



Original Contribution

SERUM ZINC IN CHILDREN WITH ENTEROCOLITIS, CHRONIC DIARRHOEA WITH MALABSORPTION SYNDROME AND TYPE 1 DIABETES

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ABSTRACT

BACKGROUND: Serum zinc concentration is the most widely used marker of zinc status in humans with different diseases and conditions.

METHODS: For determination of zinc concentration in blood serum spectrophotometric method with chromogen 4-(2-pyridylazo) resorcinol sodium salt was used.

RESULTS: Zinc level in blood serum was measured in children with acute enterocolitis, ($12.87 \pm 2.42 \mu\text{mol l}^{-1}$), chronic diarrhoea with malabsorption syndrome ($8.59 \pm 2.30 \mu\text{mol l}^{-1}$), Type 1 diabetes ($10.29 \pm 2.31 \mu\text{mol l}^{-1}$), and control groups of healthy children. The zinc levels in all diseases were significantly lower compared to those in healthy controls.

CONCLUSION: The results suggest that zinc status should be of concern when diagnosing and treating these diseases although further investigations are needed. With its good analytical parameters and accuracy of the measurements the method proposed is suitable for clinical practice and scientific investigations. In the present study it was applied for the first time in children with different diseases.

Key words: *serum zinc, enterocolitis, chronic diarrhoea, malabsorption, type 1 diabetes*

INTRODUCTION

Serum zinc is the most widely used index of zinc status in humans (1) Changes in serum zinc concentrations were found in different diseases and conditions – hepatitis, anaemia, hypertonic disease, hyper- and hypothyroidism, leukaemia and other malignancies, AIDS, ischaemia, diabetes, nutritional disorders, allergy etc (1–32).

The zinc balance is of great value for normal physiology, growth and development of children. Zinc is known to influence cell division. Zinc is needed as a cell membrane stabilizer (32). Zinc is a cofactor for over 100 enzymes, regulating cell growth and hormone levels; it seems to be involved in the proper storage and release of insulin, growth and

repair of tissues, wound healing, the ability to taste food, the production of prostaglandins, mineralisation of bone, blood clotting, the function of vitamin A, including regulation of gene transcription and growth factor metabolism. Zinc stores are small and during periods of rapid growth, insufficient zinc intake results in reduced growth velocity (17, 32).

The instrumental method most often used for determination of the serum zinc is flame atomic absorption spectrometry – FAAS (1, 6, 8-24, 32). The main disadvantages of this method are high matrix effects, high costs and suboptimal load of equipment. Spectrophotometric methods offer greater sensitivity, but are tedious and subject to numerous interferences (1). Like FAAS – methods, they also required preliminary preparation of reagents (1, 8, 9, 25–29, 33). At the same time, low cost of equipment and possibility of automation of procedures are suitable for clinical and laboratory investigations even in small laboratories.

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The necessity of knowledge of zinc status for diagnosing and treatment of different diseases leads to the aim of the present study – to offer fast, available and suitable for clinical and laboratory investigations spectrophotometric method for determination of serum zinc and to apply in the investigations of children with some diseases.

MATERIAL AND METHODS

Samples of blood serum; patients and controls

The collected venous blood samples were placed into sterile, closed tubes, untreated with heparine, EDTA, citrate etc. After two hours standing and 10 min centrifugation at 3500 rpm the separated serum was put in closed plastic laboratory vessels and was kept at -18°C in the fridge.

Blood serum samples, obtained from persons of different age, sex and health status, were used to investigate the analytical parameters of the method.

For determination of serum zinc in children - patients and controls of healthy children the following conditions were maintained:

- samples were obtained on an empty stomach between 8 AM and 9.30 AM;
- ethical approval was obtained from the institutional research ethics committee and the parents of all subjects gave

written informed consent prior to enrolment in the study.

The patients were all children residing in the vicinity of the Pleven University Hospital.

This study provides data for serum zinc concentrations measured in a group of 65 children between 1 year and 4 years of age – 25 children with acute enterocolitis, 19 children with chronic diarrhoea and malabsorption, and control group of 21 healthy children.

Children with chronic diarrhoea had loose stools 3 - 4 times a day for more than 3 – 4 weeks and proved malabsorption due to different diseases. Patients with chronic diarrhoea and malabsorption also presented with growth retardation, distended abdomen, eczemas, and frequent respiratory infections.

This study also provides data for serum zinc concentrations measured in a group of 29 children between 7 years and 16 year of age – 17 children with diabetes (Type I), and control group of 12 healthy children.

The patients with diabetes were treated with a standard dose of human insulin obtained from Novo Nordisk Industry, Copenhagen, Denmark. The mean duration of diabetes was (3.64±3.22) year. All subjects were given 2 to 4 subcutaneous doses of insulin per day. **Table 1** presents diagnosis, number and age of children involved in the study.

Table 1. Number and age of the investigated patients with enterocolitis, diabetes, and control groups.

<i>Variables</i>	<i>Patients</i>	<i>Controls</i>
Acute enterocolitis(number)	25	
Chronic diarrhea (number)	19	21
Age	1 month to 4 year	1 month to 4 year
Diabetes (number)	17	12
Age	7 to 16 year	7 to 16 year

Methods

Analytical method for determination of zinc in blood serum

The essence of the analytical method and methodological changes

After careful study of the literature an improved version of the method for determination of zinc in blood serum, adapted from Lampugnani et al (27), was proposed for this study.

The zinc quantity in blood serum was determined by spectrophotometric method after deproteinisation of samples. The

chromogen used was 4-(2-pyridylazo) resorcinol sodium salt (PAR-Na). The results of spectrophotometric measurement of zinc in blood serum were compared with those obtained by atomic absorption methods. The method was probated in a random group of patients aged 2 to 88 years irrespective of their health condition (27).

This method was modified to achieve maximum simplicity, better repeatability and sensitivity. The volume of blood serum samples and the number of reagents were reduced (from 7 solutions for reagents to 2).

The low limit of detection, hitherto

provided by the method of Lampugnani et al (27), allowed five-fold decrease in the volume of blood serum needed. This is especially important in investigations on children. The number of reagents was decreased, though the number of substances reacting in chromogen reaction and its proportions were the same as in the original method. This modification diminished the number of measurements of the volumes of reagents and respectively the possibility of errors that provided better repeatability. At the same time preparation of reagents and all other technical procedures became easier and faster. In our modification the standard solution of zinc oxide was replaced with zinc nitrate.

Reagents

All solutions were prepared from analytical – reagent grade substances and double distilled water (DDW). Laboratory glassware was washed with distilled water, immersed for 12 h in 10% HNO₃, and then washed ten times with distilled water and five times with double distilled water. After drying in a laboratory dryer glassware was kept in air-proof containers.

Preparing Reagent A: To 150 ml solution containing 1 M HCL (Merck) and 20 % trichloroacetic acid (TCAA), CCl₃COOH (Merck) in proportion 2:1, 10 ml cupferon solution C₆H₉N₃O₂ (Merck) with concentration 50 g l⁻¹ was added. The cupferon solution and reagent A were prepared immediately before use.

Preparing Reagent B: 2.83334 g hydroxylamine hydrochloride, NH₂OH.HCl (Fluka); 0.01667 g sodium thiocyanate NaSCN (Sigma – Aldrich); 4.104 g sodium hydroxide, NaOH (Merck); 3.048 g sodium tetraborate decahydrate, Na₂B₄O₇·10H₂O (Fluka) were measured, analytically transferred, and double distilled water was added up to 100 ml in a volumetric flask.

Chromogen solution: 1.0 g l⁻¹ of PAR–Na - 4-(2-pyridilaso)–resorcinol, sodium salt (Merck); 0.10 g PAR–Na was dissolved in 100 ml of double distilled water in a volumetric flask.

Zinc standard solution, Zn(NO₃)₂ (Merck) with concentration 1000 mg l⁻¹ Zn in 0.50 mol l⁻¹ HNO₃, was used as initial standard solution. Working solutions were prepared for any serial measurements by successive tenfold dilutions of the initial solution.

The laboratory equipment

pH–meter type RHM – 64 “Research”, spectrophotometer “Specol 11”, centrifuge T 30 – Janetzki, analytical scales and laboratory glassware.

Analytical procedure for determination of zinc in blood serum

Reagent A (0.8 ml) was added to serum sample (0.5 ml). After centrifugation for 10 minutes at 3000 rpm, and 0.06 ml 0.10 % solution of PAR–Na were added to 1.00 ml supernatant and 2.00 ml Reagent B. The absorbance of the resulting solution was measured at 490 nm in a 1-cm cell vs the reagents blank solution.

The zinc concentration was estimated by calibration graphs (linearity from 0.00 to 80.00 μmol l⁻¹ Zn II) or by parallel procedure using 0.50 ml standard solution, equivalent to 2.00 μg ml⁻¹ (30.6 μmol l⁻¹) Zn (II).

Smaller volumes of blood serum required proportionally decreased volumes of other reagents.

Zinc status

Zinc status was defined by serum zinc levels with international values between (25) 11.6 μmol l⁻¹ and 23.0 μmol l⁻¹. Serum/plasma zinc ratios are not useful in mild zinc deficiency when values are often within the normal range (1). For this reason serum zinc was measured in control groups of healthy children and in cases of significance, assessed by the use of the appropriate statistical method; zinc levels were considered as decreased or increased, although values were within the reference values. Zinc values below 10.71 μmol l⁻¹ in morning samples of blood serum were accepted by World Health Organization as zinc deficiency (1).

Statistical analysis

Statistical analysis of results was made using Statgraphics Plus for Windows. All values are expressed as mean ± s.d. The Student’s t-test and ANOVA were used to assess differences between study groups.

RESULTS AND DISCUSSION

Analytical control of the results of serum zinc measurements

A quantitative control was provided as zinc measured two times, and Sero – norms

(Randox Ltd) with known concentration of zinc was also used with every serial of samples.

Analytical parameters of the method for determination of zinc in blood serum

The results of the measurements of some analytical parameters of the modified method with 0.5 ml analytical sample for determination of zinc in blood serum are shown on **Table 2**.

Table 2. Analytical parameters

Analytical parameters	Results
Limit of detection	1.49 $\mu\text{mol l}^{-1}$
Relative Standard Deviation (n=10):	3.10 % - 3.30 %
Regression equation of the studied method vs method of Lampugnani et al; (n=20):	$A=0.002 + 0.999 \cdot C_{\text{Zn(II)}}$
Correlation coefficient, r^2 ;	0.9880
t- test, p:	$p > 0.05$, the results provided by both variants of the method are statistically undistinguishable

Analytical recovery

In spite of precisely proven reliability of the method of Lampugnani et al. and (Randox Ltd) used, precision of the modifying method was tested by adding and measuring known

quantities of zinc to five mixed samples of blood serum. T-test presented $p < 0.05$, i.e. there was no significant difference between added and measured zinc concentrations (**Table 3**).

Table 3. Analytical recovery in the proposed method for Zn added to serum samples

C (Zn in blood serum), $\mu\text{mol l}^{-1}$, (n = 6)*	C (Zn added), $\mu\text{mol l}^{-1}$, (n = 6)*	C (Zn in blood serum + Zn added), $\mu\text{mol l}^{-1}$, (n = 6)*	Recovery %
18.15 \pm 0.37	4.92	23.08 \pm 0.55	105.6
17.23 \pm 0.46	6.15	23.50 \pm 0.50	101.95
15.40 \pm 0.40	8.60	22.65 \pm 0.48	98.40
12.16 \pm 0.30	10.20	21.80 \pm 0.62	97.50
9.03 \pm 0.41	8.60	19.60 \pm 0.45	111.17

(n = 6)* - every mixed sample with and without Zn addition was analysed six times
n – number; C - concentration

Serum zinc in children with enterocolitis and chronic diarrhoea and malabsorption syndrome

Serum zinc levels were measured in children

with acute enterocolitis (AE), chronic diarrhoea and malabsorption syndrome (CD) and group of healthy controls. The results are shown on **Table 4**.

Table 4. Serum zinc in children with enterocolitis, chronic diarrhoea and control group

Groups → Parameters ↓	Control group (C)	Patients with acute enterocolitis (AE)	Patients with chronic diarrhoea (CD)
Serum Zn: ($\bar{x} \pm \text{sd}$) $\mu\text{mol l}^{-1}$	18.90 \pm 5.75	12.87 \pm 2.42	8.59 \pm 2.30
C(min) – C(max), $\mu\text{mol l}^{-1}$	16.05 - 22.65	8.57 – 15.75	3.75 – 13.16
Statistical significance, p (%):	p_{2-3} (n = 46) < 0.001 (99.99%)	p_{3-4} (n = 44) < 0.001 (99.99%)	p_{2-4} (n = 40) < 0.001 (99.99%)

\bar{x} – mean value, sd – standard deviation

p_{2-3} , p_{3-4} , p_{2-4} - statistical significance between zinc concentrations in blood serum of studied children, respectively: C-AE, AE -CD, C-CD

Serum zinc measured in patients with acute enterocolitis is (12.87 \pm 2.42) $\mu\text{mol l}^{-1}$.

We found significant lower serum zinc

concentrations of (8.59 \pm 2.30) $\mu\text{mol l}^{-1}$ in patients with chronic diarrhoea and malabsorption syndrome. These low

concentrations can be characterized as zinc deficiency.

Several authors mention that nutritional zinc deficiency is widely spread all over the world (6, 17, 31, 32). Zinc is released from food as free ions during digestion. These ions may then bind to endogenously secreted ligands before their transport into the enterocytes in the duodenum and jejunum. Specific transport proteins may facilitate the passage of zinc across the cell membrane into the portal circulation (2).

Important risk factors related to zinc deficiency are common use of grain proteins, low content of zinc in breast milk and weaning food (17, 20, 32). We consider that this is one of the main reasons for zinc insufficiency in studied children with chronic diarrhoea and malabsorption.

A recent research in the area of Saudi Arabia showed that serum zinc concentrations measured in healthy children vary in a range of $0,5 \mu\text{mol l}^{-1}$ to $13,9 \mu\text{mol l}^{-1}$; concentrations which are comparatively low according to the internationally accepted standards (17). Other researches on metabolism demonstrate excessive faecal loss of zinc in patients with diarrhoea (31).

Considerable amount of zinc is secreted through biliary and intestinal tract but most of it is reabsorbed (enterohepatic cycle). In chronic diarrhoea reabsorption by damaged

enterocytes is impaired and leads to increased faecal losses of zinc (2).

In the control group of healthy children serum zinc concentrations vary between $16,05 \mu\text{mol l}^{-1}$ and $22,65 \mu\text{mol l}^{-1}$. Those concentrations are in the range of the internationally accepted standards.

Serum zinc in studied patients with acute enterocolitis is between $8,57 \mu\text{mol l}^{-1}$ and $15,75 \mu\text{mol l}^{-1}$. Some of the children in this group show serum zinc levels below the value $10,7 \mu\text{mol l}^{-1}$. This is zinc deficiency due to gastrointestinal losses. Several researches cited by Hotz et al showed that zinc supplementation therapy decreased the duration of acute diarrhoea and lowered the average stool frequency per day (31). Zinc supplementation treatment results in a 47 % reduction of duration of acute diarrhoea (6). Within an appropriate dose, zinc supplementation increased weight gain in children recovering from severe malnutrition and decreased the risk of severe complications (17, 20, 31).

Serum zinc in children with Type 1 diabetes

Serum zinc Zn (II) was measured in children with Type 1 diabetes and the control group of healthy children. The results are shown on **Table 5**.

Table 5. Serum zinc and mean glycated haemoglobin in children with diabetes and control group

Groups→ Parameters ↓	Control group - C	Patients with diabetes - D
Serum Zn: (x ±sd) $\mu\text{mol l}^{-1}$	18.28 ±2.32	10.29±2.31
C(min) – C(max), $\mu\text{mol l}^{-1}$	16.24 - 21.42	3.06 – 14.08
Statistical significance, p(%), n=29:	p < 0.001 (99.99 %)	
Mean glycated haemoglobin (%)		11.8±1.7

The serum zinc in diabetic children was lower than in controls ($p < 0.001$) and in 52.94 % of the patients Zn (II) was below $10,71 \mu\text{mol l}^{-1}$ that indicated zinc deficiency. It is important to point out that diabetics have poor metabolic control of the disease – mean glycated haemoglobin value is 11.8 % (recommended value < 6.5 %).

It is clear that the predominant effect on zinc homeostasis in diabetes is hypozincaemia, which may be the result of hyperzincuria or decreased gastrointestinal absorption of zinc or both (33).

Zinc is a cofactor for superoxide dismutase, which is an intracellular antioxidant enzyme. It has been suggested that as pancreatic beta cells have low antioxidative

enzyme activities, zinc is also capable of modulating insulin action and it also improves hepatic binding of insulin. Abnormal zinc metabolism, therefore, could play a role in the pathogenesis of diabetes and its complications (29).

With its good analytical parameters and accuracy of measurements, the method for determination of serum zinc is suitable for clinical practice and scientific investigations. In the present study it was applied for the first time in clinical cases. The results suggest that zinc status should be of concern in the diagnosis and therapy of acute enterocolitis, chronic diarrhoea with malabsorption, and insulin dependant diabetes in children.

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