PLASMA CONCENTRATION OF ZINC IN CHILDREN AGED 0-3 YEARS

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Abstract

Purpose: Our research aimed at establishing the values of plasma zinc level and the presence of zinc deficiency, in healthy children aged 0-3 years in Bihor County, Romania. Methods: A total of 96 healthy children aged 0-3 years were included in the study during 2009-2011. Plasma concentration of zinc was determined using the 5-Br-PAPS endpoint colorimetric method.

Results: Mean plasma concentration of zinc in the study group was 15.33±1.49 μmol/l, higher for children who had less than 3 episodes/year of acute respiratory infections compared to those who experienced more than 3 episodes/year (15.89±1.46 μmol/l versus 14.2±0.76 μmol/l, p<0.001). Conclusions: In healthy children, aged 0-3 years, from Bihor County we recorded a mean plasma concentration of zinc of 15.33 ± 1.49 μmol/l, a value falling within normal limits. Children with more than 3 episodes of acute respiratory infections/year had lower values of mean plasma concentration of zinc than those with maximum 3 episodes/year. The children in our study showed no zinc deficiency.

Key words: zinc, diarrhea, acute respiratory infection, children.

Introduction

Zinc modulates the specific and nonspecific immune response in the human body and plays a role in the metabolism of the nucleic acids, in the enzyme activity (a catalytic, regulatory and structural role), entering the composition of over 300 enzymes (1), being necessary for growth and development processes (2).

Its presence in the human body in a very small amount (about 1.5 to 2.5 g) (3, 4) associated with storage failure causes a continuous need for dietary intake.

Zinc requirement is 2 mg/day for children aged 0-6 months and 3 mg/day for children aged 7 months-3 years (3). A part of ingested zinc becomes absorbed and then can be used by the human body. Its absorption is inhibited by the presence of phytates, oxalates, polyphenols, fibers, divalent cations and antibiotics such as floroquinolones and tetracyclines (4).

Sources of zinc are the foods rich in protein such as meat, seafood, eggs, dairy, wheat germs and brewer's yeast. These foods are usually expensive, leading to the prevalence of zinc deficiency of 30% in children worldwide (especially in developing countries), at ages when there is a maximum vulnerability for this deficiency.

The first reference to human zinc deficiency was made in 1958 by Bert L. Vallee et al (5), in a study on zinc metabolism in patients with Laennec cirrhosis. Later, in 1961 Prasad et al described the clinical characteristics of zinc deficiency in Iranian children (6).

Zinc deficiency determines the decrease of the immune system's defense capacity (7).

Zinc deficiency is associated with the stunting rate, if there is deficiency, the rate of stunting is higher (8). The rate of stunting is calculated according to the formula (Cstunt/Ctotal)*100, where Cstunt is the number of children under 5 years showing height growth retardation and Ctotal is the total number of investigated children aged under 5 years. It is considered to be a stunting growth when the height of the child is below the average height specific for that age and gender with 2 standard deviations (SD). In 2002 Romania recorded on the national scale a 12.8% stunting rate, according to the latest data reported in 2013 by the World Health Organization (WHO) (9). According to this data, Romania belongs to the list of countries with a low prevalence of zinc deficiency (less than 20%) (10).

The exact level of zinc deficiency in children from developed and medium developed countries is not precisely known (11). Therefore it is important to know the situation in Romania, in the group most vulnerable to zinc deficiency, respectively toddlers (0-3 years).

Our research aimed at establishing the plasma zinc level and the presence of zinc deficiency, in healthy children aged 0-3 years in Bihor County, Romania.

Patients and methods

The source population included 98 healthy children, aged 0-3 years, who addressed in 2009-2011 the office of the 'Gavril Curteanu' Municipal Hospital of Infectious Diseases Oradea, for laboratory investigations on demand.

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Patients included in the study were clinically healthy children aged 0-3 years who addressed during the study period the hospital's laboratory in order to perform routine medical tests for inclusion in the community (nursery, kindergarten), for analysis on demand or who came for the mandatory inspection at 2 years after acute viral hepatitis A.

Patients with a history of illness during the last three months, those who have followed previous therapy with zinc, calcium, copper, iron, laxatives, antacids containing magnesium, and those who did not have the written consent of parents were excluded from the study.

To determine the sample cases of healthy children (n) under the age of 3 years, we used the valid formula for studies in which the tracked feature is an alternative (present case: healthy-sick), with 95% probability, error limit of 0.1 and community volume of 32,420 (data provided by Bihor County Statistics Department) (12,13). The sample will include at least 96 at a population of over 10,000.

During the study period, 98 children were included in the study, 2 children were excluded at the parents' request, leaving 96 children in the study. For each patient a written consent of parents or legal guardian was requested. All procedures involving human subjects were approved by the Ethics committee of the hospital.

Children included in the study were clinically examined by a MD Infectious Disease Specialist, the day of sample collection. There were declared clinically healthy only children who after the history and physical examination showed no symptoms or clinical signs of disease.

For each child included in the study a questionnaire form was filled in, Table no. 1.

Minimum family monthly income required to ensure satisfactory conditions for the growth and development of the child was considered to be 330 EURO, (2 minimum wages at the time of the study). For the distribution of cases by determination period, the 4 seasons of the year were taken into account, namely: Winter: December to February; Spring: March to May; Summer: June to August; Autumn: September to November. Children's diet was considered excessive for the 5 types of food, if they have exceeded the recommendations of the nutrition guidelines by age group (14, 15).

Plasma concentration of zinc (PCZ) was determined to the subjects included in the study. Venous blood sampling was done in basal conditions, during the time segment 7-9.30, after fasting, from the median cubital vein with Becton-Dickinson (BD) holder-vacutainers closed system, in BD vacutainers for heavy metals containing heparin lithium as anticoagulant. PCZ determination was obtained from the plasma after centrifugation. A colorimetric method was used to determine the PCZ. This method is based on the reaction between zinc and 2 - (5-Bromo-piridilazo) -5 - (N-propyl-N-sulfo-propylamino)-phenol (5-Br-PAPS). The inter-assay coefficient of variability of the method was 2.95% and the corresponding intra-assay coefficient of variability was 1.46%. The detection limit of the method was 0.61 µmol/l. Reading was performed using the CX5 Beckman Synchron automatic analyzer (Beckman Coulter Inc, USA).

No._____		
1. Determination date dd / mm / yy		
2. Demographic data		
	place of residence	
	age	
	sex	
	area of origin	
3. Pathological medical history no. of episodes/year		
	acute respiratory tract infections	
	acute diarrheal disease	
4. Socio-economic parameters		
	number of family members	
	family monthly income	

5. Child nutrition considered excessive, amount/day (It will be considered as excessive food the one that was consumed by the child in quantities that exceeds the value in the grey area of the table, according to the child's age)

Age	Dairy		Cereals		Meat		Fruits and Vegetables		Chocolate	
0-4 months	>1liter						>100ml			
4-6 months	>1liter		>50g							
6-8 months	>800g		>60g		>50g		>250g			
8-12 months			>100g				>100ml			
1-2 years	>500g		>200g		>100g		>400g		>10g	
2-3 years										

Table no.1. Questionnaire form
dd-day, mm-month, yy-year

NO.	PARAMETER	ABSOLUTE NUMBER	%
1.	**Gender**		
	Female	43	44.7
	Male	53	55.3
2.	**Area of origin**		
	Urban	45	46.8
	Rural	51	49.2
3.	**Age**		
	0-6 months	14	14.6
	7-12 months	25	26.0
	13-24 months	28	29.2
	25-36 months	29	30.2
	mean age	19.5±10.48 months	
4.	**Pathologic personal history**		
	Acute respiratory infection	72	75
	≤ 3 episodes/year	41	42.7
	>3 episodes/year	31	32.3
	Acute diarrheal disease	42	43.7
	≤ 1 episode/year	32	33.3
	>1 episodes/year	10	10.4
5.	**Socio-economic parameters**		
	number of family members		
	≤ 4 members	73	76.1
	>4 members	23	23.9
	family monthly income		
	≤1500RON	33	34.3
	>1500RON	63	65.6
6.	**Predominant food or in excess**		
	1. Predominant food		
	dairy	14	14.5
	grain	11	11.5
	vegetables and fruits	23	23.9
	meat	11	11.4
	2. Excess food		
	chocolate	4	4.1
7.	**Period for determining the plasma concentration of zinc**		
	winter	26	27.1
	spring	22	22.9
	summer	19	19.8
	autumn	29	30.2

Table no.2. Characteristics of subjects included in the study

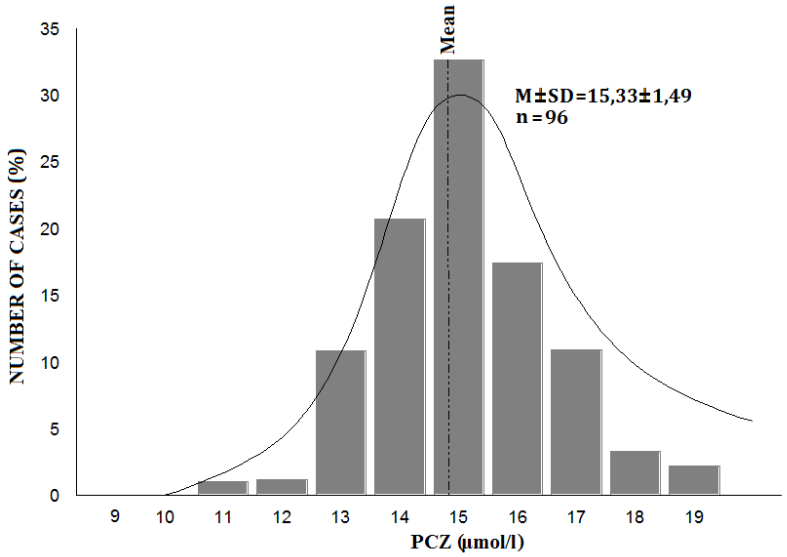

Figure no. 1. Plasma concentration of zinc in the group of healthy children
PCZ-plasma concentration of zinc, M-mean, SD-standard deviation, n-total number of children

Parameter		Mean PCZ (µmol/l)	p
Gender			
	female	15.19±1.31	0.31
	male	15.51±1.69	
Area of origin			
	rural	15.10±1.51	0.14
	urban	15.54±1.45	
Diet			
	1. Predominant food		
	meat	16.19±1.89*	
	dairy	15.67±1.55	*p
	cereals	15.57±1.84	
	vegetables and fruits	15.24±1.16	
	2. Excess food		
	chocolate	15.23±1.47	
Socio-economic parameters			
	1. Number of family members		
	≤4 members	15.52±1.62	0.19
	>4 members	15.08±1.30	
	2. Monthly family income		
	≤330 EURO	15.26±1.36	0.65
	>330 EURO	15.40±1.64	
Season			
	winter	15.66±1.12**	
	spring	15.40±1.26	
	summer	15.22±1.22	**p
	autumn	15.28±1.05	

Table no.3. Mean plasma concentration of zinc according to gender, area of origin, diet, socio-economic parameters and season
**p - the value of p for children who eat mostly meat versus other foods (dairy p=0.487, grains p=0.326, vegetables and fruits p=0.393, chocolate p=0.304), **p – the value of p for children from group winter versus other seasons (spring p=0.45, autumn p=0.2, summer p=0.22), PCZ -plasma concentration of zinc*

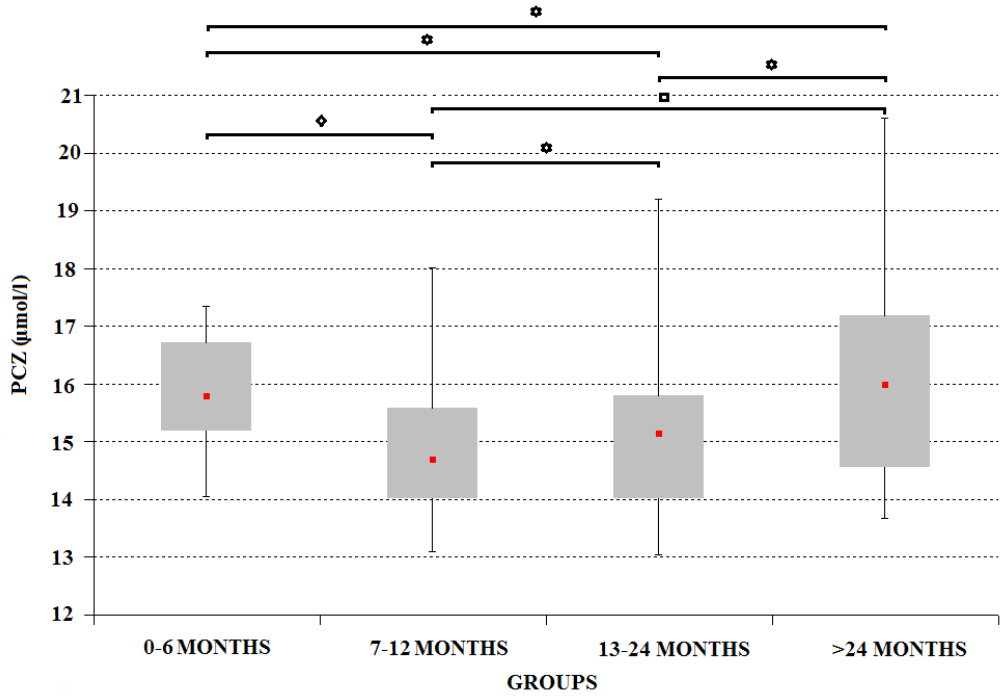

Figure no. 2. Box plot diagram of plasma concentration of zinc by age
The boxes represent the interquartile rang. PCZ-plasma concentration of zinc, M-mean, SD-standard deviation. Means PCZ are indicated by red solid square. The whiskers connect the most distant values of PCZ in each study group. Mean PCZ±SD was 15.77±1.01µmol/l (age group 0-6 months), 14.69±1.18µmol/l (age group 7-12 months), 15.15±1.58µmol/l (age group 13-24 months), 15.97±1.58µmol/l (age group >24 months). Statistical differences: ✿p>0.05; ◊p=0.005; ◘p<0.001

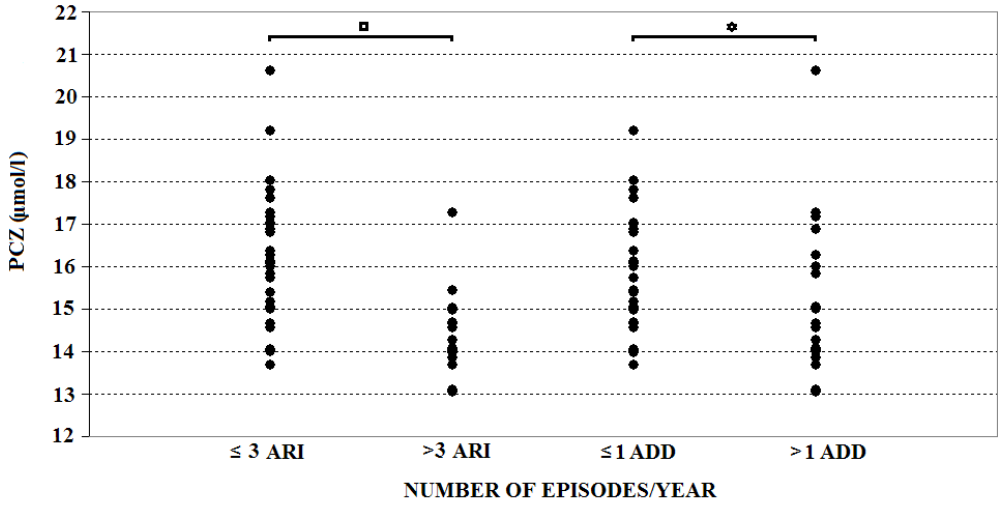

Figure no. 3. PCZ according to the pathological medical history
PCZ-plasma concentration of zinc, M-mean, SD-standard deviation, ADD-acute diarrheal disease, ARI-acute respiratory infection. The black solid circles represent the PCZ values.
Mean PCZ±SD was 15.89±1.46µmol/l (group ≤3ARI/year), 14.23±0.76µmol/l (group >3ARI/year), 15.49±1.44µmol/l (group ≤1ADD/year), 15.09±1.56µmol/l (group >1ADD/year). Statistical differences: ✿p>0.05; ◘p<0.001.

Reference values for population aged 0-3 year were 49.5 to 99.7 µg/dl (7.6 to 15.3 µmol/l) for neonates and 3.8 to 110 µg/dl (9.8 to 16.8 µmol/l) for older children (16).

Statistical analysis was performed using EPIINFO application, version 6.0, software program of the Center for Disease Control and Prevention (CDC) in Atlanta and WHO, adapted to the processing of medical statistics. There were determined means, standard deviations and tests of statistical significance (Student's t test) (12, 13). Harvard Graphics software program, version 3.0 was used for drawing the graphs.

Results

Mean value of PCZ (M) in the group of children was 15.33±1.49 µmol/l. In 80.20% of the children we recorded normal values for their age, ranging from 9.8 to 16.8 µmol/l, while the PCZ values were above the upper limit of normal in 19.8% of children. There were no low levels of PCZ, Figure no.1. Over 80% of children had values in the range M±SD (80.44%) and 90.22% between M±2SD. PCZ coefficient of variation is 9.71%.

There is no statistically significant difference between the mean PCZ of groups depending of gender, area of origin, children diet, socio-economic parameters and season, Table no.3.

The highest mean PCZ value was recorded in the age group>24 months, significantly higher than in the age group 7-12 months and compared to the age group 13-24 months, but slightly higher than in the age group 0-6 months. Mean PCZ value in the age group 0-6 months was significantly higher than in the age group 7-12 months, but insignificantly higher than in the age group 13-24 months. There were no statistically significant differences between the mean PCZ value in the age group 7-12 months than in the age group 13-24 months, Figure. no. 2.

Subjects with more than 3 episodes of acute respiratory infections (ARI)/year had a statistically significant lower value of PCZ as compared to those who had less than 3 episodes/year. There are no significant differences between those who had an episode of acute diarrheal disease (ADD)/year and those who had more than 1 episode/year, Figure. no. 3.

Discussion

Zinc is an essential micronutrient in children's growth and development. Our survey revealed that the mean PCZ, in Bihor County, in young children was 15.33±1.49 µmol/l (100.19±9.7 µg/dl), value falling within the range of normal reference values of the laboratory, age-appropriate for the subjects. A percentage of 80.20% of PCZ values was within this range. Minimum normal value of PCZ with the method studied was 9.8 µmol / l. In a 2012 study conducted on a sample group of 2115 healthy children aged 0.5 -18 years in Utah, United States, Chia-Ni Lin et al. found a mean PCZ of 89 ± 17µg/dl (18). There is no data so far concerning the value of PCZ in the healthy children aged 0-3 years in Europe (19, 20). There was no records of a child aged 0-3 years, in our study, with PCZ values lower than 9.98 µmol/l. According by WHO, zinc deficiency is defined by decreased of the value of PCZ below the 9.98 µmol/l (4). A percentage of 19.8% of children had PCZ over the normal maximum value of 16.8 µmol/l. The specialized literature indicates that increased levels of PCZ can be found in cases of increased intake of zinc or accidental exposure to zinc. Intake of quantities of 10 to 15 times the recommended daily dosage according to age and gender may cause side effects, but the medical literature doesn't mention the minimum PCZ value that can cause them to occur (21). There is no zinc deficiency in the general population of Bihor County, in this age group and for the period studied. There is no data so far concerning the value of PCZ in the pediatric population in Romania. Globally, Romania is considered as having a low prevalence (12.8%) of zinc deficiency for children under 5. The estimation was made in 2007 by WHO, using the nutritional stunting rate, last calculated for Romania in 2002 (10).

Children older than 24 months had a significantly higher PCZ mean compared to children aged 7-12 months, 13-24 months, but not significantly different from the children aged 0-6 months. Mean PCZ value in the age group 0-6 months was significantly higher than in the age group 7-12 months. These differences are explained by the fact that until the age of 6 months the infant diet is almost exclusively made up of breast milk, which represents an important source of zinc, the intestinal absorption of zinc is not influenced by the presence of phytates, fibers or oxalates from vegetables and fruits (22). After the age of 6 months the child's nutrition includes vegetables, fruits and grains that decrease the intestinal absorption of zinc and after the age of 24 months the child's nutrition coincides with that of the adult, meat being an important source of protein and zinc, respectively. Chia-Ni Lin et al. reported in 2012 that there were not found any statistically significant PCZ values different according to age in a study conducted on a group of 2115 healthy children in the United States. The results were explained by the authors by the fact that in this region fortification of foods with zinc has been used since 1987 (18).

Children with more than 3 episodes of ARI/year had a mean value of PCZ significantly lower than those who had less than 3 episodes/year. Decrease in zinc concentration distorts the functioning of the immune system, leading to the body's propensity to infections. Studies in the literature confirm that the normal level of zinc in the body protects the child from ARI (23, 24, 25). We have not found statistically significant differences between the mean PCZ in children with 1 episode of ADD/year and those with more than 1 episode/year. Literature data refer only to the effect of zinc administration to prevent ADD, the results being discordant, Walker CL. et al supports the idea of decreased incidence of acute diarrhea after zinc intake (26), while Boran P. et al denies the favorable effects of zinc on the development and severity of the illness (27). Our results can be explained by the fact that the comparison was made at a low incidence of diarrheal episodes/year (less than 1/year versus over 1/year), the small number of children with more than 1 episode of acute diarrhea/year didn't allow stratification in this segment.

We obtained slightly elevated values of the PCZ mean in males versus females, however the difference was not statistically significant. The data are consistent with the literature (15, 28).

The group of children from urban areas had a higher PCZ mean compared to those in rural areas, the difference could be explained by a lower socio-economic level in rural areas correlated with lower access of children from these areas to food rich in zinc, which are generally more expensive. Differences between PCZ values in urban versus rural areas are not statistically significant. The data are consistent with the literature in different parts of the world (26, 29).

Meat is an important source of zinc. PCZ values in children who eat particularly these kinds of food were slightly higher than in those who have a diet based mostly on vegetables and fruits, cereals, milk or chocolate consumed in excess (30). Grains contain large amounts of zinc that are lost through processing, fruits and vegetables contain small amounts of zinc which is why the body can not properly absorb the zinc in vegetable proteins compared to the zinc in animal proteins (4). Zinc ion absorption is inhibited by the presence of oxalates, polyphenols, phytates and fibers.

Mean values of PCZ were higher in children from families with a higher socio-economic level (children from families with up to four members, and those from families with monthly income over 330 EURO). The difference can be explained by the fact that foods rich in zinc are generally more expensive, access to them is restricted by the socio-economic level of families in which the person concerned. The results are consistent with the literature (26), but the differences are not statistically significant.

We recorded higher mean values of PCZ in winter compared to spring, summer and autumn, which can be explained by the more frequent consumption during this period of food of animal origin (rich in zinc), to the detriment of the food of vegetable origin (available mainly in the warm season of the year). Zinc absorption is influenced by its concentration in the food as well as by the animal or vegetable origin of the food. Its absorption from plant proteins is very low compared to that from animal protein (4). Differences were not found statistically significant.

This study is the first of its kind in Romania, conducted on human subjects aged 0-3 years (11).

Conclusions

1. In healthy children, aged 0-3 years of Bihor County we recorded a mean PCZ of 15.33±1.49 µmol/l, a value falling within normal limits.
2. We didn't find zinc deficiency in children tested.
3. We recorded elevated values of PCZ in children over 24 months, compared with children in the age groups 7-12 months and 13-24 months. The age group 0-6 months had an increased mean PCZ versus the age group 7-12 months.
4. Children with more than 3 episodes of ARI/year had lower PCZ values than those with maximum 3 episodes / year.

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Abbreviations:

WHO – World Health Organisation
PCZ – Plasma concentration of zinc
BD – Beston-Dickinson
CDC – Center for Disease Control
M – Mean
SD – Standard deviation
ARI – Acute respiratory infections
ADD – Acute diarrheal disease

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