



DOI: 10.2478/rmlm-2014-0042

The relationship between micronutrients and anthropometric measurements in malnourished children

Relația între micronutrienți și măsurătorile antropometrice la copiii malnutriți

Lidia Man¹, Adrian Man^{2*}, Cristina Oana Mărginean¹, Ana Maria Pitea¹,
Maria Despina Baghiu¹

1. University of Medicine and Pharmacy Tirgu-Mures, Romania; Pediatrics Clinic I,
Emergency Clinical County Hospital Tirgu-Mures, Romania;

2. Department of Microbiology, University of Medicine and Pharmacy Tirgu-Mures, Romania;
Central laboratory, Clinical County Hospital of Tirgu-Mures, Romania

Abstract

Purpose: to evaluate the serum levels of micronutrients in children with nutritional disorders, and to find if there is a direct correlation between them and the anthropometric measurements. **Materials and methods:** the study was conducted on 125 children (0-18 years); the working group consisted in children with Z-score < -2 standard deviations for at least one anthropometric measurement, while the children without growth disorders were considered as controls. Thus, for each anthropometric measurement, we had different working/control groups that were used for the assessment of correlation with laboratory findings. We followed eight anthropometric parameters and their relation with five of the micronutrients (Ca, Mg, Fe, Zn and Cu). **Results:** no statistical differences were found in micronutrients serum levels between genders or provenance. Most mean serum levels of micronutrients were lower in the children with Z-scores < -2 standard deviations (except Cu). Mg and Ca were positively correlated with most of the anthropometric measurements. For Fe, Zn and Cu, we found no correlation with any of the anthropometric measures. Differences in mean serum levels were found for Mg, with lower values in children with low weight-for-age and triceps-skinfold-thickness, and for Cu, with higher levels in children with low triceps-skinfold-thickness. The red blood cell indices were positively associated with Fe and Zn levels. **Conclusions:** correlations between the serum level of micronutrients and anthropometric evaluation scores were found for Mg and Ca, but not for Fe and Zn, which were instead directly correlated with red blood cells indices. Mg, Fe and Zn tend to present small serum values in children with growth deficits. Considering the costs, the routine evaluation of Zn and Cu serum levels in growth disorder suspicion is not justified in our geographic area.

Keywords: micronutrients; growth deficits; nutritional deficiency; anthropometric measurements

*Corresponding author: Adrian Man, Department of Microbiology, University of Medicine and Pharmacy Tirgu-Mures, Romania; e-mail: adrian.man@umftgm.ro

Rezumat

Scop: evaluarea nivelelor serice de micronutrienți la copii cu tulburări de nutriție, și aprecierea corelației dintre acestea și măsurătorile antropometrice. **Material și metode:** Studiul a fost realizat pe 125 copii (0-18 ani); lotul de lucru a constat în copii cu scoruri $Z < -2$ deviații standard pentru cel puțin o măsurare antropometrică, copii fără tulburări de creștere fiind considerați lot de control. Astfel, pentru fiecare măsurare antropometrică am avut diferite loturi de lucru/control, folosite pentru evaluarea corelației cu rezultatele de laborator. Am urmărit opt parametri antropometrici și relația lor cu cinci micronutrienți (Ca, Mg, Fe, Zn și Cu). **Rezultate:** nu s-au găsit diferențe semnificative între nivelurile serice de micronutrienți nici în funcție de sex, nici în funcție de mediul de proveniență. Cel mai frecvent, nivelurile serice medii de micronutrienți au fost mici la copiii cu scoruri $Z < -2$ deviații standard (cu excepția Cu). Mg și Ca s-au corelat pozitiv cu majoritatea măsurătorilor antropometrice. Pentru Fe, Zn și Cu nu am găsit nici o corelație, cu niciuna dintre măsurile antropometrice. Diferențe între nivelurile serice medii au fost găsite pentru Mg, cu valori mai scăzute la copii cu greutate scăzută pentru vârstă și pliu tricripital redus, respectiv pentru Cu, cu niveluri mai ridicate la copii pliu tricripital redus. Indicii eritrocitari au fost corelați pozitiv cu nivelurile de Fe și Zn. **Concluzii:** corelații între nivelul seric de micronutrienți și scorurile de evaluare antropometrică au fost găsite pentru Mg și Ca, dar nu pentru Fe și Zn, care în schimb s-au corelat direct cu indicii eritrocitari. Mg, Fe și Zn tind să prezinte valori serice mici la copiii cu deficite de creștere. Având în vedere costurile, evaluarea de rutină a nivelurilor serice de Zn și Cu la copii cu suspiciune de tulburări de creștere nu este justificată în zona noastră geografică.

Cuvinte cheie: micronutrienți; deficite de creștere; deficite nutriționale; măsurători antropometrice

Received: 1st September 2014; Accepted: 16th November 2014; Published: 28th November 2014.

Introduction

Many children present to the pediatrics office for nutritional and/or growth disorders. If not properly diagnosed, or confirmed and left without a proper treatment, these conditions can lead to severe consequences: low immune responses with high susceptibility for infections, impaired wound healing and intellectual development, neuropsychic disorders, delayed physical and sexual development (1–4).

According to the definition, a micronutrient is “an essential nutrient, as a trace mineral or vitamin, that is required by an organism in minute amounts”, in about generally less than 100 mg/day (5). Micronutrients play a primary role in human metabolism and are essential for the maintenance of good health. The micronutrients include calcium, chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, selenium and zinc (6). Calcium (Ca) is the most abundant microelement in the human body,

mainly found as calcium phosphate inside bones, with an important structural role. The plasmatic Ca is bound to albumin or chelated, about half remaining as free ionized Ca (7). Magnesium (Mg) has many physiological functions, especially enzymatic and structural ones (8). Only 1% of total Mg is found in serum, mainly in ionized form, bound to proteins or in complexes with anions. Thus, poor nutrition can lead to a global Mg deficit, but with quasi-normal serum values (9). Iron (Fe) is required for adequate erythropoietic function, oxidative metabolism and cellular immune responses. More than 75% of the Fe in the body is found as part of hemoglobin, myoglobin and enzymes, the rest being stored in liver, reticuloendothelial system and bone marrow. The Fe homeostasis is maintained mostly by its internal turnover (10,11). Zinc (Zn) has antioxidant action in red blood cells, major importance in hematopoiesis, in stabilizing the biomembranes and is a structural component of enzymes (12,13). Only less than 0.1% of total

body Zn concentration is found in serum, the balance of its absorption-excretion being achieved in the gastro-intestinal tract. Contrarily to Mg, a low Zn intake can lead to serum, bone, liver and testes Zn depletion, while the concentration in muscles and skin are not affected, due to its very high turn-over (14). Copper (Cu) is mainly bonded to ceruloplasmin and similarly to Mg and Zn, maintains the proper functioning of enzymes and production of hemoglobin, acting both as an antioxidant or a pro-oxidant (15). Many micronutrients are interrelated, so a deficiency of one can lead to associated micronutrient disorders. For example, Mg deficiency can lead to acute Ca deficiency by interfering with parathormone activity (16); excessive Zn intake interferes with the intestinal absorption of Cu (17); Cu is needed for Fe homeostasis (18).

Data on the prevalence of micronutrient deficiencies in children from Romania is limited. Though many micronutrients can be easily evaluated with minor investment in all children who present suspicion of nutritional deficits, some micronutrients are more difficult to assess; this is mainly due to the need of good infrastructure represented by the Atomic Absorption Spectroscopy (AAS) system as conventional method of detection; other recently developed equipment, such as the Anodic Stripping Voltammetry system may be available in the near future (19,20). Nowadays, the related costs for Zn and Cu serum detection are not negligible (~11€ retail price/probe in Romanian laboratories), as they are 4-5 times higher than those for Ca, Mg or Fe.

Currently, there is a high interest in these micronutrients due to their use as diagnosis markers, therapeutic agents and why not, due to the commercial claims for such substances.

Purpose

The aim of the study is to evaluate the serum levels of micronutrients in children that present low measurement values in the anthropometric

evaluation compared to children with normal anthropometry and to find whether there is a direct correlation between them. An associated purpose was to assess the red blood cell (RBC) indices in relation to the anthropometrics and serum levels of micronutrients.

Materials and methods

The current prospective study was conducted on children admitted to Pediatrics Clinic I within the Emergency Clinical County Hospital in Tirgu-Mures for minor pathology or for reevaluation following acute conditions, during March 2013 – July 2014. The working group consisted of children (0-18 years old) who after the primary evaluation of weight and height were suspected to present growth disorders (Z -score $< -2SD$ for at least one anthropometric measurement). The control group consisted of children who were admitted for the same suspicion, but was not confirmed following the anthropometric evaluation (Z -score $> -2SD$ for the same anthropometric measurement) and children without growth disorders who presented for reevaluation, following the acute condition that required hospitalization. Thus, for each anthropometric measurement, we had different working/control groups that were used for the assessment of correlations with the laboratory findings.

The main exclusion criteria from both groups were: the presence of any inflammatory process or chronic diseases that could influence nutritional status, including infections, children on whom complete clinical and laboratory tests could not be performed and the absence of informed consent from parents. All children were evaluated as the admission protocol requires, following the weight and height. The parent's consent was asked for other anthropometric measurements such as Waist Circumference (WC), Hip Circumference (HC), Mid-Upper Arm Circumference (MUAC) and Triceps Skin Fold thickness (TSF), as well as for blood sampling for the

evaluation of additional micronutrients serum levels: Ca, Mg, Fe, Zn and Cu. The study is in compliance with the principles of the Helsinki Declaration and the protocol was approved by the Ethics Committee within the University of Medicine and Pharmacy of Tirgu-Mures as well as by the Hospital administration.

The anthropometric calculations were performed using Growth Analyser 3.5 software, using growth references for Romania: the Netherlands 1997 for body-mass-index for age (BMI_z), weight for age (WFA_z), height for age (HFA_z), weight for height (WFH_z), waist circumference for age (WCA_z), hip circumference for age (HCA_z); Gerver 2001 for mid upper arm circumference for age ($MUAC_z$) and triceps skin fold thickness for age (TSF_z). All results were calculated as Z-scores. The body surface area (BSA) was also calculated.

The evaluation of serum levels of Ca, Mg and Fe was performed on a Roche Cobas® 6000 analyzer. The levels of Cu and Zn were assessed by AAS using the Analytik Jena Zeenit® 700 system. The RBC indices such as hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were assessed by SysmexXT-4000I® analyzer.

The lower and upper limits, as reference values, were considered according to the corresponding age and gender.

All statistical calculations were performed in spreadsheets and GraphPad InStat 3 software, using a significance threshold alpha of 0.05. When the laboratory values of micronutrients presented Gaussian distribution, parametric tests were used (Unpaired t test for comparison of means); exceptions were made if in certain sub-groups the distribution was non-Gaussian, when non-parametric tests were used (Mann-Whitney for comparison of medians). For comparison of categorical data we used Fisher's exact test. Pearson's and Spearman r tests were used for linear correlations.

Results

Following the inclusion and exclusion criteria, a total number of 125 children were evaluated, 68% of them (85 children) presenting one or more anthropometric parameters with values $< -2SD$. The overall mean Z-scores were -1.55 SD for BMI_z , -2.27 SD for WFA_z , -1.81 SD for HFA_z , -1.46 SD for WFH_z , -1.08 for WC_z , -1.20 SD for HC_z , -1.60 SD for $MUAC_z$ and -1.24 SD for TSF_z . Details of the individual measurements Z-scores for the two working groups (children

Table I. Anthropometric measurements for the two working groups

	< -2SD (working group)			> -2SD (control group)			p
	n	%	Mean/Median*	n	%	Mean*	
BMI_z	38	30.4%	-3.35±1.45	87	69.6%	-0.77±0.78	< 0.05
WFA_z	73	58.4%	-3.09 (-7.5 - -2.01)	52	41.6%	-0.81±0.81	< 0.05
HFA_z	56	44.8%	-3.15±1.01	69	55.2%	-0.71±0.97	< 0.05
WFH_z	35	28.0%	-3.31±1.26	90	72.0%	-0.73±0.81	< 0.05
WCA_z	28	22.4%	-2.25 (-7.5 - -2.01)	97	77.6%	-0.64±0.92	< 0.05
HCA_z	31	24.8%	-2.87±0.93	94	75.2%	-0.65±1.04	< 0.05
$MUAC_z$	50	40.0%	-2.85±0.7	75	60.0%	-0.76±0.93	< 0.05
TSF_z	32	25.6%	-2.44±0.31	93	74.4%	-0.83±0.88	< 0.05

* t-test / Mann-Whitney test (depending on normality test result)

with $< -2SD$ for anthropometric measurements and control group) can be followed in Table I.

Overall, the prevalence of low serum levels (under the lower normal limit) was 12% for Fe, 4.8% for Zn, 1.6% for Ca and Cu and 0% for Mg. The mean serum values for Ca, Fe and Zn were 2.51 ± 0.16 mmol/L, 13.82 ± 6.47 mmol/L and 90.15 ± 18.47 $\mu\text{g/dL}$ respectively. For Mg and Cu, which compared to the other micronutrients did not follow a Gaussian distribution, the median values were 0.91 mmol/L and 122.9 $\mu\text{g/dL}$ respectively. Two children (1.6%) presented two simultaneous micronutrient values under the lower limit, whereas 21 children (16.8%) presented only one low micronutrient. All other children presented normal or high values.

The genders were approximately equally distributed (52% boys, 48% girls). The growth differences between genders were observed in WFA only, values $< -2SD$ being found in 68.3% of girls and only in 49.2% of boys ($p=0.045$). No statistical differences were found in micronutrients serum levels between the two genders, though the boys presented lower mean values for Ca, Mg and Fe, and higher mean values for Zn and Cu, compared to the girls. More than half of the children (54%) had their residency in urban environment. No differences were found regarding the mean serum levels of micronutrients between the children that reside in urban and rural areas respectively (Table II). The micronutrients

mean values did not differ between age-groups, except for Ca and Fe; Ca presented lower values in children over 9 years old compared to the 0-2 years old group, whereas Fe was lower in the 1-2 years old group.

In Tables III and IV aspects regarding the relations between anthropometric measurements and micronutrients serum levels can be followed. Regardless of the anthropometric measurements, most mean serum levels of micronutrients were lower in children with Z-scores $< -2SD$ (except Cu), though in general not statistically significant.

Among the micronutrients, Mg and Ca presented the most correlations with the anthropometric measurements. Thus, Mg was positively correlated mostly with TSF_z ($r=0.293$), Body Surface Area ($r=0.271$), WFH_z ($r=0.275$), HCA_z ($r=0.223$), BMI_z ($r=0.202$), WCA_z ($r=0.180$), $MUAC_z$ ($r=0.179$), and WFA_z ($r=0.178$), all with $p<0.05$. Mg presented significantly lower levels in children with Z-scores $< -2SD$ for WFA_z and TSF_z only. Conversely, Ca was positively correlated only with $MUAC_z$ ($r=0.176$) and TSF_z ($r=0.219$), with $p<0.05$, but with no difference between the children with $< -2SD$ and controls. We found a significant negative correlation between the Mg serum level and HGB level ($r=-0.223$; $p=0.012$) and not quite significant with MCHC ($r=-0.169$; $p=0.058$). We found no correlation between Ca and RBC indices.

Table II. Differences in mean/median serum levels of micronutrients according to gender and provenance

		Ca (mmol/L)	Mg (mmol/L)	Fe ($\mu\text{mol/L}$)	Zn ($\mu\text{g/dL}$)	Cu ($\mu\text{g/dL}$)
Genders	Males	2.50 ± 0.14	0.90 (0.77-1.4)	13.61 ± 6.12	92.8 ± 20.3	131.6 ± 32
	Females	2.52 ± 0.17	0.93 ± 0.08	14.04 ± 6.88	87.2 ± 15.8	$119.4 (72.2-415.6)$
	p	0.561	0.085	0.716	0.088	0.403
Provenance	Urban	2.51 ± 0.17	0.92 (0.78-1.4)	14.38 ± 6.35	91.9 ± 19.9	$122.6 (51.4-415.6)$
	Rural	2.51 ± 0.13	0.91 ± 0.08	13.18 ± 6.6	88 ± 16.5	129.08 ± 27.9
	p	0.843	0.721	0.302	0.242	0.891

* t-test / Mann-Whitney test (depending on normality test result)

The Cu serum values are not correlated with any of the anthropometric measurements Z-scores. There are also no significant differences in Cu levels between the children with <-2SD and controls regarding any anthropometric marker, except for TSF_Z; the mean Cu values are higher in children with <-2SD for TSF_Z than in controls (145.0±61.8 µg/dL vs 125.9±25.7 µg/dL, p<0.05). We found no correlation between Cu and RBC indices.

Regarding Fe and Zn, we found no correlation with any of the anthropometric measurements; also, we did not find any statistically significant differences between the children with <-2SD and controls, for any of the anthropo-

metric measurements. Instead, we found significant positive correlations with HGB level for Zn (r=0.253; p=0.004) and Fe (r=0.384; p<0.001) serum levels. Fe was also positively correlated with MCH (r=0.316; p<0.001) and MCHC (r=0.23; p=0.009).

The overall hemoleucogram evaluation showed non-Gaussian distributions of RBC indices; HGB values varied from 6.8 g/dL to 15.5 g/dL, with a median of 12.3 g/dL; MCV values were situated between 30.6 fL and 92 fL with a median of 79 fL. MCH was situated between 15.3 pg and 33.2 pg (median of 27.3 pg), while MCHC varied between 18.5 g/dL and 37 g/dL (median of 34.2 g/dL). Regardless of the anthro-

Table III. Correlations between the z-scores of the anthropometric measurements and serum level of micronutrients

Anthropometric		Micronutrient mean/median serum levels				
Evaluation		Ca	Mg	Fe	Zn	Cu
(Z-scores)		(mmol/L)	(mmol/L)	(µmol/L)	(µg/dL)	(µg/dL)
BMI _Z	r	0.104	0.202	0.041	-0.020	-0.012
	p	0.246	0.023	0.648	0.827	0.888
WFA _Z	r	0.161	0.178	0.036	0.019	-0.049
	p	0.073	0.047	0.693	0.835	0.585
HFA _Z	r	0.129	0.063	0.021	0.044	0.007
	p	0.151	0.485	0.814	0.624	0.938
WFH _Z	r	0.078	0.275	0.073	-0.026	-0.004
	p	0.386	0.001	0.419	0.774	0.963
WCA _Z	r	0.124	0.180	-0.037	-0.174	-0.025
	p	0.169	0.044	0.684	0.052	0.781
HCA _Z	r	0.113	0.223	0.019	-0.104	-0.028
	p	0.211	0.012	0.831	0.251	0.754
MUAC _Z	r	0.176	0.179	0.050	-0.007	-0.081
	p	0.049	0.046	0.578	0.942	0.365
TSF _Z	r	0.219	0.293	0.078	-0.094	-0.074
	p	0.014	0.001	0.390	0.295	0.407
BSA	r	-0.009	0.271	0.043	0.047	0.001
	p	0.919	0.002	0.637	0.606	0.985

p-value assessed by Pearson / Spearman r test (depending on normality test result)

Table IV. Mean/median serum level of micronutrients in children with < -2SD and controls for all anthropometric measurements

	Z-score	Ca		Mg		Fe		Zn		Cu	
		(mmol/L)		(mmol/L)		(μmol/L)		(μg/dL)		(μg/dL)	
		Mean	p	Mean/ Median	p	Mean	p	Mean/Median	p	Mean/ Median	p
BMI _z	< -2SD	2.51±0.16	0.818	0.903±0.08	0.214	13.38±6.59	0.612	89.9±16	0.919	134.4±55.7	0.496
	> -2SD	2.51±0.16		0.924±0.09		14.02±6.46		90.3±19		129.2±28.9	
WFA _z	< -2SD	2.50±0.15	0.259	0.903±0.08	0.032	13.77±6.36	0.914	89.3±17	0.551	133.4±46.0	0.376
	> -2SD	2.53±0.18		0.938±0.1		13.90±6.70		91.3±20		127.1±25.7	
HFA _z	< -2SD	2.51±0.14	0.931	0.913±0.08	0.588	13.63±5.72	0.770	88.5±17	0.365	129.1±31.0	0.919
	> -2SD	2.51±0.17		0.922±0.09		13.98±7.07		91.5±19		123 (72.2-415.6)	
WFH _z	< -2SD	2.51±0.16	0.809	0.900±0.09	0.168	13.18±5.79	0.493	89.5±17	0.819	119 (51.4-415.6)	0.786
	> -2SD	2.51±0.16		0.925±0.09		14.07±6.74		90.4±19		129.4±28.8	
WCA _z	< -2SD	2.49±0.14	0.452	0.893±0.06	0.099	14.65±6.31	0.446	95±18	0.085	129.0±35.3	1.000
	> -2SD	2.52±0.16		0.91 (0.77-1.4)		13.58±6.54		85 (40-195)		123 (72.2-415.6)	
HCA _z	< -2SD	2.51±0.15	0.972	0.897±0.1	0.127	13.33±6.22	0.630	91.2±17	0.475	124 (51.4-415.6)	0.518
	> -2SD	2.51±0.16		0.925±0.08		13.98±6.58		84.5 (40-195)		128.1±27.6	
MUAC _z	< -2SD	2.50±0.15	0.417	0.909±0.09	0.399	13.43±6.5	0.586	88.6±16	0.457	136.8±53.1	0.156
	> -2SD	2.52±0.17		0.923±0.09		14.08±6.49		91.2±20		126.7±24.9	
TSF _z	< -2SD	2.48±0.13	0.182	0.889±0.06	0.023	12.89±5.92	0.348	89±17	0.684	145.0±61.8	0.016
	> -2SD	2.52±0.17		0.92 (0.77-1.4)		14.14±6.66		90.6±19		125.9±25.7	

* t-test / Mann-Whitney test (depending on normality test result)

pometric measurements, the mean/median levels of the studied RBC indices did not significantly differ between the children with < -2SD Z-scores and controls.

Discussions

Regarding the term “malnutrition”, this is a “condition that occurs when your body does not get enough nutrients” (21). It can develop both

in children and adults and it is primarily suspected by low WFH_z in children and low BMI_z in adults (under -2SD) (22). Our approach was not to have only one working group, but several, one for each of the anthropometric measurements. The idea is that, for example, a child with normal WFH_z score can present low WFA_z or MUAC_z scores or other lowered anthropometric measurements. These low scores reflect a certain degree of malnutrition, don't they? If one choos-

es only WFH_z as the single criteria for malnutrition, won't some of the true malnourished children be missed?

In our study, all anthropometric Z-score mean values were under -1SD, so we can consider that most of the children presented some degree of growth deficiencies (though not malnourished), which can be due to height, weight or body fat distribution disorders. Nevertheless, the serum mean values of all micronutrients were within normal ranges.

Similar results to ours were found in Brazil, with low Zn levels in very few children aged 2-5 years, with no differences in mean levels between genders (23). A study conducted in Greece supports our findings of no differences in Cu and Zn levels between genders, but instead they found positive and negative correlations with age for Zn and Cu respectively (24). Instead, in Nepal, Zn deficiency was associated with both sex and age (25). The positive correlation between the Zn and Fe levels and HGB was also described in other studies (26,27). Iron deficiency is mainly reflected through low values of HGB, MCV, MCH, and MCHC, so the positive correlation is explainable (28). Thus, there is a variety of results, which most probably are depending on the population origin and dietary habits.

Knowing that Cu is mandatory for good functioning of iron transport (as the oxidation of Fe^{2+} into Fe^{3+} is carried by ceruloplasmin, which needs Cu for its stability (18,29)), we noticed the lack of correlation (or even small negative correlations) between Cu and RBC indices; nevertheless, negative correlations have been previously reported (30). Regarding the relation to anthropometric measurements, the Cu serum values do not seem to vary, though significantly higher values were found in children with < -2SD, but only for TSF_z . Normally, Cu levels are lower in severe malnourished children (31,32), but in other studies, the wasted (not severe malnourished) children were found to have higher

Cu values compared to normal children (33), similar to our findings; the difference is that they suspected this to be a consequence of the increase in ceruloplasmin level following infections or inflammation, conditions which we have excluded in our group of children.

Low levels of Mg can cause anemic syndrome and can be confused with Fe deficiency. Aside Cu, we found Mg to also present a negative correlation, but only with HGB and partly with MCHC. Instead, most studies present a positive correlation between Mg and RBC indices (34,35).

Elevated Cu/Zn ratios are described to be associated with malnutrition (36), but according to our results, the Cu/Zn did not differ between the children with BMI < -2SD and controls. Furthermore, regardless of the number of concomitant anthropometric parameters with values < -2SD, we found no differences in the micronutrients serum levels and between the median values of RBC indices, which were within normal ranges in both groups. Thus, it seems that there is a need for more severe deficiency in food intake, in order to be reflected as micronutrient imbalance. Depletion in micronutrients is possible when severe protein-energy depletion conditions are associated, such as Kwashiorkor or Marasmus (31), that are rarely observed in our area (37). The correlation between micronutrients and growth status as assessed by anthropometric measurements can be seen in several chronic conditions. This is supported by Singla et al., who found positive correlation between serum Zn and HFA, but only in children with severe malnutrition (32); by Purice et al. who described low Zn levels in children with Down syndrome (38); or by Amare et al. who found high percentage of Zn deficiency in school children from Northwestern Ethiopia, but as we also found, not related with their nutritional status (33). Zn and Cu levels were both found to be low in 42% and respectively 1% of children from low-income families (39).

In Europe, in 2011, the risk of inadequate micronutrients intake was assessed to be about 11% to 20% for Zn, Fe and Cu. Ca, vitamins C and D, folic acid, selenium and iodine present the most inadequate intakes, though there no data was available from our region (40). Two years later, another micronutrient mapping in Europe presented a low risk of inadequate intake in all age groups and genders (41). Thus, it is understandable why we did not find extremely low serum values for these micronutrients in our region.

To our knowledge, this is the first study in Romania that assessed the micronutrients variation in generally healthy children, without chronic conditions. Several micronutrient-related national studies were published, but most of them presenting vitamins and iodine deficits. Our findings showed that Ca, Mg and Fe are the only micronutrients that present some correlations with nutritional status, thus with the anthropometric measurements, being performed in almost all children admitted to hospital, as part of their routine evaluation. The lack of correlation between anthropometrics and serum levels of Zn and Cu make us consider that the benefit/cost ratio for these micronutrients is low, due to high costs related to AAS method needed for their determination. Instead, any anemic syndrome must be thoroughly evaluated, following both Fe and other micronutrients balance, especially Mg.

The study has its limitations, mainly because of the relatively small number of children for which Zn and Cu were assessed, but also because of the small serviced area. Nevertheless, our results raise the need for further research regarding Zn and Cu deficiencies in other areas of Romania, especially as this problem is largely discussed in the international literature.

Further and larger studies are needed in order to understand the relation between micronutrients and children growth and to find what is

the degree of shortage in micronutrients, beside macronutrients, that will lead to growth disorders.

Conclusions

There is a correlation between the serum level of micronutrients and anthropometric evaluation scores for Mg and Ca, but not for Fe and Zn, which are instead directly correlated with RBC indices. In terms of micronutrients, Mg, Fe and Zn tend to present small serum values in children with growth deficits, but only Mg presented significant differences, solely in children with WFA_z or TSF_z under $-2SD$. Considering the costs, the routine evaluation of Zn and Cu serum levels as additional diagnostic markers aside Ca, Mg and Fe in order to support a suspicion of nutritional disorder (due to out-of-range anthropometric measurements) is not justified, at least in our geographic area. This may not be true in other areas, in which the nutritional deficit is higher, or in children who present associated chronic conditions that may lead to micronutrients depletion.

Acknowledgments

This paper is partially supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/CPP107/DMI1.5/S/80641.

Abbreviations

AAS - Atomic Absorption Spectroscopy
 BMI_z - body-mass-index for age Z score
BSA - body surface area
 HCA_z - hip circumference for age Z score
 HFA_z - height for age Z score
HGB - hemoglobin
MCH - mean corpuscular hemoglobin

MCHC - mean corpuscular hemoglobin concentration

MCV - mean corpuscular volume

MUAC_Z- mid upper arm circumference for age Z score

RBC – red blood cells

SD – standard deviations

TSF_Z- triceps skin fold thickness for age Z score

WCA_Z- waist circumference for age Z score

WFA_Z- weight for age Z score

WFH_Z- weight for height Z score

References

- Mărginean CO, Man L, Pitea AM, Man A, Mărginean CL, Cotoi OS. The assessment between IL-6 and IL-8 and anthropometric status in malnourished children. *Romanian J Morphol Embryol Rev Roum Morphol Embryol*. 2013;54(4):935–8.
- Brown JL, Pollitt E. Malnutrition, poverty and intellectual development. *Sci Am*. 1996 Feb;274(2):38–43. DOI: 10.1038/scientificamerican0296-38
- Stechmiller JK. Understanding the role of nutrition and wound healing. *Nutr Clin Pract Off Publ Am Soc Parenter Enter Nutr*. 2010 Feb;25(1):61–8. DOI: 10.1177/0884533609358997
- Belluscio LM, Berardino BG, Ferroni NM, Ceruti JM, Cánepa ET. Early protein malnutrition negatively impacts physical growth and neurological reflexes and evokes anxiety and depressive-like behaviors. *Physiol Behav*. 2014 Apr 22;129:237–54. DOI: 10.1016/j.physbeh.2014.02.051
- Micro-nutrient | Define Micro-nutrient at Dictionary.com [Internet]. [cited 2014 Aug 15]. Available from: <http://dictionary.reference.com/browse/micro-nutrient>
- Sadava DE, Hillis DM, Heller HC, Berenbaum M. *Life: The Science of Biology*. Ninth Edition. Sunderland, MA: MPS / W. H. Freeman & Co; 2012. 1266 p.
- Goldstein DA. Serum Calcium. In: Walker HK, Hall WD, Hurst JW, editors. *Clinical Methods: The History, Physical, and Laboratory Examinations* [Internet]. 3rd ed. Boston: Butterworths; 1990 [cited 2014 Aug 15]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK250/>
- Swaminathan R. Magnesium metabolism and its disorders. *Clin Biochem Rev Aust Assoc Clin Biochem*. 2003 May;24(2):47–66.
- Jahnen-Dechent W, Ketteler M. Magnesium basics. *Clin Kidney J*. 2012 Feb 1;5(Suppl 1):i3–i14. DOI: 10.1093/ndtplus/sfr163
- Munoz M, Villar I, Garcia-Erce JA. An update on iron physiology. *World J Gastroenterol WJG*. 2009 Oct 7;15(37):4617–26. DOI: 10.3748/wjg.15.4617
- Pantopoulos K, Porwal SK, Tartakoff A, Devireddy L. Mechanisms of mammalian iron homeostasis. *Biochemistry (Mosc)*. 2012 Jul 24;51(29):5705–24. DOI: 10.1021/bi300752r
- King LE, Fraker PJ. Zinc deficiency in mice alters myelopoiesis and hematopoiesis. *J Nutr*. 2002 Nov;132(11):3301–7.
- Powell SR. The antioxidant properties of zinc. *J Nutr*. 2000 May;130(5S Suppl):1447S–54S.
- King JC, Shames DM, Woodhouse LR. Zinc Homeostasis in Humans. *J Nutr*. 2000 May 1;130(5):1360S–1366S.
- Osredkar J, Sustar N. Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance. *J Clin Toxicol S*. 2011;3:2161–0494. DOI: 10.4172/2161-0495.S3-001
- Yamamoto M, Yamaguchi T, Yamauchi M, Yano S, Sugimoto T. Acute-onset hypomagnesemia-induced hypocalcemia caused by the refractoriness of bones and renal tubules to parathyroid hormone. *J Bone Miner Metab*. 2011 Nov;29(6):752–5. DOI: 10.1007/s00774-011-0275-7
- Hoffman HN, Phyllyk RL, Fleming CR. Zinc-induced copper deficiency. *Gastroenterology*. 1988 Feb;94(2):508–12.
- Sharp P. The molecular basis of copper and iron interactions. *Proc Nutr Soc*. 2004 Nov;63(4):563–9. DOI: 10.1079/PNS2004386
- Jothimuthu P, Wilson RA, Herren J, Pei X, Kang W, Daniels R, et al. Zinc Detection in Serum by Anodic Stripping Voltammetry on Microfabricated Bismuth Electrodes. *Electroanalysis*. 2013 Feb;25(2). DOI: 10.1002/elan.201200530
- Pei X, Kang W, Yue W, Bange A, Heineman WR, Papaty I. Improving Reproducibility of Lab-on-a-Chip Sensor with Bismuth Working Electrode for Determining Zn in Serum by Anodic Stripping Voltammetry. *J Electrochem Soc*. 2014 Feb 1;161(2):B3160–B3166. DOI: 10.1149/2.022402jes
- Board ADAME. Malnutrition. *PubMed Health* [Internet]. 2013 Apr 13 [cited 2014 Aug 23]; Available from: <http://www.ncbi.nlm.nih.gov/books/PMH0001441/>
- Centers for Disease Control and Prevention, World Food Programme. *A Manual: Measuring and Interpreting Malnutrition and Mortality*. World Food Programme; 2005. 231 p.
- Ferraz IS, Daneluzzi JC, Vannucchi H, Jordão Jr AA, Ricco RG, Del Ciampo LA, et al. Zinc serum levels and their association with vitamin A deficiency in pre-school children. *J Pediatr (Rio J)*. 2007 Dec;83(6):512–7. DOI: 10.2223/JPED.1725 DOI: 10.1590/S0021-75572007000800006
- Arvanitidou V, Voskaki I, Tripsianis G, Athanasopoulou H, Tsalkidis A, Filippidis S, et al. Serum copper and zinc concentrations in healthy children aged 3–14 years in Greece. *Biol Trace Elem Res*. 2007 Jan;115(1):1–12.

- DOI: 10.1385/BTER:115:1:1
25. Nepal AK, Gelal B, Mehta K, Lamsal M, Pokharel PK, Baral N. Plasma zinc levels, anthropometric and socio-demographic characteristics of school children in eastern Nepal. *BMC Res Notes*. 2014;7:18. DOI: 10.1186/1756-0500-7-18
 26. Temiye EO, Duke ES, Owolabi MA, Renner JK. Relationship between Painful Crisis and Serum Zinc Level in Children with Sickle Cell Anaemia. *Anemia* [Internet]. 2011 [cited 2014 Aug 15];2011. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3065914/>
 27. Cole CR, Grant FK, Swaby-Ellis ED, Smith JL, Jacques A, Northrop-Clewes CA, et al. Zinc and iron deficiency and their interrelations in low-income African American and Hispanic children in Atlanta. *Am J Clin Nutr*. 2010 Apr 1;91(4):1027–34. DOI: 10.3945/ajcn.2009.28089
 28. Wians FH, Urban JE, Keffer JH, Kroft SH. Discriminating Between Iron Deficiency Anemia and Anemia of Chronic Disease Using Traditional Indices of Iron Status vs Transferrin Receptor Concentration. *Am J Clin Pathol*. 2001 Jan 1;115(1):112–8. DOI: 10.1309/6L34-V3AR-DW39-DH30
 29. Sedlak E, Zoldak G, Wittung-Stafshede P. Role of Copper in Thermal Stability of Human Ceruloplasmin. *Biophys J*. 2008 Feb 15;94(4):1384–91. DOI: 10.1529/biophysj.107.113696
 30. Hegazy AA, Zaher MM, El-Hafez MAA, Morsy AA, Saleh RA. Relation between anemia and blood levels of lead, copper, zinc and iron among children. *BMC Res Notes*. 2010 May 12;3(1):133. DOI: 10.1186/1756-0500-3-133
 31. Gautam B, Deb K, Banerjee M, Ali MS, Akhter S, Shahidullah SM, et al. Serum zinc and copper level in children with protein energy malnutrition. *Mymensingh Med J MMJ*. 2008 Jul;17(2 Suppl):S12–15.
 32. Singla PN, Chand P, Kumar A, Kachhawaha JS. Serum, zinc and copper levels in children with protein energy malnutrition. *Indian J Pediatr*. 1996 Apr;63(2):199–203. DOI: 10.1007/BF02845244
 33. Amare B, Moges B, Fantahun B, Tafess K, Woldeyohannes D, Yismaw G, et al. Micronutrient levels and nutritional status of school children living in Northwest Ethiopia. *Nutr J*. 2012;11:108. DOI: 10.1186/1475-2891-11-108
 34. Zhan Y, Chen R, Zheng W, Guo C, Lu L, Ji X, et al. Association between serum magnesium and anemia: china health and nutrition survey. *Biol Trace Elem Res*. 2014 Jun;159(1-3):39–45. DOI: 10.1007/s12011-014-9967-x
 35. Shi Z, Hu X, He K, Yuan B, Garg M. Joint association of magnesium and iron intake with anemia among Chinese adults. *Nutr Burbank Los Angel Cty Calif*. 2008 Oct;24(10):977–84. DOI: 10.1016/j.nut.2008.05.002
 36. Guo C-H, Chen P-C, Yeh M-S, Hsiung D-Y, Wang C-L. Cu/Zn ratios are associated with nutritional status, oxidative stress, inflammation, and immune abnormalities in patients on peritoneal dialysis. *Clin Biochem*. 2011 Mar;44(4):275–80. DOI: 10.1016/j.clinbiochem.2010.12.017
 37. WHO | Romania [Internet]. WHO. [cited 2014 Aug 15]. Available from: <http://www.who.int/nutgrowthdb/database/countries/rou/en/>
 38. Purice M, Maximilian C, Dumitriu I, Ioan D. Zinc and copper in plasma and erythrocytes of Down's syndrome children. *Endocrinologie*. 1988 Jun;26(2):113–7.
 39. Schneider JM, Fujii ML, Lamp CL, Lönnerdal B, Zidenberg-Cherr S. The prevalence of low serum zinc and copper levels and dietary habits associated with serum zinc and copper in 12- to 36-month-old children from low-income families at risk for iron deficiency. *J Am Diet Assoc*. 2007 Nov;107(11):1924–9. DOI: 10.1016/j.jada.2007.08.011
 40. Roman Vi-as B, Ribas Barba L, Ngo J, Gurinovic M, Novakovic R, Cavelaars A, et al. Projected prevalence of inadequate nutrient intakes in Europe. *Ann Nutr Metab*. 2011;59(2-4):84–95. DOI: 10.1159/000332762
 41. Mensink GBM, Fletcher R, Gurinovic M, Huybrechts I, Lafay L, Serra-Majem L, et al. Mapping low intake of micronutrients across Europe. *Br J Nutr*. 2013 Aug 28;110(4):755–73. DOI: 10.1017/S000711451200565X