Colon Mucosal Parameters in Apparently Healthy Adult

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Abstract

The objectives of the study were to determine age related changes in colon mucosal parameters in apparently healthy adult. The study also investigated the correlation between the colon mucosal parameters in different age group. A total of 48 subjects from which 65 samples were collected participated in the study. The tissue sample of study was biopsy materials of five different parts of the human colon taken from archive of Minsk Consultative-Diagnostic Center, Republic of Belarus. The samples were stained with Harris' haematoxylin and subjected to morphometric analysis using Leica application suite program and ImageJ. Age group comparison of colon mucosal parameters of apparently healthy adults showed that there is significant difference between the stromal and epithelial area. It was observed that in both the stromal and epithelial area the significant differences lies between 18-50 age group and 51-61 age group, 62-70 age group and 51-61 age group. Correlation analysis of the mucosal parameters according to age showed that stromal area negatively correlates significantly (r> 0.6, P <0.001) with epithelial to stromal area ratio in all the age groups. The epithelia area on the other hand strongly and moderately correlated with epithelial to stromal area ratio only in 18-50 and 51 to 61 age groups respectively. However, for the 62 to 70 age group the significant correlation was observed with general number of inflammatory cell. In conclusion, none linear age related changes were observed in the mucosal parameters. Some intra colonic parameter correlation was also observed, however, this show age dependency.

Keywords: Age dependency, colon mucosa, histology, morphometric analyses.

Introduction

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. Histologically, the surface and the crypt of the colon mucosa are lined by a simple epithelium made up of columnar cells. Colonic glands are regularly spaced and straight cells, their base approximately touching the lower limit of the mucosa, the muscularis mucosae. The supportive stroma called lamina propria (connective tissue frame work). It extends from the sub-epithelial basement membrane to the muscularis mucosae

which forms boundary between the mucosal and the submucosal layers (Lewin *et al.,* 1992; Levine and Haggitt, 1997).

Subjective and morphometic analyses are two methods commonly used in assessment of the mucosal integrity. Morphometric analysis was considered to be more superior then the subjective analysis. This is simply due to the fact that morphometric analyses are quantitative assessments which give higher level of repeatability and reliability compared to the subjective assessment. The eye piece graticule are often used for simple quantitative methods that involve the use of linear measurements (*Rubio and Kock, 1981;* Rubio *et al.,* 1984). However, the use of sophisticated computerized image analysis systems for the assessment of colon mucosa biopsy specimens have also gained more grounds (Thompson et *al.,* 1985; Brown *et al.,* 1985; Allen et al., 1987; Graham 1988) and more so when there is need to differentiate between different types of inflammatory bowel diseases (Thompson *et al.,* 1985).

Various disease conditions were reported to affect the mucosa of the colon, justifying it's susceptible to pathological processes (Liu and Crawford, 2005). There is a paucity of quantitative data concerning normal mucosal parameter that may be useful as a reference guide in the interpretation and understanding of histopathological changes occurring in the tissue.

In a similar attempt, previous study documented some histological changes in the rectal mucosa (*Milosevic et al., 2007*). The goal of the present work were to find the age related changes in colon mucosal parameters in apparently healthy adult and to investigate the correlation between the colon mucosal parameters in the different age groups.

Materials and Methods
Study population
A total of 48 subjects drawn from various hospitals in Minsk participated from which 65 samples were collected in the study. Participants with pathological condition affecting the colon were exempted from the research. The research was approved by Minsk Consultative-Diagnostic Center, Republic of Belarus, Ethics Committee.

Tissue samples
The tissue samples of study were biopsy materials of five different parts (ceacum, ascending, transverse, descending, sigmoid and rectum) of the human colon taken from archive of Minsk Consultative-Diagnostic Center, Republic of Belarus. In the preparation, the biopsy of patients without any pathological changes endoscopically was considered.

Tissue preparation and morphometric measurements
The biopsy materials were processed routinely for staining and sectioned at 5 micrometer thickness using a microtome. Dewaxed sections were rinsed using alcohol and then in water. Tissues were stained with Harris" haematoxylin stain for 10 minutes then washed in running tap water for 1 minute, differentiated in acid alcohol for 10 seconds, washed in running tap water for 5 minutes and stained with Eosin for 4 minutes. Slides were washed in running tap water for 10 seconds dehydrated with increasing concentrations of alcohol, cleared, mounted and viewed under microscope *(Leica DM2500, Germany).* using micro photo camera Leica DFC425, each of these groups were subjected to morphometric analysis using the Leica application suite program and ImageJ software.

Statistical Analyses

The data was represented using descriptive statistics (mean ± standard deviation, range, confidence interval and median). The Shapiro Wilki test indicated not normally distributed data (P < 0.05). Kruskal Wallis test, Dunn's multiple comparisons and Benferoni correction was used to determine the differences in colon mucosal parameters between the age groups. Pearson's correlation was employed to establish the relationship of colon mucosal parameters for each age group. SPSS version 20 (IBM Corporation, Armonk, NY) was used for the statistical analyses.

RESULTS

Figure 1 show a section of the transverse colon mucosa of apparently healthy adult (57 years). The tissue parameters (Such as Crypt length, stromal area epithelial area,no of inflamatory cell in stroma and in the epithelia)of the mucosa were well developed. The surface epithelia (SE) showing downward growth into the supporting lamina propria (LP) to form the crept, lined with crypt epithelia (CE) were clearly demonstrated. The lumens (L) of the crypt were also prominent.
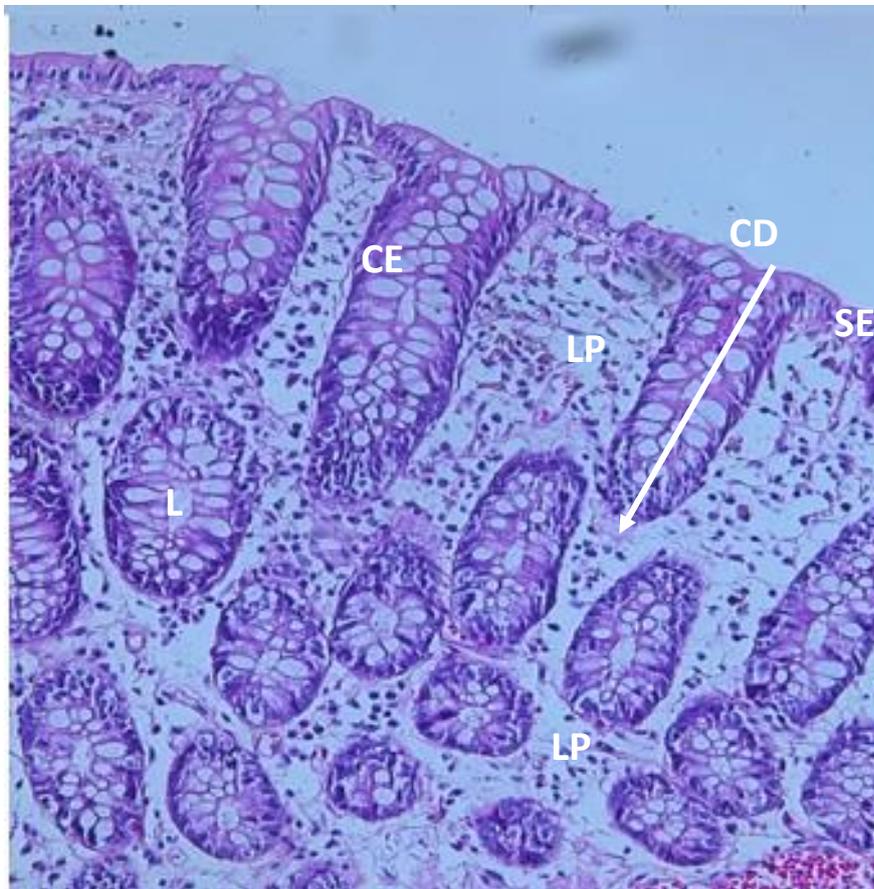

Figure 1: section of the transverse colon mucosa of apparently healthy adult (57years). LP: lamina propria, SE: surface epithelium, L: lumen of the crypt; CE: crypt epithelium; crypt depth [H & E staining. x 200]

Significant changes were observed between age groups 18-50 and 51-61 years as well as between ages 51-61 and 62-70 years, but this was not presented histologically in the results for comparison. All age groups should be presented so as to see the significant and insignificant changes observed.

Table 1 shows descriptive statistics of the colon mucosal parameters according to age groups. Changes in the mean values of the mucosal parameters with ages were observed. A non linear

change in mean values characterized the mucosal parameters, except for epithelial to stromal area ratio which increased steadily with age.

Table 1: Descriptive statistics of the colon mucosal parameters according to age groups

Variables	Age Groups	Mean	Standard deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower	Upper		
Crypt Depth	18-50 (n=20)	1220.65	1307.106	608.91	1832.39	322	6578
	51-61 (n=14)	789.64	291.92	621.09	958.19	387	1240
	62-70 (n=31)	992.26	334.881	869.42	1115.09	413	1637
Stromal Area (mm²)	18-50 (n=20)	0.027	0.004	0.0251	0.0289	0.0184	0.033
	51-61 (n=14)	0.0326	0.0054	0.0295	0.0357	0.0223	0.0436
	62-70 (n=30)	0.027	0.0060	0.0235	0.029	0.0143	0.0429
Epithelial area (mm²)	18-50 (n=20)	0.0261	0.0054	0.0235	0.0286	0.0167	0.0357
	51-61 (n=14)	0.0321	0.0056	0.0289	0.0353	0.0253	0.0438
	62-70 (n=31)	0.0282	0.0081	0.0252	0.0312	0.0095	0.058
Epithelial to stromal area ratio	18-50 (n=20)	0.9909	0.2737	0.8628	1.119	0.5542	1.5816
	51-61 (n=14)	1.0068	0.2232	0.878	1.1357	0.6006	1.3426
	62-70 (n=30)	1.06201	0.3877	0.4806	2.7596	0.58	2.6623
General number of cells	18-50 (n=20)	447.9	112.411	395.29	500.51	287	675
	51-61 (n=14)	467.46	68.861	425.85	509.07	339	639
	62-70 (n=31)	449.71	146.926	395.82	503.6	235	822

From Figure 2 age group comparison of colon mucosal parameters of apparently healthy adults showed that there was statistically significant difference (P < 0.05) in the stromal area (Fig. 2B) and epithelial area (Fig. 2C) with respect to age groups. It was observed that in both the stromal and epithelial areas the significant differences lies between 18-50 age and 51-61 age groups, 62-70 age and 51-61 age groups.
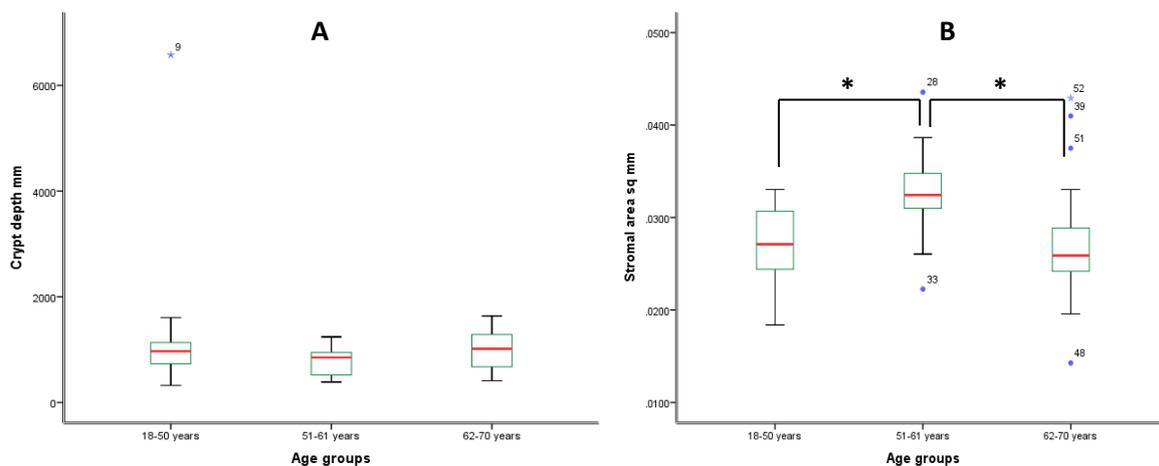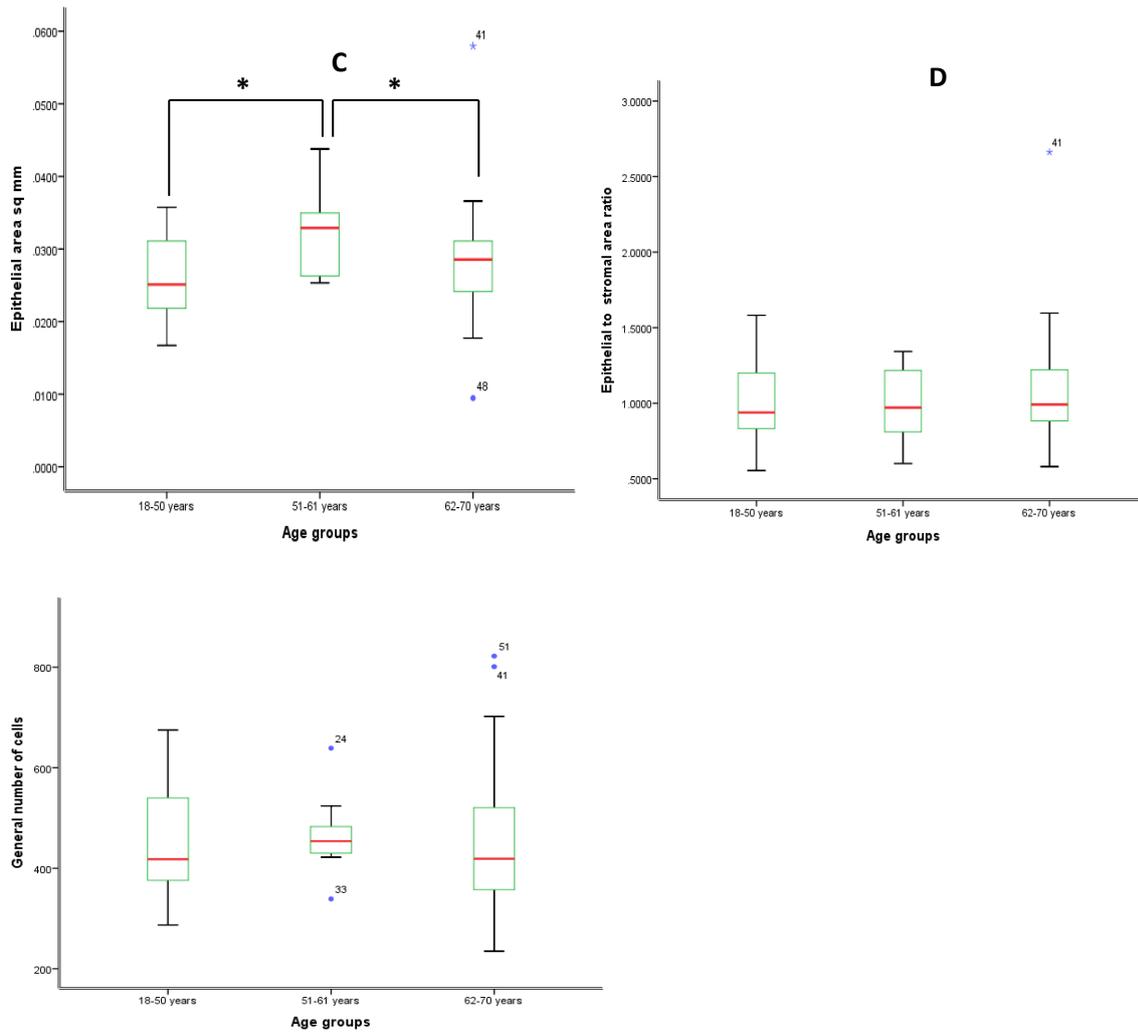
Figure 3: Age group comparison of colon mucosal parameters of apparently healthy adults. * P < 0.05

Correlation analyses of the mucosal parameters according to age groups (Table 2) showed a significant negative correlation between the stromal area and epithelial to stromal ratio in all the age groups. The epithelia area on the other hand strongly and moderately correlated with epithelial to stromal area ratio only in 18-50 and 51 to 61 age groups respectively. However, for the 62 to 70 age group significant correlation was observed with respect to the general number of cells.

Table 2: Pearson's correlation matrix of colon mucosal parameters according to age groups

Age Group (years)		Stromal Area (mm2)	Epithelial area (mm2)	Epithelial to stromal ratio	General Number of cell
18-50	Crypt Depth	0.248	0.406	0.125	0.295
	Area of epithelial stromal (mm²)	1	-0.057	-0.647**	0.372
	Epithelial area (mm²)		1	0.781**	0.195
	Epithelial to stromal ratio			1	-0.074
51-61	Crypt Depth	-0.343	0.129	0.448	-0.563*
	Area of epithelial stromal (mm²)	1	0.068	-0.676**	0.150
	Epithelial area (mm²)		1	0.666**	-0.091
	Epithelial to stromal ratio			1	-0.219
62-70	Crypt Depth	0.161	0.180	-0.180	-0.096
	Area of epithelial stromal (mm²)	1	0.009	-0.637**	0.179
	Epithelial area (mm²)		1	0.277	0.373*
	Epithelial to stromal ratio			1	-0.038

*p <0.05, **p <0.01

Discussion

Age has long been considered as a confounding variable that may alter the morphological architecture of tissues and organs (Lakatta et al., 1987; Olivetti et al., 1995; Forman et al., 1997). The main objectives of this study were to determine the age-related changes in colon mucosal parameters in apparently healthy adults. The study also investigated the correlation between the colon mucosal parameters in the different age groups.

The significant differences in the stromal and epthelial areas with respect to age groups indicate the significance of age as it relates to changes in mucosal parameters. In support to the present finding it was reported that there is decrease in mucosal parameters (crypt depth, area of epithelial stromal, epithelial area) in elderly males with no such difference among elderly females (Milosevic´ et al., 2007). Since it is a known fact that risk of colorectal cancer increases with age; for males and females an age of 68 years and 72 years respectively were considered as the median age for colon cancer diagnosis (Howlader et al., 2016). It may therefore be suggested that an increase in the quantitative mucosal parameter may be considered as a risk factor associated with the occurrence of colorectal cancer. However, there is need to define the age limit at which the colon parameter will reach their peak before starting to decrease. This may require population specific research for ruling out the effect of ethnicity amongst others.

The present study seems to highlight the influence of age on the mucosal parameters. This provides the need of careful interpretation in the pathological changes that may occur in the mucosal. To streamline the changes in the mucosal parameters Jenkins et al. (1988) graded the changes, the increase in lamina propria cellularity was considered as the most powerful discriminate. It was also reported that 50% decrease in the height and area of surface epithelium and that of crypt cell height ratio were recorded (Zaitoun et al., 1991). Difference in mucosal parameters was previously reported in the literature, for example it demonstrated

that control subjects were different from the untreated patient population (Zaitoun et al., 1991).

Interestingly, none linear changes observed in the present study may indicate the re-emphasize the important of age as factor that need to be put into consideration when interpreting pathological changes. However, it is important to note that some of the structures were not affected by age, therefore any significant morphometric changes may be in favor of pathological changes. Correlation analyses of the mucosal parameters according to age groups shows with correlation between the mucosal parameters. It was documented that correlation analyses of different findings assessed by quantitative morphometry may be valuable as reported in the previous studies (Powell-Tuck et al., 1982; Floren et al., 1987; Riley et al., 1988; Mulder et al., 1988). Also, based on histological criteria for this comparison, no significant correlation was reported (Zaitoun et al., 1991). However, the correlation between parameters observed in the current study may indicate the dependency of other colon parameter on one another.

It is important to note that the quantitative assessment of mucosa of the colon using computerized methods resulted to more accurate differentiation between normal and abnormal mucosa of the colon (Thompson et al., 1985). This was suggested to be useful in differentiation normal colonic mucosa from adenoma and adenocarcinoma (Graham et al., 1988). Morphometric analysis was also reported to be used to differentiate other pathological conditions (Allen et al., 1987; Allen et al., 1988). Therefore, it can stated that this study provide additional information needed in reaching conclusion about pathological condition in the colon.

Conclusion

In conclusion, there was no linear age related changes were observed in the mucosal parameters. Some age dependent intra colonic parameter correlations were also observed.

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Conflict of Interest: There is no conflict of interest

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