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ORIGINAL ARTICLE

MORPHOMETRIC ANALYSIS OF SUBMUCOSAL AND MYENTERIC NERVE PLEXUSES IN CHRONIC INFLAMMATORY BOWEL DISEASE IN CHILDREN

MORFOMETRIJSKA ANALIZA SUBMUKOZNIH I MIJENTERIČKIH NERVNIH PLEKSUSA U HRONIČNOJ INFLAMATORNOJ BOLESTI CREVA KOD DECE

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Abstract

Introduction: Chronic inflammatory bowel diseases (IBD) include ulcerative colitis (UC) and Crohn's disease (CD), characterized by relapses and an insufficiently clarified etiology. The enteric nervous system (ENS) consists of neuronal cells distributed along the gastrointestinal tract, organized into the submucosal Meissner's plexus and myenteric Auerbach's plexus. The glial cell index (GCI) represents the ratio of the number of glial cells to ganglion cells within the ganglia of the ENS.
Aim: The aim of this study is a morphometric analysis of the ENS in intestinal samples from surgically treated pediatric patients with CD and UC.
Material and methods: Histological slides from the archives of the Institute of Pathology, Faculty of Medicine, University of Belgrade, were analyzed. In the study groups of UC and CD, there were five participants in each group, while the control group included four participants. The study included an analysis of intestinal wall sections from pediatric patients with IBD (experimental group) and those with uncomplicated familial adenomatous polyposis syndrome (control group). Using the ImageJ software, five microphotographs of submucosal and myenteric nerve plexuses stained with Masson trichrome and captured with an Olympus DP70 digital camera at x400 magnification were analyzed.
Results: A significant difference in the GCI value in the myenteric plexus of the colon was observed between the experimental and control groups. No significant difference in the GCI value in the submucosal plexus of the colon was found between the two groups. A minimal difference in the GCI value within the experimental group was observed in the submucosal plexus sections.
Conclusion: This study presents a unique analysis of glial cells in CD, UC, and the control group in the submucosal and myenteric plexuses. The results indicate a disruption of GCI in the ENS in IBD, which likely represents the morphological substrate for intestinal motility disorders in these conditions.

Keywords:
Crohn's disease,
ulcerative colitis,
morphometry,
enteric nervous system

The authors declare no conflicts of interest.

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Sažetak

Uvod: Hronična inflamatorna bolest creva (IBD) obuhvata ulcerozni kolitis (UC) i Kronovu bolest (CD), koje karakterišu relapsi i nedovoljno razjašnjena etiologija. Enterički nervni sistem (ENS) sastoji se od nervnih ćelija raspoređenih duž gastrointestinalnog trakta, organizovanih u submukozni Majsnerov i mijenterički Auerbahov pleksus. Indeks glijalnih ćelija (GCI) predstavlja odnos broja glijalnih i ganglijskih ćelija unutar ganglija ENS-a.
Cilj: Cilj ovog rada je morfometrijska analiza ENS-a u uzorcima creva hirurški lečenih pedijatrijskih pacijenata od CD i UC.
Materijal i metode: Analizirani su histološki preparati iz arhive Instituta za patologiju Medicinskog fakulteta Univerziteta u Beogradu. U ispitivanim grupama, UC i CD, bilo je po pet ispitanika, dok je kontrolna grupa obuhvatila četiri ispitanika. Studija je obuhvatila analizu preseka zida creva pedijatrijskih pacijenata sa IBD-om (eksperimentalna grupa) i pacijenata sa nekomplikovanim porodičnim adenomatoznim polipoznim sindromom (kontrolna grupa). Pomoću softvera *ImageJ* analizirano je pet mikrofotografija submukoznog i mijenterickog nervnog pleksusa, obojenih Mason-trihromnim bojenjem, snimljenih digitalnom kamerom *Olympus DP70* na velikom mikroskopskom uvećanju (x400).
Rezultati: Uočena je značajna razlika u vrednosti GCI u mijenterickom pleksusu debelog creva između eksperimentalne i kontrolne grupe. Nije primećena značajna razlika u vrednosti GCI u submukoznom pleksusu debelog creva između ove dve grupe. Minimalna razlika u vrednosti GCI unutar eksperimentalne grupe uočena je u preseku submukoznog pleksusa.
Zaključak: Ova studija predstavlja jedinstvenu analizu glijalnih ćelija u CD, UC i kontrolnoj grupi u submukoznom i mijenterickom pleksusu. Rezultati ukazuju na poremećaj GCI u ENS-u kod IBD-a, što verovatno predstavlja morfološki supstrat za poremećaje motiliteta creva kod ovih stanja.

Ključne reči:
Kronova bolest, ulcerozni kolitis, morfometrija, enterički nervni sistem

Introduction

Inflammatory bowel disease (IBD) does not represent a single entity, but implies a common name for Crohn's disease (CD) and ulcerative colitis (UC). These are chronic relapsing inflammatory disorders of unknown etiology. They occur in genetically predisposed individuals due to a pathological local immune response directed at the intestinal microbiota, but probably also at some self-antigens. While CD can affect any part of the gastrointestinal tract, from the esophagus to the anus (although most commonly in the ileum), UC affects the colon exclusively (1). Both diseases most commonly begin during adolescence and early youth (2).

More than 150 genes have been identified to be involved in the pathogenesis of IBD (3). The hereditary factor in the pathogenesis of CD appears to be stronger than that in UC (4). Studies indicate a positive family history in 25 - 30% of cases, while twin studies show a concordant occurrence of IBD, especially in monozygotic twins (5).

The most important factors of the external environment in the pathogenesis of IBD, which can largely explain the different geographical distribution, are the differences in nutrition and hygiene between individual populations. The increase in the incidence of IBD in developing countries may be related to the adoption of Western dietary habits, which include a diet rich in fat and carbohydrates (6). Treatment of the disease is pharmacological, however, surgical resection is sometimes necessary (7).

The enteric nervous system (ENS) regulates motility and other functions of the gut. It is organized into two plexuses, myenteric (Auerbach's) and submucosal (Meissner's and Henle's) (8). Enteric plexuses are damaged in various systemic chronic diseases such as: diabetes mellitus, Alzheimer's disease, Parkinson's disease, multiple sclerosis, but also in IBD, which significantly affects intestinal motility (9). The enteric nervous system (ENS) is impaired and disrupted to varying degrees and in different ways in IBD (8). So far, different methods that have been applied various studies in the analysis of ENS significantly complicate the comparison of cellular elements. As the most powerful quantitative descriptor of intestinal nerve plexuses, the glial index, i.e. the glial cell index (GCI) has been described. This parameter represents the quotient of the number of glia and ganglion cells in the ENS ganglia (10).

The aim of this study is a morphometric analysis of the ENS in intestinal samples from surgically treated pediatric patients with CD and UC.

Material and methods

Histological slides from the archives of the Institute of Pathology, Faculty of Medicine, University of Belgrade, were analyzed. A total of five participants were included in each of the UC and CD groups, while the control group consisted of four participants. The study included an analysis of intestinal wall sections from pediatric patients with IBD (experimental group) and those with uncomplicated familial adenomatous polyposis syndrome (control

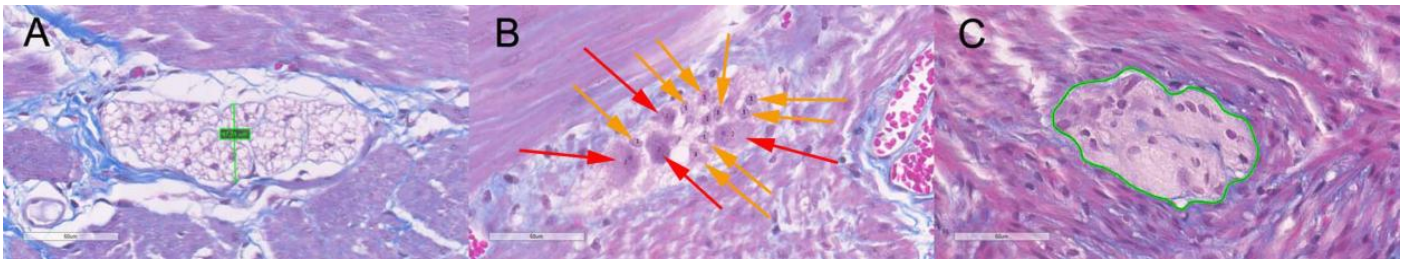

Figure 1. Morphometric analysis: nerve measurement method (A), analysis of ganglion composition: glial cells (orange arrow) and ganglion cells (red arrow) (B) and myenteric plexus ganglion surface (C)

group). For morphometric analysis, hematoxylin-eosin (HE) stained preparations of longitudinally oriented intestinal sections were selected and new sections from the corresponding paraffin block were stained with Masson trichrome method. Using the ImageJ software, five microphotographs of submucosal and myenteric nerve plexuses, stained and captured with an Olympus DP70 digital camera at x400 magnification (high microscopic magnification, high power field - HPF), were analyzed per participant. This study analyzed the number of ganglion cells of the submucosal and myenteric nerve plexus at 5HPF, the GCI was calculated for each ganglion, the thickness of the nerves in the submucosa and the intermuscular zone, as well as the surface of the myenteric ganglia were measured (**figure 1**).

All results are tabulated. Descriptive statistics methods were applied. Student's t-test was used to test the significance of the difference. Values for $p < 0.05$ were taken as the level of significance.

Results

The material from 5 patients (3 girls and 2 boys) with CD, with an average age of 15.9 ± 0.70 years, and 5 patients (3 boys and 2 girls) with chronic UC, with an average age of 12.5 ± 4.87 years, was analyzed. The age of patients with CD and UC did not differ significantly ($t = -1.181$; $p = 0.127$). The average age of the control group was 15.2 ± 2.77 years.

The results of the morphometric analysis in the submucosal plexus of the large intestine are shown in **table 1**. The median GCI in the colon in all analyzed cases of CD was 2 (0.5 - 8.67), in UC it was 3.33 (1.33 - 10), and in the control group it was 2.83 (1 - 12).

Myenteric plexus parameters obtained by morphometric analysis are shown in **table 2**. The median GCI in the colon in all analyzed cases of CD is 4.50 (1 - 14), in UC it is 4.55 (1 - 10), while in the control group it is 8.25 (3 - 18).

Analysis of the submucosal and myenteric plexus in the small intestine was possible only in cases of CD, because in other cases, the samples of the small intestine were not satisfactory. The results of this analysis are presented in **table 3**.

Discussion

Changes in enteric nerve plexuses can occur during IBD. Certain studies suggest the occurrence of enteric nerve plexus abnormalities due to an inflammatory process; however, it is increasingly certain that these abnormalities precede the inflammatory process (8). Developmental anomalies of the ENS, caused by genetic defects, can lead to the onset of IBD, but also drastically modify its course and contribute to the severity of the disease (11). Glial cells, although insufficiently studied, can be seen as the equivalent of astrocytes in the central nervous system: they have a trophic and protective role, but also an important role during the immune and inflammatory response. Expression of cytokines, cytokine receptors and antigen-presenting role enables them to do this. Some studies indicate a significant disruption of GFAP+ (Glial fibrillary acidic protein) glial cells, in segments of the intestine not affected by inflammation, in CD, but not to such an extent in UC. Extensive gliosis was found in both CD and UC, but this phenomenon was present to a significantly lesser extent in CD. These results indicate a weaker response to inflammatory signals in patients with CD. Disruption of this type can be one of the potential reasons for increased mucosal permeability and vascular dysfunction, which favors the development of inflammation (12). Studies indicate an increased number of GFAP and S100β (S100 calcium-binding protein B - S100β) glial cells in the Peyer's plaques of patients with CD, compared to the surrounding lamina propria and compared to the control group (13).

The great importance of glial cells in preserving the integrity of the mucous membrane of the gastrointestinal tract has been observed. Depending on the concentration of proinflammatory cytokines used in the experiment, the response of glial cells is different. In the case of relatively low doses of IL-1β, IL-10, or a combination of both of these cytokines, glial cell proliferation is inhibited. On the other hand, high doses of IL-10 or a combination of IFN-γ and lipopolysaccharide (LPS) have the exact opposite effect. Cytokines and LPS also affect the expression level of glial markers. In glial cell culture, GFAP expression was induced by the application of TNF-α, IL-1β, LPS, or a combination of LPS and IFN-γ, but also by entero-invasive *Escherichia coli*. Several studies performed on samples from patients with UC and CD show increased expression of GFAP and S100β in inflamed areas, compared to those not affected by inflammation (14).

All of the above clearly point to the impaired integrity of the ENS in the context of IBD, and the involvement of all cell types.

Table 1. The GCI - the number of ganglion cells and the thickness of nerves (μm) in the submucosa of the colon in CD, UC and the control group

N	GCI Med (min - max)			Number of ganglion cells / 5 HPF			Nerve thickness (μm) Med (min-max)		
	CD	UC	CG	CD	UC	CG	CD	UC	CG
1	5.00 (0.50 - 6.00)	5.00 (2.67 - 6.50)	3.67 (1.60 - 8.00)	10	16	10	47.27 (36.44 - 61.69)	42.99 (25.13 - 58.66)	24.81 (14.25 - 31.80)
2	2.00 (1.00 - 6.33)	3.33 (1.50 - 8.00)	1.75 (1.00 - 6.00)	14	12	17	22.08 (14.01 - 36.45)	26.64 (24.11 - 57.56)	36.95 (30.68 - 40.15)
3	1.50 (0.50 - 4.00)	1.67 (1.33 - 6.00)	4.30 (2.00 - 8.00)	19	13	8	48.11 (37.05 - 60.08)	27.80 (26.50 - 31.10)	19.21 (14.69 - 31.75)
4	1.50 (1.00 - 8.67)	2.75 (1.33 - 8.00)	2.00 (1.00 - 12.00)	11	18	9	22.65 (14.57 - 49.08)	36.80 (27.63 - 47.32)	22.31 (14.76 - 30.06)
5	2.00 (1.50 - 3.33)	5.00 (1.33 - 10.00)		16	14		46.64 (39.10 - 54.32)	25.30 (22.06 - 31.38)	

GCI - glial cell index; CD - Crohn's disease; UC - Ulcerative colitis; CG - Control group; HPF - high power field

Table 2. GCI, number of ganglion cells, nerve thickness (μm) and area of ganglia (μm²) in the myenteric plexus of the colon in CD, UC and the control group

N	GCI Med (min - max)			Number of ganglion cells / 5 HPF			Nerve thickness (μm) Med (min-max)			Area of ganglia (μm²) Med (min-max)		
	CD	UC	CG	CD	UC	CG	CD	UC	CG	CD	UC	CG
1	6.50 (3.00 - 14.00)	7.00 (4.33 - 10.00)	9.00 (3.50- 13.00)	9	10	8	71.20 (59.43 - 97.59)	58.91 (24.87 - 54.18)	41.00 (33.19 - 48.81)	5363.83 (2445.04 - 12737.81)	2330.30 (1091.26 - 6860.95)	5551.73 (2649.64 - 6390.09)
2	3.00 (1.20 - 5.25)	3.50 (1.50 - 9.00)	7.50 (4.80- 17.50)	15	7	14	34.26 (25.53 - 42.59)	53.45 (39.38 - 49.25)	42.44 (30.82 - 47.22)	2141.71 (655.20 - 5952.69)	1137.42 (657.40 - 4920.65)	6839.48 (52.87.67 - 8757.77)
3	8.50 (2.50 - 10.67)	3.25 (1.00 - 6.00)	11.00 (5.00- 18.00)	16	10	10	49.71 (24.29 - 59.57)	22.74 (18.63 - 31.22)	63.52 (37.04 - 82.47)	5880.50 (3550.53 - 11490.04)	2572.80 (735.40 - 5359.90)	5716.83 (3952.25 - 15658.79)
4	4.50 (2.33 - 10.50)	5.50 (1.80 - 10.00)	7.00 (3.00- 13.00)	13	14	5	67.00 (59.54 - 130.09)	38.23 (32.93 - 92.45)	48.06 (25.42 - 60.57)	4174.97 (2083.96 - 12515.53)	5666.20 (3591.97 - 11349.79)	3344.50 (2065.33 - 9228.58)
5	4.00 (1.00 - 7.00)	4.55 (4.50 - 10.00)		10	10		49.49 (37.14 - 64.02)	37.35 (15.98 - 44.39)		3228.40 (1122.97 - 4342.56)	4242.19 (1893.15 - 6486.50)	

GCI - glial cell index; CD - Crohn's disease; UC- Ulcerative colitis; CG - Control group; HPF - high power field

Table 3. Results of morphometric analysis in the submucosal and myenteric plexus of the small intestine in CD

	N	GCI Med (min - max)	Small intestine - CD		Area of ganglia (μm²) Med (min - max)
			Number of ganglion cells per 5 HPF	Nerve thickness (μm) Med (min - max)	
Submucous plexus	1	3.50 (1.00 -11.00)	12	36.19 (31.39 - 52.73)	
	2	2.25 (1.50 - 5.50)	18	27.60 (16.68 - 39.00)	
	3	2.00 (1.00 - 10.00)	13	19.40 (14.91 - 58.17)	
	4	1.67 (0.50 - 2.00)	13	39.69 (28.81 - 60.46)	
	5	14.23 (11.87 - 27.69)	15	1.50 (1.00 - 2.67)	
Myenteric plexus	1	4.50 (2.00 - 7.00)	8	58.97 (38.83 - 65.68)	1303.13 (484.93 - 4041.71)
	2	4.40 (3.00 - 8.00)	22	52.06 (30.03 - 93.39)	5772.85 (1399.92 - 10849.51)
	3	4.63 (4.00 - 8.00)	10	40.42 (29.93 - 62.41)	2922,41 (1493.24 - 6828.75)
	4	3.00 (1.50 - 7.50)	12	82.41 (74.14 - 92.05)	2827.15 (973.34 - 7450.68)
	5	3.00 (2.50 - 11.00)	9	31.58 (18.15 - 41.17)	2949.95 (1253.29 - 5243.29)

GCI - glial cell index; CD - Crohn's disease; HPF - high power field

In the submucosal plexus of parts of the intestine not affected by inflammation in CD, a decrease in the number of glial cells was observed. Studies indicate that myenteric plexitis is present in 75% of patients with CD (especially in sections of tissue affected by inflammation) and 64% of patients with UC. Plexitis is a common finding in CD, which is understandable considering the transmural nature of the inflammation. On the other hand, in UC, especially the one refractory to therapy, it is possible to extend the inflammatory process to the submucosal layer. There is also some degree of increase in neuron body dimensions in patients with CD. Data from experimental animal models support the fact that this phenomenon is caused by parenteral nutrition of patients (8).

This study did not establish a significant deviation of the GCI in the submucosa of the colon of patients with CD from the GCI of patients with UC, although the median glial index in the submucosa of patients with UC is slightly higher compared to those with CD. There is no significant difference in the median GCI in the submucosa between the experimental and control groups (**table 1**). On the other hand, GCI values in the myenteric plexus of the large intestine of patients with CD almost coincide with GCI values of those with UC, while glial index values in the myenteric plexus of the control group are almost twice as high (**table 2**).

The discrepancy between literature data and those resulting from this research can be explained, first of all, by a small sample, which means that the confirmation of commonly known data requires the inclusion of a larger number of respondents. The patients whose samples were processed are of pediatric age, and the specifics of the pediatric population should be taken into account. We should not ignore the process of active inflammation that was present, but also the potential therapeutic effect on the results. For more precise results, the research needs to be conducted on a larger sample.

Conclusion

This study presents an informative presentation of ENS morphometry in relatively common autoimmune diseases today, the frequency of which is increasing in developing countries. This paper provides a detailed account of a unique analysis of glial cells in three important categories: CD, UC, and familial adenomatous polyposis syndrome, in the submucosal and myenteric plexuses. The results obtained in this study, which should provide a basis for further research in this area, where GCI does not significantly differ in the submucosal plexus of the colon between the mentioned pathological conditions, and that in the myenteric plexus, GCI is twice as high in non-inflammatory conditions, compared to inflammatory, highlight the direction of further research. Such results draw attention to the importance of GCI determination in IBD in both mentioned plexuses, as well as comparison with other non-inflammatory conditions.

Literature

1. Kummar V, Abbas KA, Fausto N, Mitchell NR, editors. Robbins basic pathology: Oral cavity and gastrointestinal tract. 8th ed. Belgrade: Data Status; 2010.
2. Rosen MJ, Dhawan A, Saeed SA. Inflammatory Bowel Disease in Children and Adolescents. JAMA Pediatr. 2015; 169(11):1053-60.
3. Abraham BP, Ahmed T, Ali T. Inflammatory Bowel Disease: Pathophysiology and Current Therapeutic Approaches. Handb Exp Pharmacol. 2017; 239:115-46.
4. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007; 448(7152):427-34.
5. Younis N, Zarif R, Mahfouz R. Inflammatory bowel disease: between genetics and microbiota. Mol Biol Rep. 2020; 47(4):3053-63.
6. Altajar S, Moss A. Inflammatory Bowel Disease Environmental Risk Factors: Diet and Gut Microbiota. Curr Gastroenterol Rep. 2020; 22(12):57.
7. Paredes Méndez JE, Junes Pérez SI, Vargas Marcacuzco HT, Alosilla Sandoval PA, Gutiérrez Córdova IB, Fernández Luque JL, et al. Manejo médico quirúrgico de la enfermedad inflamatoria intestinal moderada-severa [Medical and surgical management of moderate-to-severe inflamatory bowel disease]. Rev Gastroenterol Peru. 2021; 41(2):79-85.
8. Villanacci V, Bassotti G, Nascimbeni R, Antonelli E, Cadei M, Fisogni S, et al. Enteric nervous system abnormalities in inflammatory bowel diseases. Neurogastroenterol Motil. 2008; 20(9):1009-16.
9. Niesler B, Kuerten S, Demir IE, Schäfer KH. Disorders of the enteric nervous system - a holistic view. Nat Rev Gastroenterol Hepatol. 2021; 18(6):393-410.
10. Jankovic R. Analysis of glial cell index and interstitial cells of cajal in colorectal biopsies of children with hirschsprung disease and related disorders [Dissertation]. Belgrade: Faculty of Medicine, University of Belgrade; 2016.
11. Lake JI, Heuckeroth RO. Enteric nervous system development: migration, differentiation, and disease. Am J Physiol Gastrointest Liver Physiol. 2013; 305(1):1-24.
12. Cabarrocas J, Savidge TC, Liblau RS. Role of enteric glial cells in inflammatory bowel disease. Glia. 2003; 41(1):81-93.
13. Biskou O, Meira de-Faria F, Walter SM, Winberg ME, Haapaniemi S, Myrelid P, et al. Increased Numbers of Enteric Glial Cells in the Peyer's Patches and Enhanced Intestinal Permeability by Glial Cell Mediators in Patients with Ileal Crohn's Disease. Cells. 2022; 11(3):335.
14. Pochard C, Coquenlorge S, Freyssinet M, Naveilhan P, Bourreille A, Neunlist M, et al. The multiple faces of inflammatory enteric glial cells: is Crohn's disease a gliopathy?. Am J Physiol Gastrointest Liver Physiol. 2018; 315(1):1-11.