EVALUATION OF ZINC AND COPPER CONCENTRATIONS AND THE TOTAL ANTIOXIDANT CAPACITY OF BLOOD PLASMA IN PATIENTS WITH MALABSORPTION SYNDROME

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Abstract

Disturbance of the antioxidative-oxidative balance is a predisposing factor in the development of a variety of diseases. Microelements, including zinc and copper, are components of active enzyme regions participating in anti-oxidative systems. Patients with malabsorption syndrome may suffer from diseases related to the chronic deficiency of micro- and macroelements and vitamins, but also experience health problems due to an impaired antioxidative plasma defence, i.e. illnesses caused by oxidative stress. The aim of the study was to assess zinc and copper concentrations as well as the total antioxidant capacity of blood plasma in 33 patients hospitalized in the Department of Internal Medicine, Metabolic Diseases and Dietetics, presenting symptoms of intestinal malabsorption such as weight loss, abdominal pain and diarrhoea. Blood donors made up the control group. Statistically significantly lower concentrations of zinc and copper were found in the study group as compared to the control group for women and men together as well as for women and for men separately ($p < 0.05$). A significantly lower FRAP (Ferric Reducing Ability of Plasma) value as a measure of blood plasma the total antioxidative capacity was found in the study group of men in comparison with men from the control group ($p < 0.05$). Patients showed deficiencies of the two microelements despite normal values of BMI (Body Mass Index) and laboratory parameters such as haemoglobin, protein, albumin and iron concentrations. Regardless of the nutritional state assessment, patients with malabsorption syndrome should receive adequate supplementation of vitamins and microelements.

Keywords: microelements, antioxidative plasma defence, impaired intestinal absorption.

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INTRODUCTION

Recently, many studies have reported disturbances of the antioxidative -oxidative balance as a predisposing factor in the development of a variety of diseases. Excessive production of free oxygen radicals causes oxidative stress, which has an effect on the initiation of inflammatory processes in the body, increasing the risk of neoplasms, cardiovascular diseases, as well as accelerating the ageing process. Free oxygen radicals, defined as chemical compounds containing one unpaired electron, are highly reactive and easily react with cellular components, activating degenerative processes in the body (ZABŁOCKA, JANUSZ 2008). They cause damage within proteins, nucleic acids, lipids and carbohydrates. Among all reactive oxygen and nitrogen species, superoxide anion radical (O_2) as well as products of its conversion such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH-) and peroxynitrate (ONOO–) have the most significant importance in the initiation of pathological processes. Production of free radicals is regulated by two anti-oxidative systems: enzymatic and non-enzymatic. The enzymatic system encompasses superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), glutathione reductase (GSSGR) and ceruloplasmin. The non-enzymatic system consists of low molecular weight compounds such as blood serum proteins, coenzyme Q10, flavonoids, vitamins A, C, E and many others (RAHMAN 2007).

Zinc is as a coenzyme and activator that belongs to a group of over 100 enzymes such as dehydrogenase, carbonic anhydrase, alkaline phosphatase, superoxide dismutase (Zn-SOD) and DNA and RNA polymerase. It is responsible for nucleic acid metabolism, protein and DNA biosynthesis and gene expression. It plays a crucial role in the body's antioxidative defence as it slows down the oxidation of unsaturated fatty acids, decelerates the synthesis of superoxides and hydroxyl radicals by transitional metals and, by being a component of superoxide dismutase (Zn-SOD), and participates in the elimination of free oxygen radicals. (PUZANOWSKA-TARASIEWICZ et al. 2008). The overall amount of zinc in the body oscillates between 2 and 4 g, up to 98% of which is intracellular zinc, contained in muscles, the liver, the pancreas, kidneys, bones, skin, hair and erythrocytes (RABIEJ et al. 2006). An adequate amount of zinc in serum is defined as a zinc concentration within the international reference values of 11.6 µmol l^{-1} - 23 µmol l^{-1}, whereas zinc values of less than 10.71 µmol^{-1} are defined by the WHO as a zinc deficiency (ANGELOVA et al. 2006). In practice, however, symptoms of dietary zinc deficiency may be noticed even when laboratory values do not show any abnormalities (BAE-HARBOE et al. 2012).

Clinically, an abnormally low level of zinc in the body is manifested by abnormalities in reaching height and puberty, skin dysfunction, disturbances in production of antibodies, lack of appetite, body mass loss, psychological and neuro-sensory disturbances as well as an impaired sense of taste (CROFION et al. 1983, PUZANOWSKA-TARASIEWICZ et al. 2008). Currently, there are

numerous methods for determination of the zinc content in serum, hair, erythrocytes and leukocytes. Unfortunately, none of these techniques is fully satisfactory. The most popular indicator in zinc deficiency assessment is the determination of zinc concentration in serum (ANGELOVA et al. 2006). However, there are many factors which influence the level of zinc in serum, for example its concentration decreases with age, tends to be higher during the morning hours, but decreases parallel to a decline in the albumin concentration in the presence of inflammation (GUERRIERI et al. 1986).

Copper is a component of active enzyme regions participating in oxidation-reduction processes, such as those of cytochrome oxidase, lysyl oxidase, ascorbic oxidase, superoxide dismutase or ceruloplasmin. As a cofactor of many enzymes, it is engaged in the intracellular respiration, prostaglandin synthesis, angiogenesis, the production of connective tissue and red blood cells, transmission of nervous tissue impulses and in the immunological response. The total content of copper in the body is around 80-150 mg, and the element is mostly accumulated in muscles, the liver, bones and the brain. Metallothionein is responsible for the regulation of absorption, intestinal transport and liver capture of copper. After the intestinal absorption and transfer to blood serum, copper ions are bound to albumin. Copper is accumulated in hepatic lysosomes or bound to apoceruloplasmin, forming ceruloplasmin, which binds even up to 90% of copper subsequently secreted to serum (STERNLIEB, JANOWITZ 1964., HUFF et al. 2007). The most common symptoms of copper deficiency are anaemia, rupture of blood vessels, bone fractures, height retardation, cardiovascular system disturbances, disturbances in nervous, vascular, reproductive systems, increase in the cholesterol level as well as depressed humoral and cellular immunity (KOCHANOWSKA et al. 2008).

Patients with malabsorption syndrome, due to the deficient absorption of different nutritional components, suffer from diseases related to chronic deficiency of micro- and macroelements and vitamins. In addition, as a result of a less efficient antioxidative defense, they are more likely to develop illnesses caused by oxidative stress, such as neoplasms or cardiovascular diseases.

Aim of the study

The aim of the study was to assess serum zinc and copper concentrations and the total antioxidant capacity of plasma in patients with malabsorption syndrome. Healthy people who were blood donors made up the control group.

SUBJECTS AND METHODS

Researched group

The group was composed of 33 patients, 23 women and 10 men, aged 20 to 67 years old, with symptoms of malabsorption syndrome, who were hospitalized in the Department of Internal Medicine, Metabolic Diseases and Dietetics. All patients were being diagnosed because of symptoms such as abdominal pain, body mass loss and episodes of diarrhoea. At the end of the diagnostic examination, they were diagnosed with the following diseases: coeliac disease, lactose intolerance and inflammatory bowel disease.

The control group was composed of 20 healthy women and 10 healthy men aged from 23 to 57 years old, whose blood samples were obtained from the Regional Blood Donation and Blood Treatment Center. Permission for conducting the research was obtained from the Poznan University of Medical Sciences Bioethical Commission.

Methods

From all the patients, fasting venous blood was drawn into vials with sodium edetate. The samples were subsequently centrifuged for 10 minutes with a centrifugal force of 800 g. Blood serum specimens were stored in a freezer at -80°C until analyses, such as determinations of copper and zinc concentrations and the total antioxidative potential of serum. The determinations were performed by flameless absorption spectroscopy on a Perkin-Elmer Zeeman 3030 spectrometer. As reference materials for the evaluation of standard curves of the assayed bio-elements, Seronom Trace Elements Serum (Nycomed Pharma As), powdered tissues of animal muscles (IAEA, Vienna, Austria) and bovine liver (National Bureau of Standards 1577a) were used. Three measurements were made for every trial. The determination error was 3-5%. The total antioxidative potential of blood serum was determined with a FRAP method, using the reduction capability of Fe+3 to Fe+2 in serum.

The reactions occurred in low pH (3.6), leading to the creation of colourful, blue-violet complex Fe+2-tripirydyl-s-triazyn (TPTZ). The absorption of this colourful complex was measured by spectrophotometry at a wavelength of 535 nm. The results were processed with the use of Labsystem software.

Blood morphology, sodium, potassium, iron, albumin, total protein concentrations and anti-transglutaminase antibodies were evaluated in the hospital's central laboratory as standard measurements required during a diagnostic process. Gastroscopy, colonoscopy, abdominal ultrasonograph as well as hydrogen breath test detecting lactose intolerance were performed to confirm malabsorption syndrome.

RESULTS

The diagnosis of malabsorption syndrome was confirmed in all the patients. More specifically, 9 patients had lactose intolerance, 10 patients had celiac disease, 7 patients had celiac disease and lactose intolerance, 3 patients had Crohn's disease and 4 patients had Crohn's disease and lactose intolerance. Coeliac disease was confirmed by histopathological examination of samples obtained by duodenum biopsy and/or positive anti-transglutaminase antibodies in 17 patients. Lactose intolerance was verified by hydrogen breath test in 20 patients. Colonoscopy with the histopathological examination of intestinal biopsy samples as well as typical endoscopic features confirmed the diagnosis of Crohn's disease in 7 patients.

Laboratory parameters

The mean concentrations in the studied group were as follows: haemoglobin $12.7\pm1,5$ g dl^{-1} (12.2 ± 1.2 g dl^{-1} in women and 13.9 ± 1.7 g dl^{-1} in men), albumin 4.2 ± 0.8 g dl^{-1}, total protein 6.9 ± 0.9 g dl^{-1}, iron 86.5 ± 53.3 µg dl^{-1}, sodium 139.4 ± 2.8 mEq l^{-1} and potassium 4.1 ± 0.4 mEq l^{-1}. These results were within the normal ranges for the laboratory reference values.

Body mass index

BMI in the researched group of women and men was 20.9 ± 4.3, being 21.2 ± 4.7 for women and 20.3 ± 3.4 for men.

Age

The tested group: age of women: 44.9 ± 17.9 years, age of men: 44.8 ± 20 years.

The control group: age of women: 34.8 ± 11.2 years, age of men 37.1 ± 11.8 years.

Zinc

The concentration of zinc in the tested group of women and men was 11.7 ± 0.8 mmol l^{-1}, with 11.5 ± 0.8 mmol l^{-1} for women and 11.9 ± 0.8 mmol l^{-1} for men.

In the whole control group, the concentration of zinc was 13.5 ± 1.2 mmol l^{-1}, being 13.6 ± 1.3 mmol l^{-1} for women and 13.6 ± 1.1 mmol l^{-1} for men.

A statistically significantly lower concentration of zinc was found in the analyzed group than in the control group for women and men together (Figure 1) as well as for women and for men separately ($p < 0.05$).
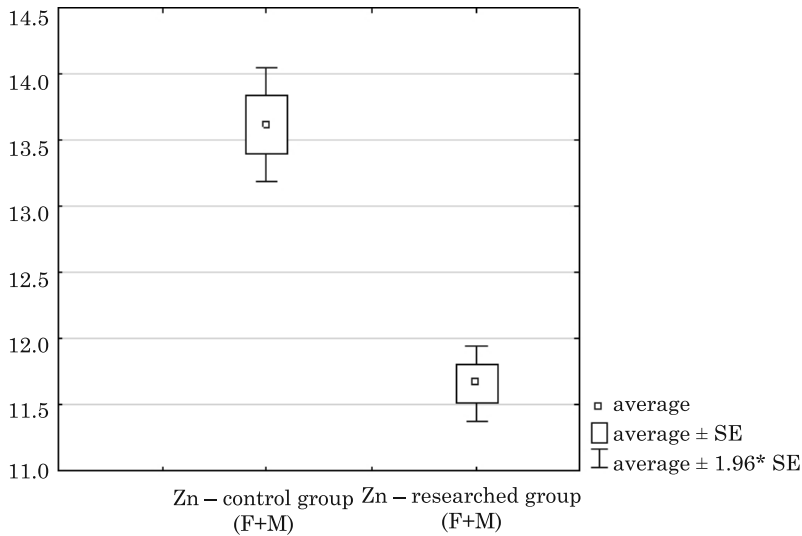
Fig.1. Comparison of zinc concentrations (mmol l⁻¹) in the researched
and control group of both women (F) and men (M) together

Copper

Copper concentration in the researched group of women and men was 12.3±1.6 μmol l⁻¹ (11.8±1.3 μmol l⁻¹ in the women's group and 13.5±1.6 μmol l⁻¹ in the men's group). In the control group of women and men, the copper concentration was 16.3±1.3 μmol l⁻¹, (16.7±1.5 μmol l⁻¹ in women and 15.5±0.8 μmol l⁻¹ in men). A statistically lower serum concentration of copper was found in the researched group as compared to the control group, for women and men taken together as well as for women and for men separately ($p < 0.05$) – Figures 2-4.

Total antioxidative capacity

The FRAP value in the researched group of women and men was 932.9±225.6 μmol l⁻¹, 887.0±196.4 μmol l⁻¹ in the women's group and 1038.3±262.7 μmol l⁻¹ in the men's group. In the whole control group, the FRAP value was 1026.6±325.6 μmol l⁻¹, 905.7±138.9 μmol l⁻¹ in women and 1439.9±472.8 μmol l⁻¹ in men. A significantly lower FRAP value was found in the men of the study group in comparison with men from the control group ($p < 0.05$) – Figure 5. No statistically significant difference was found between the women of the study group and women's control, or between the groups of women and for men together.

One possible limitation of the study is a slightly older age of the patients compared to the control group, which may have influenced the outcome. Another shortcoming is the low number of patients examined and control group of blood donors.
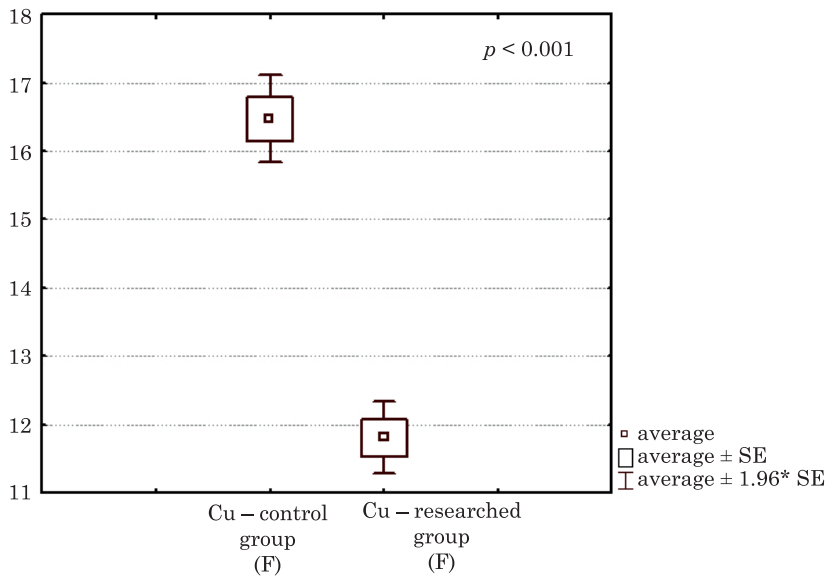
Fig.2. Comparison of copper concentrations (μmol l^{-1})
in the researched and control group of women (F)

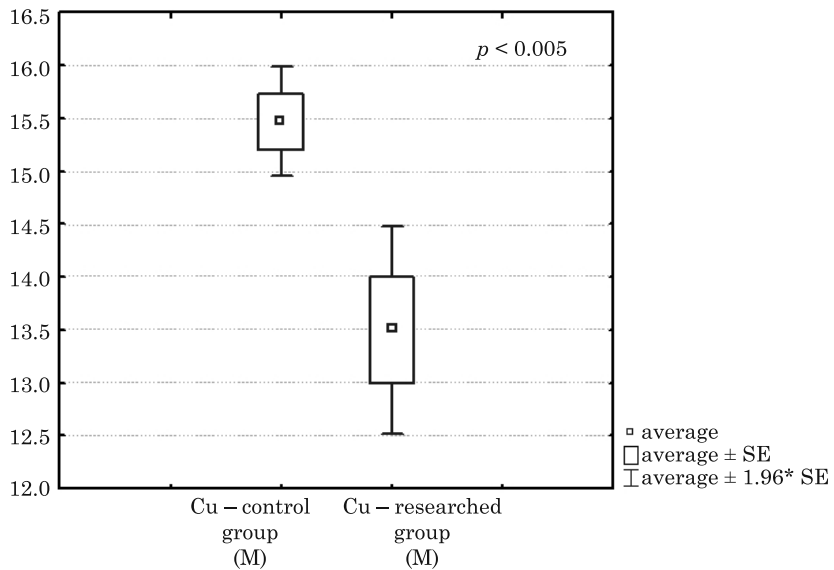Fig. 3. Comparison of copper concentrations (μmol l^{-1})
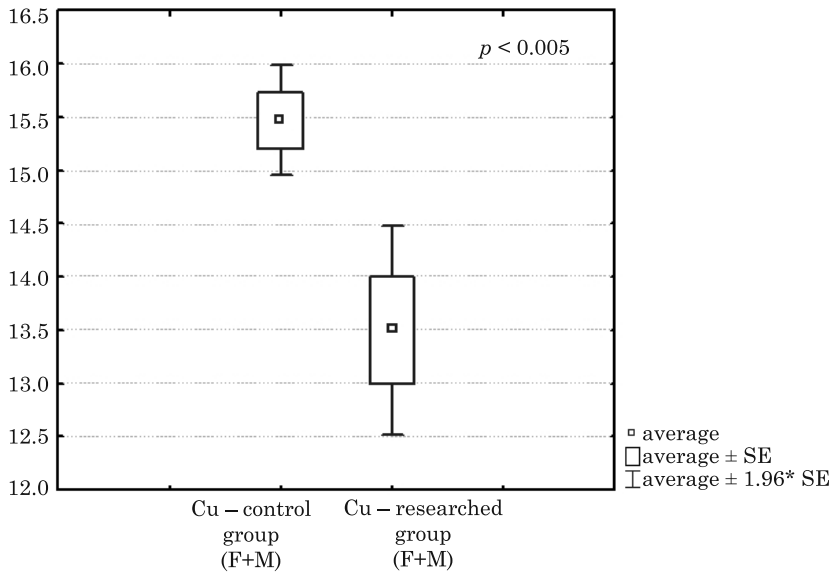in the researched and control group of men (M)

Fig.4. Comparison of copper concentrations (μmol l^{-1}) between researched group and control group of women (F) and men (M)
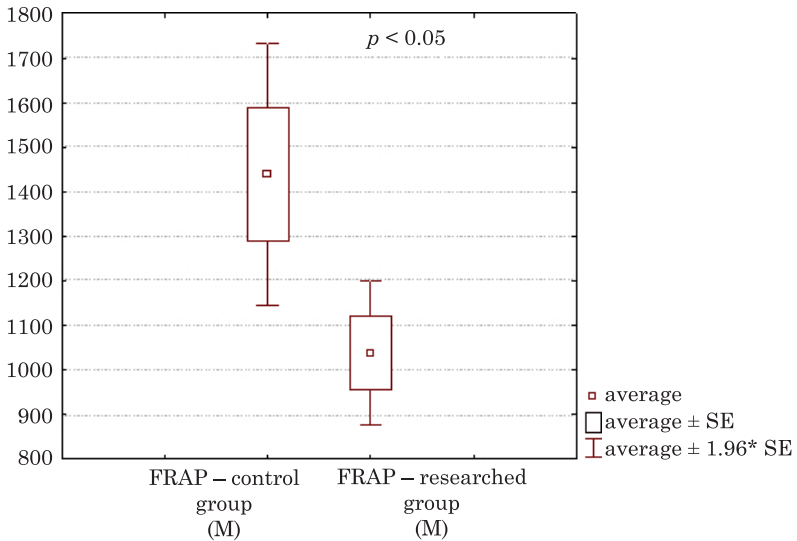

Fig. 5. Comparison of FRAP values between the researched and control men's (M) groups

DISCUSSION

Weight loss, abdominal pain or chronic diarrhoea may be symptoms of malabsorption syndrome caused by many diseases affecting the digestive

system. In a clinical setting, it is usually possible to assess the amount of iron, sodium, potassium, phosphorus, magnesium and calcium but copper, zinc and many other trace elements or vitamins that take part in the antioxidative-oxidative balance are not usually measured because of the difficult diagnostic methods or high costs.

As recent studies indicate, cases of deficiency of copper and zinc among patients with gastroenterological problems are well-documented, but the extent of the problem has not been fully researched (SENYALI et al. 2008, SAPER, RASH 2009). The study by AYESHA et al. (2006) dating back to the year 2000 points out that a deficiency of zinc, whose average amount was 58.86 µg dl^{-1}, appeared in 68% of children with coeliac disease (especially newly diagnosed cases), while the copper level in the same group was close to values detected in healthy children. Similar results were reported about 40 years ago by SOLOMONS et al. (1976), where the zinc level below 70 µg 100 ml^{-1} occurred in 90% of adults suffering from coeliac disease and the average concentration of that microelement in the same patients fell to a level of 51.1±12.2 µg 100 ml^{-1} vs. 74.3±9.7 µg l00 ml^{-1} in the control group. This study did not show a statistically significant difference in the amount of serum copper of patients with coeliac disease as compared with the healthy group (102.6±67.2 µg 100 ml^{-1} vs. 105.0±27 µg 100 ml^{-1}). Contrary results were revealed by Botero-Lopez et al. (2011) in a study conducted on a group of 73 patients with coeliac disease and 36 healthy adults. Deficient amounts of zinc appeared in 20% of the patients but the average concentration of the element in serum in all the distinguished groups was similar: 85 µg dl^{-1}, 80 µg dl^{-1} vs. 90 µg dl^{-1}, respectively. Copper deficiency was determined in 15% of coeliac disease patients, and its concentration, both in typical and atypical forms of the disease, was lower than in the control group (105 µg^{-1} dl, 97.5 µg dl^{-1} vs. 125 µg dl^{-1}, respectively).

Some earlier studies showed that patients suffering from inflammatory bowel diseases (IBD) were prone to Fe, Ca, Mg, Zn as well as vitamin A, D and B$_{12}$ deficiencies. Deficiencies of macro- and microelements occur much more commonly in Crohn's disease – CD (25%-69%) than in patients with ulcerative colitis – UC (1%-32%) because most of the mineral components are absorbed in the small intestine, which is affected by inflammation in the course of the former disease (MULLIN 2012). In patients with ulcerative colitis, the incidence of malnutrition is less frequent, but nutritional deficiencies may develop during periods of disease exacerbation (HARTMAN et al. 2009). Like in the case of coeliac disease, the severity and incidence of zinc and copper deficiencies in patients with IBD have not been unequivocally estimated and numerous studies show discrepant results. According to HARTMAN et al. (2009), zinc deficiencies may occur in 15.2-62% patients with IBD (mainly with newly diagnosed disease) while copper deficiencies affect up to 84% of those patients. Similarly, FILIPPI et al. (2006) reports that a low zinc concentration is claimed to appear in almost 3% of patients with UC as well as 8.5-65% of patients with CD. AINLEY et al. (1988) point out that the level

of zinc in serum of patients with CD and UC is lower (13.5±2.2 µmol l^{-1} and 14.6±2.4 µmol l^{-1}, respectively) than in the control group (15.4±2.0 µmol^{-1} l). OJUAWO and KEITH (2002) showed that the level of copper in the serum of patients with IBD falls within the normal range and is statistically higher in patients with CD (22.7±5.49 mmol l^{-1}) than in patients with UC (17.6±5.15 mmol l^{-1}) and in the control (20.76±4.06 mmol l^{-1}) ($p < 0.01$). The same study showed that children suffering from CD had a lower concentration of zinc (11.01±2.49 mmol l^{-1}) in comparison with the control group (13.6±1.63 mmol l^{-1}), although this concentration level fell within the lower borderline of the normal level. No statistically significant difference in the zinc level was seen in the serum of children with UC and the control group.

With the normal concentrations of zinc 11.5-18.5 µmol l^{-1}, the serum zinc concentration in all patients from the study group was at the lower borderline level, but fell below it in women from the study group. In the control group, the serum zinc concentration was normal.

The copper concentration in serum in the researched group was also at the lower borderline of the normal level, with the normal copper concentration being 11-22 µmol l^{-1}, while in the control group it was higher – in the middle of the normal range.

The FRAP value, testifying the antioxidative defense ability, was lower in persons with absorption disturbances. The lowering of this value, among others, resulted from the microelement deficiency in this group of patients.

The dependency between zinc metabolism and digestive tract diseases continues to be analyzed. Numerous studies show that lower zinc values correlate with the degree of damage to the intestinal mucosa (CROFION et al. 1983). Although both of the elements are absorbed mainly in the upper part of the small intestine, studies show that zinc deficiency is more frequently observed in patients than copper deficiency. This can be explained by the fact that both minerals are transported by the same protein, metallothionein, which has greater affinity for copper than for zinc (MULLIN 2012). It is the opinion of some researchers that deficiencies of nutritional components in patients with coeliac disease and inflammatory bowel diseases only partially result from absorption disturbances. As the study by TRAN et al. (2011) shows, the degree of zinc absorption in children with coeliac disease does not fall below the normal level. The deficiency of this element results to a greater degree from a depleted store of exchangeable zinc, which is being used up, for example, in the healing process of intestinal mucosa or as a result of inflammation (CROFION et al.1983).

Zinc and copper deficiency also results from an increased intestinal loss of minerals in the course of diarrhoea, e.g. due to carbohydrates intolerance, food allergies, inflammatory bowel disease, coeliac disease or chronic pancreatitis. There is a mutual dependency between diarrhoea and zinc deficiency. Diarrhoea predisposes to a worse absorption of nutritional components during digestion disturbance. On the other hand, increasing zinc deficiency

is a factor which causes chronic diarrhoea (Guerrieri et al. 1986). Unabsorbed fatty acids are also responsible for the lower quantity and inferior bioavailability of microelements in patients with absorption disturbances. Fatty acids bind free zinc and copper, and cause their loss with diarrhoea. In physiological conditions, zinc and copper in serum are bound to proteins. Protein malabsorption results in a lower concentration of binding proteins, frequent in those patients (Walker et al.1973, Solomons et al. 1976).

A lowered supply of copper and zinc with food ought to be added to the aforementioned causes of deficiency of these elements in patients with digestive tract diseases. As a result of their illness, many patients develop anorexia. At the time of exacerbation, patients are often advised to resort to an easily digestible diet, which often eliminates products high in copper and zinc (Baczewska-Mazurkiewicz, Rydzewska 2011, Ryżko et al. 2011). Beshgetoor and Hambidge (1998) claim that disturbed reabsorption of Cu and Zn from bile is responsible for the deficiency of these minerals. This mechanism seems likely because earlier studies showed that the insufficient production of cholecystokinin and secretin in coeliac disease decreases the secretion of bile.

CONCLUSIONS

The above study included patients with suspected malabsorption syndrome, who presented symptoms such as abdominal pain, chronic diarrhoea and body mass loss, common in the course of different diseases, such as coeliac disease and inflammatory bowel disease. In this group of patients, the concentrations of zinc and copper in blood serum were at or below the lower borderline of the norm, being significantly lower than in the control group. Deficiency of those elements in the body leads to the development of many diseases, including illnesses due to disturbances of the oxidative-antioxidative balance. In the studied group, a lower FRAP value was observed than in the control group. The patients showed deficiencies of the two microelements despite normal values of BMI, albumin, total protein, iron, haemoglobin, sodium and potassium concentrations. In view of the presented research results, it seems extremely important to recommend adequate supplementation of vitamins and microelements, if possible administered parenterally, to patients with malabsorption syndrome regardless of the nutritional state assessment.

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