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Erythrocyte transketolase deficiency in patients suffering from Crohn's disease

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Abstract. – OBJECTIVE: Aim of the study was to assess the possible vitamin B1 deficiency in relation to the exacerbation of Crohn's disease (CD) in adult patients.

PATIENTS AND METHODS: Forty-nine Crohn's disease (CD) patients with different disease activity (The Crohn's Disease Activity Index-CDAI) were included in the study. Anthropometrical and biochemical parameters, i.e., high sensitive C-reactive protein, were assessed. The spectrophotometric method was used to measure the transketolase activity (TK) in erythrocytes. The normalized transketolase activity ratio (NTKZ) and the percentage of activation with thiamine pyrophosphate (%TPP) were also evaluated.

RESULTS: The mean values of BMI were close to cut-off: 18.5 kg/m^2, indicating a poor nutritional status in CD patients. The patients with moderate-to-severe active CD had a statistically significant higher value of CDAI and hsCRP concentrations compared to those being in the asymptomatic remission or at the mildly active stage of the disease. The level of NTKZ and %TPP were statistically different between the analyzed groups, showing the deficit of vitamin B1 in the group of moderate-to-severe active CD patients (Mean ± SD; NTKZ: 1.99 ± 0.87 *vs.* 1.54 ± 0.62 U/g Hb; % of TPP: 0.15 ± 0.78 *vs.* 54.90 ± 38.80).

CONCLUSIONS: Vitamin B1 deficiency is part of the Crohn's disease manifestation in moderate-to-severe active patients.

Key Words:
Inflammatory bowel disease, Transketolase, B-vitamins, Deficiency.

Introduction

Along with the clinical manifestation of Inflammatory Bowel Disease (IBD) usually co-existing with malnutrition, patients with Crohn's Disease (CD) may be characterized by vitamin deficiency[1]. Particularly, protein-calorie malnutrition may worsen the vitamin B1 (thiamine) absorption being carried out with albumin[2]. The low concentration of thiamine in serum is related to reduced food intake with high nutritional density due to anorexia or fear of eating from abdominal pain, exudation from intestinal mucosa interrupted enterohepatic circulation, malabsorption (i.e., resection or bypass, stagnant loop syndrome from strictures fistulae or surgically created blind loops) or effect of medical therapy/parenteral nutrition[3]. The proper value of vitamin B1 in the IBD patients is of high importance, as thiamine plays a role in the function of nervous and cardiovascular system (Wernicke-Korsakoff syndrome and/or beriberi); therefore, can directly influence treatment effect and health status of IBD patients[4]. The good sources of thiamine are pork, fish, seeds, nuts, beans, green peas, tofu, brown rice, squash, asparagus, and seafood[5]. However, products rich in vitamin B1, are not well tolerated by patients with IBD and thus are consumed in small amount[6]. Additionally, the ongoing inflammatory process in the gastrointestinal tract may further complicate thiamine absorption (low concentration – by passive transport or high concentration – possible by facilitating diffusion), which takes place in proximal small intestine, especially in the jejunum[7]. The daily requirement of vitamin B1 is 1.2 mg[5]; however, it is suspected that IBD patients do not meet the recommended value. There is inconsistency in information about vitamin B1 levels in IBD patients during the different stages of the manifested disease. Therefore, this study aimed to assess the possible vitamin

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B1 deficiency in the relations to exacerbation of Crohn's disease in adult patients.

Patients and Methods

Forty-nine CD patients, diagnosed between 2013-2015, were included in the study. The study was conducted during the patients' medical visits in the University Clinic of Gastroenterology in Poznan while the selection of the patients was based on the following inclusion criteria: diagnosed CD, age >18 and willingness to participate in the study. Exclusion criteria were as follow: celiac microscopic diseases of intestinal inflammation, bacterial infections of the digestive tract (*Salmonellosis, Shigellosis, Yersiniosis, Campylobacteriosis*), parasitic and viral diseases, drug abuse, use of long-term parenteral nutrition or dialysis, Furosemide and Theophylline treatment in the last 3 months, legal incompetence or limited legal competence, diagnosed chronic alcoholism and experienced of the intense vomiting during the recruitment process. The disease activity in CD patients was assessed with the use of The Crohn's Disease Activity Index (CDAI) and interpreted as follow: 0 to 149 points: asymptomatic remission, 150 to 220 points: mildly to moderately active Crohn's disease, 221 to 450 points: moderate-to-severe active Crohn's disease, 451 to 1100 points: severely active to fulminant disease). All patients agreed to participate in the study and signed appropriate informed consent. The Ethical Committee at the University of Medical Sciences in Poznan (Poland) approved the research protocol (No. 42/09). The research met the requirements of the Helsinki Declaration.

The body weight and body high with an approximation of 0.1 kg and 0.5 cm (Seca digital scale 763; Seca, Hamburg, Germany) were assessed to calculate Body Mass Index (BMI).

The biochemical assessment was performed by a certificated laboratory in the Department of Neurochemistry and Neuropathology at the Poznan University of Medical Sciences according to standardized procedure and good laboratory practice. Blood samples were taken after 14-hours of fasting. The concentration of a high sensitivity C-reactive protein (hsCRP) was measured using ELISA test (eBioscience, San Diego, CA, USA) and white blood cells (WBC) by Synchron CX 7 Delta (Beckman, Fullerton, CA, USA). The spectrophotometric method in the modification by Bayoumi and Rosalki[8] and Smeets et al[9] was used to measure the transketolase activity in erythrocytes (TK) being expressed as units per gram of hemoglobin. One unit of transketolase produces 1.0 μmol of glyceraldehyde 3-phosphate from xylulose 5-phosphate per minute at 25°C, in the presence of ribose 5-phosphate, thiamine pyrophosphate and Mg^{2+} using a coupled system with α-GDH/TPI[10]. The absorbance was measured using the Cecil CE spectrophotometer (Cecil Instruments Limited, Cambridge, Cambridgeshire, UK). The normalized transketolase activity ratio (NTKZ) was assessed according to the following formula:

$$NTKZ = TK/[0.6066 - (0.002045 \times age)] \text{ [U/g Hb]}$$

***Abbreviation:** *NTKZ, normalized transketolase activity ratio in erythrocytes*, *TK, activity of transketolase in erythrocytes,* U/g Hb, units/g of hemoglobin

The percentage of TK activation with thiamine pyrophosphate (TPP) was calculated with the use of the following formula:

$$\% \text{ activation of TPP} = \frac{(\text{Activity TK after addition of TPP}}{\text{Activity TK without TPP)}} \times 100\%$$

***Abbreviation:** TPP, activation with thiamine pyrophosphate; *TK, activity of transketolase in erythrocytes*

Data interpretation: normal value: 0-15%; > 15% - deficit of vitamin B1.

Statistical Analysis

The data were presented as means ± SDs. The normality of the distribution was checked by the Shapiro-Wilk test. Comparisons between the groups were assessed with the use of the Kruskal-Wallis test. The statistical significance was set up at the level of 0.05. All calculations were performed using the Statistica 10 software (TIBCO Software Inc., Palo Alto, CA, USA).

Results

The patients with moderate to severely active Crohn's disease were characterized by the statistically significant higher value of CDAI and hsCRP concentration compared to those being in the asymptomatic remission or at the mildly active stage of the disease ($p<0.0001$). The mean values of BMI were close to cut-off: 18.5 kg/m^2, indicating the poor nutritional status in the studied patients (Table I). The level of NTKZ and %TPP were statistically different between

Table I. Characteristics of patients (n=49).

Analyzed parameters	Asymptomatic remission to mildly active Crohn's disease (n = 26)	Moderately to severely active Crohn's (n = 23)	*p*-value
Age (years)	34.81 ± 11.86	36.56 ± 10.03	0.4113
Sex (% of male)	50.00	42.37	–
BMI (kg/m^2)	18.95 ± 2.76	18.91 ± 2.02	0.8805
CDAI (points)	232.04 ± 104.20	345.26 ± 106.85	0.0004
CRP (mg/l)	28.07 ± 20.60	68.44 ± 56.03	< 0.0001
WBC (10^3)	9.04 ± 3.49	9.35 ± 3.89	0.8021

*BMI, Body Mass Index; CDAI, The Crohn's Disease Activity Index; hsCRP, high sensitivity C-reactive protein; WBC, white blood cells.

the analyzed groups showing the deficit of vitamin B1 in the group of moderate to severely active Crohn's disease patients (% of TPP>15) (Table II).

Discussion

We showed that vitamin B1 deficiency is related to moderate and severely active Crohn's disease characterized by poor nutritional status and ongoing process of inflammation.

Inadequate dietary intake, malabsorption, and disease's activity are directly related to malnutrition observed in CD patients[11]. Thus, in the clinical practice, different strategies of parenteral nutrition (PN) and enteral nutrition (EN) are looked into, where the nutritional supplementation with either an elemental or polymeric diet may be equally effective in active CD patients[12,13]. Thiamine is critical for the activity of four key enzymes in cellular metabolism; pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (α-KGDH) in the tricarboxylic acid (TCA) cycle, transketolase (TKT) within the pentose phosphate pathway (PPP) and branched-chain alpha-keto acid dehydrogenase complex (BCKDC) which is involved in amino acid catabolism[14]. Furthermore, vitamin B1 is converted into the active diphosphate, thiamine pyrophosphate form intracellularly being involved in macronutrient metabolism[14,15]. It was already considered that use of the thiamine in the dietary supplement is important for improving the well-being of groups being at risk of thiamine deficiency, i.e., those affected by IBD, when the proper dose of the easily absorbed form (such as benfotiamine) would be used[16,17]. Thiamine antivitamins should also be considered as useful additional agents in the therapy of cancer[16].

In the experimental studies by Bruce et al[18] has been suggested that thiamin deficiency can result in the endogenous formation of genotoxic alpha-oxoaldehydes (glyoxals) and the induction of colon cancer, showing the clinical importance of the current study. As a typical Western diet provides a low level of vitamin B1, patients with IBD may also be exposed to an increased risk of colon cancer or other types of cancers. However, we have to keep in mind that thiamine is usually

Table II. The activity of erythrocyte's transketolase in IBD patients (n=49).

The activity of erythrocyte's transketolase in IBD patients (n = 49)	The activity of erythrocyte's transketolase in IBD patients (n = 49)	The activity of erythrocyte's transketolase in BD patients (n = 49)	The activity of erythrocyte's transketolase in IBD patients (n = 49)
TK (U/g Hb)	1.065 ± 0.47	0.82 ± 0.31	0.0545
NTKZ (U/g Hb)	1.99 ± 0.87	1.54 ± 0.62	**< 0.0001**
%TPP activity	0.15 ± 0.78	54.90 ± 38.80	**< 0.0001**

*TK, activity of transketolase in erythrocytes; NTKZ, normalized transketolase activity ratio in erythrocytes; %TPP, % activation with thiamine pyrophosphate.

supplemented in processed foods and readily consumed in over-the-counter vitamin and nutritional supplements in Western countries with high cancer incidences, while in Asian and African countries characterized by food consumption rich in thiaminases (a natural thiamine-degrading enzyme) exposure of vitamin B1 may be reduced[19]. Nevertheless, it has been indicated that inflammation may also play a role in the pathogenesis of colorectal cancer as the colorectal cancer risk is associated with biochemical markers for B-vitamin deficiency, insulin resistance, and colonic inflammation[20]. Moreover, it was highlighted that hsCRP concentration, being elevated among persons who subsequently develop colon cancer, may provide a general assessment of ongoing inflammation and therefore is itself associated with risk of colon cancer[21]. This perspective shows that the population-based importance for further investigation of B-vitamin deficiency as well as the probable protective effect of a well-balanced diet in patients suffering from IBD against cancer. In our study, patients with moderate and severe active CD showed a high deficit in vitamin B1 expressed as 54.90±38.80 %TPP activity. We noticed that the method of TK activity analysis and mode of its expression plays a role in the detection of deficiency. Normalized transketolase activity ratio in erythrocytes and percentual activation of TK with thiamine pyrophosphate seem to be more sensitive in the diagnosis of thiamine deficits.

As indicated by Ghashut et al[2], red cell concentrations of vitamin B1, B2, and B6 are likely to be more reliable measurements of vitamin status in the presence of a systemic inflammatory response. It is important to recognize nutritional deficiencies at an early stage, initiate appropriate treatment, and do not let many patients suffer unnecessarily from the consequences of deprivation of nutrients[3]. Thiamin deficiency can induce insulin resistance often observed in CD patients, which may be related to an oxidative stress[22,23]. The interventions with n-3 fatty acids together with thiamin would be effective and could provide a benefit approaching patients with weight loss[24]. We have to remember that thiamin deficiency is also one of the refeeding syndrome risk factors being very often presented in CD patients, and therefore, it should be recognized at the beginning of nutritional intervention[25]. Another target for health interventions for IBD is fatigue, being commonly manifested in these groups of patients. According to a review of Artomt et al[26], fatigue may be partially eliminated by solution-focused therapy with the use of thiamine and exercise. So, there are different levels of beneficial effect of thiamine action, and therefore, the proper concentration of vitamin B1 in humans should be of high interest among professionals as well for medicine as for nutritional sciences.

Conclusions

Vitamin B1 deficiency is part of the Crohn's disease manifestation in moderately and severe active patients. It seems that diet supplied to these patients should include the proper dose and form of vitamin B1, which may improve health status and the effect of medical treatment. Moreover, normalization of TK activity and its response to thiamine pyrophosphate seems to be a more proper method in the detection of vitamin B1 deficiency.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

DMW: data collection, data interpretation, and manuscript writing, SM: laboratory test analysis, critically manuscript reviewing, JK: critically manuscript reviewing, AD: data collection and critically manuscript reviewing, AW: data analysis, MSM: study design, data interpretation, and manuscript writing.

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