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Original Contribution

ZINC DEFICIENCY AND CLINICAL LABORATORY INDICATORS IN CHILDREN WITH MALABSORPTION SYNDROME

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ABSTRACT

The aim of this study was to investigate the correlation between serum concentrations of zinc and different clinical indicators in children with malabsorption syndrome. The study population consisted of 24 patients with malabsorption syndrome and a group of 30 healthy children as controls. Serum levels of zinc and iron of patients (9.18±2.60 μmol/L, 5.22±3.76 μmol/L) showed significant differences compared with serum concentrations in healthy children ($p<0.05$). We have found correlations between the following micronutrient concentrations and clinical indicators of the patients: Hb/Ht (r = 0.84, p = 0.0001); Hb/Fe (r = 0.66, p = 0.0004); Zn/Hb (r = -0.46, p = 0.037); Zn/Ht (r = -0.44, p = 0.04); Zn/Fe (r = - 0.69, p = 0.0001).

The *inverse* correlation between zinc and haematological indicators suggests that the intake of zinc and iron containing medications should be performed according to therapeutical programs either way: for prevention or for treatment of zinc and iron deficiency in children.

Key words: serum zinc, iron, malabsorption syndrome.

INTRODUCTION

Zinc and iron belong to the group of 10 essential micronutrients which are usually studied in different medical conditions and diseases (1-3). Low serum levels of zinc and iron, as well as zinc and iron deficiencies, are often reported in patients with acute enterocolitis, malabsorption syndrome, pneumonia and other diseases (2-7). Low activity levels of GPx were observed in patients with pneumonia and acute enterocolitis (1-3). Low zinc concentrations in patients with enterocolitis and malabsorption syndrome were significantly improved after zinc supplementation to the diet of those patients for a period of 30 days (4-6).

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The manifestations of severe zinc deficiency in humans include bullous pustular dermatitis, alopecia, diarrhea, emotional disorder, weight loss, intercurrent infections due to cell-mediated immune dysfunctions, hypogonadism in males, neurosensory disorders, and problems with healing of ulcers (7). Prolonged diarrhea, weight loss, infections, neurosensory disorders, problems with healing of ulcers in patients with malabsorption syndrome is an episode of complex process of physiogical, hematological and biochemical changes due to bacterial and viral infections, parasites, food intolerances or allergies, reactions to medications (7). It is known that the level of serum iron is a very important clinical parameter for control and therapy of the patients with malabsorption syndrome. In addition to other clinical parameters, glutathione peroxidase activity (GPx) has been studied as important marker for the oxidative damage in patients with malabsorption syndrome (8). To our knowledge, there are no enough data

concerning serum GPx activity at malabsorption syndrome.

Since micronutrients metabolism and their influence on different medical conditions and diseases are not thoroughly studied yet, the aim of the present research was to investigate the correlation between serum zinc concentrations and some clinical laboratory parameters such as serum iron, GPx, Hb, Er, and Ht in children with malabsorption syndrome.

MATERIAL AND METHODS

Subjects

Study population consisted of 24 patients with malabsorption syndrome and a group of 30 healthy controls. Malabsorption syndrome is defined according to the definition given by the World Health Organization. The subjects with malabsorption syndrome and the control group were matched by age (0.5 – 4 years, 1.91±1.11 years) and social status. All children were patients of the University Hospital in Pleven, Bulgaria.

Venous blood samples were collected for analysis from all children after both their parents had signed a written letter of consent. The research was carried out according to the principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional research ethics committee of the University of Medicine–Pleven.

While enrolled in the study, both groups were not treated with any medications containing zinc or iron.

Determination of serum zinc and zinc status

Zinc in blood serum was determined according to method described by of Lampugnani et al. (9) with our modifications published recently (6). After deproteinization of the samples, with chromogen 4-(2-pyridylazo) resorcinol sodium salt, zinc quantity was determined spectrophotometrically. Absorption of the samples at 490 nm was proportional to the zinc (II) concentration.

Zinc status was defined by serum zinc levels in a reference region of 11.6 μmol/L to 23.0 μmol/L (10). According to the World Health Organization, all zinc values bellow 10.71 μmol/L in morning samples of blood serum were defined as zinc deficiency (11).

Clinical laboratory methods

All children were examined for complete blood count by analyzer MIKROS – 18 (ABX). All clinical chemistry parameters – albumin, total protein, iron – were measured by COBAS INTEGRA 400 (Roche) analyzer. Electrolytes – sodium, potassium and chloride were measured by ion- specific electrode (ISE) analyzer AVL 998.

Glutathione peroxidase activity was measured on a chemistry analyzer (Hitachi 704), with test kit Ransel – Randox laboratories Ltd for the quantitative determination of GPx in whole blood (12). The activity of GPx was assessed by decrease in absorption at 340 nm. GPx was expressed as U/L.

Serum iron was studied within the ranges of investigated clinical and laboratory indicators. Iron was measured by COBAS INTEGRA 400 (Roche) analyzer, which utilizes the pherozine method (13). Reference values of serum iron vary between 9.0 and 21.0 μmol/L. Iron values below 8.0 μmol/L are considered as iron deficiency.

Statistical analyses

Excel (Microsoft Corporation, Redmond, WA) and Statgraphics Plus (Manugistics, Rockville, MD) for Windows were applied in order to be analyzed statistically the results of the study. All values were expressed as mean (x) ± standard deviation (SD). Student's t-test, F-ratio, and one-way analysis of variance (ANOVA) were used to assess differences between study groups (least significant difference, Tukey's honest significant difference, Scheffé, Bonferroni, Newman-Keuls, and Duncan (normal distribution) and Kruskal-Wallis (K-W) tests (non- normal distribution). Correlation analysis was performed for selected data sets, and data were considered significant when p value was less than 0.05.

RESULTS

Serum concentrations of zinc and iron, and whole blood glutathione peroxidase activity are presented in **Table 1.** Patients showed statistically significant lower levels of serum zinc and serum iron, as compared to the control group and normal values ($p<0.0001$, **Table 1**). Values of GPx activity were more than twice, in some cases more than three times, lower than the reference values **(Table 1).**

Results of the other investigated clinical laboratory indicators and reference values are shown in **Table 2**. As is seen, patients showed significantly lower levels of Hb, g/L; (K-W = 13.66, $p^{a,b} = 0.0002$; n = 10), Ht (F = 33.37, $p^{a,b} = 0.0001$; n = 18), Na^+ (K-W = 13.84, $p^{a,b} = 0.0001$; n = 7), K^+ (F = 74.2, $p^{a,b} = 0.0001$; n =

5) and total protein, ($F = 15.00$, $p^{a,b} = 0.0008$; $n = 5$) when were compared to the standard values **(Table 2)**. The other studied clinical and laboratory indicators of the patients were within standard values with small exceptions **(Table 2)**.

Significant correlation was found for the values of serum Zn with those of Hb ($r = -0.46$, $p = 0.037$), Ht ($r = -0.44$, $p = 0.04$) and Fe ($r = -0.69$, $p = 0.0001$). Significant correlation was established for the serum Fe with Hb ($r = 0.66$, $p = 0.0004$) and Ht ($r = 0.64$, $p = 0.001$) values, as well.

R-squared ($R = r^2.100$) represents the extent (%) of correlation in between the studied parameters within a pair. We found that R-squared was about 40% in both pairs – Hb and Fe, and Ht and Fe. Hb and Ht also showed significant correlation in between ($r = 0.84$, $p=0.0001$). R-squared showed that 70.6 % changes in Hb values were due to changes in Ht values and vice-versa - 70.6 % changes in Ht values were related to changes in Hb values. R-squared showed also that 21.2% changes in Zn levels were due to changes in Hb levels, and 19.4% changes in Zn levels were due to changes in Ht levels and vice versa.

The correlation found between the values of serum zinc and iron shows that 47.6 % changes in the levels of Zn were due to changes in Fe levels and vice versa. Correlations between serum Zn or serum iron values and the rest studied parameters were not found.

However, we found that low levels of serum Zn were associated with low levels of GPx. Although the correlation between the two parameters was not significant ($r=0.38$, $p = 0.0746$), it was quite close to significance ($p < 0.05$).

Body mass and height of the children with malabsorption syndrome were followed through the study period and compared to those at birth. Results are presented on **Table 3**. It was observed growth retardation, expressed in reduced body mass and height during the study period and at birth, as well.

Table 1. *Serum zinc, iron and GPx of children*

Studied groups	Serum zinc, μmol/L, x ± sd, (n), m, RSD %	Serum iron, μmol/L, x ± sd, (n), m, RSD %	GPx, U/L, x ± sd, (n), m, RSD %
Children – patients	9.18 ± 2.60 (23), 8.42, 28.37	4.31 ± 2.27 (22), 4.01, 52.7	1783.67 ± 89.49 (24), 1801.87, 4.98
Children from control group[(1)], normal values[(2)] and reference values[(3)]	18.33[(1)]±1.77 (30), 18.31, 9.66 11.60[(3)] – 23.00	15.14[(2)]±0.71, (2), 15.14, 5.56 9.00[(3)] - 21.00	4171[(3)]-10881
t, F[1]; *p*	t = 15.21; 0.0001	F = 43.25; 0.0001	

[1] ANOVA; t, F – ratio, m – median, n – number of studied subjects, RSD – Relative Standard Deviation

Table 2. *Clinical laboratory indicators of children - patients*

Indicators; reference values	Under standard values[a], **n**	Within standard values[b], **n**	All studied subjects; **n = 24**
Hb, g/L;	93.3±12.3,	118.1±11.3,	107.75±16.92
108 – 140	**10**	**14**	
Er, 10^{12} /L;	3.64. ±0.17,	4.68±0.35	4.59±0.53
4.1 – 5.5	**3**	**21**	
Ht ;	0.31±0.025,	0.40±0.040,	0.33±0.044
0.36 – 0.46	**18**	**6**	
Total protein, g/L;	58.8±3.74,	70.42±6.31,	68.01±7.52
63 – 86	**5**	**19**	
Albumins, g/L;	35.6±2.0,	42.05±2.30,	41.78±2.61
37 – 56	**1**	**23**	
Na^+, mmol/L;	134±1.37,	138.39±1.93,	137.20±2.63
136 – 145	**7**	**17**	
K^+, mmol/L;	2.78±0.07,	4.82±0.23,	4.88±0.59
3.8 – 5.4	**5**	**19**	
Cl^-, mmol/L;	58.88±4.04,	102.83±1.94,	102.30±2.60
96 – 106	**3**	**21**	

Table 3. *Body mass and height of investigated children*

	n	At birth		During investigation	
		Average	Ranges (from – to)	Average	Ranges (from – to)
Body mass (x±sd) kg	24	2.57±0.745	0.930 – 4.15	8.43±1.75	2.6 – 11.0
Height (x±sd) cm	24	47.5±4.12	36.0 – 52	78.8±3.22	72 – 83

DISCUSSION

Scientific views of micronutrients' importance for children's health and nutrition have changed recently. Many authors now emphasize significant long-term effects that zinc and iron have on children's health (1, 14, 15).

Disorders in plasma zinc levels have been reported for individuals in periods of their growth or while following a dietary regimen (2, 6). Zinc deficiency is considered as one of the most severe consequences of diarrhea along with anemia, acrodermatitis enteropatica, mental retardation, behavioral disorders, nail changes, alopecia, growth retardation, and common infections (2, 5, 7, 16, 17). Twice lower average values of serum zinc of our patients comparing to those of the healthy controls, we could explain by the above reported findings. Iron deficiency found in our patients with malabsorption syndrome we explained by well known fact that pathological processes in the gastrointestine, blood loss from chronic diarrhea, etc. cause iron deficiency which impairs general cell growth and proliferation of tissues like the nervous system and gastrointestinal tract (18, 19). Usually patients with anemia have low levels of Hb and Ht. In addition to serum iron deficiency some of the studied children showed Hb and Ht under the standard values. Low levels of serum zinc and iron found in some of studied patients we have related to low dietary intake and excessive faecal loss of trace elements in children with diarrhea.

It is well known that Zn deficiency causes growth retardation in children. Such patients are shorter or thinner for their age and in most cases their diets are lacking in protein and rich in phytate and fiber (20-22). In our patients along with diarrhea we observed also severe weight deficit, growth and neuropsychological development retardation and frequent infections. The development in height and weight of some 3-4 years old children with malabsorption syndrome corresponds to that of a year old child.

Several environmental factors, like feed composition, trace element status and vitamin intake, are known to affect blood GPx activity (3). The decreased GPx activity, is a marker of enhanced oxidative stress that results in suppression of T-cells immunity, decreased resistance to infections and increased morbidity (23-25). We assume that the twice lowered GPx activity obtained for our patients with malabsorption syndrome in comparison with the reference values was probably due to the disturbance of transition element status (Fe and Zn) and also is related to the frequent infections observed in our patients. This our assumption is also supported by our other finding that low levels of serum zinc were associated with low levels of GPx. Such low level of GPx is considered as selenium deficiency and is related to high risk for arterial hypertension, non-specific intestinal disorders, asthma, pneumonia, etc (3).

Based on the reverse correlation between the values of Zn and Hb, Zn and Ht, and Zn and Fe for the patients demonstrated by the present study, we suppose that an increase in Hb, Ht and Fe could result in a decrease of serum zinc levels, while the increase in serum zinc would lower Hb, Ht and Fe. In the last few years the results of different studies on introducing zinc supplemented therapy in humans have been controversial – some investigators did not discover any effect, others found little or positive effect of zinc on clinical indicators and diarrhea, while others still stated that: zinc supplementation has a negative effect on iron status, iron supplementation does not affect zinc status and iron intake increases zinc deficiency (20, 21, 26-31). Despite controversial findings and reports, it is estimated that large amounts of iron in supplements (greater than 25 mg) may decrease zinc absorption (32). Therefore, taking iron supplements between meals will help to decrease its effect on zinc absorption (32).

CONCLUSIONS

Having in mind all our results obtained and especially results concerning correlation between the values of GPx activity and serum Zn, we suggest that micronutrient supplemented therapy, especially zinc supplementation, introduced to children with malabsorption syndrome could improve their antioxidant status.

Moreover, our results reveal existence of complex relationships between hematological indicators – iron, Ht, Hb and zinc in children with malabsorption syndrome.

It is obvious, that further investigations should to be done in order to study correlations between micronutrients and clinical laboratory indicators in patients with different diseases related to micronutrient deficiencies. Such investigations would allow to be applied more proper therapy to those patients

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