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A deficiency or developmental defect in paneth cells may contribute to the pathogenesis of appendicitis in children

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Abstract

Aim: The aim of this study was to investigate whether there were differences in the Paneth cells between children with acute appendicitis (AA) and those with normal appendix (NA), and to reveal the distribution and morphological changes in Paneth cells in appendix inflammation.

Materials and Methods: The data of 63 patients who underwent appendectomy diagnosed with acute appendicitis between January 2021 and December 2022, including age, gender, operative diagnosis, and postoperative histopathological examination results, were analyzed retrospectively. To evaluate the distribution and changes of Paneth cells throughout AA and NA groups, samples with hematoxylin and eosin (H&E)-stained sections were obtained from the Department of Histopathology's archives. Selected blocks were stained with Masson-Trichrome. The number of Paneth cells and the degree of granular density in the appendicitis tissues were statistically evaluated and compared with the results of the control group.

Results: A total of 63 appendectomies were performed, including 31 incidental appendectomies and 32 performed for acute appendicitis. There were no statistically significant differences between the groups that underwent surgery for AA and the NA in terms of gender and age ($p>0.05$). It was observed that the number of Paneth cells and granule density decreased significantly in acute appendicitis (AA) group ($p<0.05$).

Conclusion: Reduction or developmental deficiency in Paneth cells, may results in the loss of protective secretion, and may increase the appendix's susceptibility to bacterial infection by allowing organisms to adhere and penetrate the mucosa. The resultant enhancement of infection may contribute to the pathogenesis of appendicitis.

Introduction

The vermiform appendix is generally recognized as a primitive portion of the gut. The significance of the appendix in forming and preserving gut-associated lymphoid tissue (GALT) and its connection with intestinal flora is a major area of research [1,2]. Although humans do not use the appendix to house cellulose-degrading bacteria, its form and position in the digestive tract may serve as a "safe host" for healthy colonic flora. After gastrointestinal diseases, the appendix may act as a reservoir where normal microbial diversity can be restored quickly [3,4].

The appendix wall comprises mucosa, submucosa, muscularis externa, and serosa, the same as the intestinal wall structure. However, the occurrence, number, and function of cells within these layers differ between the appendix and colon, as demonstrated by the presence of lymphoid follicles in the mucosa and submucosa of the appendix [5]. Similar to the colon, Lieberkühn crypts are seen in the appendix mucosa. These crypts contain Paneth cells, typically found in the small intestine near their base [6], which primarily produces antimicrobial peptides [7]. Josef Paneth was the first scientist who described Paneth cells in 1888 as cytoplasmic granule-rich epithelial cells found at the base of small intestinal crypts (also known as "crypts of Lieberkühn") [8], which play a crucial part in the regulation of host immunity, as well as defense against a variety of intestinal microbes [9].

Paneth cells' role in the small intestine's pathophysiology has yet to be fully elucidated. Recent evidence suggests that these molecules, were previously believed to play a phagocytic role; however, recently it has been shown that, they contribute to innate mucosal protection through the expression of a wide range of antimicrobial

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and immune regulatory molecules, including lysozyme, α-defensin, secretory phospholipase-A2, α1-antitrypsin and tumor necrosis factor [10,11]. Paneth cell defensins have a direct role in eliminating pathogens and providing a symbiotic relationship with the normal gut microbiome [12,13]. Differences in the secretory profile induced by diseases can impair the intestinal barrier and increase the risk of pathogen transmission [14].

When the role of Paneth cells in AA is considered, a decrease in Paneth cells may suppress mucosal immunity in the appendix. This study aimed evaluates changes in the number and morphology of Paneth cells and its role in the infection and inflammation of the appendix. This also explains the underlying mechanism of bacterial migration in AA.

Materials and Methods

This study received approval from Malatya Turgut Ozal University Non-Invasive Human Research Ethics Committee (Date: 10.01.2023, Decision No: 2023/3) and was conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. Informed consent of the subjects was weived by the ethics commiteebecause the sample consisted of medical records. Demographic data and histopathology reports of the groups were obtained from the medical registry system of Malatya Turgut Ozal University, Training and Research Hospital. We retrospectively analyzed the data of 63 patients. These appendicitis group (AA) consisted of 32 patients who had undergone open appendectomy for confirmed acute appendicitis. The control group (NA) included 31 patients who had selected similar age and gender profiles and who had undergone incidental appendectomy (e.g., adhesions, exploratory laparotomy/trauma laparotomy, Amyand's hernia, ovarian cyst, ovarian torsion, intussusception, and Meckel's diverticulum) and the pathologic examination showed that the appendix tissues were normal. The diagnosis of acute appendicitis was confirmed by the clinical, radiological, surgical, and histopathological characteristics of the patients.

In both groups, children with a known history of hematological or metabolic disease, or diseases requiring regular drug use, and any histopathological finding (gangrene/perforated appendicitis, mucocele, tumor) were excluded from the study.

Preparation of tissues for histopathological study

To evaluate the distribution and changes of Paneth cells throughout NA and AA groups, samples with hematoxylin and eosin (H&E)-stained sections were obtained from the archives of Pathology Department. The pathologists involved in the study confirmed the histological appearance of all sections to be consistent with the final diagnosis (T.SF.). Selected blocks' histochemistry was stained with Masson-Trichrome. The number and granule density of Paneth cells stained by Masson-Trichrome rated better than H&E.

The sections were quantitatively counted Paneth cells and evaluated using qualitative granular morphology. Vertically located crypts (between seven and 30 in number) were selected for Paneth cell counting. Fully observable crypts from the lumen of the appendix vermiformis to the lamina propria were recorded, and the total number of Paneth cells was divided by the number of crypts. Findings were recorded to show the count of Paneth cells in each crypt. The crypt was divided into three equal zones on the longitudinal axis. While Zone1 crypt represents the base, zone 2 refers to the middle part, and zone 3 refers to the appendix lumen (Figure 1). Paneth cell granule morphology was evaluated using a three-point scale (normal, mildly depleted granules, and severely depleted granules) (Figure 2).

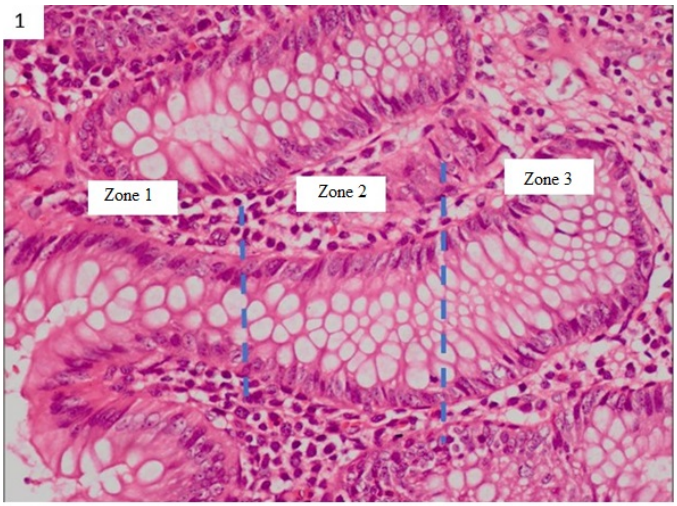

Figure 1. Evaluated crypts. (H&E,40x).

Statistical analysis

Descriptive statistics for dichotomous variables were presented in frequencies and percentages, while continuous variables were presented using mean ± standard deviation (SD) or median and interquartile range (IQR) values depending on the data distribution. The Shapiro-Wilk test was employed to assess the normal distribution of data. Data were analyzed using Mann Whitney U and Pearson chi-square tests with an exact approach when appropriate. The significance level was set at $p<0.05$. The American Psychological Association (APA) 6.0 format was used to report the statistical analysis results. All analyses were conducted using IBM SPSS Statistics 28.0 for Windows (New York, USA).

Results

The data from 63 patients were analyzed. The patient group consisted of 21 boys (65.63%) and 11 girls (34.38%), while the control group comprised 21 boys (67.74%) and ten girls (32.26%). Regarding gender, there was no significant difference between the study groups ($p>0.05$, see Table 1). The median age of the patients was 12.5 (IQR =7) years, while the median age of the healthy control cases was 12 (IQR=6.5) years. Likewise, no significant age-related difference was observed between the groups ($p>0.05$, see Table 1).

Paneth cells in each crypt were recorded to show the count. The crypt was divided into three equal zones on the longitudinal axis. While zone 1 crypt is the base, zone 2

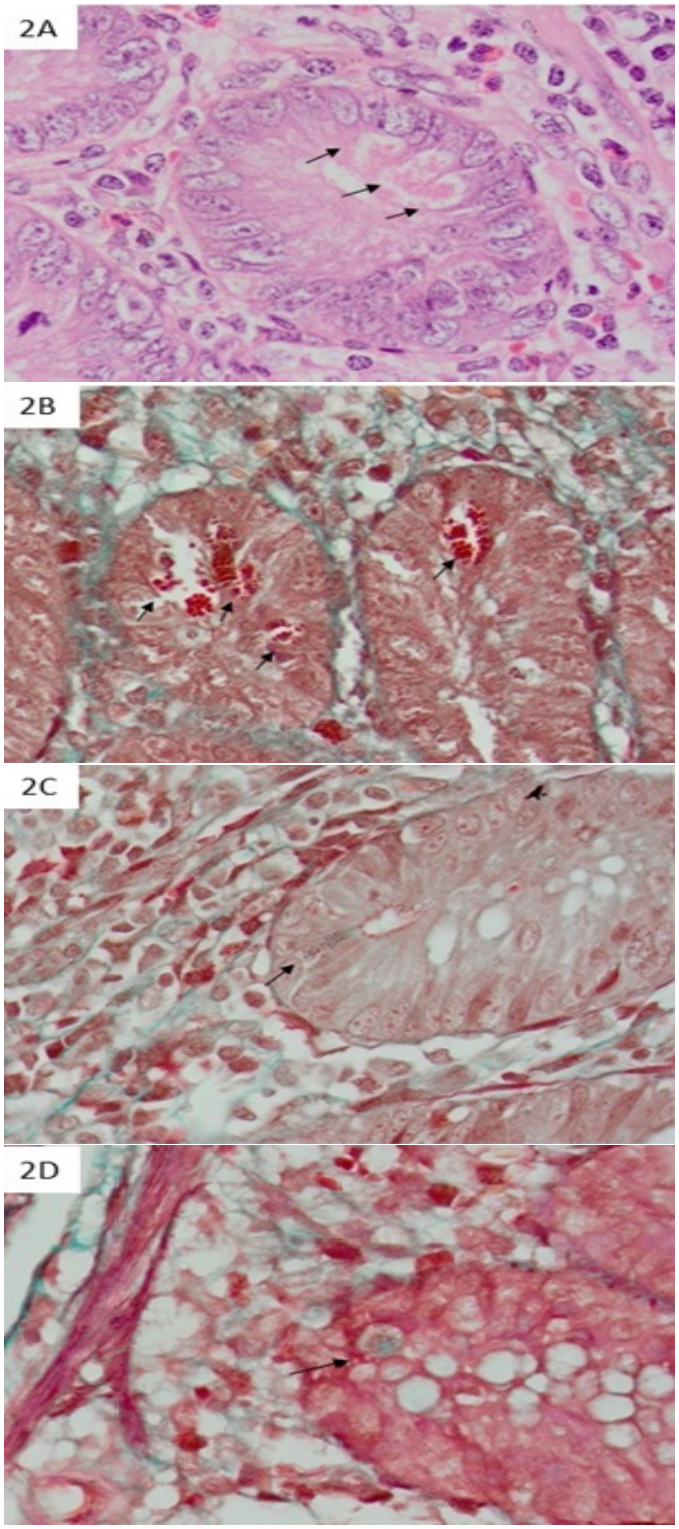

Figure 2. 2A-B: Paneth cells with normal granule density (arrows) (H&E, Masson-Trichrome, 100x), 2C: Paneth cell (arrow) with slightly reduced granules (Masson-Trichrome, 100x), 2D: Paneth cell (arrow) with severely reduced granules (Masson-Trichrome) Trichrome, 100x).

represents the middle part, and zone 3 represents the appendix lumen. The median value was 0.155 (IQR=0.165) in zone 1, and 0 (IQR=0) in zones 2 and 3. While there were statistically significant difference between the groups in terms of paneth cell number variables of zone 1 (IQR: 0.165, $p<0.05$), no statistically significant difference was found for zones 2 and 3 ($p>0.05$, see Table 2).

Table 1. Comparison of the groups according to demographic variables.

Variable	Groups		AA	NA	Total	p
Gender	Girl	n	11	10	21	1*
		%	34.38%	32.26%	33.33%	
	Boy	n	21	21	42	
		%	65.63%	67.74%	66.67%	
	Total	n	32	31	63	
		%	100.0%	100.0%	100.0%	
Age	IQR		7	6.5		0.443#
	Median (Min-Max)		12.5(7-18)	12(7-18)	12 (2-18)	

AA: Acute appendicitis. NA: normal appendices; n: frequency; %: percent; IQR: interquartile range .*: Pearson chi-square test with the exact approximation. #: Mann Whitney U test.

Table 2. Descriptive statistics for continuous variables among the study groups.

Variables	Group		
Paneth cell count	NA (n=31) Median (IQR)	AA(n=32) Median (IQR)	p*
zone1	0.78 (0.68)	0.155 (0.165)	<0.001
zone2	0 (0.05)	0 (0)	0.030
zone3	0 (0)	0 (0)	1

NA: normal appendices, AA: Acute appendicitis, Data are summarized by the median (IQR; interquartile range); *: Mann-Whitney U test.

Table 3. Descriptive statistics for categorical variables among the study groups.

Variables	Categories	Group NA(n=31)	Group AA (n=32)	p*
Granular density depletion	Normal	26 ^{a} (83.87%)	0 ^{b} (0.00%)	<0.001
	Mild	5 ^{a} (16.13%)	14 ^{b} (43.75%)	
	Severe	0 ^{a} (0.00%)	18 ^{b} (56.25%)	

NA: normal appendices. AA: Acute appendicitis. Data are summarized by number (percentage); *: Pearson chi-square test with the exact approximation; a.b: Different letters for variable categories in each row indicate significant differences ($p<0.05$). Normal; granule density normal; mild; granule slightly decreased; severe; granules were expressed as severely decreased.

Paneth cell morphology was evaluated qualitatively according to granule densities. The granule density, categorized as normal (Figure 2A-2B), mild (Figure 2C), and severe (Figure 2D), is shown in Table 3. When group variable categories and granule density variable categories were compared, there was a statistically significant difference between the groups ($p<0.05$). In acute appendicitis group, 56.25% (n:18) of patients showed a severe decrease in granular density and 83.87% (n:26) of control group had normal granular density.

Discussion

It is still unclear whether the alterations in morphology and number of Paneth cells are due to human intestinal

disease or vice versa. In necrotizing enterocolitis, Paneth cells that produce lysozyme are nonexistent [15]. On the other hand, in congenital zinc deficiency, such as acrodermatitis enteropathica, the cytoplasmic granules of Paneth cells are irregular. In the latter, some Paneth cells, but not all, have empty secretory granules [16]; some have small and differently shaped granules [17]. It has been suggested that a decline in Paneth cell counts in coeliac disease indicates individuals who are tolerant to gluten abstinence [18,19]. The proliferation of colonic Paneth cells in inflammatory bowel disease (IBD) may result in an adaptive response in which alpha-defensins act as a protective barrier to stop secondary mucosal infection in the inflamed bowel [20].

Acute appendicitis is the most frequent cause of acute abdominal pain in children, which requires urgent appendectomy [21]. Luminal obstruction due to lymphoid hyperplasia, parasites, tumors, or facilities is responsible for the inflammation of the appendix. Limited capillary blood flow due to the accumulation of intestinal secretions damages the mucosal epithelial barrier, leads to the swelling of the appendix lumen, and results in bacterial invasion into the appendix wall [22,23]. The theory of luminal obstruction is unable to explain the majority of appendicitis cases [24]. Therefore the theory of microbial overgrowth and invasion as a result of luminal obstruction needs to be more convincing. Alternatively, a primary infectious event is also suggested [25]. Our data suggest that granular depletion of Paneth cells can lead to acute appendicitis by causing mucosal damage. In our opinion this is a very new data and can be considered as a new theory for the pathogenesis of acute appendicitis.

The differentiated epithelial cell types, including goblet cells, enteroendocrine cells, cluster cells, enterocytes, and Paneth cells, show the complex structure of the human intestine [26,27]. The Paneth cell, situated at the crypt's base, has many granules in its cytoplasm that are made up of growth factors like epidermal growth factor, transforming growth factor-α, and Wnt ligands as antimicrobial peptides like defensins and lysozyme. These components act against microorganisms and regulate the gut microbiota and the stem cells. Pathologies that affect the Paneth cells may compromise the function of immune system and the preservation of the stem cell niche. Crohn's disease, necrotizing enterocolitis, and graft-versus-host disease cause Paneth cells to apoptosis and release their peptides into the crypt lumen, causing a diseased organ vulnerable to infections and dysbiosis. Intestinal cell renewal is additionally slowed down [28].

Changes in the granule morphogy in the appendix tissue of the AA group suggests that intestinal mucosal invasion by pathogens due to decreased Paneth cell secretion, may contribute to the development of appendicitis [29]. Loss of Paneth cells could disrupt the stem cells, which are essential for intestinal epithelial renewal and development [30]. In this study, the Paneth cell count in zone 1 for the appendicitis group was significantly lower than that in the control group ($p < 0.001$, see Table 2). This suggests that the intestinal microbiota is impaired, and the mucosal defense is compromised in the AA group. In the AA group, Paneth cell granules' density decreased significantly compared to the control group ($p < 0.001$, see Table 3).

The limitation of the study was that the control group was small due to the low number of patients with appendectomies who were evaluated to have normal histopathology.

Conclusion

To explain the cause of compromised immunity and mucosal inflammation, we discussed the histopathological features of Paneth cells in appendicitis. Paneth cells are essential in preventing intestinal inflammation with their bactericidal effects and protective properties for intestinal flora. The decrease in Paneth cells in the appendix mucosa may lead to appendicitis by causing proliferation of pathogens in the lumen. Although there is a bulk of research about Paneth cells in the literature, further research is needed to reveal its relationship with microbiota and acute appendicitis.

Ethical approval

The study was started after the approval of Malatya Turgut Ozal University, Faculty of Medicine Non-Invasive Ethics Committee (Date: 10.01.2023, Decision No: 2023/3).

Declaration of conflicting interests

The authors declare that they have no conflict of interest.

Financial disclosure

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