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PROCENA REGENERACIJE SLUZNICE DEBELOG CREVA U ULCEROZNOM KOLITISU PUTEM LINEARNIH MERENJA

EVALUATION OF LARGE INTESTINAL MUCOSA REGENERATION IN ULCERATIVE COLITIS USING LINEAR MEASUREMENTS

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Sažetak - Ulcerozni kolitis je hronična inflamatorna bolest creva, koja klinički i histološki prolazi kroz tri faze: aktivnu fazu i fazu regresije i remisije. Nakon faze aktivnosti nastaje određeni stepen atrofije sluznice debelog creva, čija je procena ponekad otežana i tada kvantifikacija pojedinih parametara sluznice može poslužiti kao pomoćna metoda. Cilj rada bio je da odredimo morfološke parametre linearne mikrometrije, koji bi poslužili u proceni regeneracije sluznice debelog creva u ulceroznom kolitisu i upotrebljivosti metode u rutinskom radu. Merenja su urađena na rutinskom bioptičkom materijalu nakon kvalitativne histološke analize i određivanja tipa i faze bolesti. Merenjem smo odredili: broj kripti na jedinicu dužine, visinu epitela kripti, dijametar kripti, lumena kripti i intersticijuma i izračunali smo količnik dijametra kripti i dijametra intersticijuma. Analizom izmerenih parametara dobija se uvid u prisustvo i stepen regeneracije i/ili atrofije sluznice, naročito praćenjem parametara na nivou epitela kripti. Linearna merenja se mogu primeniti u proceni regeneracije i atrofije u sluznici debelog creva.
Ključne reči: Ulcerozni kolitis; Biopsija; Linearni modeli; Sluznica creva; Regeneracija

Summary - *Ulcerative colitis is a chronic inflammatory condition characterized by three phases: active, regression and remission phase. The active phase is followed by atrophy of the large intestinal mucosa. Although its evaluation is sometimes difficult, quantification of ceratin mucosal parameters can be used as an accessory method. The aim of the study was to determine the parameters of linear micrometry in order to estimate the regeneration of the large intestinal mucosa in ulcerative colitis, and to evaluate the efficiacy of this method in everyday work. The measurements were performed on routine bioptic samples after qualitative histologic analysis and determination of the type and stage of the disease. The measurements were carried out to determine: the number of crypts per unit length, the height of crypt epithelium, diameter of crypts, their lumen and interstices; also, the quotient between the diameters of crypts and interstices was calculated. The analysis of the measured parameters points to presence and degree of regeneration and or atrophy of mucosa, particularly by following the parameters of crypt epithelium. Linear measurements can be used in estimation of regeneration and atrophy of large intestinal mucosa.*
Key words: *Colitis, Ulcerative; Biopsy; Linear Models; Intestinal Mucosa; Regeneration*

Uvod

Ulcerozni kolitis (UK) je inflamatorna bolest creva nepoznate etiologe. Brojni uzročnici šu okrivljeni za nastanak bolesti, a kao najverovatniji se navodi autoimuni poremećaj. Bolest se klinički i patohistološki karakteriše smenom tri faze: aktivnom fazom bolesti, fazom regresije i fazom remisije [1]. Aktiva faza UK praćena je ulceracijama na sluznici debelog creva i izraženim kliničkim tegobama. Tokom faze regresije intezitet tegoba i patohistološke promene na sluznici se smanjuju, a u fazi remisije završavaju sa više ili manje uspešnom regeneracijom. S obzirom da su matične ćelije za obnovu žlezdanog i površnog epitela smeštene u dnu kripti [2,3], regeneracija u sluznici creva podrazumeva migraciju epitelnih ćelija, ekstrakriptalnu proliferaciju ćelija i formiranje novih kripti [4]. Stepen obnove zavisi od odbrambene i funkcionalne sposobnosti sluznice debelog creva. U najboljem slučaju nastaje kompletna regeneracija sluznice i žlezdanih komponenti. Pojedini autori smataraju da posle aktivne faze u sluznici postoji barem minimalni stepen atrofije [5].

Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease of unknown etiology. Numerous factors have been suspected to cause it, the most probable being an autoimmune disorder. The disease is clinically and histopathologically characterized by three phases: active phase, regression phase, and remission phase [1]. The active phase of UC is associated with ulcerations of the large intestine mucosa and is clinically manifested by discomfort. During the regression phase, the intensity of discomfort and of histopathologic changes in the mucosa decrease and in the remission phase they disappear, with more or less successful regeneration. Since mother cells, which initiate the regeneration of the glandular and surface epithelium, are located at the bottom of crypts [2,3], mucosal regeneration is manifested by migration of the epithelial cells, extracryptal cell proliferation and formation of new crypts [4]. The degree of regeneration depends on the defensive and functional capacities of the large intestine mucosa. The best possible outcome is that mucosa and glandular compo-

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Skraćenice

UK - ulcerozni kolitis
IBD - inflamatorna bolest creva

Atrofija sluznice može biti različitog stepena (visokog, srednjeg, niskog stepena) i histološki se verifikuje kao smanjenje broja intestinalnih žlezda ili kripti, poremećaj rasporeda kripti i/ili smanjenje dubine kripti, fuzija i dilatacija kripti [6,7]. Funkcionalno se manifestuje kao poremećaj mukozne barijere i smanjenom produkcijom mucina i lokalnih imunoloških derivata [8]. U slučajevima kada atrofija sluznice postoji, pacijent nema značajne tegobe, ali je funkcija njegove sluznice fokalno smanjena srazmerno stepenu atrofije.

U bioptičkim uzorcima atrofije visokog i srednjeg stepena promene su uočljive i patolog ih može verifikovati u svom nalazu, dok se atrofije nižeg stepena teže uočavaju i njihova procena zavisi od "oka" patologa. U slučajevima niskostepenih atrofija primenom kvantifikacija može se utvrditi postojanje i stepen atrofije, kao i stepen regeneracije sluznice u različitim fazama bolesti [1,9].

Linearna mikrometrija je jedna od metoda kvantifikacije u histologiji i patohistologiji i predstavlja prenošenje klasičnih linearnih merenja i razmera iz makrosveta na mikrosvet. Linearna merenja se rade pomoću linearnog ili okularnog mikrometra, koji je okrugla staklena pločica sa ugraviranim podeocima linearnog mikrometra. Postoje različita merila, a obično se upotrebljava mikrometar od 5mm sa 50 podeoka (1 podeok = 1/10 mm). Pre svakog linearnog merenja mora se izbaždariti mikroskop, odnosno odrediti mikrometarski količnik za svaku upotrebljenu kombinaciju okulara i objektiva. Dobijeni količnik je stalan za stalnu dužinu tubusa mikroskopa i za stalne veličine okulara i objektiva. Za linearna merenja moraju se znati vrednosti mikrometraskog količnika za svako povećanje. Merenje se radi tako što se prebroji ukupan broj podeoka okularnog mikrometra, koji prepokrivaju ispitivanu fazu i dobijeni broj podeoka pomnoži sa količnikom za dati objektiv. Vrednosti se izražavaju u mm ili μm [10].

U našem radu izmereni su morfološki parametri za sluznicu debelog creva i izračunati su količnici linearne mikrometrije za normalnu sluznicu debelog creva i sluznicu creva tokom pojedinih faza ulceroznog kolitisa, sa ciljem da se odredi prisustvo atrofije u sluznici, proceni da li rezultati linearnih merenja mogu dati uvid u stepen regeneracije sluznice i proveri opravdanost upotrebe linearnih merenja u rutinskom radu.

Materijal i metode

Ispitivanje je urađeno na rutinskom bioptičkom materijalu dobijenom tokom endoskopije 20 pacijenata kod kojih je kvalitativnom patohistološkom analizom postavljena dijagnoza pojedinih faza UK i nespecifičnog kolitisa (ukupno četiri grupe). Slu-

Abbreviations

UC - ulcerative colitis
IBD - inflammatory bowel disease

nents regenerate completely. Some authors hold that after the active phase of UC, mucosa is characterized by at least some degree of atrophy [5].

The degree of mucosal atrophy may vary from high, medium to a low degree, and is histologically verified as a decreased number of intestinal glands or crypts, irregular distribution of crypts and/or reduction of the depth of crypts, their fusion and dilatation [6,7]. It is functionally demonstrated as an impairment of the mucosal barrier and a reduced production of mucine and local immunological derivates [8]. In spite of mucosal atrophy, a patient may feel no significant discomfort, but the function of his mucosa is focally decreased proportionally to the degree of atrophy.

In bioptic samples with high and medium degrees of atrophy, the changes are noticeable and verifiable, whereas low-degree atrophies are less easy to notice, and their estimation depends on a pathologist's "eye". Low atrophy, is determined by quantification of the presence and degree of atrophy, as well as the degree of mucosal regeneration in different phases of the disease [1,9].

Linear micrometry is a quantification method in histology and pathology by which the classical macroscopic linear measurements and proportions are turned into microscopic ones. Linear measurements are carried out by means of a linear or ocular micrometer, which is a small, round glass plate with engraved grades. Among various micrometers, the most frequently used is the 5 mm long, with 50 grades (1 grade = 1/10 mm). Before each measurement, a microscope must be checked and set, i.e. the quotient for each combination of the ocular and objective must be determined. The obtained quotient is permanent for the constant length of the microscope tube and for the constant dimensions of ocular and objective. Linear measurements require precise values of the micrometer quotient for each magnification. The measurement consists of counting the total number of ocular micrometer grades of the investigated area, and of multiplication of the obtained number with the quotient of the corresponding objective. The values are expressed in mm or μm [10].

In this paper morphologic parameters of the large intestine mucosa were measured and quotients of linear micrometry for normal large intestine mucosa and mucosa in some phases of UC were counted in order to determine mucosal atrophy, to estimate whether the results of linear measurements could point to the degree of mucosal regeneration, and to justify the usefulness of linear measurements in routine work.

znica debelog creva u nespecifičnom kolitisu se u kvantitativnim ispitivanjima koristi kao normalna sluznica, jer označava blagu nespecifičnu inflamaciju sluznice debelog creva, bez narušavanja opšte arhitektonike i bez atrofije žlezda.

Uzeti materijal je pripremljen standardnom histološkom tehnikom: fiksiran u 70% alkoholu, kalupljen u parafinu, rezan na isečke debljine 5μm i bojen haematoksilinom i eosinom. Na ovako pripremljenom materijalu na povećanju od 10x10, uradena su linearna merenja sa linearnim mikrometrom sa 100 podeoka, na dužini od 1000μm lamine muskularis mukoze. Merenje je urađeno na 4 vidna polja u dva nesusedna histološka reza. Sve linearne vrednosti su izražene u mikronmetrima.

U sve četiri grupe odredili smo: dijametar celokupne kripte, visinu epitela kripti, dijametar lumena kripti, dijametar intersticijuma i ukupan broj kripti na navedenoj dužini lamine muskularis mukoze i izračunali smo količnik dijametra kripti i dijametra intersticijuma.

Rezultati

a) Srednja vrednost dijametara kripti

Srednja vrednost dijametara kripti u normalnoj sluznici (Dkn) debelog creva je Dkn=(66,50 ± 2,98) μm;

Srednja vrednost dijametara kripti u aktivnoj fazi (Dka) bolesti je Dka=(69,46 ± 4,56)μm;

Srednja vrednost dijametara kripti u fazi regresije (Dkrg) je Dkrg=(86,96 ± 8,39)μm;

Srednja vrednost dijametara kripti u fazi remisije (Dkr) je Dkr=(75,32 ± 6,70)μm.

Srednja vrednost dijametara kripti u fazi aktivnosti brojčano se povećava u odnosu na normalnu sluznicu (razlika nije statistički značajna), da bi u fazi regresije dostigao maksimalnu vrednost (statistički značajna razlika vrednosti između aktivne faze i faze regresije bolesti, na nivou $p<0,01$). U fazi remisije vrednost dijametra kripti se u odnosu na fazu regresije smanjuje (statistički značajna razlika na nivou $p<0,05$), ali ostaje veća u odnosu na aktivnu fazu bolesti i normalnu sluznicu, pri čemu je razlika statistički značajna samo u odnosu na normalnu sluznicu debelog creva.

b) Srednja vrednost visine epitela kripti

Srednja vrednost visine epitela kripti u normalnoj sluznici (Dekn) debelog creva je Dekn=(44.50 ±3,63)μm;

Srednja vrednost visine epitela kripti u aktivnoj fazi (Deka) bolesti je Deka=(44,10±3,85)μm;

Srednja vrednost visine epitela kripti u fazi regresije (Dekrg) je Dekrg=(55,85±5,15)μm;

Srednja vrednost visine epitela kripti u fazi remisije (Dekr) je Dekr=(52,96±2,80)μm.

Srednja vrednost visine epitela kripti u fazi aktivnosti povećava se u odnosu na normalnu sluznicu (nema statističke značajnosti), da bi u fazi regresije dostigla maksimalnu vrednost (statistički značajna razlika vrednosti visine epitela u aktivnoj fazi i fazi

Material and methods

The investigation was carried out on routine bioptic material obtained during endoscopy from 20 patients in whom qualitative histopathologic analyses led to diagnoses of ulcerative colitis and nonspecific colitis (a total of four groups). Large intestinal mucosa in nonspecific colitis is regarded as normal in quantitative investigations, because it presents with a mild nonspecific inflammation of the large intestine, without any impairment of the structure and without glandular atrophy.

The biopsy samples were investigated with standard histological technique: fixation in 70% ethyl alcohol, paraffin embedment, cutting into sections of 5 μm, staining with Haematoxyline and Eosine. This material, with the magnification of 10x10, was linearly measured with a linear micrometer of 100 grades, at the length of 1000 μm of the mucosal lamina muscularis. The measurement was performed in four sight fields in two non-neighbouring histologic sections. All linear values are expressed in micrometers.

In all four groups, we determined the diameter of the whole crypt, the diameter of crypt lumen, the diameter of interstice, as well as the total number of crypts along the mucosal lamina muscularis. We also counted the quotient of the crypt diameter and the interstice diameter.

Results

a) Mean crypt diameter

The mean crypt diameter in normal mucosa (Dcn) of large intestine was Dcn=(66.50±2.98) μm

The mean crypt diameter in the active phase (Dca) was Dca=(69.46±4.56) μm

The mean crypt diameter in regression phase (Dcrg) was Dcrg=(86.96±8.39) μm

The mean crypt diameter in remission phase (Dcr) was Dcr=(75.32 ±6.70) μm

The mean crypt diameter in the active phase of the disease increased compared with normal mucosa (the difference was statistically insignificant), reaching the maximum value in the regression phase (statistically significant difference between the value in the active phase and the value in regression, $p<0.01$). In remission, the crypt diameter decreased in comparison with the regression phase (statistically significant difference, $p<0.05$), but it remained higher in comparison with the active phase of the disease and with the normal mucosa, the difference being statistically significant only in respect to the normal large intestine mucosa.

b) Mean value of the epithelial crypt height

The mean value of the epithelial crypt height in normal mucosa (Decn) of large intestine was Decn=(44.50±3.63) μm

The mean value of the epithelial crypt height in the active phase (Deca) of the disease was Deca=(44.10±3.85) μm

Tabela 1. Srednje vrednosti dijametara linearnih merenja izraženih u μm (X = SE) za normalnu sluznicu debelog creva i sluznicu creva u svim fazama ulceroznog kolitisa

Table 1. *Mean diameter values using linear measurments expressed in μm (X = SE) for normal large bowel mucosa and for large bowel mucosa in all phases of ulcerative colitis*

	Dk(μm)	Dek(μm)	Dl(μm)	Di(μm)	Dk/Di
Normalna	66,50	44,50	12,66	33,32	2,00
Normal	±2,98	± 3,63	± 3,18	± 2,55	± 0,18
Aktivnost	69,46	44,10	24,40	113,68	0,70
Active	±4,56	± 3,85	± 4,62	± 9,78	± 0,124
Regresija	86,96	55,85	31,10	106,88	0,94
Regression	± 8,39	± 5,15	± 6,10	± 12,36	± 0,154
Remisija	75,32	52,96	17,52	77,83	1,10
Remission	± 6,70	± 2,80	± 2,73	± 8,00	± 0,157

regresije na nivou p<0,01). U fazi remisije srednja vrednost visine epitela kripti neznatno je manja u odnosu na isti parametar u fazi regresije (nema statističke značajnosti) i statistički značajno veća u odnosu na srednje vrednosti visine epitela u aktivnoj fazi i u normalnoj sluznici creva (p<0,01).

c) Srednja vrednost dijametara lumena kripti

Srednja vrednost dijametara lumena kripti u normalnoj sluznici (Dln) debelog creva je Dln= (12,66 ±3,18)μm;

Srednja vrednost dijametara lumena kripti u aktivnoj (Dla) fazi bolesti je Dla=(24,40±4,62)μm;

Srednja vrednost dijametara lumena kripti u fazi regresije (Dlrg) je Dlrg=(31,10±6,10)μm;

Srednja vrednost dijametara lumena kripti u fazi remisije (Dlr) je Dlr=(17,52±2,73)μm.

Srednja vrednost dijametara lumena kripti u fazi aktivnosti povećava se u odnosu na normalnu sluznicu (dvostruko povećanje vrednosti), da bi u fazi regresije dostigao maksimalnu vrednost. U fazi remisije srednja vrednost dijametra lumena kripti u odnosu na aktivnu fazu i fazu regresije se smanjuje, ali ostaje veća u odnosu na normalnu sluznicu debelog creva.

Razlike u parametrima su statistički značajne za p<0,01, izuzev razlike srednjih vrednosti dijametara lumena u aktivnoj fazi i fazi regresije.

d) Srednja vrednost dijametara intersticijuma

Srednja vrednost dijametara intersticijuma u normalnoj sluznici (Din) debelog creva je Din= (33,32±2,55)μm;

Srednja vrednost dijametara intersticijuma u aktivnoj fazi (Dia) bolesti je Dia=(113,68±9,78)μm;

Srednja vrednost dijametara intersticijuma u fazi regresije (Dirg) je Dirg=(106.88±12,36)μm;

Srednja vrednost dijametara intersticijuma u fazi remisije (Dir) je Dir=(77,83±8.00)μm.

Srednja vrednost dijametara intersticijuma u fazi aktivnosti bolesti ima maksimalne vrednosti i oko 3,5 puta je veća u odnosu na isti parametar za normalnu sluznicu creva. U fazi regresije dijametar intersticijuma se lako smanjuje u odnosu na aktivnu fazu, ali je i dalje oko 3 puta veći u odnosu na normalnu sluznicu debelog creva.

The mean value of the epithelial crypt height in regression (Decrg) was Decgr=(55.85±5.15) μm

The mean value of the epithelial crypt height in remission (Dekr) was Dekr=(52.96±2.80) μm

The mean value of the epithelial crypt height in the active phase of the disease increased in comparison with normal mucosa (without statistical significance), but in regression it reached the maximum value (statistically significant difference between the value in the active phase and the value in regression, p<0.01). In remission, the mean value of the epithelial crypt height was slightly lower compared with the same parameter in regression (without statistical significance), but it was statistically significantly higher in comparison with the mean values of epithelial height in the active phase and in normal large intestine mucosa (p<0.01).

c) Mean value of crypt lumen diameters

The mean value of crypt lumen diameters in normal mucosa (Dln) of large intestine was Dln=(12.66 ±3.18) μm

The mean value of crypt lumen diameters in the active phase (Dla) of the disease was Dla=(24.40± 4.62) μm

The mean value of crypt lumen diameters in regression (Dlrg) was Dlrg=(31.10 ±6.10) μm

The mean value of crypt lumen diameters in remission (Dir) was Dir+(17.52±2.73) μm

The mean value of crypt lumen diameters in the active phase increased in comparison with normal mucosa (twofold increase), and it reached its maximum in regression. In remission, the mean value of crypt lumen diameters decreased, compared with the values in the active and regression phases, but it remained higher than that in the normal large intestine mucosa. Differences in parameters were statistically significant (p<0.01), except for the difference between the mean values of lumen diameters in the active and regression phases.

d) Mean value of interstice diameters

The mean value of interstice diameters in normal large intestine mucosa (Din) was Din=(33.32±2.55) μm

The mean value of interstice diameters in the active phase (Dia) of the disease was Dia= (113.68±9.78) μm

The mean value of interstice diameters in regression (Dirg) was Dirg=(106.88±12.36) μm

The mean value of interstice diameters in remission (Dir) was Dir=(77.83±8.00) μm.

The mean value of interstice diameters reached its peak in the active phase of the disease, and it was 3.5 times higher than it is in normal large intestine mucosa. In regression, interstice diameter slightly decreased in comparison with that in the active phase, but it was still three times longer comaperd with the normal large intestine mucosa. In remission, the mean value of interstice diameters decreased (about 1.5 times lower than in the active and regresion phases), but it remained about 2 times

U fazi remisije srednja vrednost dijametra intersticijuma se smanjuje (oko 1,5 puta je manja u odnosu na aktivnu fazu i fazu regresije), ali ostaje oko 2 puta veća u odnosu na normalnu sluznicu debelog creva. Razlike u parametrima su statistički značajne za $p<0,01$, izuzev razlike srednjih vrednosti dijametara intersticijuma u aktivnoj fazi i fazi regresije.

e) Količnik srednjih vrednosti dijametara kripte i dijametra intersticijuma (Dk/Di)

Količnik srednje vrednosti dijametra kripti i intersticijuma u normalnoj sluznici debelog creva je Dkn/Din=2,00±0,179;

Količnik srednje vrednosti dijametra kripti i intersticijuma u aktivnoj fazi bolesti je Dka/Dia=0,70 ±0,124;

Količnik srednje vrednosti dijametra kripti i intersticijuma u fazi regresije je Dkrg/Dirg=0,94± 0,154;

Količnik srednje vrednosti dijametra kripti i intersticijuma u fazi remisije je Dkr/Dir=1,10±0,157.

U aktivnoj fazi bolesti količnik dijametara kripti i intersticijuma se za oko 3 puta smanjio u odnosu na isti parametar za normalnu sluznicu. U fazi regresije i remisije vrednosti količnika se povećavaju u odnosu na količnik u aktivnoj fazi, ali su 2,1, odnosno 1,8 puta manji u odnosu na količnik za normalnu sluznicu debelog creva. Razlike u parametrima su statistički značajne za $p<0,01$, izuzev razlika količnika srednjih vrednosti dijametara kripti intersticijuma u fazi regresije i remisije.

f) Broj kripti po jedinici dužine (na 1000 μm lamine muskularis ili lamine proprije sluznice)

U normalnoj sluznici debelog creva prosečan broj kripti po jedinici izmerene dužine je 11,3;

U aktivnoj fazi bolesti prosečan broj kripti po jedinici izmerene dužine je 5,4;

U fazi regresije prosečan broj kripti po jedinici izmerene dužine je 6,3;

U fazi remisije prosečan broj kripti po jedinici izmerene dužine je 9,5.

Poređenjem prosečnih vrednosti kripti po jedinici dužine normalne sluznice sa istim parametrom u pojedinim fazama bolesti i poređenjem parametara između faza pokazali su da su sve razlike statistički značajne ($p<0,01$, odnosno $p<0,05$).

Diskusija

U svakodnevnom radu patologa neophodno je da se niz detalja videnih na pločici uklopi u jednu sliku i na osnovu njih postavi dijagnoza. S obzirom da je ovo relativno subjektivna metoda, u rutinskoj histopatologiji i histologiji javila se želja za kvantifikacijom pojedinih struktura radi njihove reproducibilnosti i mogućnosti komparacije sa drugim parametrima [11]. Do danas kvantifikacija u rutinskom radu u patohistologiji nije zaživela, jer zahteva dodatno vreme i angažovanost, te se uglavnom primenjuje u eksperimentalnim i laboratorijskim uslovima.

Kod inflamatorne bolesti creva (IBD) su često patohistološke promene jasne, ali problem nastaje u

higher than in the normal large intestine mucosa. Differences were statistically significant ($p<0.01$), except for the difference in the mean values of interstice diameters in the active and regression phases.

e) Quotient of mean values of crypt diameters and interstice diameter (Dk/Di)

The quotient of mean values of crypt diameters and interstice diameter in normal large intestine mucosa was Dcn/Din=2.00±0.179;

The quotient of mean values of crypt diameters and interstice diameter in the active phase of the disease was Dca/Dia=0.70±0.124;

The quotient of mean values of crypt diameters and interstice diameter in regression was Derg/Dirg=0.94±0.154;

The quotient of mean values of crypt diameters and interstice diameter in regression was Der/Dir=1.10±0.157.

In the active phase of the disease, the quotient of crypt diameters and interstice diameter decreased 3 times in comparison with normal mucosa. In regression and remission, the quotient rose comparing with the active phase, but they were 2.1 and 1.8 times lower than the quotient in the normal large intestine mucosa. Differences were statistically significant ($p<0.01$), except for the differences in quotients of mean values of crypt interstice diameters in regression and remission.

f) Number of crypts per unit length (per 1000 μm of the mucosal lamina muscularus or lamina propria)

In the normal large intestine mucosa, the average number of crypts per unit length was 11.3;

In the active phase of the disease, the average number of crypts per unit length was 5.4;

In regression, the average number of crypts per unit length was 6.3;

In remission, the average number of crypts per unit length was 9.5.

The comparison of average values of crypts per unit length in the normal mucosa with the same parameters in certain phases of the disease, as well as comparison of parameters in different phases, has revealed that all the differences were statistically significant ($p<0.01$ and $p<0.05$).

Discussion

In everyday routine work of a pathologist, it is necessary that all details visible in a glass plate fit into a picture which will point to a diagnosis. Since this is a relatively subjective method, in routine histopathology and histology, there has emerged a need to quantify some structures for the purpose of their reproducibility and comparison with other parameters [11]. However, the quantification method has not been introduced in histopathologic routine work so far, because it requires extra time and engagement, so it is applied mostly in experimental and laboratory conditions.

graničnim slučajevima ili slučajevima sa minimalnim promenama, jer se tada kvalitativno IBD teško razlikuje od ostalih tipova akutnih kolitisa [12]. U navedenim slučajevima kvantifikacija i poređenje sa parametrima za normalnu sluznicu može pomoći u postavljanju tačnije dijagnoze [9,13-15].

U histopatologiji i histologiji kvantifikacija se može uraditi linearnim i/ili stereološkim merenjima.

Tokom merenja treba primeniti jedan konzistentan pristup u mestu uzimanja biopsije, obradi materijala, orjentaciji preseka, preciznoj definiciji šta se meri i uvesti standardizaciju procedure merenja [16-18].

U našem radu primenili smo metodu linearne mikrometrije, koja se smatra lakšom i pristupačnijom, zahtevajući manje vremena, ali koja je po nekim autorima, manje pouzdana u odnosu na stereološka merenja, jer daje uvid u dve dimenzije.

Analizom pojedinačnih linearnih parametara kroz pojedine faze bolesti dobili smo paradoksalne rezultate u odnosu na očekivane i u odnosu na kvalitativnu patohistološku analizu. Tokom bolesti, kvalitativnom patohistološkom analizom utvrđeno je da postoji smanjeni broj žlezda, a vrednosti dijametra kripti pokazuju porast vrednosti u odnosu na normalne, te se stiče utisak da se žlezdana komponenta povećala umesto da se smanji. Vrednosti visine epitela kripti pokazuje istu paradoksalnu dinamiku promena kao i dijametar kripti. Dijametar intersticijuma, pokazuje smanjeni dijametar u aktivnoj fazi i fazi regresije, da bi se u fazi remisije povećao, ali ne i dostigao vrednosti za normalnu sluznicu. Dijametar lumena kripti je pokazivao neočekivane maksimalne vrednosti u fazi remisije.

S obzirom na navedene paradoksalne vrednosti navedenih parametara, uveli smo dodatni parametar- broj kripti na jedinicu površine, koji je jasno pokazao da u određenom prostoru broj kripti tokom bolesti opada i da se njihov broj u fazi remisije ne vraća na referentne vrednosti.

Zaključivši da pojedinačni parametri ne daju uvid u stanje sluznice tokom bolesti, korelirali smo nekoliko parametara zajedno i uveli smo količnik dijametra kripti sa dijametrom intersticijuma. U prvom aktu korelirali smo dijametar kripti i intersticujuma i visinu epitela u odnosu na ukupan broj kripti unutar faza bolesti i tada smo dobili rezultate koju su ukazivali da atrofija sluznice postoji (smanjenje broja kriptiatrofija, koja je praćena dilatacijom celokupne kripte). Interesantan parametar je visina epitela kripti. Maksimalne vrednosti smo dobili u fazi regresije, da bi se u fazi remisije vrednosti smanjile, ali su vrednosti znatno veće u odnosu na referentne. Ovo povećanje vrednosti visine epitela u odnosu na normalne vrednosti mogu se objasniti prisustvom regeneratornog tipa epitela (ćelija u deobi i ćelije sa intezivnom sintezom povećavaju svoju visinu) ili sa druge strane kompenzatornom hipertrofijom epitela.

Takođe smo poredili srednje vrednosti dijametara lumena i visinu epitela kripti u odnosu na srednju

In inflammatory bowel disease (IBD), histopathologic changes are often clear, but problems arise in boundary cases or in cases with minimal changes, when IBD qualitatively hardly differs from other types of acute colitis [12]. In these circumstances, quantification and comparison with normal mucosa parameters can be helpful in making a more precise diagnosis [9,13-15].

In histopathology and histology, quantification can be carried out using linear and/or stereologic measurement.

Measurements should follow a consistent approach in regard to the place of biopsy, preparation of material, orientation of the cross section, precise definition, and a standardization of measurement should be introduced [16-18].

In our study, we applied linear micrometry, which is considered to be easier and more convenient, less time-consuming, but which is, after some authors, less reliable in comparison with stereologic measurement because it gives an insight into two dimensions only.

Analyzing certain linear parameters in some phases of the disease, we obtained paradoxical results with respect to the expected ones and to the qualitative histopathologic analysis. Qualitative histopathologic analysis showed that, during the disease, the number of glands decreased, whereas the crypt diameters increased in comparison with normal ones, so that one can get an impression that the glandular component increased instead of declined. Heights of crypt epithelia showed the same paradoxical dynamics of changes as crypt diameters. Interstice diameters decreased in the active and regression phases, but failed to reach the value of normal mucosa. Diameters of crypt lumina had unexpected maximum values in the remission phase.

Due to paradoxical values of mentioned parameters, we introduced an additional parameter - the number of crypts per unit surface, which clearly showed that in a certain area the number of crypts decreased during the disease and did not regain the referential values in remission.

Since individual parameters do not point to the quality of mucosa in the course of the disease, we correlated several parameters and introduced the measurement of the quotient of crypt diameter and interstice diameter. First we correlated the diameters of crypts and interstice and the epithelial height in relation to the total number of crypts in some phases of disease, and at that point we obtained the results indicating atrophy (decrease in the number of crypts - atrophy, followed by dilatation of the whole crypt). The height of crypt epithelium is an interesting parameter. The maximum values were obtained in regression; they decreased in remission, but were significantly higher in comparison with the referential ones. This increase in epithelial height in comparison with normal values can be explained by presence of regenerative epithelium (cells in mitosis and cells with intensive synthesis increase their heights) or by a compensated hypertophy of the epithelium.

vrednost dijametra kripti. Navedeni međuodnos parametara ukazivao je na prisustvo i stepen regeneracije epitela i žlezda.

Navedeni parametri, analizirani na ovaj način dali su potpuniju sliku šta se dešava sa celokupnom sluznicom i kriptama tokom pojedinih faza bolesti. Iz dobijenih rezultata se može dokazati prisustvo atrofije (dilatacija kripti tj. povećane vrednosti dijametra lumena u fazi remisije i smanjene vrednosti dijametra intersticijuma) kao i prisustvo i stepen regeneracije u sluznici (povećane vrednosti visine epitela u fazi remisije).

Dobijeni rezultati pokazuju da linearna merenja daju dobar uvid u tok i ishod ulceroznog kolitisa i da u procenu obavezno treba uključiti veći broj parametara, odnosno pratiti međuodnos parametara. Slično zapažanje izneo je Rubio u svom radu i predložio je skoring sistem na više izmerenih parametara. Od svih korištenih parametara smatrao je da su najbolji parametri rastojanje između kripti (u našem radu diametar intersticijuma) i broj kripti na određenoj površini (u našem radu broj kripti po jedinici dužine) [19].

Zaključak

Linearna merenja dijametara kripti, njihovih epitela i lumena, dijametar intersticijuma, kao i međuodnosi navedenih parametara, daju dobar uvid u regeneraciju sluznice i omogućuju lakše praćenje regeneratornih promena, posebno na nivou epitela.

We also compared the mean values of lumen diameters and the height of crypt epithelium with the mean value of crypt diameter. The interrelationship of these parameters pointed to the presence and degree of epithelial and glandular regeneration.

Mentioned parameters, thus analyzed, shed more light on the processes taking place in the whole mucosa and crypts during some phases of the disease. The obtained findings can point to the presence of atrophy (dilatation of crypts, i.e. increased values of lumen diameters in the remission phase and decreased values of interstice diameters), as well as to the degree of the mucosal regeneration (increased values of the epithelial heights in remission).

Our results show that linear measurements may give a good insight into the course and outcome of ulcerative colitis and that they should necessarily include a considerable number of parameters, as well as analysis of their interrelationship. Similarly, Rubio has proposed a scoring system for several parameters. He holds that the best parameters are the distance between crypts (interstice diameter in our work) and the number of crypts in a certain area (the number of crypts per unit length in our work) [19].

Conclusion

Linear measurements of crypt diameters, their epithelia and lumina, their interstice diameters, as well as interrelationship of these parameters, offer a valid insight into regeneration of the large intestine mucosa and facilitate the estimation of regenerative changes, particularly in the epithelium.

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