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Relationships between haematological parameters, biochemical markers of iron metabolism, and trace elements in Paediatric patients under 3 years with iron deficiency Anaemia

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Abstract
Iron deficiency causes disbalance of other micro- and macroelements that leads to disruption of the exchange of most micronutrients and development of characteristic clinical symptoms. Interaction and correlation between trace elements and haematological parameters is still not clear. *Aim.* To investigate the relationship between haematological parameters, biochemical markers of iron metabolism and trace elements in children under 3 years with Iron-deficiency anaemia (IDA). *Materials and Methods.* Investigation comprises 86 patients from 0 to 3 years of age with clinical and laboratory signs of IDA. 38 children-patients are of the University Hospital, Medical University – Pleven, Bulgaria – I group, and 48 are patients of the Sumy Regional Child's Clinical Hospital, Sumy, Ukraine – II group. Comparison group includes 25 healthy children at the same age. Haematological parameters and the red cell indices were examined by analyzer MICROS – 18 (ABX). The serum erythropoietin (sEPO) and ferritin (sFR) levels were determined by ELISA. Serum content of trace elements was determined

spectrophotometrically (I group) or by atomic absorption spectrophotometry (II group). To evaluate a relationship between markers of iron metabolism and haematological parameters we have used Spearman's correlation coefficients. *Results.* Formation of IDA is caused not only by violation of iron metabolism, but also other trace elements that directly or indirectly may affect both iron exchange and erythropoiesis regulation through the erythropoietin production. We found significant correlations between the level of chromium and transferrin saturation ($r = -0.382$, $p = 0.018$), zinc – with sEPO ($r = 0.543$, $p = 0.036$), copper – with sFR ($r = -0.561$, $p = 0.029$), and sEPO ($r = -0.739$, $p = 0.0016$), and cobalt – with sEPO ($r = 0.769$, $p = 0.0021$), that indicate the role of trace elements in the pathogenesis of IDA. Thus, if the routine therapy of IDA is ineffective, a concomitant micronutrient disorder should be considered, with an evaluation of trace elements level, and, if necessary, a correction should be carried out.

1. Introduction

Iron-deficiency anaemia (IDA) is the most common form of anaemia worldwide [1]. Causes of iron deficiency include poor iron intake, chronic blood loss, impaired absorption, or any combination of the three [2]. Iron-deficiency anaemia accounts for approximately one-half of anaemia cases [4]. The global prevalence of anaemia is 24.8% and 1.62 billion people are affected. The highest prevalence is in preschool-aged children – 47.4% worldwide, but Africa and Asia have the highest prevalence of anaemia among this category – 64.6% and 58.0%, respectively [3]. Despite WHO initiative, global incidence of IDA had not decreased during last decade [1].

Iron deficiency is probably the most common deficiency in children both in developing and industrialized nations. Iron is a key micronutrient in IDA pathogenesis and prognosis, but iron deficiency causes disbalance of other micro- and macroelements that leads to disruption of the exchange of most micronutrients and development of characteristic clinical symptoms. Iron, zinc, copper, etc. are some of the inorganic elements that are necessary for normal growth and sustained biological activities [5].

The role of some micronutrients in IDA is still not completely discovered, as well as their relationship. Zinc (Zn) is essential for normal iron metabolism, influences the growth and affects the development and integrity of the immune system [6]. Many studies have shown that zinc deficiency can cause T-cell dysfunction and impair cellular immune functions [7, 8]. Also zinc, as well as copper, is cofactor of the antioxidant enzyme, superoxide dismutase, which detoxifies the toxic superoxide radical [9]. Some data show decreased serum zinc levels during IDA [10, 11], but another report about normal zinc level in these patients [12]. Also some experimental data show low iron concentration in organs and blood during zinc deficiency [13]. But study of iron supplementation among anaemic patients finds effectively increased iron status while adding zinc to iron supplementation [14]. The administration of zinc along with iron presumably increases production of proteins and globin related to haematopoiesis in the bone marrow [15]. In case of iron deficiency, a zinc ion is alternately incorporated into the porphyrin ring complex of haemoglobin, thereby increasing the concentration of zinc protoporphyrin. That's why zinc protoporphyrin may be an indicator for the assessment of iron status [16].

Copper (Cu) plays vital role as a structural and catalytic component of metalloenzymes, it is an essential nutrient required for growth and development [17], and it is needed to absorb and utilize iron [18]. The characteristic haematological effects of copper deficiency are anaemia, both microcytic, normocytic or macrocytic, neutropenia [19], and more rarely – thrombocytopenia [20]. The peripheral blood and bone marrow aspirate findings in copper deficiency can mimic myelodysplastic syndrome [21]. But there are different data about the Cu level during the IDA. Turgut et al. have not found significant differences of Cu blood concentration in children with IDA [22], while Ece et al. report about elevation of copper level in comparison to non-anaemic patients [23]. However, a lot of researchers show decreasing Cu level in children with IDA [5, 24, 25].

The biological function of chromium (Cr) is not fully known yet. Cr interacts with the thyroid metabolism in humans and stimulate the DNA-dependant RNA synthesis [26]. Cr acts with insulin on the first step in the metabolism of sugar entry into the cell, and facilitates the interaction of insulin with its receptor on the cell surface. Cr has a lot of interactions with some metals, for example Zn, and vanadium and iron supplementation has been shown to decrease the absorption of Cr [27]. On the other hand, absorption of Cr was elevated in Zn-deficient rats and was reduced by zinc supplementation [28]. The interaction between Cr and iron is not completely understood, but some data show iron deficiency in patients with high Cr intake [29].

Cobalt (Co) is a key constituent of cobalamin, also known as vitamin B_{12}, which is the primary biological reservoir of this metal. Also Co can stimulate erythropoietin in kidney and maintains red blood cell production. Lack of Co causes vitamin B_{12} deficiency anaemia. But there is few information about Co level in IDA, and some authors report about higher blood concentrations of Co and cadmium in patient with low iron stores [30].

Nickel (Ni) is one of the essential elements for mammals [31]. Khan and Moheman (2006) report that nickel interacts with iron found in the haemoglobin, helps in the oxygen transport, and stimulates the metabolism. It is regarded as a key metal in several plants and animal enzyme systems. Nickel is involved in the transmission of genetic code (DNA, RNA), and it is also present in certain enzyme systems that metabolize sugars. Schnegg and Kirchgessner [32] report that nickel deficiency in rats led to reduced iron content in organs, reduced haemoglobin and haematocrit values, and anaemia. But there are no data about Ni concentrations in the organism of anaemic children, as well as their correlations with

haematological parameters.

Thus, a lot of trace elements play key roles in the pathogenesis of IDA, due to synergism and antagonism with iron. But their interactions and correlations with haematological parameters are still not clear.

The aim of our research was to investigate the relationship between haematological parameters, biochemical markers of iron metabolism and trace elements in children under 3 years with IDA.

2. Materials and Methods

2.1. Investigation Design

Our investigation comprises 86 patients from 0 to 3 years of age with clinical and laboratory signs of IDA. 38 children are patients of the University Hospital, Medical University – Pleven, Bulgaria – I group, and 48 are patients of the Sumy Regional Child's Clinical Hospital, Sumy, Ukraine – II group. Comparison group includes 25 healthy children at the same age.

All children were enrolled in the study after informed consent from their parents or guardians. Ethical approval was obtained from the Institutional research ethics committees.

WHO haemoglobin thresholds were used to identify patients as anaemic (haemoglobin level < 110 g/l). IDA was defined as haemoglobin values less than WHO guidelines with the presence of two or more of the following parameters: low mean corpuscular volume (MCV), serum ferritin below 12 μg/l, and transferrin saturation (SatTf) less than 16% [33].

2.2. Blood Sampling

Fasting venous blood samples were obtained for analysis in the morning from all children into sterile tubes untreated with heparin, EDTA, citrate, etc. After two hours standing and centrifugation at 3500 rpm for 10 minutes, blood serum was separated. The serum samples were put in closed plastic laboratory vessels for haematological analysis or stored at – 18ºC until trace element analysis.

2.3. Haematological Analysis

Haematological parameters – haemoglobin (Hb), haematocrit (Ht), red blood cell (erythrocyte) count (RBC), and the red cell indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW), were examined by analyzer MICROS – 18 (ABX). The reticulocyte count was determined by microscopic examination of a peripheral blood smear stained with a supravital dye.

The serum erythropoietin (EPO) levels were determined by ELISA using a kit of reagents "Pro Con EPO HS" produced by "Protein Contour" (Russia), and serum ferritin levels – with the same methods using a kit of reagents "UBI MAGIVEL FERRITIN" produced by "United Biotech Inc." (USA).

2.4. Trace element Analysis

In I group, serum content of trace elements iron, zinc, copper, and chromium was determined spectrophotometrically: ferozine method [34] for serum iron by COBAS INTEGRA 400 (Roche) analyzer, spectrophotometric methods using GIESSE diagnostics (Italy) tests for serum zinc and AUDIT diagnostics (Ireland) tests for serum copper, and spectrophotometric method [35] with our modifications for serum chromium. Serum concentrations of zinc, copper, and chromium were determined by a spectrophotometer DR2800 (Hach Lange, Germany).

In II group, the content of trace elements iron, zinc, copper, cobalt, and nickel in blood serum and erythrocytes was determined by atomic absorption spectrophotometry (AAS) on a spectrophotometer C-115M1 (JSC "Selmi", Ukraine) [36, 37].

2.5. Statistical Analysis

Statistical data processing was performed using statistical packet SPSS v. 13.0 and Excel for Windows. Statistical significance was indicated by p-values < 0.05. To evaluate a relationship between biochemical markers of iron metabolism and haematological parameters we have used a correlation analysis with calculation of the correlation coefficients by Spearman.

3. Results and Discussion

Results of investigated clinical laboratory indicators in patients with IDA and the respective reference values are presented in table 1. All key haematological indicators of anaemic children are significantly lover that the control group. Low serum trace element concentrations of zinc, copper, cobalt, and nickel, mainly due to inadequate dietary intake, malabsorption problems, and trace element interactions, were found in both studied groups (I and II group) of children with IDA. Profiles of trace elements iron and zinc in blood serum do not differ in the two examined groups by the means of two analytical methods applied. Increased serum concentrations of copper in II group in comparison to control subjects are probably due to metabolic changes resulting from adaptation mechanisms in IDA.

In order to study the causes for trace element deficiencies of iron, zinc, copper, and chromium in children with IDA, as well as the influence of trace element status of zinc, copper, and chromium on the development of IDA, we have investigated the relationship between markers of trace element status, serum concentrations of macroelements, and haematological parameters as continuous quantitative variables.

Table 1. *Haematological parameters and content of trace elements in blood serum and erythrocytes of children with IDA.*

Parameter	I group with IDA (n=38)	II group with IDA (n=48)	Comparison group (n=25)
Haemoglobin (g/l)	86.87±14.06*	89.79±1.23*	119.15±2.41
Haematocrit (l/l)	0.276±0.031*	0.301±0.004	0.3442±0.006
RBC ($\times10^{12}$ cells/l)	4.51±0.61*	3.58±0.05	4.07±0.12
Rtc (‰)	–	5.5±0.87*	7.86±0.98
MCV (fl)	62.03±10.18*	74.66±1.08	82.52±1.17
MCH (pg)	19.97±4.26*	24.77±0.39	29.82±0.56
MCHC (g/l)	318.92±19.95*	323.47±4.34	365.17±3.6
RDW (%)	16.18±2.07	–	–
WBC($\times10^9$/l)	11.75±4.4*	6.3±0.81	6.5±0.59
PLT ($\times10^9$/l)	467.68±163.11*	250.8±22.7	243.2±21.6
Serum Fe (μmol/l)	4.01±1.36*	9.23±0.86*	22.92±1.83
TIBC (μmol/l)	79.22±16.91	–	–
SatTf (%)	5.19±2.25	–	–
Erythrocyte Fe (mcg/mg ash)	–	15.58±1.13*	31.56±1.65
Serum ferritin (ng/ml)	–	9.42 ± 0.75*	38.67 ± 4.18
Serum erythropoietin, (mU/ml)	–	32.67 ± 2.49*	15.29 ± 4.26
Serum zinc (μmol/l)	10.62 ± 4.37*	11.02±1.79*	17.96±1.06
Serum copper (μmol/l)	11.42 ± 3.97*	23.80±0.76*	16.50±0.71
Serum chromium (μmol/l)	0.93 ± 0.67	–	–
Serum cobalt (μmol/l$\times10^{-3}$)	–	5.74±0.76*	9.16±0.61
Serum nickel (μmol/l$\times10^{-3}$)	–	8.99±0.868*	14.35±1.09

Notes: * Significantly different from the control group, $p<0.05$.

Table 2. *Results of correlation analysis of the relationship between biochemical markers of iron metabolism and haematological parameters in children with IDA.*

Indicator	Serum Fe (μmol/l), n = 38	TIBC (μmol/l), n = 38	SatTf (%), n=38	Erythrocyte Fe (mcg/mg ash), n=48	Serum ferritin (ng/ml), n=48	Serum erythropoietin, (mU/ml), n=48
Hb (g/l)	0.361**	-0.448**	0.468**	0.822**	0.514**	-0.814**
Hct (l/l)	0.263**	-0.299**	0.325**	0.641*	0.191	-0.535*
RBC($\times10^{12}$/l)	- 0.180*	0.341**	-0.307**	0.410*	-0.525*	-0.275
MCV (fl)	0.401**	-0.635**	0.598**	0.728**	0.831**	-0.070
MCH (pg)	0.402**	-0.646**	0.602**	0.835**	0.601**	-0.616*
MCHC (g/l)	0.322**	-0.501**	0.471**	0.219	0.178	0.229
RDW (%)	-0.372**	0.324**	-0.430**	0.283	-0.452*	-0.154
WBC($\times10^9$/l)	-0.167*	-0.198**	-0.073	0.134	0.589*	-0.176
PLT ($\times10^9$/l)	-0.302**	0.239**	-0.325**	0.365*	0.651*	0.021

Notes: * Significance level, $p<0.05$;
** Significance level, $p<0.01$.

We found statistically significant correlations between biochemical markers of iron metabolism – serum and erythrocyte iron, TIBC, transferrin saturation, and serum ferritin, and haematological parameters – haemoglobin, haematocrit, RBC count, RBC indices – MCV, MCH, MCHC, and RDW, in studied patients with IDA (Table 2). It is worthy of note that the correlations of markers of iron metabolism with haemoglobin, RBC indices MCV and MCH are strong.

In parallel with lowering the values of serum and erythrocyte iron, serum ferritin, and SatTf, and increasing of TIBC, a decrease in values of haematological parameters Hb, Hct, MCV, MCH, and MCHC was observed with progression of ID. Serum iron, serum ferritin and SatTf showed negative, TIBC and erythrocyte iron – positive correlations with the RBC count and the RBC index RDW (Table 2).

There were statistically significant weak negative correlations between WBC count and serum level of iron, and WBC count and TIBC, but positive strong between WBC and serum ferritin. Observed statistically significant correlations between laboratory parameters of iron metabolism and platelet count were negative weak for serum iron, weak positive for TIBC, and negative moderate for transferrin saturation. A positive strong correlation between platelet count and serum ferritin was observed (Table 2).

Level of serum erythropoietin which is a main regulator of RBC production, had statistically significant negative correlations with haemoglobin, haematocrit and such RBC indices as MCH.

Statistically significant correlations between biochemical markers of iron metabolism (serum iron, transferrin saturation, erythrocyte iron, serum ferritin) and haematological parameters Hb, Hct, red cell indices express the progression of anaemia severity and changes in RBC morphology, in parallel with development of ID. The relationship between severity of ID and reduction in RBC size (microcytosis), and haemoglobin content of the RBC (hypochromia), quantitatively determinable with RBC indices MCV and MCH is most pronounced. In a document of the WHO (2001), it is reported that MCV and MCH are the most sensitive indicators for ID from RBC indices [38].

The elevation in values of RDW with increasing severity of ID reflects the presence of anisocytosis – differences in the

size of RBC (Table 1). According Thomas (2005), RDW is markedly increased with worsening of ID [39]. The negative sign of the relationship between indicators of iron status SFe and SatTf, and the RBC count (Table 1) is probably related to the mean value of RBC above the lower limit of the reference values, which is observed in the study group of patients.

Observed correlations between TIBC and haematological parameters Hb, Hct, RBC count, RBC indices have opposite signs to relationships with SFe and SatTf. This reflects the elevation in values of TIBC with progression of ID, due to increase in unoccupied binding sites on plasma transferrin [40, 41]

Negative correlation between TIBC and WBC count may be explained by the increase in the value of WBC in infectious processes, which are involved in the development of anaemia with normal or reduced TIBC [42]. Negative correlation between SFe and WBC count may be explained by lowering serum levels of iron in conditions of infectious processes mediated by the hormone hepsidin [43-45]. High positive correlation between serum ferritin and WBC may be related to the role of ferritin in the acute phase response [46, 47]. In the absence of inflammation, the concentration of SFR describes the size of the total body iron stores. Normal or slightly decreased WBC count together with low serum ferritin level is typical for IDA [48].

In the studied patients, progression of ID is associated with an increase in platelet count. In iron-deficient conditions, stimulation of erythropoiesis with increased production of erythropoietin occurs [49]. This molecule has a structural homology with thrombopoietin and cross-reacts with the megakaryocyte receptor of thrombopoietin [41]. Absence of correlations between sEPO, RBC and PLT in anaemic children may be due to deficiencies of other micronutrients needed for proper formation of erythrocytes and platelets, such as zinc, copper, chromium, etc.

At the same time, high levels of sEPO typical for children with IDA negatively correlate with the level of haemoglobin, haematocrit and MCH. In anaemic patients hypoxia and iron depletion induce increase in erythropoietin synthesis and marked suppression of hepcidin [50]. But if serum hepcidin is still elevated, it suppresses intestinal iron uptake and violates the normal haemoglobin synthesis [51].

According to the aim of our research, we examined the relationship between serum concentrations of particular trace elements – zinc, copper, chromium, cobalt, and nickel, as well as the relationship between serum levels of trace elements and biochemical markers of iron metabolism, macroelements and haematological parameters.

Table 3. *Correlations between serum levels of trace elements, biochemical markers of iron metabolism and macroelements in group I (n = 38) and group II (n = 48) patients.*

Group I			
Micronutrient	**Indicator**	**Correlation coefficient, r**	**Significance level, p**
SFe (μmol/l)	SNa (mmol/l)	0.357	0.028
SFe (μmol/l)	SK (mmol/l)	-0.307	0.061
SZn (μmol/l)	SNa (mmol/l)	0.343	0.035
SCr (μmol/l)	SFe (μmol/l)	-0.318	0.052
SCr (μmol/l)	TIBC (μmol/l)	0.302	0.065
SCr (μmol/l)	SatTf (%)	-0.382	0.018
Group II			
Micronutrient	**Indicator**	**Correlation coefficient, r**	**Significance level, p**
SFe (μmol/l)	SZn (μmol/l)	0.611	0.016
SFe (μmol/l)	SCu (μmol/l)	-0.854	0.0005
SFe (μmol/l)	SCo (μmol/l)	0.209	0.513
SFe (μmol/l)	SNi (μmol/l)	0.084	0.776
SFe (μmol/l)	SFR (ng/ml)	0.785	0.0003
SFe (μmol/l)	SEPO (mU/ml)	0.768	0.0005
SZn (μmol/l)	SCu (μmol/l)	-0.614	0.046
SZn (μmol/l)	SCo (μmol/l)	0.229	0.354
SZn (μmol/l)	SNi (μmol/l)	0.015	0.886
SZn (μmol/l)	SFR (ng/ml)	0.336	0.221
SZn (μmol/l)	SEPO (mU/ml)	0.543	0.036
SCu (μmol/l)	SCo (μmol/l)	-0.798	0.0004
SCu (μmol/l)	SNi (μmol/l)	-0.065	0.831
SCu (μmol/l)	SFR (ng/ml)	-0.561	0.029
SCu (μmol/l)	SEPO (mU/ml)	-0.739	0.0016
SCo (μmol/l)	SNi (μmol/l)	0.900	0.0001
SCo (μmol/l)	SFR (ng/ml)	0.319	0.289
SCo (μmol/l)	SEPO (mU/ml)	0.769	0.0021
SNi (μmol/l)	SFR (ng/ml)	-0.258	0.394
SNi (μmol/l)	SEPO (mU/ml)	0.275	0.364

In the studied group of children with IDA (n = 38), we found a moderate positive correlation between serum levels of zinc and sodium (r = 0.343; p = 0.035), a moderate positive correlation between serum levels of iron and sodium (r = 0.357; p = 0.028), a moderate negative correlation between serum levels of iron and potassium (r = -0.307; p = 0.061), and correlations between serum levels of chromium and biochemical markers of iron metabolism. Relationships

described were statistically significant or close to the statistical significance. The results obtained are shown in Table 3.

In parallel with progression of ID presenting with reduction in SFe and SatTf, and increase in TIBC, there was an increase in serum chromium.

Trace elements zinc and iron, macronutrients sodium and potassium are received in the organism by the food. Common causes for their insufficiencies in the studied patients are diseases with presence of malabsorption syndrome. It was found that malabsorption increases significantly risk for iron, zinc, and copper insufficiencies in affected subjects [52, 53].

The positive correlation between SFe and serum sodium found was due to the synergism between the two metals, since the insufficiencies of synergistically interacting with the iron minerals lead to development of ID [54]. In patients with malabsorption, mean serum levels of both minerals are below the lower limits of the reference ranges. In the literature, there is evidence of synergistic interactions between iron and potassium [54]. The correlation between their serum levels, which we observed, is negative. The direction of this relationship may be attributed to observed values of serum potassium within the reference values, even in patients with clinically manifested malabsorption syndrome.

We consider that the positive correlation between serum zinc and sodium is due to co-existing insufficiencies of both minerals in patients with malabsorption, in which their mean serum levels were below the lower limits of the reference ranges.

In studied patients with IDA, increasing in serum chromium with the progression of ID is observed. It is well-known that between the trivalent chromium and trivalent iron, there is a competitive antagonism for binding to apo-transferrin in the serum [54, 55]. In a study on male volunteers, negative effects of chromium-containing preparations on iron metabolism and status in long-term and high dose application are hypothesized [56]. In confirmation of this interaction, Ani and Moshtaguie (1992) reported a significant decrease in the values of SFe, TIBC, serum ferritin, Hb, and Hct after administration of chromium in experimental animals.

Both metals zinc and copper demonstrate statistically significant correlations (positive and negative, respectively) with iron, due to their important role in the absorption and metabolism of iron. The serum zinc level was significantly altered in children with iron deficiency, in whom zinc protoporphyrin is produced instead of haem and is consequently increased in erythrocytes. Measuring zinc protoporphyrin together with ferritin and haemoglobin can be used to assess the degree of iron deficiency [23]. The copper containing protein, ceruloplasmin, is the link between copper and iron metabolism. Oxidation of ferrous iron is greatly accelerated (10-100 fold) by ceruloplasmin ferroxidase [57]. Negative correlation with iron might be a result of the fact that copper is insufficiently used and accumulates excessively in tissues during iron deficiency. In experiments with iron deficiency, increases of the level of some trace elements like copper have been observed [58]. Similar results were found among children [12]. Conversely, severe copper deficiency in humans provokes iron accumulation in the liver, due to the absence of ferroxidase activity [57].

Iron stores in the liver, spleen, and bone marrow exist primarily in the form of ferritin. That's why the concentration of serum ferritin is positively correlated with the serum iron and the size of the total body iron stores [46].

Positive correlation of iron and erythropoietin shows their interaction and influence on the stem cells along the erythroid line. Iron is necessary for the production of new haemoglobin on the pro-erythroblast stage. Erythropoietin promotes the maturation of burst-forming unit – erythroid's into colony-forming unit – erythroid's and subsequently into mature erythrocytes. If erythropoietin is present without sufficient iron, there is insufficient material for red cell production [59].

Zinc competes with copper in the processes of absorption in the intestine, which may explain the presence of a negative correlation between these trace elements [23]. A study done by Turgut et al. have also found a decrease in serum Zn level and an increase in serum Cu level in the anaemic group [12].

Patients with IDA due to continuous hypoxia may demonstrate supraphysiological level of erythropoietin [60, 61]. In case of IDA, erythropoietin stimulates production of protoporphyrin IX, the precursor of the haem, but the iron that normally is inserted into the protoporphyrin lacks. Zinc, the second most abundant divalent cation in the red cells, fills the void for forming zinc protoprophyrin. However, zinc protoporphyrin does not function in oxygen delivery by haemoglobin, that's why hypoxia and erythropoietin production continue [62].

Copper is one of the main negative regulators of erythropoiesis in children with IDA. This element demonstrates statistically significant negative correlations with serum iron, zinc (as mentioned above), but also with cobalt, as well as serum ferritin and erythropoietin.

However, copper in the form of ceruloplasmin influences the oxidation of ferrous iron into ferric state [11]. These processes are important for iron stores and ferritin formation. In anaemic patients with depletion of iron stores high ceruloplasmin activity is ineffective. On the one hand, it does not increase ferritin level, on the other – may provoke elevation of oxidative blood ability and cause lipid peroxidation [63].

Erythropoietin is not only erythropoiesis stimulator, but also an anti-apoptotic, anti-inflammatory substance that has protective properties against oxidative stress, and modulatory effects on Cu and Zn levels [64]. Negative correlation between erythropoietin and copper in children with IDA may be related to the high activity of ceruloplasmin.

Cobalt and copper are absorbed by similar transport mechanisms [65] and the competition between them contributes to a negative correlation.

Positive impact of cobalt on the erythropoietin production is associated with the following mechanisms. Cobalt

stimulates erythropoietin production by activation of the transcription factor hypoxia-inducible factor-1α (HIF-1α) and HIF-2α [66]. The results of such influences are acceleration of maturation of erythroid stem cells and stimulation of haemoglobin synthesis [67]. One International erythropoietin Unit (IU) elicits the same erythropoiesis-stimulating activity as 5 μmol Co^{2+} [68].

Our investigation shows that both cobalt and nickel have indirect mechanisms of influence on the iron metabolism. Positive interaction with cobalt and other mechanisms may play role here. For example, the levels of other minerals, such as magnesium, zinc and calcium may alter nickel absorption from the gut [69]. Nickel induces the synthesis of phosphatidylserine, which stabilizes the membrane of erythrocytes and prolonges their life. Significant amounts of nickel are found in RNA and DNA, where nickel interacts with these nucleic acids and that's why may influence on the erythroblasts growth. Most of the plasma nickel is a constituent of the circulating proteins nickeloplasmin and albumin, and it is also thought to be a factor in a hormone (erythropoietin), and cell membrane metabolism [31]. Nickel deficiency increases the excretion of iron ions from the human body [70].

Results of our work suggest that serum zinc, copper, cobalt and nickel interact with iron, haematological parameters of iron metabolism and with each other in children with IDA. The differences observed might be attributed to relations between iron and different elements at the absorption, transport, and storage.

4. Conclusion

Correlations between haematological parameters (haemoglobin, haematocrit, red blood cells), erythrocyte indexes (MCV, MCH, MCHC) and biochemical markers of iron metabolism describe leader role of iron in erythropoiesis in children with IDA. But formation of IDA is caused not only by violation of iron metabolism, but also by interactions with other trace elements that may affect directly or indirectly both the iron exchange and erythropoiesis regulation through the erythropoietin production.

Thus, we found a significant correlations between the level of chromium and SatTf ($r = -0.382$, $p = 0.018$), zinc – with SEPO ($r = 0.543$, $p = 0.036$), copper – with SFR ($r = -0.561$, $p = 0.029$), and SEPO ($r = -0.739$, $p = 0.0016$), and cobalt – with SEPO ($r = 0.769$, $p = 0.0021$), that indicate the role of trace elements in the pathogenesis of IDA.

Some trace elements play significant roles in haematopoiesis and iron metabolism. Their failure or excess may lead to alteration in haem metabolism and provoke anaemia. We found that some trace element insufficiencies (zinc, cobalt, and nickel) develop in children with iron deficiency anaemia, but the question about serum copper level is still open. There is insufficiency of copper in some patients, while excess of copper in the blood is observed in other populations of children. Deficiencies or interactions between trace elements may cause disorders in the absorption, distribution, metabolism and elimination of other trace elements. Two main microelements (zinc and copper) can directly influence on the iron metabolism. Mechanism of other trace elements action (cobalt, nickel, chromium) is more complex. It is associated with a mediated effect, i.e. by other trace elements, including the processes of transport, deposition of iron, etc.

Thus, if the routine therapy of IDA is ineffective, a concomitant micronutrient disorder should be considered, with an evaluation of trace elements level, and, if necessary, a correction should be carried out.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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