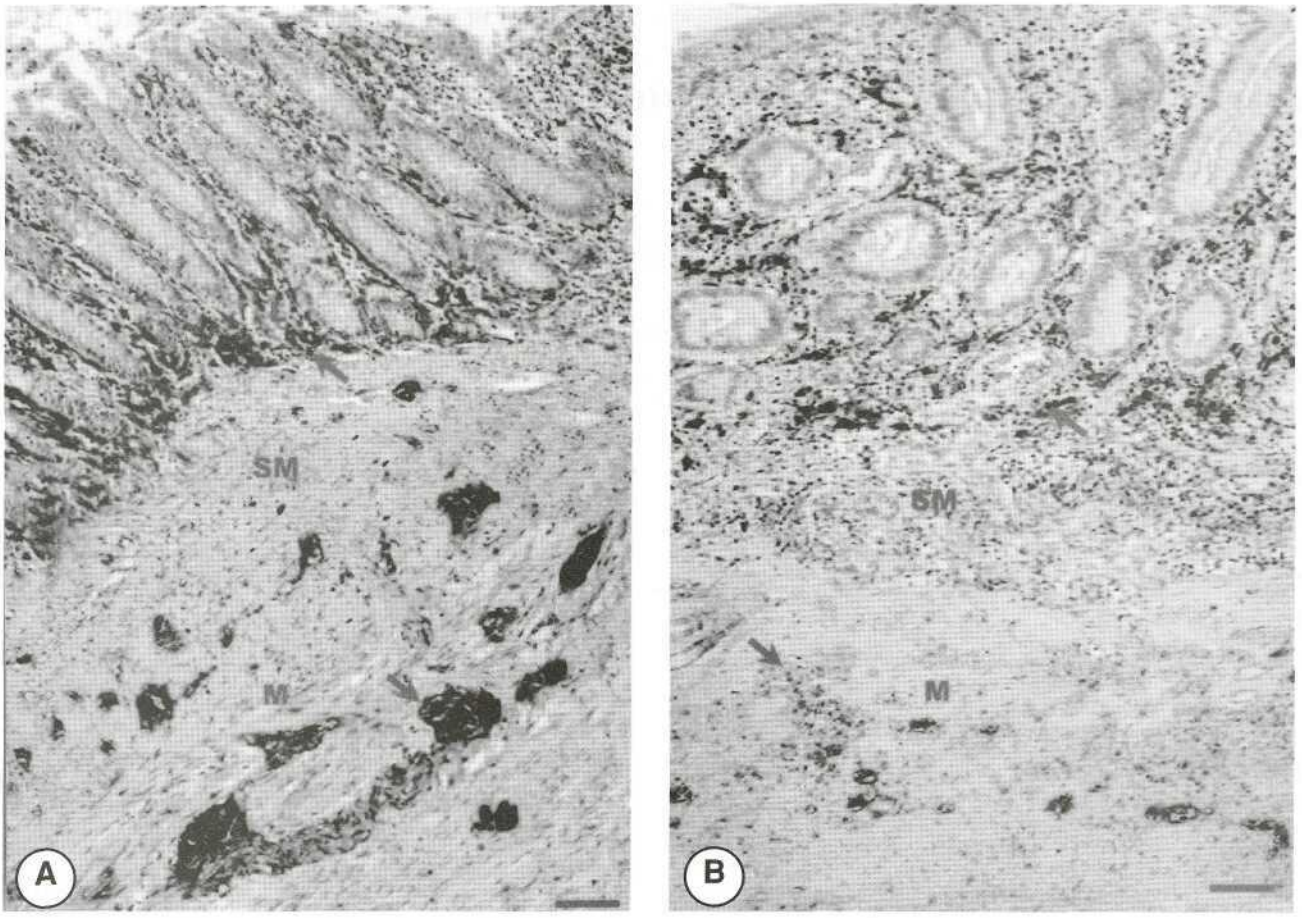

Figure 2. PGP 9.5 immunoreactivity of (A) acute appendicitis and (B) normal appendices (arrows). A significantly increased number of PGP 9.5-positive nerve fibers and ganglia and enlarged ganglia are seen in acute appendicitis.
(L: indicates lamina propria; SM: submucosa and M: muscularis)
(Scale bar -SOOum)

interesting since there are few reports of altered neural components and MCs in association with acute inflammatory conditions in the gastrointestinal system. Xiong et al (16), using similar antibody to define MCs and a similar counting procedure to our study, observed a significant increase in neural components and MCs in cases with acute appendicitis. At present, the pathophysiological basis of the neuronal hypertrophy and increased MCD in this disease is not known. Recently, an increase in neural components and neuropeptides observed in clinically suspected but histopathologically normal appendices (14-16). It has been proposed that the neuronal cell hypertrophy might represent the reparative phase of an inflammatory process to previous chronic or repeated acute injury. It is more appropriate to suggest that acute appendicitis might represent an exacerbation of an inflammatory process that already exists in the appendix (14-16). Because the existence of chronic inflammation of the appendix is not generally accepted as a pathological entity, negative appendicectomies were not included in our study. For this reason it is not possible to conclude definitively from on our findings whether or not the increase in Schwann cells and in the number and size of ganglia, and the increased MCD in acute appendicitis developed during a single episode of inflammation. However, well developed neuronal changes have been described as necessitating molecular and cellular events over time (21). For this reason, the neuronal hypertrophy and proliferation observed in our study are unlikely to have developed during a short and single episode of acute inflammation.

On the other hand, our findings are in accordance with those reported by Xiong et al (16) which suggested that MCs could be one of the important cell populations responsible for nerve proliferation and hypertrophy in cases with clinically and

histopathologically diagnosed acute appendicitis.

Several authors have suggested that neuronal proliferation or changes in the number of MCs in the appendix might be associated with fibrosis and reflect a physiologic aging phenomenon (22). In our study fibrosis was minimal or not present in all cases with acute appendicitis and histopathological findings of acute inflammation were prominent. In addition, neural proliferation and MCD were lower in the control group than in cases of acute appendicitis and no relationship was detected between neural components and MCD in the statistical analysis. Our results thus suggest that increased MCD and neural components reflect the interaction of MCs and ENS during inflammation of the appendix rather than a physiological aging phenomenon.

In conclusion, although only a limited number of cases were included in this study, the significant association of neural components and MCD indicates that MCs could be one of the important cell populations responsible for nerve proliferation and hypertrophy in acute appendicitis. However, further studies on a large number of patients including clinically suspected but histologically normal appendicectomies are necessary to further establish the relationships between MCs, neural components and MC mediators in the pathogenesis of acute appendicitis.

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