

ALTERATION OF SEROTONIN POSITIVE CELLS IN COLONIC MUCOSA OF PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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ABSTRACT:

BACKGROUND AND AIMS: THERE HAVE BEEN DESCRIBED INCREASED MUCOSAL DENSITIES OF SEROTONIN POSITIVE NEUROENDOCRINE CELLS IN INFLAMMATORY BOWEL DISEASE (IBD) . THE AIM OF OUR STUDY IS TO DETERMINE THE SEROTONIN POSITIVE NEUROENDOCRINE CELLS DENSITIES IN COLONIC MUCOSA OF PATIENTS WITH IBD.

METHODS: COLONIC BIOPSIES FROM 18 PATIENTS WITH IBD (8 ULCERATIVE COLITIS, 10 CHRON'S DISEASE) AND 16 HEALTHY CONTROLS WERE EVALUATED HISTOPATOLOGIC WITH HEMATOXYLIN-EOSIN AND IMMUNOHISTOCHEMICAL WITH SEROTONIN ANTIBODIES.

RESULTS: NE CELLS POSITIVE FOR 5-HT WERE COUNTED ON A TOTAL MEDIAN NUMBER OF 20.7 (1,80) CRYPTS IN IBD GROUP AND 25.65(2,64) CRYPTS IN CONTROLS.

THE TOTAL DENSITIES/SUBJECT OF 5-HT POSITIVE CELLS WERE SIGNIFICANTLY HIGHER IN IBD GROUP COMPARED TO CONTROLS: 0.56(0.11,1.87) FOR IBD GROUP AND 0.28(0.14,0.71) FOR CONTROLS, P=0.004.

CONCLUSION: OUR STUDY SHOWED AN INCREASED DENSITY OF SEROTONIN POSITIVE NEUROENDOCRINE CELLS IN IBD COLITIS WHEN COMPARED TO HEALTHY CONTROLS.

KEY WORDS: ENDOCRINE CELLS, INFLAMMATORY BOWEL DISEASE, SEROTONINE

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INTRODUCTION

Gastrointestinal (GI) neuroendocrine (NE) cells are components of the intestinal barrier localized in the digestive tract mucosa.

There have been described three types of NE cells: enterochromaffine (EC) cells the most frequent GI NE cells with serotonin as the main secretion product. They represent the majority of NE cells localized in the intestinal mucosa (with the exception of duodenum), the appendix and the colon (with the exception of rectum).

Other types of NE cells are represented by G cells and D cells (which secrete serotonin) localized in the antrum and duodenum, L cells (which secrete enteroglucagon) localized in the terminal ileum and in the appendix and EC-like cells from the gastric mucosa which secrete histamine⁹.

An increased number in NE cells expressing serotonin (5HT) has been observed in patients with Crohn's disease (CD) and ulcerative colitis (UC).

GI tract is the site of secretion of about 95% of serotonin of which neurons are responsible for about 10% of the secretion and the rest is secreted by the EC cells.

In the mucosa of digestive tract serotonin activates neural reflexes associated with intestinal motility, secretion and sensation through at least two receptors 5-HT₃, 5-HT₄¹⁰.

Serotonin has an important role in inflammation and in immune response. Mast cells, macrophages/ monocytes, and T cells produce serotonin. Neutrophils, eosinophils, monocytes, macrophages, dendritic cell, mast cells, and natural killer (NK) cells and lymphocytes express serotonin receptors. Serotonin activates lymphocytes, whose proliferation protects NK cells and T-helper cells and endorses the recruitment of T cells¹¹.

The role of 5-HT in adaptive immunity of GI tract and the implication in intestinal functions are interesting paths to be evaluated for the underlying mechanism of inflammatory bowel disease (IBD) and its clinical impact.

We aim to investigate the densities of 5-HT expressing NE cells in colonic mucosa of patients with IBD.

MATERIALS AND METHODS

Cases

We included 18 patients with IBD (8 with UC and 10 with CD). All patients had colonic involvement of IBD. All patients had continuous treatment for IBD from diagnosis. Only one patient had antecedents of surgery (segmental ileal resection for stenosing CD). No patient with CD had fistulising disease. In IBD group 11 patients were in clinical remission of the disease (4 with UC and 7 with CD) and 7 patients had clinical active disease (4 patients with UC and

⁹ Magdy El-Salhy, Tefera Solomon, TrygveHausken, Odd Helge Gilja, Jan Gunnar Hatlebakk, "Gastrointestinal neuroendocrine peptides/amines in inflammatory bowel disease", *World Journal of Gastroenterol* 23(28) 2017: 5068-5085, doi: 10.3748/wjg.v23.i28.5068; Ashok R., Gunawardene, Bernard M. Corfe, Carolyn A. Staton, "Classification and function of the enteroendocrine cells of the lower gastrointestinal tract", *International Journal of Experimental Pathology* 92 (2011): 219-231, doi: 10.1111/j.1365-2613.2011.00767; Rindi G, Leiter AB, Kopin AS, Bordi C, Solcia E., "The "normal" endocrine cell of the gut: changing concepts and new evidences", *Annals of the New York Academy of Science*, 1014(2004):1-12 doi:10.1196/annals.1294.001

¹⁰ Rindi G, Leiter AB, Kopin AS, Bordi C, Solcia E., "The "normal" endocrine cell of the gut: changing concepts and new evidences", *Annals of the New York Academy of Science*, 1014(2004):1-12 doi:10.1196/annals.1294.001; Ashok R., Gunawardene, Bernard M. Corfe, Carolyn A. Staton, "Classification and function of the enteroendocrine cells of the lower gastrointestinal tract", *International Journal of Experimental Pathology* 92 (2011): 219-231, doi: 10.1111/j.1365-2613.2011.00767

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3 patients with CD Controls were represented by 16 subjects :9 Females and 7 males median age 56 years, range 50-68 years) without personal medical history of gastrointestinal tract conditions who underwent colonoscopy for colorectal cancer screening. Both groups signed the study consent form.

Patients' characteristics are summarized in (table 1).

Colonoscopy

Patients with IBD who underwent colonoscopy had indication for endoscopic examination in context of their IBD.

Five biopsies were taken one from each anatomical segment of the colon (rectum, sigmoid, descending, transverse, ascending and cecum) through colonoscopy examination from each subject (IBD group and controls). Macroscopic normal tissue specimens were biopsied from controls. All biopsy samples were oriented using nitrocellulose filter.

Table 1. Patients characteristics

Patients	UC	CD
Male	4	7
Female	4	3
Age (years)	38 (23,54)	39 (25,56)
Duration of disease (years)	5 (2,13)	4 (2,8)
Phenotype E2 Montreal	2	-
Phenotype E3 Montreal	6	-
Phenotype L2B1 Montreal	-	1
Phenotype L3B1 Montreal	-	7
Phenotype L3B2 Montreal	-	2
Mayo clinical < 2	4	-
Mayo clinical >=2	4	-
CDAI < 150	-	7
CDAI > 150	-	3
Mayo endoscopic <2	4	-
Mayo endoscopic > 2	4	-
CDEIS < 3	-	6
CDEIS >=3	-	4
Conventional treatment	4	4
Anti-TNF alpha	4	6

Histopathology

Biopsies were fixed in 10 % formalin for 24 hours and prepared in addition to standard protocol: gradual dehydrated through graded alcohols, paraffin embedded, microtome sectioned at a thickness of 4 microns and stained with hematoxylin-eosin.

Immunohistochemistry

Before proceeding with the IHC protocol, the sections were deparaffinized and rehydrated. According to the type of the antibody and the technical specifications of the producer, in specific cases enzymatic or termic pre-treatment was applied. The tissue sections were cooled, washed with distilled water, treated with TRIS based solution and peroxide and left for incubation with the specific antibody between 10 and 30 minutes. Lightning-Link staining, Streptavidin and chromogen DAB were subsequently added upon sections which were previously marked and repeatedly washed with TRIS solution and dried according to standard

protocol. Hematoxylin staining is used for 1-2 minutes, the sections are afterwards dehydrated with graded alcohols and examined at the optic microscope.

For serotonin we used an polyclonal antibody, Leica, dilution 1:100

Examintion of the tissue sections

The sections were examined at optic microscope for hetoxilin-eosin staining and IHC. The diagnosis of chronic colitis and the differential diagnosis between IBD colitis and other forms of chronic colitis was established. Histological activity of IBD was established using Geboes score for UC and Global Histologic Disease Activity Score (GHAS) for CD.

The IHC technique allowed the evaluation of the presence, the distribution and the densities of NE cells which were positive serotonin. The counting of NE cells was performed manually on the most representative sections and on a maximum number of crypts available on the microscopic field.

The examination of the sections was performed by the same person (Prof.MD.Gabriel Becheanu) who was blinded about the origin of the samples from the studied groups.

Statistical analysis

Results were reported as means and standard deviations for variables with a normal distribution and median, minimum and maximum respectively for variables with an abnormal distribution. We looked for differences concerning the independent variables by outcome in bivariate analysis (Mann-Whitney *U* test or Fisher's exact test, depending on variables). Two-sided hypothesis testing was used, with a *P* value of less than 0.05 considered statistically significant. Data analyses were performed using statistical software SPSS version 20.0 from IBM Corporation, Armonk, NY, USA.

RESULTS

Histopathological examination

We obtained a total of 180 slides (90 biopsy specimens from each study group). From a total of 40 biopsy specimens in UC group 34 were represented by quiescent colitis and 6 segments with active disease out of which 4 with mild-moderate activity and 2 with severe activity. In CD group from a total of 45 biopsy specimens we found 9 segments with active disease 5 segments with mild-moderate involvement and 4 segments with severe activity. Histological examination in controls showed normal aspect of the mucosa.

Immunohistochemistry

NE cells positive for 5-HT were counted on a total median number of 20.7 (1,80) crypts in IBD group and 25.65(2,64) crypts in controls.

The total densities/subject of 5-HT positive cells were significantly higher in IBD group compared to controls: 0.56(0.11,1.87) for IBD group and 0.28(0.14,0.71) for controls, $p=0.004$. (figure 1, figure 2, figure 3)

We also evaluated the 5-HT positive cells densities of each colonic segment with higher values in IBD group. (table 2).

The median number of crypts/segment on which we made de calculation is summarized in (table 3).

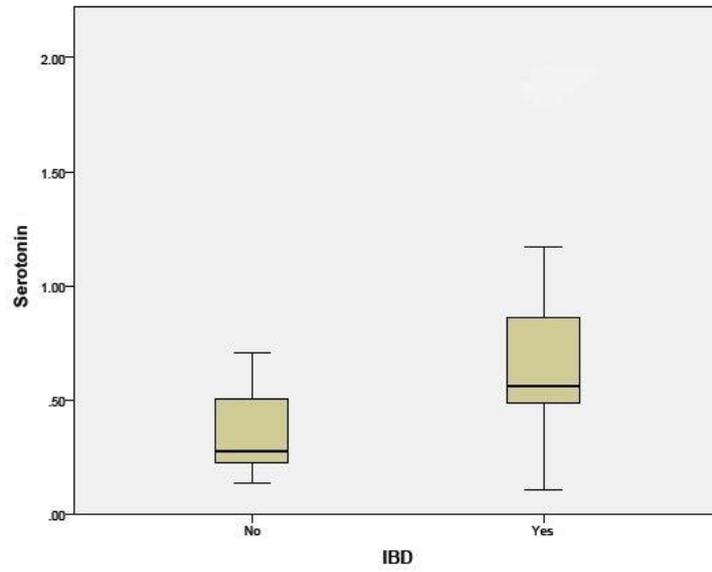


Figure1. Median densities of 5-HT positive cells in IBD and controls

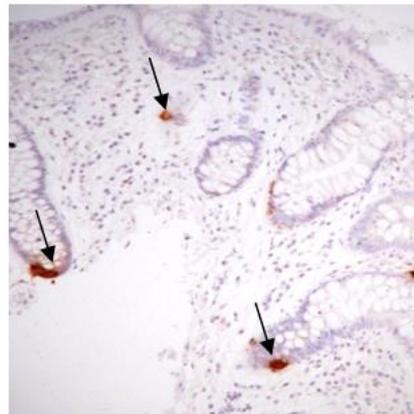


Figure 2. Immunohistochemistry with serotonin – normal colonic mucosa. 200x. (NE cells expressing serotonin arrows)

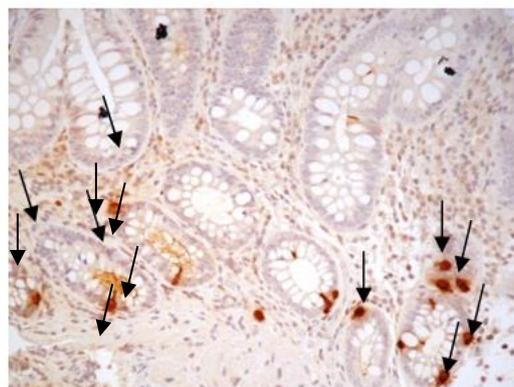


Figure 3. Immunohistochemistry with serotonin – chronic quiescent colitis. 200x. (NE cells expressing serotonin arrows)

Table2. Median 5-HT cells densities/crypt/colonic segment

Segments	IBD	Controls	P-value
Rectum	0.69(0.07,3.09)	0.39(0.17,1.47)	0.09
Sigmoidum	0.45(0.04,2.5)	0.3(0.07,1.2)	0.03
Descending	0.49(0.11,1.71)	0.19(0.03,0.65)	0.001
Transverse	0.65(0.07,1.75)	0.36(0.05,1.25)	0.02
Ascending	0.57(0.22,1.42)	0.27(0.09,0.56)	0.001

Table 3. Median number of crypts/colonic segment evaluated for 5-HT positive cells.

Segments	IBD	Controls
Rectum	28.3(9,57)	28.2(12,38)
Sigmoidum	17.7(1,80)	9.9(2,33)
Descending	23.6(5,45)	34.25(19,64)
Transverse	15.27(5,39)	29.8(12,37)
Ascending	18.66(7,35)	26.12(12,45)

According to the type of IBD we obtained a median number of 5-HT positive cells/crypt for UC of 0.7(0.23,1.87) for UC and 0.51(0.11,0.98) for CD, p value=0,16

When compared the densities of 5-HT positive cells between the group with clinically active disease and the group in clinical remission we found a median density of 5-HT positive cells of 0.59(0.49,1.87) and 0.5(0.11,1.17) in patients with clinically active disease and in patients in clinical remission respectively, p value=0.2

According to endoscopic activity the median 5-HT densities were 0.72 (0.49,1.48) in patients with edoscopic active disease and 0.54(0.11,1.87) in patients with endoscopic remission p=0.12

DISCUSSIONS

The increased density of serotonin expressing cells in colonic mucosa of patients with IBD explaining a possible correlation between gut hormones, immune reactivation and inflammatory response and being responsible of the gastrointestinal dismotility, visceral sensitivity, appetite influence via increase satiety, common findings in IBD patients¹².

Serotonin has an important role in inflammation and in immune response. Mast cells, macrophages/ monocytes, and T cells are capable produce serotonin. neutrophils, eosinophils, monocytes, macrophages, dendritic cell, mast cells, and natural killer (NK) cells and

¹² Ashok R., Gunawardene ,Bernard M. Corfe, Carolyn A. Staton,” Clasiffication and function of the enteroendocrine cells of the lower gastrointestinal tract”, International Journal of Experimental Pathology 92 (2011): 219–231,doi: 10.1111/j.1365-2613.2011.00767; Moran GW, Pennock J, McLaughlin JT,” Enteroendocrine cells in terminal ileal Crohn's disease”, Journal of Crohns and Colitis, 6(9) 2012:871-80,doi: 10.1016/j.crohns.2012.01.013; Khan WI, Ghia JE. “Gut hormones: emerging role in immune activation and inflammation”, Clinical and Experimental Immunology,161(1) 2010:19-27, doi:10.1111/j.1365-2249.2010.04150; Moran GW, Leslie FC, McLaughlin JT, “Crohn's disease affecting the small bowel is associated with reduced appetite and elevated levels of circulating gut peptides”, Clinical Nutrition, 32(3) 2013 :404-411, doi: 10.1016/j.clnu.2012.08.024

lymphocytes express serotonin receptors. Serotonin activates lymphocytes, whose proliferation protects NK cells and T-helper cells and endorses the recruitment of T cells¹³.

The pathway of 5-HT regulation in IBD is still unclear.

There is conflicting data upon the role of mucosal serotonin in the GI tract arising from both neural and mucosal sources of serotonin within the gut and the widespread and overlapping distribution of specific serotonin receptor subtypes¹⁴.

Two experiments from the same animal model were published. The authors proposed removal of colonic mucosa prior to analysis of a stereotyped motor pattern, the colonic migrating motor complex (CMMC). The published results were contradictory. Keating and Spencer reported that the CMMC persists after complete removal of the mucosa in conclusion it doesn't depend on the release of mucosal serotonin but Heredia et al stated that reported that removal of colonic mucosa abolished spontaneous CMMCs, but could still trigger CMMCs mechanically confirming that the neural circuitry was intact. Another paper published by Li et al. in 2011 used knockouts of tryptophan hydroxylase 1 (TPH1), the rate limiting enzyme for mucosal synthesis of serotonin, and TPH2 (the neural form) to delete serotonin from both sources on mice. The TPH1 knockouts did not differ from the initial form in any function measured including motility and transit, in contrast TPH2 knockout mice had alteration in every function suggesting a minor role of the intestinal mucosa release of serotonin on GI functions¹⁵.

In 1997 El Salhi showed an increased density of serotonin expressing cells in IBD group¹⁶.

However Magro et al. found lower levels of 5-HT in the mucosa of patients with UC and CD compared to controls¹⁷.

Coates and al. assessed the enteric 5-HT signalling on rectal biopsies of patients with UC compared to patients with inflammatory bowel syndrome (IBS) and normal controls and the result was a decrease in the EC expressing cells in the mucosa of patients with severe UC compared to patients with non-severe UC, IBS and controls suggesting that UC may interfere with the differentiation of EC cells. No difference in the mucosal level of 5-HT was observed suggesting that intraluminal concentration is not influenced by the cells number¹⁸.

Our study demonstrated increased colonic densities in 5-HT positive cells in UC and CD when compared to subjects with normal colonic mucosa assessed on a minimum of 10 crypts. We did not find statistical significant difference between 5-HT positive cells according to clinical and endoscopical activity of IBD.

¹³ Magdy El-Salhy, Tefera Solomon, Trygve Hausken, Odd Helge Gilja, Jan Gunnar Hatlebakk, "Gastrointestinal neuroendocrine peptides/amines in inflammatory bowel disease", *World Journal of Gastroenterol* 23(28) 2017: 5068-5085, doi: 10.3748/wjg.v23.i28.5068

¹⁴ Costedio MM, Hyman N, Mawe GM, "Serotonin and its role in colonic function and in gastrointestinal disorders", *Diseases of the Colon and Rectum*, 50(3) 2007:276-388

¹⁵ Li Z., Chalazonitis A., Huang Y.-Y., Mann J. J., Margolis K. G., Yang Q. M., Kim D. O., Côté F., Mallet J., Gershon M. D., "Essential roles of enteric neuronal serotonin in gastrointestinal motility and the development/survival of enteric dopaminergic neurons", *Journal of Neuroscience*, 31(2011), 8998–9009 , doi: 10.1523/JNEUROSCI.0203-11.2011

¹⁶ M. El SALHI, A.Danielsson, R.Stenling, L.Grimelius, "Colonic endocrine cells in inflammatory bowel disease", *Journal of Internal Medicine* (242)1997: 413–419

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¹⁸ Coates, Matthew D. et al., "Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome", *Gastroenterology*, 126(7) 2004 : 1657 – 1664, doi:10.1053/j.gastro.2004.03.013

There are limitations to our study. We included a small number of patients. Other markers such as serum and fecal 5-HT levels that could better evaluate the clinical impact of mucosal alterations were not included in our study.

Further studies are needed in order to understand the behaviour of serotonin positive cells in IBD with potential implication in clinical management of the disease.

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All authors report no potential conflict of interest.

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