ORIGINAL ARTICLE

CONTENTS

Morphological and functional features of the colonic mucus barrier in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis

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ABSTRACT

Aim: The purpose was to identify the morphological and functional features of the colonic mucus barrier in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

Materials and Methods: In the research, three groups were formed. Group 1 included fragments of the mucous membrane of the large intestine, which were collected from 12 people during autopsies. The results of autopsies and histological examination of the material did not reveal any gastrointestinal pathology. Group 2 included biopsies of the mucous membrane of the large intestine from the area of the diverticulum of 34 patients with symptomatic uncomplicated diverticular disease. Group 3 included biopsies of the mucous membrane of the large intestine of 26 patients with acute uncomplicated diverticulitis. Histological (hematoxylin and eosin staining), histochemical (PAS reaction) and immunohistochemical (mouse monoclonal antibodies to Mucin 2 (MUC2) and Mucin 4 (MUC4)) staining methods were used. A morphometric study was also carried out.

Results: In patients with diverticular disease, the authors identified disturbances in the morphofunctional state of the mucus barrier of the colon, the structure and function of goblet cells contained in its mucous membrane, characterized by a decrease in the thickness of the mucus layer covering the surface of the mucous membrane; a decrease in the size and number of goblet cells with a decrease in their mucus-producing ability; a change in the mucin profile, characterized by a violation of the content of MUC2 and MUC4. These changes were greatest in patients with acute uncomplicated diverticulitis compared with patients with symptomatic uncomplicated diverticular disease.

Conclusions: The identified disturbances in the morphofunctional state of the mucus barrier of the colon, structural and functional changes in goblet cells may be one of the mechanisms for the development of acute uncomplicated diverticulitis and symptomatic uncomplicated diverticular disease.

KEY WORDS: colonic mucus barrier, symptomatic uncomplicated diverticular disease, acute uncomplicated diverticulitis, morphological and functional features

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INTRODUCTION

The large intestinal epithelia are covered by a mucus layer which consists of about 30 core proteins, including mucins, antimicrobial peptides, secreted immunoglobulin A [1, 2]. It is a viscous gel-like layer, forming inner firm and outer loose mucus layers, moreover, the latter accounts for more than 80% of the thickness [3-5]. This layer has a primary role in intestinal protection against mechanical, chemical, biological attacks and contributes to the maintenance of intestinal homeostasis [6].

Among the intestinal mucus layer components, mucins are the most important [1]. Goblet cells are single-cell glands that produce and secrete mucins. It is known that mucin is a high-molecular-weight glycoprotein, the synthesis process of which includes dimerization in the endoplasmic reticulum, Golgi glycosylation, and finally oligomerization [7]. There are currently 22 known mucins which named from MUC1 through MUC22 [8]. All mucins are divided into membrane-associated (MUC1, MUC3A/B, MUC4, MUC12, MUC13, MUC15, MUC17, MUC20, MUC21), which perform a protective function and take part in intracellular signal transmission, and secretory. Secretory mucins are categorized into two subclasses: gel-forming and nongelforming mucins. MUC2, MUC5AC, MUC5B, MUC6 and MUC19 are gel-forming mucins involved in protection, transportation, lubrication, and hydration, and MUC7, MUC8, MUC9 are nongel-forming mucins [8, 9]. MUC1, MUC2, MUC3 and MUC4 are the predominant mucins detected in the large intestine mucous membrane [10].

The lesion, abnormal proliferation and differentiation of goblet cells, as well as the deficiency synthesis and

secretion of mucins, result in intestinal mucosal barrier dysfunction [11]. The latter is an important determinant of intestinal diseases (inflammatory bowel disease, irritable bowel syndrome, colorectal cancer), disorder in extraintestinal organs [8].

Diverticular disease is a common, gradually progressive chronic gastrointestinal condition with increasing prevalence which manifests in early adulthood and progresses with advancing age with respect to its anatomical extent and diverticula size [12, 13]. Disturbances in the morphological and functional state of the colon mucus layer may be one of the mechanisms for the development of various variants of diverticular disease. There is no information in the literature about the morphological features of the colon mucus barrier in patients with diverticular disease, which makes this study relevant.

AIM

The purpose was to identify the morphological and functional features of the colonic mucus barrier in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

MATERIALS AND METHODS

In the research, three groups were formed. Group 1 included fragments of the mucous membrane of the large intestine, which were collected from 12 people during autopsies on the basis of the Pathology Department of the Municipal Non-Profit Enterprise of the Kyiv Regional Council «Kyiv Regional Clinical Hospital». The results of autopsies and histological examination of the material did not reveal any gastrointestinal pathology. Group 2 included biopsies of the mucous membrane of the large intestine from the area of the diverticulum of 34 patients with symptomatic uncomplicated diverticular disease. Group 3 included biopsies of the mucous membrane of the large intestine of 26 patients with acute uncomplicated diverticulitis. Patients of groups 2 and 3 were treated in the Gastroenterology Department of the Feofaniya Clinical Hospital of the State Administration of Affairs for the period from 2019 to 2022.

Biopsies of the colon mucosa were fixed in a 10% solution of neutral buffered formalin (pH 7.4) for 24-48 hours. After fixation, the material was processed according to the standard method in an Excelsior AS apparatus (Thermo Fisher Scientific, UK), embedded in paraffin blocks on a HistoStar apparatus (Thermo Fisher Scientific, UK), from which serial histological sections with a thickness of 2-3 μm were prepared. The latter were stained with hematoxylin and eosin. PAS reaction was performed.

An immunohistochemical study was performed on Super Frost Plus adhesive slides (Menzel, Germany). For high-temperature processing of epitopes of antigens, citrate buffer with pH 6 and EDTA buffer (pH 8) were used. The UltraVision Quanto HRP detection system, DAB Quanto chromogen manufactured by Thermo Fisher Scientific (USA) was used. Immunohistochemical study was performed using mouse monoclonal antibodies to MUC2 (clone Ccp58, Master Diagnostica, Spain), MUC4 (clone 8G7, Master Diagnostica, Spain).

Hematoxylin and eosin-stained microscope specimens were examined using a ZEISS Primostar 3 microscope (Carl Zeiss, Germany) with a built-in digital color camera, a BRESSER Science TFM-301 Trino microscope with a BRESSER Full HD camera (Bresser GmbH, Germany).

Using the Labscope program, a morphometric study was carried out, during which in groups 1-3 it was determined the thickness of the layer of PAS-positive secret located above the surface epithelium of the colon mucosa in the field of view of the microscope ×400; the absolute number of goblet cells in the intestinal glands in the field of view of the microscope ×200. The severity of the PAS reaction in goblet cells of the intestinal glands, the expression of MUC2 and MUC4 in the epithelium of the intestinal glands and surface epithelium were assessed in the field of view of the microscope ×400 by determining the brightness coefficient in the Lab color model using the computer program "Analysis of color properties of raster images" [14, 15].

Indicators in groups 1-3 were processed statistically using the PAST program (version 4.15, Natural History Museum, University of Oslo, Norway). Average values of indicators in groups were compared using the Student's t-test and the Mann-Whitney U-test. Differences of indicators were considered significant at $p<0.05$.

RESULTS

When performing a PAS reaction it was noted in all groups in the mucous membrane of the colon a PAS-positive secret located in the form of a layer above the surface epithelium; in goblet cells of the surface epithelium and intestinal glands of varying degrees of severity.

In group 1, a uniformly expressed layer of PAS-positive secretion was determined over the surface epithelium (Fig. 1), the average thickness of which was (12.08±0.59) μm (Fig. 2). In group 2 and, especially, in group 3, an unevenly expressed layer of PAS-positive secretion was detected above the surface epithelium of the mucous membrane. In group 3, in the part of the visual fields where erosive and ulcerative changes were determined, this layer was not identified. The average thickness of

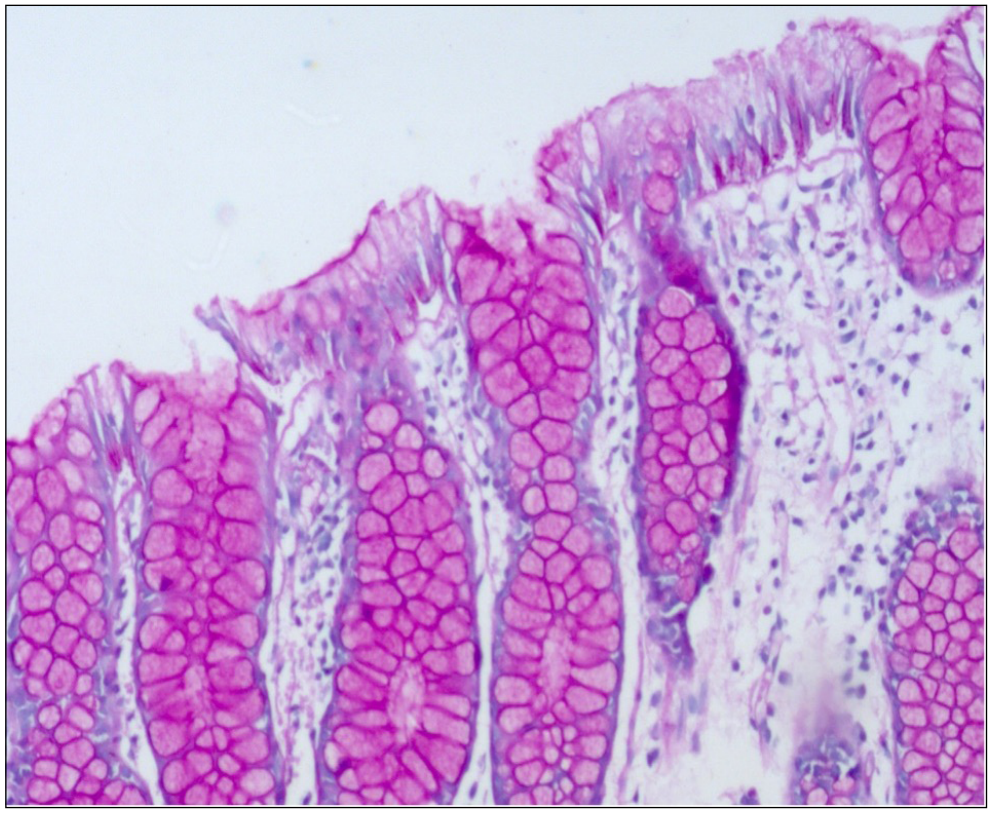

Fig. 1. Group 1. A uniformly expressed layer of PAS-positive secretion over the surface epithelium. PAS-positive reaction in goblet cells of the colon mucosa in the form of their uniform staining. PAS reaction, × 100.

this layer in group 3 was significantly ($p<0.05$) smaller than in group 2 (Fig. 2). The thickness of the PAS-positive secretion layer in groups 2 and 3 was significantly ($p<0.05$) smaller than the corresponding indicator in group 1.

During the subsequent analysis of the PAS reaction, the morphofunctional state of goblet cells in groups 1-3 was assessed. In all groups, it was identified a pattern of distribution of goblet cells in the surface epithelium and epithelium of the intestinal glands. Thus, in the epithelium of the intestinal glands the content of goblet cells was high.

In group 1, in the surface epithelium and intestinal glands, goblet cells were characterized by the presence of one or two vacuoles filled with evenly distributed PAS-positive secretion, the average brightness coefficient of which was 0.77±0.005 (Fig. 3). This secretion was also detected in the lumen of the intestinal glands. The average absolute number of goblet cells in the intestinal glands was 43.83±1.03 (Fig. 4).

In group 2, the number of goblet cells in the intestinal glands was significantly ($p<0.05$) lower compared to group 1 (Fig. 4). Also, compared with group 1, goblet cells in group 2 were characterized by smaller sizes and the content of a larger number of small vacuoles. The latter contained PAS-positive secretion or granules of varying intensity of staining. The average value of the brightness coefficient in the vacuoles of goblet cells was significantly ($p<0.05$) higher compared to group 1, which indicated a decrease in the ability of these cells to mucus produce (Fig. 3).

In group 3, compared to group 1, goblet cells were smaller and contained several small vacuoles (Fig. 5). The latter contained PAS-positive secretion or granules. When performing the PAS reaction, only the contours of the vacuoles were stained in most fields of view. In such goblet cells, survey microscopy revealed atrophic and degenerative changes of varying severity. The average value of the brightness coefficient in the vacuoles of goblet cells was significantly ($p<0.05$) greater than the corresponding indicators of groups 1 and 2, which indicated inhibition of mucus secretion by these cells (Fig. 3). In group 3, the average value of the absolute number of goblet cells in the intestinal glands was significantly ($p<0.05$) lower compared to the corresponding indicators of groups 1 and 2 (Fig. 4).

In groups 1-3, an immunohistochemical reaction with monoclonal antibodies to MUC2 and MUC4 revealed their membrane expression in the surface epithelium and epithelium of the intestinal glands. In group 1, the expression of MUC2 and MUC4 was uniformly expressed (Fig. 6). In group 2 and, especially, in group 3, uneven and reduced expression of these monoclonal antibodies was determined (Fig. 7).

The results of determining the brightness coefficient in immunohistochemical reactions with monoclonal antibodies to MUC2 and MUC4 are shown in Table 1. The table shows that the indicators of groups 2 and

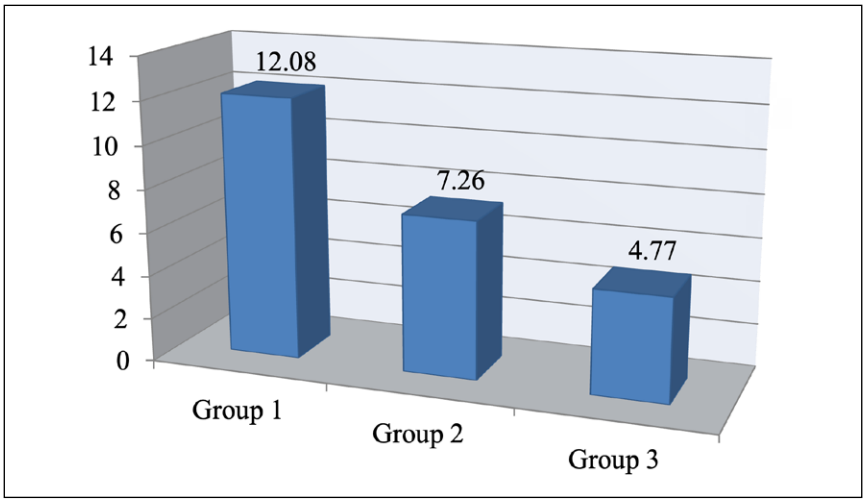

Fig. 2. Average values of the thickness of the PAS-positive secretion layer on the surface of the colon mucosa in groups 1-3.

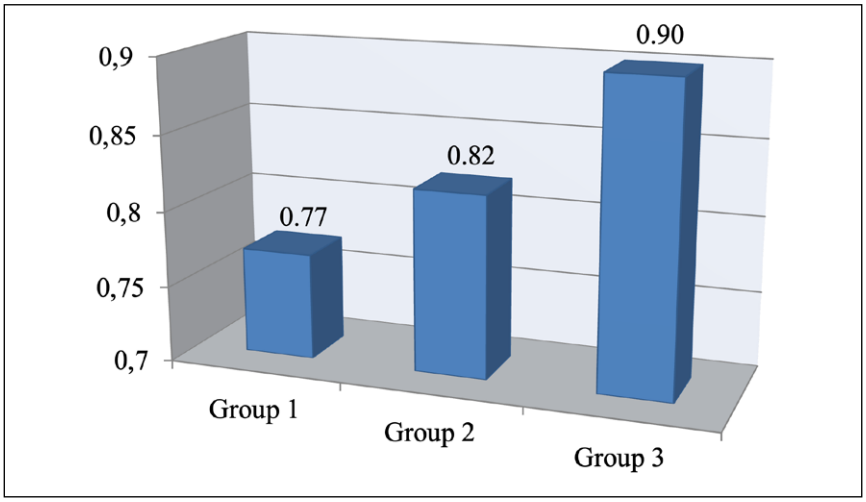

Fig. 3. Average values of the brightness coefficient of PAS-positive secretion in goblet cells in groups 1-3.

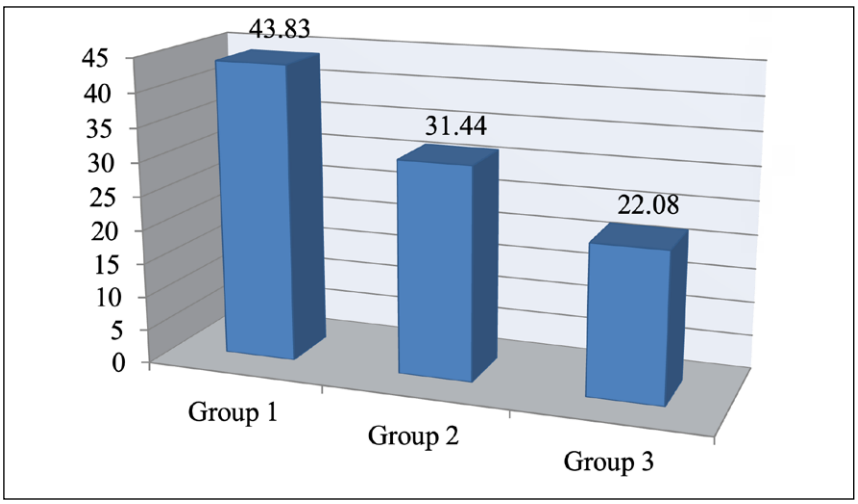

Fig. 4. Average values of the absolute number of goblet cells in the intestinal glands in groups 1-3.

3 were significantly ($p<0.05$) increased compared to group 1, which indicated a decrease in the expression of these monoclonal antibodies. Moreover, the most pronounced decrease in the expression of MUC2 and MUC4 was observed in group 3 compared to group 2.

It is interesting that in groups 1 and 2 the brightness coefficient took a significantly ($p<0.05$) lower value in the reaction with MUC4 compared to the reaction with MUC2, which indicated a higher content of MUC4. In group 3, no such dependence was detected, which indicated an equal content of such mucins in the mucus.

DISCUSSION

In a comprehensive morphological study conducted by the authors, for the first time, disturbances in the morphofunctional state of the mucus barrier and goblet cells in the colon in patients with symptomatic uncom-

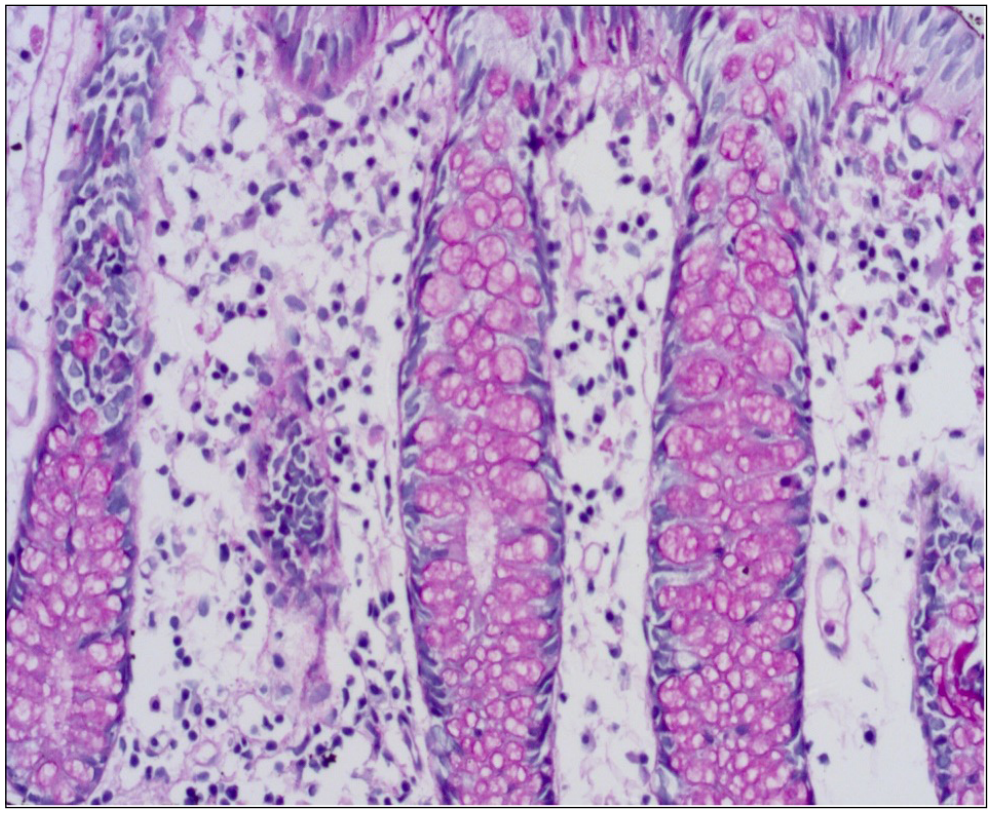

Fig. 5. Group 3. Secretions or granules in goblet cells of varying intensity of staining. Staining in part of the goblet cells only the outline of the vacuoles. PAS reaction, × 200.

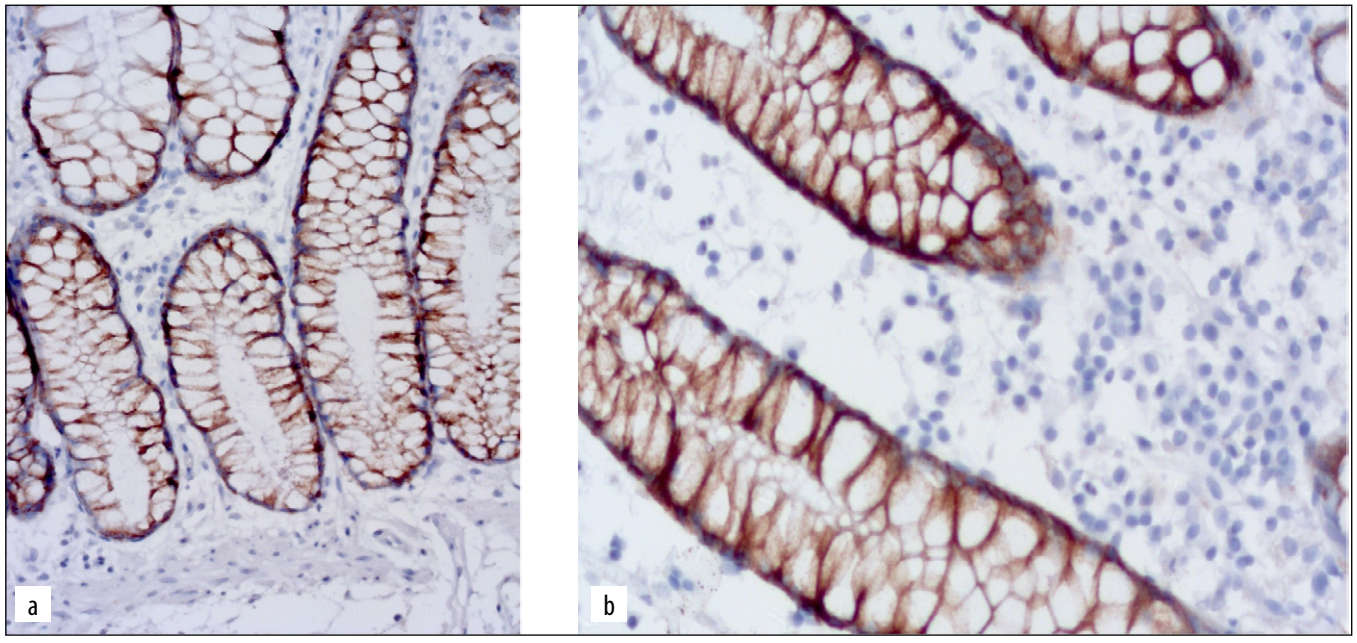

Fig. 6. Group 1. Uniform expression of MUC2 (a) and MUC4 (b) in the colon mucosa. Immunohistochemical reaction with monoclonal antibodies to MUC2 and MUC4, a) ×200, b) ×200.

plicated diverticular disease and acute uncomplicated diverticulitis were identified and analyzed. In patients with symptomatic uncomplicated diverticular disease and, especially, in patients with acute uncomplicated diverticulitis, the identified disorders were characterized by a decrease in the thickness of the mucus layer covering the surface of the colon mucosa; a decrease in the number of goblet cells, characterized by reduced size and containing several small vacuoles; decreased mucus production by goblet cells; decrease in the content of MUC2 and MUC4 in mucus. Patients with symptomatic uncomplicated diverticular disease showed a predominance of MUC4 in mucus, and patients with acute uncomplicated diverticulitis showed equal levels of MUC2 and MUC4.

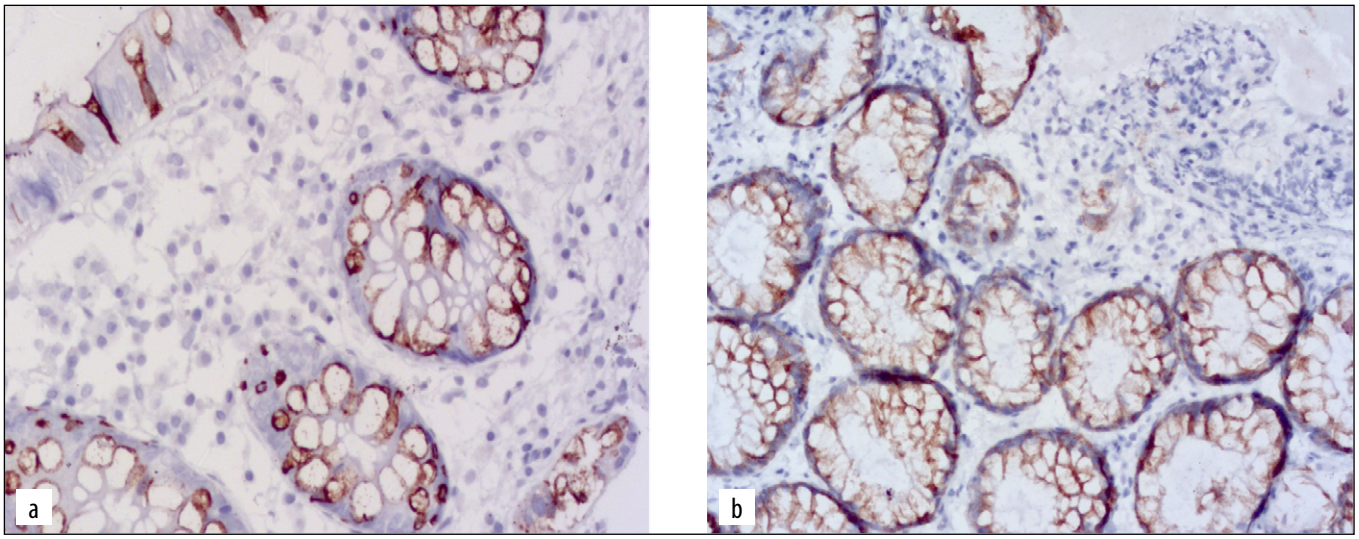

Fig. 7. Reduced and unevenly expressed expression of MUC2 (a) in group 2 and MUC4 (b) in group 3 in the colon mucosa. Immunohistochemical reaction with monoclonal antibodies to MUC2 and MUC4, a) ×200, b) ×200.

Table 1. Average brightness coefficient values in groups 1-3 in immunohistochemical reactions with monoclonal antibodies to MUC2 and MUC4

Reaction name	Group number		
	1	2	3
Reaction with monoclonal antibody to MUC2	0.42±0.007	0.49±0.006 [1]	0.57±0.009 [1,2]
Reaction with monoclonal antibody to MUC4	0.39±0.012 [3]	0.45±0.004 [1,3]	0.57±0.008 [1,2]

Note: 1 – significance of differences compared to the indicator of group 1; 2 – significance of differences compared to the indicator of group 2; 3 – significance of differences compared to the indicator in reaction with monoclonal antibody to MUC2.

The intestinal mucus gel layer is an integral structural component of the intestine used for protection, lubrication, and transport between the luminal contents and the epithelial cells [16]. The violations identified by the authors in the morphofunctional state of the mucus layer of the colon and goblet cells contained in the mucous membrane and producing mucus may underlie in the development of symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

Changes in goblet cell functions and in the chemical composition of intestinal mucus are detected in response to a broad range of luminal insults, including alterations of the normal microbiota [17]. Our previous studies revealed changes in the composition of the microbiota in the intestines in patients with symptomatic uncomplicated diverticular disease [18].

CONCLUSIONS

In patients with diverticular disease, the authors identified disturbances in the morphofunctional state of the mucus barrier of the colon, the structure and function of goblet cells contained in its mucous membrane, characterized by a decrease in the thickness of the mucus layer covering the surface of the mucous membrane; a decrease in the size and number of goblet cells with a decrease in their mucus-producing ability; a change in the mucin profile, characterized by a violation of the content of MUC2 and MUC4. These changes were greatest in patients with acute uncomplicated diverticulitis compared with patients with symptomatic uncomplicated diverticular disease. The identified disturbances in the morphofunctional state of the mucus barrier of the colon, structural and functional changes in goblet cells may be one of the mechanisms for the development of acute uncomplicated diverticulitis and symptomatic uncomplicated diverticular disease.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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