Medicine

NEUROENDOCRINE CELLS ALTERATIONS IN COLONIC MUCOSA OF PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Corina MEIANU[1]
Carmen PREDA[2]
Gabriel BECHEANU[3]
Cosmin CIORA[4]
Doina PROCA[5]
Mona DUMBRAVA[6]
Alexandru LUPU[7]
Mircea DICULESCU[8]

ABSTRACT:

BACKGROUND AND AIMS: SEVERAL PUBLISHED STUDIES ON HUMAN AND ANIMAL MODELS SHOWED INCREASED DENSITIES OF NEUROENDOCRINE CELLS (NE)CELLS IN COLONIC MUCOSA OF INFLAMMATORY BOWEL DISEASE (IBD) COLITIS. THE AIM OF OUR STUDY IS TO DETERMINE THE (NE) 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 CHROMOGRANIN A (CGA) AND SYNAPTOHYSIN(SYN) ANTIBODIES.

RESULTS: NE CELLS WERE COUNTED ON A TOTAL MEDIAN NUMBER OF 24.1(7,56) CRYPTS FOR CGA, 24(5,57) FOR SYN IN IBD GROUP AND 29 (11,64) CRYPTS FOR CGA AND 29,2(11.52) CRYPTS FOR SYN IN CONTROLS.

THE TOTAL DENSITIES/SUBJECT OF NE CELLS WERE SIGNIFICANTLY HIGHER IN IBD GROUP COMPARED TO CONTROLS FOR BOTH CGA AND SYN: 1.45(0.72,2.78) AND 0.86(0.43,1.73) FOR CGA (P= 0,006), 0.89(0.22,1.63) AND 0.63(0.28,1.28) FOR SYN (P=0,025)

[1] Fundeni Clinical Institute, Bucharest, Romania;

[2] Fundeni Clinical Institute, Bucharest, Romania; "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

[3] Fundeni Clinical Institute, Bucharest, Romania; "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

[4] Fundeni Clinical Institute, Bucharest, Romania;.

[5] Fundeni Clinical Institute, Bucharest, Romania;

[6] Fundeni Clinical Institute, Bucharest, Romania;

[7] Fundeni Clinical Institute, Bucharest, Romania; "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

[8] Fundeni Clinical Institute, Bucharest, Romania; "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

CONCLUSIONS: OUR STUDY SHOWED AN INCREASED DENSITY OF CGA AND SYN POSITIVE NEC IN PATIENTS WITH IBD.

INTRODUCTION

Inflammatory bowel disease (IBD) which includes ulcerative colitis (UC) and Crohn's disease (CD) is a condition of unknown aetiology thought to arise from an interaction between genetic, environmental and immunological factors that create an abnormal response against a luminal antigen possible a component of the microbiota.

IBD is a chronic, relapsing condition with reduced quality of life and important social and economic burden.

Different paths have been explored for determining the aetiology of IBD. Abnormalities concerning mucosal cells in the epithelium of patients with IBD have determined a recent focus of understanding the correlation with IBD physiopathology.

Intestinal homeostasis is maintained by a system called '' intestinal barrier'', a dynamic structure that separates intestinal contents from the host tissues and allows interactions between the resident bacterial flora and the mucosal immune system. It also acts like a filter between pathogens and lamina propria[9]

When the integrity of the intestinal epithelial layer is broken, the permeability of the epithelium is increased which allows a high influx of intestinal contents and/or a high burden of microorganisms is thought to initiate and maintain a sustained inflammatory response, which is considered to be one of the mechanisms underlying IBD[10].

The gastrointestinal neuroendocrine (NE) cells are localized in the mucosa of the gastrointestinal tract and are present in all segments with the exception of esophagus, comprising less than 1% of all epithelial cells. Along the intestinal epithelial cells they are distributed isolated from one another interspersed by non-endocrine epithelial cells[11].

NE cells present secretory vesicles large dense core vesicles (LDCV) and synaptic-like microvesicles (SLMV). Gut endocrine cells are recognized by the expression of several "general" markers, including the LDCV marker chromogranin A, a matrix soluble glycoprotein and the SLMV marker synaptophysin, a membrane glycoprotein. There are at least 15 different types of NEC classified upon the hormone they produce and the structure of their secretory granules[12].

---

[9] Roda G, Sartini A, Zambon E, et al. "Intestinal epithelial cells in inflammatory bowel diseases", World Journal of Gastroenterology: 16(34) 2010:4264-4271, doi:10.3748/wjg. v16.i34.4264.

[10] Kim, Duk Hwan, and Jae Hee Cheon. "Pathogenesis of Inflammatory Bowel Disease and Recent Advances in Biologic Therapies." Immune Network 17.1 (2017): 25–40

[11] 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

[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

A NE cell can produce up to 7 different hormones and two hormones can be found in the same type of NE cell. These hormones include somatostatin, serotonin, peptide YY, glicentine, oxyntomodulin, glucagon-like peptide 1 and glucagon-like peptide 2 and have endocrine and paracrine functions with different functions including gut absorbtion and secretion, motility, modulation of immune response like lymphocyte proliferation, immunoglobulin production by inducing T helper cells differentiation, cytokine secretion, cells activation and phagocytosis[13].

There were reports of an increased distribution of NE cells in the mucosa of patients with IBD also case reports of neuroendocrine neoplasms arising in IBD patients.

The present study investigates the densities of 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. 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).

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

---

[13] 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; Magdy El-Salhy, Tefera Solomon, Trygve Hausken, Odd Helge Gilja, Jan Gunnar Hatlebakk, "Gastrointestinal neuroendocrine peptides/amines in inflammatory bowel disease", World Journal of Gastroenterology 23(28) 2017: 5068-5085, doi: 10.3748/wjg.v23.i28.5068; 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; May CL, Kaestner KH, " Gut Endocrine Cell Development", Molecular and cellular endocrinology,323(1) 2010:70-75. doi:10.1016/j.mce.2009.12.009.

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

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.

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 antibodies for cromogranin A (CgA) and synaptophysin (Syn) for 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. Hematoxilyn saining is used for 1-2 minutes. The sections were afterwards dehydrated with graded alcohols and examined at the optic microscope.

The antibodies clones that were used were: IMPATH, monoclonal, clone MRQ-40, dilution 1:500 for Syn and Cell Marque, monocolonal, clone LK2H10, dilution 1:100 for CgA.

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 were 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 for the general markers of differention CgA and Syn. 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. Histologcal examination in controls showed normal aspect of the mucosa.

Immunohistochemistry

NE cells were counted on a total median number of 24.1(7,56) crypts for CgA, 24(5,57) for Syn in IBD group and 29 (11,64) crypts for CgA and 29,2(11.52) crypts for Syn in controls.

The total densities/subject of NE cells were significantly higher in IBD group compared to controls for both CgA and Syn: 1.45(0.72,2.78) and 0.86(0.43,1.73) for CgA (p= 0,006), 0.89(0.22,1.63) and 0.63(0.28,1.28) for Syn (p=0,025). (figure 1)

We also evaluated the NE cells densities of each colonic segment with significantly higher values for IBD group in CgA (table 2).

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

According to the type of IBD we obtained a median number of NE cells/crypt for UC of 1.33(0.92,2.66) for CgA, 0.88(0.5,1.32) for Syn and 1.48(0.72,2.78) for CgA and 0.89(0.22,1.63) for Syn for CD, p =0.89, p=0.9.

The median NE cells densities were analysed according to disease duration as follows: disease duration of less than 4 years 1.53(0.8,278) for CgA, 0,95(0.68,1.63) for Syn, disease duration

of more than 4 years 1.37(0.72,2) for CgA and 0.87(0.22,1.43) for Syn, p=0,33, p= 0,093 respectively.

According to endoscopic activity of the disease the median NE cells densities were 1.36(0.72,2.78) for CgA, , 0.87(0.22,1.63) for Syn in the segments with endoscopic activity and 1.43(0.72,2.78) for CgA and 0.88(0.2,2.78) for Syn in the segments with quiescent colitis, p =0,26, p=0,8).
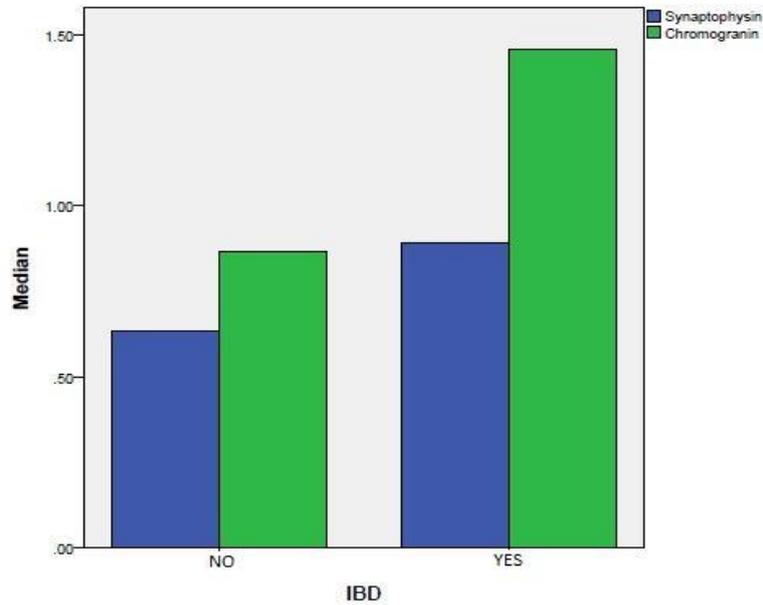

Figure 1. Median NE cells densities

Table 2. Median NE cells densities/crypt/colonic segment

Segments	IBD		Control		P value	
	CRO	SYN	CRO	SYN	CRO	SYN
Rectum	2.28(0.37,4.73)	0.99(0.07,2.28)	1.01(0.41,2.11)	0.67(0.23,1.53)	0.001	0.046
Sigmoidum	1.61(0.61,2.63)	0.66(0.2,1.38)	0.99(0.44,1.91)	0.73(0.34,1.36)	0.006	0.87
Descending	1.29(0.45,2.6)	0.66(0.21,1.8)	0.79(0.25,1.85)	0.63(0.18,1.15)	0.048	0.55
Transverse	1.65(0.25,3.25)	0.77(0.21,2.88)	0.92(0.23,2.58)	0.7(0.2,1.48)	0.036	0.79
Ascending	1.35(0.82,5.8)	0.89(0,4.42)	0.9(0.19,2.06)	0.61(0.21,1.35)	0.01	0.154

Table 3. Median number of crypts/colonic segment

Segments	IBD		Control	
	CRO	SYN	CRO	SYN
Rectum	28.7 (12,56)	28.2(9,57)	28.3(12,40)	28.1(12,40)

Sigmoidum	26.6(16,56)	26.5(13,56)	26.3(11,40)	27(11,40)
Descending	23.7(8,46)	24.4(9,45)	35.1(19,64)	35.3(20,50)
Transverse	20.7(8,42)	21(8,35)	29.4(12,52)	29.3(12,52)
Ascending	20.8(7,38)	19.9(5,35)	26.3(12,45)	26.4(13,45)

DISCUSSIONS

The present study identified that the densities of NE cells are higher in patients with IBD than in controls with normal colonic mucosa.

The presence of NE cells, both in patients with IBD and in those without histopathological changes, is easier to evaluate using CgA compared to SYN. CgA has a more intense, diffuse and uniform tissue signal. NE cells positive for SYN are present in a significantly lower number of cells with weak-moderate signal.

The number of NE cells positive for CgA is, in all studied cases, higher than the number of NE cells positive for Syn. NE cells have a uniform distribution in normal colonic mucosa and a non-uniform distribution in colonic mucosa with active IBD.

In normal mucosa, NE cells have a predominantly basal distribution being localised at the level of intestinal crypts, are rarely superficial, isolated NE cells can be found in the superficial epithelium. They are not organized in clusters or in micronodules in comparison with the inflamed mucosa where NE cells exhibit high but non-uniform density, with clustered areas in some crypts, frequently forming groups and nests of 3 to 5 cells. (figure 2, figure 3)

In the inflamed mucosa NE cells are frequently superficially arranged, also can be found in the surface epithelium. We did not find NE cells dysplasia in the studied group.
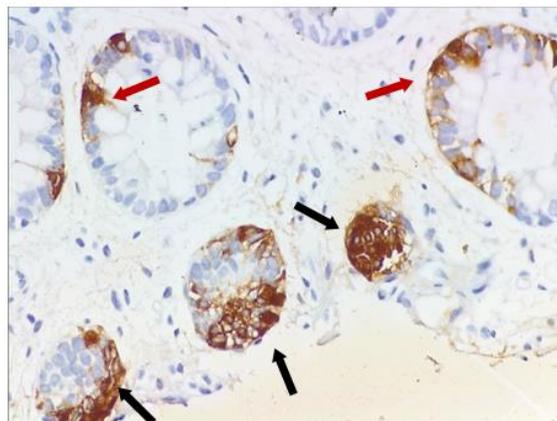

Figure 2. Immunohistochemistry with CgA: micronodules of NE cells (black arrows) localised at the base of crypts. Hyperplastic NE cells (red arrows). Chronic active colitis. 200x
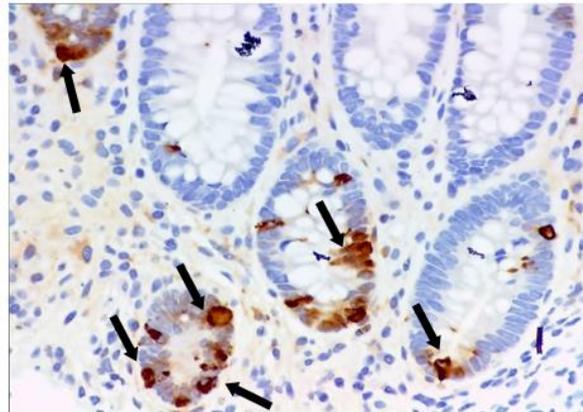
Figure 3. Immunohistochemistry with CgA: NE cells in groups of 3-4 cells at the base of the crypts (black arrows). Chronic active colitis. 200x

CgA is a general marker of differentiation for NE cells. All of the gastrointestinal (GI) endocrine cell types produce members of the granins family (including chromogranins A and B) that are co-stored and co-released from the GI endocrine cells[14].

Ferrero et al. stated in a mouse model study that Cg A prevents the vascular leakage induced by tumour necrosis factor (TNF). Cg A inhibits TNF induced vascular permeability by preventing endothelial cytoskeleton rearrangements. Secretion of CgA could contribute to the regulation of endothelial barrier function and protection of vessels against plasma leakage in inflammatory diseases[15].

In a study that evaluated the serum concentration of CgA in patients with IBD showed higher values of CgA serum concentrations than in non-IBD group and a decrease in the value in patients under biologic treatment in comparison to those under conventional treatment indifferent of the activity of the disease[16].

We didn't find statistical significant differences regarding NE cells densities when subjects were divided accord to disease type, disease duration or endoscopic activity probably due to the small sample groups. We were interested in finding out if the changes in NE cells are reversible with disease duration taking in consideration that all patients were under treatment for IBD from diagnosis.

After proximal small bowel, the rectum is the location with the next greatest frequency of NECs and the only location in the GI tract where NECs are occasionally seen adjacent to each other or in clusters[17]. In our study higher densities of NE cells were observed in rectum in both IBD and controls independent of the endoscopic activity in IBD group.

[14] Bartolomucci A, Possenti R, Mahata SK, Fischer-Colbrie R, Loh YP, Salton SRJ, "The Extended Granin Family: Structure, Function, and Biomedical Implications". Endocrine Reviews, 32(6) 2011:755-797, doi:10.1210/er.2010-0027

[15] Ferrero E, Magni E, Curnis F, Villa A, Ferrero ME, Corti A.," Regulation of endothelial cell shape and barrier function by chromogranin A", Annals of the New York Academy of Science, 971(2002):355–358, doi: 10.1111/j.1749-6632.2002.tb04495

[16] Zissimopoulos A, Vradelis S, Konialis M, Chadolias D, Bampali A, Constantinidis T, Efremidou E, Kouklakis G, " Chromogranin A as a biomarker of disease activity and biologic therapy in inflammatory bowel disease: a prospective observational study", Scandinavian Journal of Gastroenterology,49(8) 2014: 1-8, doi: 10.3109/00365521.2014.920910

[17] 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

We found 2 similar studies in literature that evaluated the NE cells densities in patients with IBD.

In 1997 El Salhi include a number of 17 patients with UC, 11 patients with CD and showed an increased density of both chromogranin A expressing cells in IBD group[18].

Chojnacki and colleagues evaluated the number of enterochromaffin cells in 30 patients with active ulcerative proctitis and 30 patients with active ulcerative colitis and found and increased number of enterochromaffin cells in these groups on rectal respectively colonic biopsies compared to healthy controls[19].

Both studies evaluated the NE cells densities on a number of 10 microscopic fields.

Our study confirmed the increased densities of NE cells in patients with IBD positive for CgA that were counted on a median number of a minimum 20 crypts.

We may conclude that NE cells hyperplasia in IBD is an intestinal epithelial change due to chronic inflammation. Further we intend to enlarge our study group in order to evaluate the reversibility of the NE alteration in the disease course, the treatment influence and the clinical implication of NE cells alterations in IBD

---

[18] M. El SAlhi, A.Danielsson, R.Stenling, L.Grimelius, "Colonic endocrine cells in inflammatory bowel disease", Journal of Internal Medicine 242(1997): 413–419
[19] Chojnacki C, Wiśniewska-Jarosińska M, Kulig G, Majsterek I, Reiter RJ, Chojnacki J., "Evaluation of enterochromaffin cells and melatonin secretion exponents in ulcerative colitis", World Journal of Gastroenterology, 19(23) 2013:3602-3607,doi:10.3748/wjg.v19.i23.3602

REFERENCES

1. **Roda G, Sartini A, Zambon E, et al.** "Intestinal epithelial cells in inflammatory bowel diseases", World Journal of Gastroenterology: 16(34) 2010:4264-4271, doi:10.3748/wjg. v16.i34.4264.
2. **Kim, Duk Hwan, and Jae Hee Cheon.** "Pathogenesis of Inflammatory Bowel Disease and Recent Advances in Biologic Therapies." Immune Network 17.1 (2017): 25–40.
3. **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.
4. **Magdy El-Salhy, Tefera Solomon, Trygve Hausken, Odd Helge Gilja, Jan Gunnar Hatlebakk,** "Gastrointestinal neuroendocrine peptides/amines in inflammatory bowel disease", World Journal of Gastroenterology 23(28) 2017: 5068-5085, doi: 10.3748/wjg.v23.i28.5068
5. **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
6. **May CL, Kaestner KH,** " Gut Endocrine Cell Development", Molecular and cellular endocrinology,323(1) 2010:70-75. doi:10.1016/j.mce.2009.12.009.
7. **Bartolomucci A, Possenti R, Mahata SK, Fischer-Colbrie R, Loh YP, Salton SRJ**, "The Extended Granin Family: Structure, Function, and Biomedical Implications". Endocrine Reviews, 32(6) 2011:755-797, doi:10.1210/er.2010-0027.
8. Ferrero E, Magni E, Curnis F, Villa A, Ferrero ME, Corti A.," Regulation of endothelial cell shape and barrier function by chromogranin A", Annals of the New York Academy of Science, 971(2002):355–358, doi: 10.1111/j.1749-6632.2002.tb04495.
9. **Zissimopoulos A, Vradelis S, Konialis M, Chadolias D, Bampali A, Constantinidis T, Efremidou E, Kouklakis G**, " Chromogranin A as a biomarker of disease activity and biologic therapy in inflammatory bowel disease: a prospective observational study", Scandinavian Journal of Gastroenterology,49(8) 2014: 1-8, doi: 10.3109/00365521.2014.920910.
10. **M. El SAlhi, A.Danielsson, R.Stenling, L.Grimelius,** "Colonic endocrine cells in inflammatory bowel disease", Journal of Internal Medicine 242(1997): 413–419
11. **Chojnacki C, Wiśniewska-Jarosińska M, Kulig G, Majsterek I, Reiter RJ, Chojnacki J.**, "Evaluation of enterochromaffin cells and melatonin secretion exponents in ulcerative colitis", World Journal of Gastroenterology, 19(23) 2013:3602-3607,doi:10.3748/wjg.v19.i23.3602.