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Serum trace elements profile in the pediatric inflammatory bowel disease progress evaluation

Anna Stochel-Gaudyn[a,*], Krzysztof Fyderek[a], Paweł Kościelniak[b]

[a] Department of Pediatrics, Gastroenterology and Nutrition, Polish-American Children's Hospital, Jagiellonian University Medical College, Wielicka 265, Krakow 30-663, Poland

[b] Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, Kraków 30-387, Poland

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ABSTRACT

Introduction: Inflammatory bowel disease (IBD), a chronic inflammatory disorder of gastrointestinal tract, arises from complex interaction between genetics, environment, gut microbiota and mucosal immune response. Along with clinical, endoscopic and radiological evaluation various biomarkers are needed as an additional diagnostic tool, as well as to predict disease course and therapeutic outcomes.

Aim: The aim of this study was to evaluate clinical value of essential trace elements (ETEs) serum concentration profile in the assessment of pediatric IBD diseases development.

Materials and methods: Concentration of five ETEs: iron (Fe), zinc (Zn), copper (Cu), manganese (Mn) and selenium (Se) in serum of 41 children with newly diagnosed IBD (27 CD and 14 UC) and 20 healthy controls were determined by inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectrometry (AFS) at the moment of diagnosis and after one year of treatment.

Results: The obtained results revealed significant differences in serum concentration profile of studied ETEs' for IBD pediatric patients and healthy controls. Decrease of iron, zinc and selenium and increase of copper and manganese serum concentration were observed in IBD patients at the time of diagnosis. The changes were reversible and after one year of treatment the studied ETEs serum concentration profile resembled much more that observed for healthy controls. Correlations between studied ETEs levels within cases (IBD, CD, UC) were also found to be different from those in healthy controls (HC).

Conclusion: Although much more studies are required on the subject our results demonstrate a clinical value of ETEs serum concentration profile in pediatric IBD patients regarding disease development.

1. Introduction

Inflammatory bowel disease (IBD) is a group of chronic inflammatory conditions of the gastrointestinal tract (GI) characterized by periods of remission and exacerbation. The clinical symptoms are mixed, with unknown initial cause and multifactorial etiology including genetic and environmental factors [1–4]. Within IBD we can distinguish two main entities: Crohn's disease (CD) affecting the whole GI tract and ulcerative colitis (UC) limited to the colon. Despite clinical, radiological, endoscopic and histopathological evaluation additional biomarkers are needed as a diagnostic tool in case of uncertain diagnosis as well as for assessment of disease progress [5–7].

Weight loss and malnutrition are common complications in majority of IBD patients, leading to deficiencies in both macronutrients and micronutrients including trace elements [8–11]. Deficiencies may be a result of inadequate consumption, compromised intestinal absorption or ongoing disease. Dyshomeostasis of any of ETEs' may affect its structural, catalytic, regulatory or other functions, and in consequence lead to disease onset or exacerbation. The pediatric population, due to its' poor body storages and developing status, seems to be especially sensitive [12–21]. The serum concentration of some ETEs have been extensively studied in various clinical conditions. For inflammatory bowel disease most studies were devoted to adults population whereas studies conducted among children were scarce and selective, leading sometimes to contradictory results. According to our knowledge there was no attempt to discuss changes in serum concentration of various ETEs collectively as a characteristic profile for IBD patients [14–16,22–62].

The aim of present study was to determine concentration levels of five ETEs such as iron, zinc, copper, manganese and selenium in blood

* Corresponding author.
E-mail address: a.stochel-gaudyn@uj.edu.pl (A. Stochel-Gaudyn).

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serum of children with IBD diseases (CD and UC) at the moment of diagnosis and after one year of treatment in the comparison with healthy control group. We wanted to evaluate the clinical value of ETEs serum concentration profile and recognize the possibility of using such profiles for the assessment of the disease progress as well as for differentiation between CD and UC cases. Correlations between studied ETEs levels within cases (IBD, CD, UC) and healthy controls (HC) as well as their correlations with some clinical parameters in cases were assessed as the supporting information.

2. Materials and methods

2.1. Ethics

The study was carried out in accordance with guidelines of the Declaration of Helsinki. The study protocol was approved by the Jagiellonian University Bioethical Committee and informed consent to participate in the study was obtained from all patient's parents or legal guardians and patients more than 16 years of age enrolled in the study.

2.2. Subjects

In a prospective cohort study a total of 41 children with newly diagnosed IBD (17 boys and 27 girls), patients of the Clinic of Pediatrics, Gastroenterology and Nutrition Collegium Medicum Jagiellonian University, were recruited. In the studied group Crohn's disease (CD) was diagnosed in 27 patients whereas ulcerative colitis (UC) in 14 of them. The control group consisted of 20 healthy children (9 boys and 11 girls). The mean age for both groups was 11 years. The diagnosis of IBD (CD, UC) was based on the Porto criteria, including clinical presentation as well as endoscopic, histopathological and radiological examination. The inclusion criteria were: newly diagnosed IBD, age 1–18 years and written consent of the parents or legal guardians and of patients 16 years old and above. In case when there was no consent of the parent/legal guardian or the patient itself or the patient suffered from chronic disease other than IBD or there was a history of chronic multivitamin supplementation or reduction diet, the patients' were excluded from the study.

2.3. Methods

2.3.1. Measurements of clinical parameters

Anthropometric parameters such as height (cm), weight (kg), BMI (body mass index) and CI (Coles Index) [44,45] were collected for IBD patients and healthy controls (Table 1). Additionally, the standard morphological and biochemical parameters were assessed.

2.3.2. Serum sample preparation and trace elements analysis

From every of CD and UC patient a serum sample was collected twice, at the moment of diagnosis and after one year of observation. In the control group the samples were taken only once. After serum preparation the samples were frozen and stored at minus 40C until use. Inductively coupled plasma mass spectrometry (ICP-MS, Spectrometer ICP-MS Elan CRC-e, Perkin Elmer) was used to determine Fe, Cu, Zn and Mn serum concentration whereas Hydride generation atomic mass spectrometry (HG-AFS, AFS-8220, Beijing Titan Instrumets Co) was used for Se level measurements. Each sample was mineralized prior to analysis using microwave digestion system Multiwave 3000 (ANTON PAAR). The credibility of above analytical methods in the determination of all considered analytes (Fe, Cu, Zn, Mn) in serum were verified with the use of the certified reference material (CRM) Seronorm™ Trace Elements Serum L-2 SERO, Norway).

2.4. Statistical analysis

The collected data underwent statistical analysis using R software (Core Team R. A language and environment for statistical computing. Vienna, Austria; R. Foundation for statistical computing, 2013). A basic data analysis was performed to obtain the mean values, median, range and standard deviation of the mean. Differences in the concentration of the various metals in blood serum for studied groups were determined using the Mann-Whitney U test and chi-square test. Correlation coefficients between variables were assessed using Pearsons test. Dispersion and Loess curve fitting were done for selected pairs of variables. Value of $p < 0.05$ was considered statistically significant.

Table 1
Clinical and Laboratory characteristics of study subjects.

Characteristics	Groups	
	UC	CD
N	14	27
Age	10.4 ± 5.50	12.1 ± 4.10
Weight/Height [%]	**−3.43 ± 12.8**	**−11.2 ± 15.4**
Coles' index [%]	**96.4 ± 13.4**	**89.6 ± 14.8**
Hb [g/dl]	11.6 ± 1.58	11.2 ± 1.24
Hct [%]	36.1 ± 3.60	36.3 ± 3.63
MCV [fl]	**80.9 ± 5.72**	**77.3 ± 5.27**
OB [mm]	27.6 ± 17.2	28.2 ± 19.1
CRP [mg/l]	**23.4 ± 61.0**	**30.3 ± 47.2**
Protein[mg/ml]	70.2 ± 9.83	69.3 ± 7.98
Albumin [mg/ml]	39.1 ± 5.51	37.4 ± 6.84
Ferritin [ug/l]	34.2 ± 46.2	66.2 ± 76.8
Transferin[g/l]	2.34 ± 0.69	2.30 ± 0.53
TfS [%]	12.2 ± 11.0	12.5 ± 8.69

Data are Means ± SD. The values marked with bold are considered significant as adjusted $p < 0,05$.
UC- ulcerative colitis; CD- Crohn's disease; Hb- hemoglobin; Hct- heamatocrit; MCV- mean corpuscular volume.
OB-erythrocyte sedimentation rate; CRP- C-protein; TfS- transferrin saturation.

3. Results

3.1. Clinical characteristics

Clinical characteristics of IBD (CD and UC) patients are shown in Table 1. Malnutrition occurred in up to 48.7% of children at the moment of diagnosis (CI < 90%). Among CD group it was 60% whereas in the UC group the percentage was lower, around 33.3%. Malnutrition occurred more often in girls than boys (65% vs 35%).

Mean hemoglobin concentration in both CD and UC groups was in the lower limit of the reference range (11 g/dl). MCV values as well oscillated in the lower limit of norm and were statistically lower in the CD subgroup compared to UC subgroup (77.3 fl vs 82.6 fl) ($p = 0.32$). Iron deficiency, corrected to CRP values, was present in 70.4% CD and 64.3% UC patients. Transferrin saturation below reference values was found in 74.1% children with CD and 78.6% children with UC.

3.2. Serum ETEs levels

Serum concentration of five measured ETEs (Fe, Zn, Cu, Mn, Se) at the moment of diagnosis and after one year of treatment in IBD (with separation for CD and UC subgroups), as well as for the healthy controls (HC) are listed in Tables 2 and 3. The obtained data demonstrate a significant alteration of trace elements status in serum of children with IBD in comparison to the healthy group. The most prominent, statistically significant differences in concentration of ETEs were detected for Fe, Zn, and Cu. At the moment of IBD diagnosis (baseline) the copper concentration was significantly higher than observed for healthy controls (median concentration 1.38 mg/l vs 1.06 mg/l; $p < 0.001$). On the other hand, the median concentration of zinc (0.78 mg/l vs 0.88 mg/l; $p = 0.049$) and iron (1.09 mg/l vs 1.52 mg/l; $p = 0.011$) were significantly lower for IBD group than the control group.

Table 2
Concentration of trace elements found in serum of patients with IBD, CD, UC at the time of diagnosis (baseline) and in healthy control group.

Elements	IBD Baseline	CD baseline	UC baseline	Control	p_1	p_2	p_3	p_4
Fe [mg/l]	1.41 ± 1.30 1.09 [0.73;1.47]	1.44 ± 1.34 0.89 [0.74;1.48]	1.37 ± 1.27 1.10 [0.74;1.25]	1.57 ± 0.63 1.52 [1.26;1.74]	**0.011**	**0.036**	**0.016**	0.978
Cu [mg/l]	1.41 ± 0.39 1.38 [1.14;1.59]	1.44 ± 0.44 1.43 [1.17;1.58]	1.35 ± 0.29 1.27 [1.14;1.58]	1.02 ± 0.19 1.06 [0.88;1.15]	**< 0.001**	**< 0.001**	**0.002**	0.554
Zn [mg/l]	0.85 ± 0.29 0.78 [0.68;0.94]	0.84 ± 0.33 0.77 [0.68;0.92]	0.85 ± 0.22 0.90 [0.67;1.01]	0.93 ± 0.22 0.88 [0.84;0.95]	**0.049**	**0.013**	**0.661**	0.492
Mn [µg/l]	12.9 ± 9.79 9.00 [6.20;16.5]	14.0 ± 10.7 11.1 [5.75;18.2]	10.8 ± 7.56 8.45 [6.62;13.9]	10.3 ± 9.07 6.65 [5.10;12.3]	0.160	0.152	0.381	0.690
Se [µg/l]	44.3 ± 13.6 42.0 [36.0;51.0]	45.1 ± 15.0 42.0 [36.0;51.2]	42.7 ± 10.7 41.6 [38.2;48.9]	45.1 ± 13.4 47.4 [41.4;52.8]	0.307	0.432	0.294	0.847

Data presented as mean ± SD, median [quartile]; p_1 -IBD group vs control group; p_2 - CD group vs control group; p_3 -UC group vs control group; p_4- CD vs UC. The values marked with bold are considered significant as adjusted $p < 0.05$.
IBD- inflammatory bowel disease; CD- Crohn's disease; UC- ulcerative colitis; Fe- iron; Cu- copper; Zn- zinc; Mn- manganese; Se- selenium.

Considering the Crohn's disease (CD) and the ulcerative colitis (UC) subgroups separately, generally the same trends in concentrations of studied ETEs for each of the two groups in comparison to healthy controls were observed as for the whole IBD group (Table 2), although in the case of UC the zinc concentration (median 0.85 mg/l vs 0.93 mg/l) were at the same level.

After one year of observation (Table 3) there was statistically significant decrease in mean concentrations of copper in serum of each of the studied groups of patients (IBD with $p = 0.002$; CD with $p = 0.049$; and UC with $p = 0.011$) but still the levels remained higher than in the control group. The other changes were not statistically significant although a trend of the increasing Fe and Se concentration in all studied groups of patients (IBD, CD, UC) as well as the decreasing Mn concentration in IBD and CD groups were observed. Comparing both subgroups (the CD and UC) between themselves after one year of diagnosis, there were no statistically significant differences (Table 3).

3.3. Correlations between serum ETEs within cases and HC

Correlations of serum ETEs levels within cases (IBD, CD, UC) and healthy controls (HC) at the baseline and after one year of treatment were conducted using Pearson's test (Table 4). For the IBD group at the moment of diagnosis the iron level correlated positively with zinc and manganese levels. In the CD group a positive correlation was observed only between iron and zinc levels whereas in the UC group no correlation between measured ETEs was found. After one year of treatment in both IBD and CD groups the iron levels correlated positively with manganese levels, whereas in UC group a negative correlation between the copper and selenium levels was observed. Correlations of serum ETEs levels within HC were different from that within cases.

3.4. Correlations between serum ETEs levels and clinical and biochemical parameters

Correlations of serum ETEs levels with clinical and biochemical parameters in cases at the moment of diagnosis are presented in Table 5. For the IBD group a positive correlation was observed for iron and transferrin saturation (TfS) as well as total protein concentration. Manganese correlated positively with weight, height and the concentration of transferrin, transferrin saturation (TfS) and total serum protein concentration. Selenium correlated positively with hematocrit level and TfS. A negative correlation was observed between copper and hemoglobin as well as hematocrit concentration. In the CD subgroup a positive correlation was observed for zinc and manganese with weight and height as well as for iron and manganese with total protein concentration. A negative correlation was observed between iron and CRP concentrations. In the case of the UC subgroup positive correlations between the concentration of copper in serum and the total proteins as well as albumin levels were observed. Manganese correlated positively with transferrin levels. Selenium concentration at the baseline correlated positively with MCV value.

4. Discussion

Our study shows that the ETEs serum concentration profile for IBD patients is significantly different from that of healthy controls. Disturbances in ETEs homeostasis observed on cellular and systemic levels for IBD patients are caused by various factors, including inadequate consumption, compromised intestinal absorption, direct gastrointestinal loss, or hypermetabolic state [9–17,20–31]. Characteristic for IBD disease ETEs dyshomeostasis has its clinical presentations in

Table 3
Concentration of trace elements found in serum of patients with IBD, CD, UC at diagnosis and after one year of observation.

Elements	IBD Baseline	IBD after one year	CD baseline	CD after one year	UC baseline	UC after one year	p_1	p_2	p_3
Fe [mg/l]	1.41 ± 1.30 1.09 [0.73;1.47]	1.57 ± 1.62 1.04 [0.79;1.68]	1.44 ± 1.34 0.89 [0.74;1.48]	1.48 ± 1.33 1.16 [0.82;1.66]	1.37 ± 1.27 1.10 [0.74;1.25]	1.75 ± 2.12 0.96 [0.70;1.70]	0.898	0.934	0.855
Cu [mg/l]	1.41 ± 0.39 1.38 [1.14;1.59]	1.13 ± 0.47 1.08 [0.78;1.43]	1.44 ± 0.44 1.43 [1.17;1.58]	1.19 ± 0.52 1.14 [0.82;1.46]	1.35 ± 0.29 1.27 [1.14;1.58]	1.00 ± 0.32 0.94 [0.80;1.21]	**0.002**	**0.049**	**0.011**
Zn [mg/l]	0.85 ± 0.29 0.78 [0.68;0.94]	0.85 ± 0.20 0.84 [0.71;0.95]	0.84 ± 0.33 0.77 [0.68;0.92]	0.83 ± 0.15 0.85 [0.70;0.94]	0.85 ± 0.22 0.90 [0.67;1.01]	0.89 ± 0.27 0.83 [0.75;0.94]	0.783	0.657	0.903
Mn [µg/l]	12.9 ± 9.79 9.00 [6.20;16.5]	12.1 ± 12.5 8.20 [3.84;14.7]	14.0 ± 10.7 11.1 [5.75;18.2]	12.5 ± 10.9 8.37 [4.50;17.2]	10.8 ± 7.56 8.45 [6.62;13.9]	11.4 ± 15.4 5.28 [2.30;12.5]	0.480	0.485	0.808
Se [µg/l]	44.3 ± 13.6 42.0 [36.0;51.0]	50.9 ± 19.9 48.6 [37.8;56.3]	45.1 ± 15.0 42.0 [36.0;51.2]	49.9 ± 19.9 49.1 [37.2;56.8]	42.7 ± 10.7 41.6 [38.2;48.9]	52.7 ± 20.5 47.2 [40.9;53.7]	0.098	0.336	0.091

Data presented as Mean ± SD, Median [quartile]; p_1 -IBD group at diagnosis vs IBD group after one year of observation; p_2 - CD at diagnosis vs CD after one year of observation; p_3 -UC at diagnosis vs UC after one year of observation. The values marked with bold type are considered significant as adjusted $p < 0.05$.
IBD- inflammatory bowel disease; CD- Crohn's disease; UC- ulcerative colitis; Fe- iron; Cu- copper; Zn- zinc; Mn- manganese; Se- selenium.

Table 4
Correlation coefficients between measured trace elements concentrations in serum of control and IBD, CD and UC patients groups at the time of diagnosis (baseline) and after one year of treatment (one year).

	Fe		Cu		Zn		Mn		Se	
	Baseline	one year	baseline	one year	baseline	one year	baseline	one year	baseline	one year
IBD group										
Fe	1.00	1.00								
Cu	0.02	0.08	1.00	1.00						
Zn	**0.55**	0.13	−0.14	−0.17	1.00	1.00				
Mn	**0.34**	**0.48**	0.09	0.16	0.27	0.05	1.00	1.00		
Se	0.07	0.19	−0.17	−0.02	0.12	0.19	0.01	−0.06	1.00	1.00
CD group										
Fe	1.00	1.00								
Cu	−0.09	0.15	1.00	1.00						
Zn	**0.67**	0.01	−0.22	−0.21	1.00	1.00				
Mn	0.35	**0.52**	−0.06	0.24	**0.41**	0.07	1.00	1.00		
Se	0.09	0.24	−0.22	0.22	0.25	0.13	0.04	0.09	1.00	1.00
UC group										
Fe	1.00	1.00								
Cu	0.35	−0.16	1.00	1.00						
Zn	0.39	0.28	0.08	−0.09	1.00	1.00				
Mn	0.13	0.53	0.49	−0.12	0.08	0.03	1.00	1.00		
Se	0.04	0.09	−0.03	**−0.59**	−0.10	0.20	−0.22	−0.28	1.00	1.00
Control group										
Fe	1.00									
Cu	0.01		1.00							
Zn	0.27		0.18		1.00					
Mn	**0.53**		0.18		0.02		1.00			
Se	0.30		0.16		−0.28		0.35		1.00	1.00

Statistically significant correlation coefficients ($p < 0.05$) are in bold font.
IBD- inflammatory bowel disease; CD- Crohn's disease; UC- ulcerative colitis; Fe- iron; Cu- copper; Zn- zinc; Mn- manganese; Se- selenium.

Table 5
Correlation Pearsons's coefficients between measured trace elements and clinical and laboratory parameters in serum of IBD, CD and UC patients at the time of diagnosis.

	weight	height	CRP	Hb	Hct	MCV	transferrin	TfS	total protein	albumin
IBD group										
Fe	0.07	0.04	−0.27	0.29	0.23	0,14	−0.07	**0.32**	**0.43**	0.03
Cu	−0.24	−0.18	0.11	**−0.36**	**−0.34**	−0.27	0.13	−0.14	0,21	0.16
Zn	0.20	0.14	−0.21	0.12	0.07	−0.06	−0.08	−0.00	0,18	0.14
Mn	**0.31**	**0.34**	−0.09	0.07	0.11	−0.15	**0.31**	**0.30**	**0.38**	0.15
Se	0.17	0.16	−0.10	0.25	**0.31**	0.29	0.01	**0.31**	−0.09	0.04
CD group										
Fe	0.07	0.15	**−0.44**	0.26	0.19	0.06	−0.14	0.15	**0.44**	0.37
Cu	−0.14	−0.07	0.31	−0.36	−0.35	−0.25	−0.01	−0.27	−0.10	−0.01
Zn	**0.42**	**0.41**	−0.27	0.24	0.29	−0.23	−0.12	0.06	0.24	0.16
Mn	**0.47**	**0.57**	0.06	0.10	0.12	−0.04	0.17	0.29	**0.42**	0.08
Se	0.32	0.29	−0.10	0.38	**0.47**	0.24	−0.09	0.29	−0.12	0.05
UC group										
Fe	0.03	−0.09	−0.16	0.34	0.21	0.30	0.03	0.50	0.48	0.16
Cu	−0.38	−0.37	−0.51	−0.38	−0.41	−0.27	0.50	−0.02	**0.73**	**0.55**
Zn	−0.06	−0.09	0.02	−0.26	−0.27	0.09	−0.02	−0.12	0.10	0.06
Mn	−0.16	−0.13	−0.41	0.10	0.09	−0.34	**0.65**	0.24	0.28	0.26
Se	0.08	−0.04	−0.21	−0.03	−0.07	**0.55**	0.21	0.41	−0.04	0.17

IBD- inflammatory bowel disease; CD- Crohn's disease; UC- ulcerative colitis; Fe- iron; Cu- copper; Zn- zinc; Mn- manganese; Se- selenium; Hb-hemoglobin, Hct-hematocrit, Tfs-transferrin saturation; MCV- mean corpuscular volume;CRP- C-protein.
Statistically significant correlation coefficients ($p < 0,05$) are in bold font.

extraintestinal complications like malnutrition, iron deficiency anemia or systemic inflammation [8–11,31–43]. All these complications were found in the studied by us cases (IBD, CD, UC) and were in line with the observed serum ETEs concentration profile as well as correlations between ETEs levels within cases.

In accordance with the previous data [30] we found lower levels of iron for IBD as well as separately CD and UC patients compared to the control group. Iron deficiency anemia, the most common systemic complication and extra intestinal manifestation in IBD [8,20,21,31–43] was observed by us in more than half of the cases. Also the changes in correlations between ETEs within cases in comparison to that in healthy control (HC) were in line with the disease process. In IBD group we observed statistically significant strong positive correlation for iron with zinc (especially in CD subgroup) and weak positive with manganese whereas in control group strong positive correlation for iron with manganese and weak positive for iron with zinc and selenium. The influence of manganese and zinc on iron homeostasis may be mediated through influence on its absorption, circulating transporters like

transferrin, and regulatory proteins [48–58]. Mechanisms mediating Fe and Zn interaction are strongly dependent on the levels of metals and their ratio [48,52,49–58]. The recent study of Massimo Martinelli and coworkers did demonstrate that serum hepcidin is increased in IBD children with active disease and could be responsible for iron malabsorption [57,58]. Considering iron and manganese correlations, the same intestinal transport systems for Fe and Mn could explain the fact that iron deficiency may increase the amount of transported manganese. Literature data [48–52] available for children with anemia from iron deficiency show much higher concentrations of manganese in serum in comparison with the control group what is correlating well with our results received for IBD, CD and UC groups. After one year of treatment Mn levels in IBD group as well as in subgroups CD and UC decreased in comparison with the baseline values.

Similarly to iron, mean serum zinc concentration was found to be significantly lower for examined group of children with IBD, than in the control group. The difference mostly concerned CD subgroup what is correlating well with accessible literature data [27]. Deficiency of Zn may have severe consequences as this metal is necessary for maintaining cellular structure and various functions like acid-base catalysis, immune functions, cell division and apoptosis [53–56]. Zinc is absorbed along the length of the small intestine and its increase in the cell content under disease conditions is accompanied by a decrease in the concentration of this metal in blood serum [53,55]. In the present study at the moment of diagnosis zinc correlates positively with iron (IBD group) and with manganese (CD subgroup).

At the moment of diagnosis mean serum concentration of copper was found to be significantly higher in IBD patients group than healthy control group. At the division to CD and UC subgroups the same result has been observed. Moreover, the mean level for children from the CD subgroup was much higher than for the UC subgroup. Similar data were obtained by Sturnilo [25,26] and Ringstad [22] in their studies on adults and Ojuawo and Keith [27] on pediatric IBD patients. Previous studies have shown that inflammation can increase Cu level and that there is association between systemic inflammation and high Cu level in serum. Increased serum concentration of copper can lead to radicals generation also in the colonic mucosa resulting in a continuous cycle of inflammation in IBD [59,60]. In our study after one year of treatment significant decrease of the Cu concentration in serum for the IBD group as well as CD and UC subgroups was found. The observed changes may reveal reduction of the inflammation process. In antioxidant defense, both copper and zinc are involved as cofactors of cytosolic superoxide dismutase. Decrease of copper concentration due to the reduction of inflammation process during one year therapy was probably also responsible for the decrease of Cu/Fe concentrations ratio observed by us for IBD group as well as for CD and UC subgroups.

Literature data concerning Se concentration in serum for IBD patients are ambiguous. Selenium deficiency has been associated with complications and unfavorable outcomes in various acute and chronic diseases [61,62] and could be a result of reduced intake, reduced absorption or increased mobilization for defense with free radicals which contribute to tissue injury in these conditions [27]. In some trials a lower concentration was observed for IBD [8,9] in others only for UC [26] or CD patients [22,23]. In the study of pediatric population, Ojuewo and Keith observed low serum levels of Se for both CD and UC pediatric patients [27], whereas Sikora et al. [30] did not find any significant differences in Se concentrations between IBD patients and control group. We also in our study did not observed statistically significant differences between Se concentrations for IBD group and healthy control although there was a trend to lower Se concentrations within cases than healthy controls.

5. Limitations

Several limitations should be addressed when interpreting the results of the present study. Firstly, limited number of patients in study population and healthy controls, which my influence statistical analysis. Secondly, no analysis was made after one year of treatment regarding the drugs used to receive clinical remission in cases. It is possible that although all patients received clinical remission, the dose or type of medications used to achieve that, my influence the ETEs metabolism differently. Finally, our study findings represent results of ETEs concentration in the blood serum which may not necessarily reflect their body storages.

6. Conclusion

The profile of trace elements (Fe, Zn, Cu, Mn and Se) concentration in blood serum for pediatric IBD patients at the moment of diagnosis was found to be significantly different from such a profile observed for the healthy control group. Decrease of iron and zinc and increase of copper concentration were connected with the development of IBD pathology. The changes were more significant for CD than UC patients, yet statistically not significant thus the possibility of using such a profile as a biomarker for differentiation between CD and UC pathologies in children needs much broader studies. After one year of treatment the trace elements profile of IBD patients resembled much more that characteristic for healthy control group showing its validity as clinical biomarker in disease development assessment. This tool could be additionally supported by the analysis of the changes in the ETEs correlations' profile.

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