

A quantitative morphometric study of rectal mucosa in adult and aged healthy subjects

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Summary. Rectal mucosa is relatively susceptible to pathological processes and frequently it is affected by various diseases. However, there is a notable lack of quantitative data regarding normal rectal mucosa, which would provide a reference for histoquantitative studies of the pathologically changed tissue. Therefore, we obtained the tissue from 27 healthy patients subjected to diagnostic rectoscopy during active screening for asymptomatic cancer of the large intestine, in which no disease was found. Using computer-aided morphometric analysis, we studied all structural elements of the rectal mucosa. The patients were divided into four groups according to the age and sex: adult males, elderly males, adult females and elderly females. The patients under 60 years of age were grouped as adult and those older than 60 years as aged subjects. A decreased height of surface epithelium was registered in both elderly male and female groups. This finding, however, was significant only when adult and elderly male groups were compared. The tendency towards reduction of the mucosal height was also registered comparing male adult and elderly groups. The number of crypts per 0.1 mm² of tissue increased with aging in both males and females, whereby the crypts were always more numerous in males than in females. The increase in number of crypts in male subjects was accompanied by a decrease in their diameter and perimeter. The changes associated with ageing were discrete and affected only the male subjects.

Key words: Morphometry, Rectal mucosa, Age, Gender, Human

Introduction

Rectal mucosa is relatively susceptible to pathological processes and frequently it is affected by autoimmune and malignant diseases (Liu and Crawford, 2005). Fortunately, it is easily accessible to diagnostic procedures – it can be inspected using rectoscopy and a biopsy can be taken. However, in contrast to the wealth of data on pathologically affected tissue, there is a notable lack of information regarding the normal structure of this tissue. Especially notable is the lack of quantitative data regarding the normal rectal mucosa, which would provide reference for future histoquantitative studies of the pathologically changed tissue. Therefore, we believed that it would be useful to obtain the specimens of healthy tissue from subjects showing no diseases involving the rectal mucosa, and study all of its structural elements using computer-aided morphometric analysis. Furthermore, it is well known that in many tissues and organs the structural changes occur during ageing (for example, Lakatta et al., 1987), which are further influenced by sex-related factors (for example, Olivetti et al., 1995; Forman et al., 1997). Thus, we felt that the effects of gender and ageing on the histoquantitative parameters of the healthy rectal mucosa should be studied using morphometric methods. Our study showed that the delicate changes occurred in normal rectal mucosa during ageing and affected only the male subjects.

Materials and methods

Tissue samples

Tissue samples were collected using rectoscopy from 27 healthy patients during the active screening procedure for the patients possibly suffering from as yet unidentified, asymptomatic cancer of the large intestine, in which no disease involving the rectal mucosa was

found. The material was collected at the Center for Gastroenterology and Hepatology, Zvezdara Clinical Center, Belgrade, Serbia. The study has been approved by the Ethics Committee of Zvezdara Clinical Center, Belgrade, Serbia (03/11/2003) and performed in accordance with ethical standards laid down in the 1964 Declaration of Helsinki. All persons involved received detailed verbal information and gave their informed consent prior to their inclusion in the study. The patients were divided into four groups according to age and sex: adult males (n=8; mean age=42.2 years); elderly males (n=5; mean age=72.6 years); adult females (n=6; mean age=50.2 years) and elderly females (n=8; mean age=72.0 years). The patients under 60 years of age were grouped as adult and those older than 60 years comprised the group of aged subjects.

Tissue preparation and morphometric measurements

The biopsies of the rectal mucosa were fixed in neutral buffered formalin and processed to Paraplast. Tissue sections, 3-5 μm thick, were stained with hematoxylin-eosin and used for morphometric assessment. The sections were inspected under Opton Photomicroscope III (Carl Zeiss AG, Oberkochen, Germany). The microphotographs acquired with an Olympus C3030-Z digital camera (Olympus Deutschland GmbH, Hamburg, Germany) were used for morphometric measurements. Computer-aided image analysis software Analysis 3.1 (Soft Imaging System GmbH, Münster, Germany) was used. All tissue elements of the rectal mucosa were measured. The height of the rectal mucosa (in μm) between the lamina muscularis mucosae and surface epithelium was measured using tissue samples with strictly longitudinally sectioned Lieberkühn crypts (Fig. 1) at 100x magnification. The number of Lieberkühn crypts (per 0.1 mm^2 of tissue) was measured at 100x magnification. The height of the surface epithelium and parameters of the crypts (in μm) were measured at 250x magnification. Only tissue samples with strictly cross-sectioned crypts (Fig. 2) were measured to estimate the following parameters: crypt diameter, crypt perimeter and the height of the crypt epithelium (in μm).

Tissue elements of the rectal mucosa were studied using the following methods: Mallory's trichrome staining for demonstration of collagen fibers; Weigert's method for demonstration of elastic fibers; Gomori's method for silver impregnation of reticular fibers; Periodic-acid Schiff (PAS) and mucicarmine staining for demonstration of mucus content in goblet cells, according to Bancroft and Stevens (1982).

Statistical analysis

The statistical package SPSS for Windows 12.0 (SPSS Inc., Chicago, IL, USA) was used to calculate the means and standard deviations, as well as to indicate

significant differences (Mann-Whitney *U*-test and Student's *T*-test at $P < 0.05$).

Results

The tissue elements of rectal mucosa were well developed in all groups of patients (Figs. 1,2), which enabled easy distinction and precise measuring of all selected parameters. Very rarely a mild infiltration with lymphocytes was registered (Fig. 3) and only infrequently a solitary lymphoid follicle of moderate size

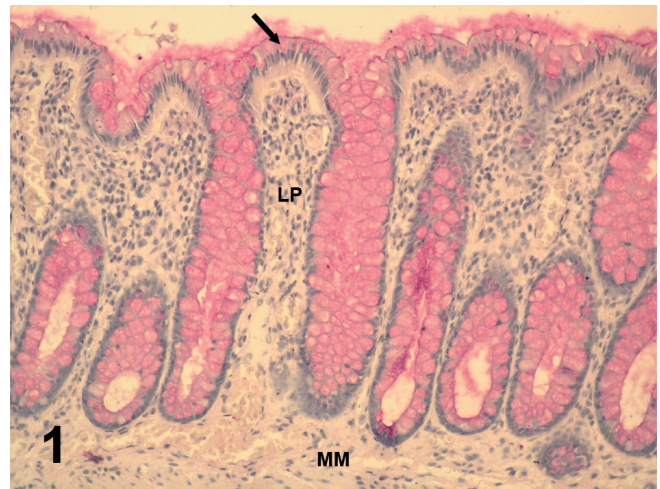


Fig. 1. Longitudinal section of the rectal mucosa. Adult (46 years), female healthy subject. MM: lamina muscularis mucosae; LP: lamina propria; arrow: surface epithelium. Mucicarmine staining. x 100

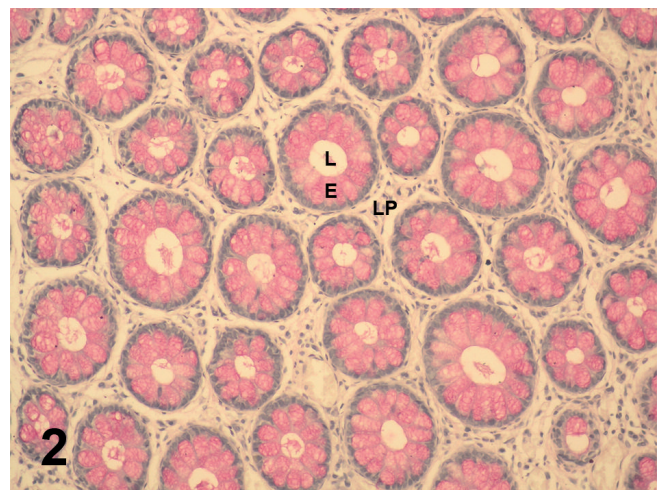


Fig. 2. Transverse section of the rectal mucosa. Adult (44 years), male healthy subject. L: lumen of the crypt; E: crypt epithelium; LP: lamina propria. Mucicarmine staining. x 100

Morphometry of human rectal mucosa

Table 1. Histoquantitative parameters of the rectal mucosa in adult and aged healthy subjects of both sexes.

Group ^a	Mucosal height (μm)	Surface epithelium height (μm)	Number of crypts (per 0.1 mm ²)	Diameter of crypts (μm)	Perimeter of crypts (μm)	Crypt epithelium height (μm)
Adult male	561.3±129.6	47.6±10.8	10.2±3.6	77.7±16.4	268.7±52.6	26.5±5.3
Elderly male	388.3±132.4	38.9±3.1 ^b	13.5±5.7	72.3±6.1	250.9±21.3	26.4±1.4
Adult female	459.8±79.3	45.9±11.1	8.2±2.0	77.7±9.2	287.5±21.1	28.8±1.6
Elderly female	649.3±222.4	41.3±9.2	10.2±2.6	76.1±10.6	271.0±34.7	26.8±5.1

^an: 5–8; ^b: statistically significant difference between adult and elderly male subjects (P<0.05).

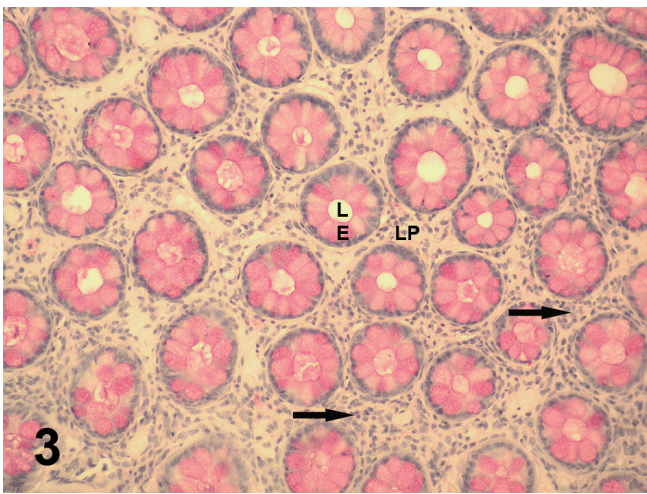


Fig. 3. Transverse section of the rectal mucosa. Elderly (76 years), male healthy subject, with mild infiltration of lamina propria with lymphocytes (arrows). L: lumen of the crypt; E: crypt epithelium; LP: lamina propria. Mucicarmin staining. x 100

could be seen (not shown).

A decreased height of surface epithelium was registered in both elderly male and female groups (Table 1). However, this finding was statistically significant only when the comparison was made between adult and elderly male groups. The tendency towards reduction of the mucosal height was also registered comparing male adult and elderly groups, but this finding was not significant (Table 1). The number of crypts per 0.1 mm² of tissue increased with aging in both males and females, whereby the crypts were always more numerous in males than in females (Table 1). The increase in the number of crypts in male subjects was accompanied by a decrease in their diameter and perimeter (Table 1; Fig. 3), which was not obvious in females. Only rarely, these changes were associated with mild lymphocyte infiltration of the connective tissue (compare Figs. 2 and 3). However, an accompanying decrease in height of the crypt epithelium was not observed (Table 1).

Discussion

Rectal mucosa is relatively susceptible to pathological processes and frequently affected by autoimmune and malignant diseases (Liu and Crawford, 2005). Still, the morphometric data on the human rectal mucosa are very infrequent, although the rare available studies of the human colon indicate that morphometry may be used successfully for separation of healthy, benign and malignant growths in adenomatous tissue (Kayser et al., 1985) or detection of mild abnormalities which otherwise may be overlooked (Salzmann et al., 1989). This induced us to perform a detailed histoquantitative analysis of the human rectal mucosa in healthy subjects. As it is well known that gender and age may affect the structural features and morphometric parameters of tissues and organs (for example, Lakatta et al., 1987; Olivetti et al., 1995; Forman et al., 1997), we formed the appropriate groups of patients that enabled us to investigate the influence of these factors.

Our data obtained in adult subjects may be compared with some rare morphometric data on the normal human rectal mucosa from persons of similar age: the diameter of crypts in the rectal mucosa (71.5 μm) obtained from healthy volunteers (Richter et al., 1993) is in accordance with that recorded in our study.

Our data obtained in elderly subjects are in good keeping with the results of earlier studies (Milošević, 1990), which were performed in persons of comparable age: mucosal height (428.0 μm) and surface epithelium height (36.3 μm).

It is generally accepted that inflammation is present if the number of cells in the lamina propria is increased (Lee et al., 1988). In our study we did not estimate the cellular density of lamina propria, but a mild increase in cell number and connective tissue fibers was evident in the groups of elderly subjects.

In this study no morphometric differences were found in biopsies of the rectal mucosa between the groups of adult and elderly females. This is in contrast to the data obtained in elderly males where the decrease in mucosal height, surface epithelium height, diameter and perimeter of crypts were observed.

Our study, which encompasses all elements of the normal human rectal mucosa, provides valuable

morphometric reference for future histoquantitative studies of this tissue under pathological conditions.

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