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Zinc concentration in preterm newborns at term age, a prospective observational study

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ABSTRACT

Objectives To determine zinc concentrations and associated factors in a population of preterm newborns at term age.

Design This analytical, descriptive, observational and prospective study was conducted in the neonatal unit of a tertiary hospital. Preterm newborn between gestational weeks 24 and 34 were included in the study. The patients were recruited close to the date of birth. Their clinical histories were collected, and the serum zinc concentrations (SZCs) at gestational weeks 37–41 were measured. This study aimed to measure SZC in a population of preterm newborns at term age, and analyse the anthropometric, clinical and nutritional parameters associated with a decrease in SZC.

Results Overall, 83 preterm subjects were evaluated, including 44 (53%) female infants and 39 (47%) male infants. The median period of gestation was 31 (IQ25–IQ75: 29–33) weeks, and the mean weight at birth was 1.523±0.535 kg. The median SZC at term was 4.4 (IQ25–IQ75: 2.6–6.9) µmol/L. There were some variables associated with zinc concentrations like bronchopulmonary dysplasia (BPD), weight at birth, z-score of length at discharge, being small for gestational age and treatment with recombinant human erythropoietin, although the unique variable that was independent of the other variables in the multivariate analysis (p 0.01) was BPD. Preterm newborn with BPD had lower SZC at term age than those without (2.7 vs 4.9 µmol/L, p 0.005).

Conclusions Zinc concentrations in this preterm population were low. BPD was significantly and negatively correlated with zinc concentrations.

Clinical trial registration NCT03532555.

What is known about the subject?

- Preterm newborns are at high risk of zinc deficiency. Few studies have measured serum zinc concentration and associated factors in preterm newborns at term.

What this study adds?

- Serum zinc concentrations were low in the preterm infants at term. Bronchopulmonary dysplasia was a risk factor for low serum zinc.

INTRODUCTION

Zinc is essential for enzyme function in individuals of all ages, including preterm infants. The typical symptoms of zinc deficiency usually develop after three continuous months of low serum zinc concentration (SZC) and include failure to thrive, poor weight gain, periorificial dermatitis, glossitis and increased susceptibility to infections.[1] Regardless of the clinical severity of this deficiency, the role played by zinc in the growth of preterm newborns is crucial because, together with other micronutrients, this mineral is directly involved in the increase in birth weight (BW), birth length (BL) and head circumference at birth.[2–4] Nonetheless, few studies to date have measured the SZC and associated factors in preterm newborns.[5–7] However, there is an increasing interest in identifying the benefits of zinc supplementation, with an increasing number of studies improving infant growth with zinc supplementation.[8–10] The most recent studies on the benefits of zinc in preterm infants demonstrated that morbidity decreased as zinc intake increased,[11] and the analytical and clinical parameters of sepsis also improved.[12]

This study aimed to measure SZC in a population of preterm newborns at term age, and analyse the anthropometric, clinical and nutritional parameters associated with a decrease in SZC.

SUBJECTS AND METHODOLOGY

Study population

This study included a population of preterm newborns born between 1 October 2016 and 31 October 2017, at gestational weeks 24–34 in a hospital with a level III neonatal unit and whose parents/guardians provided signed informed consent. Infants with severe digestive symptoms and those who received enteral supplementation with zinc were excluded.

The patients were recruited close to their dates of birth. Their clinical histories were collected and their SZC at gestational weeks 37–41 were measured, coinciding with routine blood draw.

Sample size

Using as prior data the estimates of means and SD of the zinc concentration in preterm of Itabashi study[6] and estimating a number of preterm newborns of our population of 110, 84 cases are needed to obtain an accuracy of 1.5% with EPIDAT software.[13]

Variables

The collected anthropometric and demographic variables included gender, gestational week at delivery, birth before 32 weeks of gestation, small for gestational age (SGA, defined by a z-score of weight and/or BL ≤–1.7), as well as weight, BL and head circumference at birth and corresponding z-scores (Fenton growth chart).[14] The evaluated nutritional variables included the number of days of parenteral nutrition, type of feeding (maternal, fortified breast milk, baby formula, or other types) in the 3 days before zinc measurement, enteral iron concentrations in the 3 days before zinc measurement (taking into account the contribution of medications and fortified breast milk), high iron intake (>3 mg/kg/day), enteral protein intake in the 3 days before zinc measurement (taking into account the contribution of fortified breast milk) and high protein intake (>3 g/kg/day). The evaluated nutritional development variables included weight, length and head circumference at discharge and corresponding z-scores (Fenton growth chart), weight gain (g/day) (by subtracting weight at the time of zinc measurement and weight at birth divided by the number of days), and increase in length and head circumference (difference between the value at the time of zinc measurement and the value at birth divided by the number of weeks). The evaluated clinical variables included the presence of congenital or nosocomial infections (clinical and/or bacteriological diagnosis), bronchopulmonary dysplasia (BPD),[15] and metabolic bone disease (alkaline phosphatases >600 IU, or 500 if serum phosphorus was <4 mg/dL). The number of patients treated with recombinant human erythropoietin (rhEPO) according to the judgement of the attending neonatologist was also determined.

Nutritional strategies

Parenteral nutrition is started the first day of life in all preterm <32 weeks and/or <1500 g. Over 32 weeks parenteral nutrition is used if there is a concomitant disease like sepsis, perinatal asphyxia or others that prevent an early enteral tolerance. The parenteral nutrition composition in regards to protein is started with 1.5–2 g/kg/day with daily progressive increase up to 3.2–4 g/kg/day and with adequate caloric–protein ratio from the fifth day of life. Regarding carbohydrates, we started with of 4–5 mg/kg/min with progressive increase up to 10–12 mg/kg/min according to blood glucose levels. In regard to lipids, we started with 0.5 g/kg/day in preterm infants weighing less than 1000 g and with 1 g/kg/day in larger patients and progressive increments of 1 g/kg/day up to 3 g/kg/day. This infusion (INTRALIPID 20%) is administered separately from the rest of parenteral nutrition. As for liquid contributions, we start with 60–80 mL/kg/day, progressively increasing up to 150 mL/kg/day. As for Zinc composition, parenteral nutrition brings in all cases 400 µg/kg/day.

Enteral nutrition is started after 24 hours provided there is haemodynamical stability and with volumes of 10–20 cc/kg/day and daily increases of 20–30 mL/kg/day except there is intolerance data. Enteral nutrition is attempted to be complete at 7 days of age and parenteral nutrition is usually withdrawn when 100 cc/kg/day of enteral nutrition is reached. Enteral nutrition is administered by bolus in 30 min, except intolerance, and in the case of breast milk, fortification begins when 100 cc/kg/day of enteral nutrition is reached.

Zinc measurement

Blood samples were collected in the morning after a 3-hour fast and the zinc concentration was measured. A test tube specific for the quantification of metals (BD Vacutainer, Trace Element Serum, Ref 368380; BD, Becton Drive, Franklin Lakes, USA) was used and 0.5 mL of blood was collected from each patient. The sample was analysed on the same day of collection using a spectrometric assay with the reagent 2-(5-bromo-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)-amino]-phenol (Lot No. 15375, BioSystems SA Costa Brava, 30,08030, Barcelona, Spain). The zinc concentration was measured in heparinised plasma and serum to avoid contamination from erythrocytes, platelets and leucocytes during coagulation and centrifugation.

The SZC of 20 full-term newborns measured during routine blood collection for metabolic screening was used as a laboratory control. Written informed consent was obtained also from the parents/guardians of these full term newborns.

Patient involvement

After completing the design phase of the study and verifying the standardisation of laboratory tests, patients were recruited having previously been informed of the study purpose and were asked for informed and written consent. The patients were collected according to inclusion criteria, they were informed of the tests that were going to be performed, the techniques for obtaining the samples and the analytical results obtained. In patients with serum zinc values below the normal range, parents/guardians were informed and preterm neonates were treated with zinc supplements and clinical and analytical follow-up was performed.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, V.22.0. The normality of the distribution of SZC and other continuous variables was assessed using Kolmogorov-Smirnov tests with Lilliefors

correction. Variables with normal and non-normal distributions were expressed as means and SD and as medians and interquartile values (IQ25/IQ75), respectively. Variables with non-normal and normal distributions were examined using Mann-Whitney U and Student's t-tests, respectively. The correlation between SZC and dietary supplementation was evaluated using Kruskal-Wallis tests. The SZC and the remaining continuous variables with non-normal distributions were examined using Spearman correlations. Multivariate analysis was conducted by transforming the SZC to its square root to obtain a normal distribution, whereas multivariate analysis was performed by multiple linear regression using the SZC as a dependent variable.

RESULTS

During the study period, 110 preterm subjects were admitted to the neonatal unit; of these, 92 met the inclusion criteria and 83 preterm newborns were included in the study after excluding nine newborns lost to follow-up. A total of 83 preterm subjects were evaluated, including 44 (53%) female infants and 39 (47%) male infants. Of this population, 18 newborns (21.7%) were SGA and 43 (51.8%) were born before gestational week 32. Moreover, the following conditions were reported in this sample: treatment with rhEPO (30; 36%), diagnosis of congenital or nosocomial infection (22; 26.5%), diagnosis of BPD (16; 19%) and diagnosis of metabolic bone disease (6; 7%). The most common dietary supplements provided to this population were baby formula (33; 40%); fortified breast milk (17; 20%); formula milk (15; 18%); and other formulas including hydrolysates, starter formulas, or lactose-free formulas (18; 22%). The anthropometric, nutritional and continuous variables are described in table 1.

The SZCs in the examined preterm population at term age was 4.4 (IQ25–IQ75: 2.6–6.9) µmol/L. The quality sample control group consisted of 20 newborns with a gestational age of 38.5 (38/39) weeks, BW of 3.245±0.364 kg, and an SZC of 10 (8.5–10.9) µmol/L.

The relationships between the SZC and continuous variables, including BW, BW z-score, and length at discharge z-score were analysed. There was a negative correlation between the mean weight gain at discharge (g/day) and protein intake (table 2). For dichotomous variables, there was no significant association between the SZC and gender, birth before gestational week 32, congenital or nosocomial infection, and metabolic bone disease. However, there was a positive correlation between SGA, rhEPO treatment and BPD diagnosis (table 3).

Of the significant variables in the bivariate analysis, which were included in the multivariate analysis, only BPD remained significant (p=0.01) (table 4).

Table 1 Description of continuous variables in all the 83 preterm newborn studied

	Mean (SD)	Median (IQ25–IQ75)
Anthropometric variables		
Weeks of gestation		31 (29–33)
Birth weight (kg)	1.52 (0.53)	
Birth weight z-score		0.4 (–0.9–0.5)
Birth length (cm)		41 (43–36.5)
Birth length z-score	–0.03 (1.28)	
Head circumference at birth (cm)		29 (27–31)
Head circumference at birth z-score		0.2 (–0.5–0.9)
Nutritional development variables		
Weight gain (g/day)	19.61 (7.17)	
ZWD–ZWB	–1.03 (0.70)	
Weight at discharge (kg)		2.36 (2.07–2.68)
ZWD	1.22 (1.18)	
Length at discharge (cm)		46 (44–48)
Length at discharge z-score	–0.89 (1.44)	
Increase in length (cm/week)		1 (0.60–1.20)
Head circumference at discharge (cm)		32 (31–33)
Head circumference at discharge z-score	–0.52 (1.13)	
Increase in head circumference (cm/week)		0.6 (0.5–0.87)
Nutritional variables		
Parenteral nutrition (days)		4 (0–9)
Iron intake (mg/kg/day)	4.43 (2.21)	
Protein intake (g/kg/day)	3.35 (0.89)	
Zinc intake (mg/kg/day)		1.30 (0.91–1.52)

Variables with normal distributions are represented as means (SD), while variables with non-normal distributions are shown as medians (IQ25–IQ75). Kolgorov-Smirnov with Lilliefors correction test to study normality.
ZWD-ZWB, z-score of weight at discharge minus the z-score of weight at birth.

DISCUSSION

The SZCs in our examined preterm population when they reached term age, were significantly lower than those reported in previous studies.[5–7] These differences can be explained by several factors. First, previous analytical methods of zinc measurement (mass spectrometry) differed from those used in our hospital. Second, the types of neonatal unit differed and comparable studies were published more than a decade ago. Supplementation strategies for newborns have changed considerably by reducing the number of days of parenteral nutrition to decrease the need for venous access and associated infections, as well as early initiation of enteral nutrition to improve weight gain. Zinc is required for weight gain; however, blood concentrations of this mineral are

Table 2 Correlation analysis of different variables with zinc concentration

	Spearman rank correlation	P value *
Anthropometric variables		
Weeks of gestation (SEM)	0.15	–
Birth weight (kg)	0.26	0.01
Birth weight z-score	0.24	0.02
Birth length (cm)	0.17	–
Birth length z-score	0.15	–
Head circumference at birth (cm)	0.15	–
Head circumference at birth z-score	0.10	–
Nutritional development variables		
Weight gain (g/day)	−0.20	0.050
Weight at discharge (kg)	0.07	–
Weight at discharge z-score	0.14	–
Length at discharge (cm)	0.15	–
Length at discharge z-score	0.22	0.040
Increase in length (cm/week)	0.05	–
Head circumference at discharge (cm)	−0.06	–
Head circumference at discharge z-score	0.5	–
Increase in head circumference (cm/week)	−0.06	–
Nutritional Variables		
Parenteral nutrition (days)	−0.19	–
Iron intake (mg/kg/day)	−0.12	–
Protein intake (g/kg/day)	−0.26	0.01
Zinc intake (mg/kg/day)	−0.07	–

*Spearman correlation test.

Table 3 Analysis of the relationships between zinc concentration in preterm newborns and the dichotomous variables of small for gestational age, recombinant human erythropoietin treatment, bronchopulmonary dysplasia, congenital or nosocomial infection, preterm bone disease, gender and birth before 32 weeks of gestation

	Median (IQ25–IQ75) of serum zinc concentration (µmol/L)	P value*
Small for gestational age		
Yes (n=18)	3.3 (1.7–4.3)	**0.011**
No (n=65)	4.9 (3.0–7.6)	
Treatment with recombinant human erythropoietin		
Yes (n=30)	3.8 (2.1–4.9)	**0.020**
No (n=53)	5 (3.0–7.7)	
Bronchopulmonary dysplasia		
Yes (n=16)	2.7 (1.8–4.3)	**0.005**
No (n=67)	4.9 (3.0–7.7)	
Congenital or nosocomial infection		
Yes (n=22)	4.3 (2.1–6.4)	0.37
No (n=61)	4.4 (2.8–7.0)	
Metabolic bone disease		
Yes (n=6)	3.9 (2.4–5.1)	0.36
No (n=77)	4.4 (2.7–7.0)	
Gender		
Male (n=39)	4.4 (2.8–7.6)	0.33
Female (n=44)	4.2 (2.2–6.4)	
Birth before 32 weeks of gestation		
Yes (n=43)	4.2 (2.2–5.6)	0.13
No (n=40)	4.9 (3.0–7.7)	

*Mann-Whitney U test.

decreased when the amount of zinc used by the body is higher than the consumed amount.

There was a positive association between nutritional status, BW, BW z-score and SGA. A similar finding of a positive correlation between SZC, gestational week at delivery, and SGA was reported by Wulf in a retrospective analysis of 226 preterm newborns.[16] Furthermore, while SZC was linked to BW, they were not independent of each other in multivariate analysis. In contrast, Wulf observed clinical symptoms in newborns with zinc deficiency (<7.6 µmol/L). In the present study, there was no significant relationship between SZC and the gestational week at delivery and the association with BW did not persist in multivariate analysis, indicating that other variables might influence this relationship.

There was a positive relationship between SZC and z-score for the length at discharge. This novel result, which a priori seems reasonable because of the theoretical effects of zinc on infant growth,[17 18] underscores the limited number of studies to date to evaluate these parameters in preterm newborns. Marriot *et al* found no significant correlation between BL and SZC.[5] Previous studies provided higher amounts of zinc to preterms, resulting in higher growth. For instance, Friel *et al* evaluated 52 preterm subjects who were randomised and received 2.2 mg/kg/day of zinc, bmjopen-2018-028465 reported that the BL was higher in the supplemented group.[8] A similar result was reported by Díaz-Gómez *et al*.[9] Our results suggest the importance of maintaining an adequate SZC to increase the growth rate.

Table 4 Multiple regression analysis of significant independent variables with zinc concentration (µmol/L) as the dependent variable

Variable	B coefficient	P value
Bronchopulmonary dysplasia	**− 0.43**	**0.01**
Birth weight z-score	0.09	0.29
Protein intake (g/kg/day)	− 0.10	0.19
Length at discharge z-score	− 0.02	0.79
Weight gain (g/day)	− 0.00	0.47

There was an inverse correlation between mean weight gain (g/day) and SZC (r=−0.208). Our findings were consistent with the findings of Itabashi in a study of 118 preterm infants.[6] Itabashi hypothesised that preterm infants with higher weight gain require more zinc, which consequently reduces its concentration in the blood.

In this study, we observed a negative association between SZC and protein intake. In contrast, Marriot reported no significant correlation between these two variables in newborns.[5] No other studies to date have evaluated the relationship between these two variables in newborns. This finding is contrary to our expectations because a previous study reported a positive link between these variables; that is, the plasma zinc concentration increased with increased protein intake, although the study investigated the absorption of zinc-rich protein foods by adults, which may have produced biassed results.[19] However, this finding suggests that a higher SZC is necessary because protein absorption and hydrolysis involve zinc-dependent enzymatic processes; thus, a high intake of protein would decrease the SZC.

The observed association between SZC and BPD has not been reported previously, although it is known that nutrition is essential for the treatment of preterm newborns with BPD. Moreover, zinc stimulates epithelial development and tissue repair and protects against infections,[20] suggesting its involvement in BPD; therefore, high SZCs are required in tissues, consequently, decreasing its circulating concentrations. There is an ongoing study on Zn intervention to prevent BPD.[21] The objective of that study is to determine whether enteral zinc supplementation leads to improve growth in infants at risk for BPD.

We have found in our preterm population very low SZC when they reached term age. This finding, seems very interesting as it would reinforce the results previously published on growth and weight gain by supplementing preterm newborns with zinc with DBP and poor growth.[22] DBP preterm infants should be monitored for zinc concentration as they are at high risk of insufficient zinc levels and should be aware of possible clinical association with these low Zn levels.

Contributors RV-G conceived and developed the present job. VB developed and directed the present proyect. MJ disigned and directed the job. CVG contribuited to the analysis. II-F helped in the statiscal analisis. JP-R directed the job and supervised results.

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Competing interests None declared.

Patient consent for publication All data were collected and stored and written informed consent was obtained from the parents/guardians.

Ethics approval All experimental procedures were performed in accordance with the ethical guidelines of the Declaration of Helsinki and the study was approved by the Institutional Research Ethics Committee.

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Data availability statement Data may be obtained from a third party and are not publicly available. All data relevant to the study are included in the article or uploaded as supplementary information.

REFERENCES

1. Finch CW. Review of trace mineral requirements for preterm infants: what are the current recommendations for clinical practice? *Nutr Clin Pract* 2015;30:44–58.
2. Koletzko B, Poindexter B, Uauy R. *Nutritional care of preterm infants: scientific basis and practical guidelines*. Basel, Switzerland: Karger, 2014: 314.
3. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. *Am J Clin Nutr* 2007;85:614S–20.
4. Cheong JLY, Hunt RW, Anderson PJ, *et al*. Head growth in preterm infants: correlation with magnetic resonance imaging and neurodevelopmental outcome. *Pediatrics* 2008;121:e1534–40.
5. Marriott LD, Foote KD, Kimber AC, *et al*. Zinc, copper, selenium and manganese blood levels in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2007;92:F494–F497.
6. Itabashi K, Saito T, Ogawa Y, *et al*. Incidence and predicting factors of hypozincemia in very-low-birth-weight infants at near-term postmenstrual age. *Biol Neonate* 2003;83:235–40.
7. Obladen M, Loui A, Kampmann W, *et al*. Zinc deficiency in rapidly growing preterm infants. *Acta Paediatr* 1998;87:685–91.
8. Friel JK, Andrews WL, Matthew JD, *et al*. Zinc supplementation in very-low-birth-weight infants. *J Pediatr Gastroenterol Nutr* 1993;17:97–104.
9. Díaz-Gómez NM, Doménech E, Barroso F, *et al*. The effect of zinc supplementation on linear growth, body composition, and growth factors in preterm infants. *Pediatrics* 2003;111:1002–9.
10. Loui A, Raab A, Wagner M, *et al*. Nutrition of very low birth weight infants fed human milk with or without supplemental trace elements: a randomized controlled trial. *J Pediatr Gastroenterol Nutr* 2004;39:346–53.
11. Terrin G, Berni Canani R, Passariello A, *et al*. Zinc supplementation reduces morbidity and mortality in very-low-birth-weight preterm neonates: a hospital-based randomized, placebo-controlled trial in an industrialized country. *Am J Clin Nutr* 2013;98:1468–74.
12. Banupriya N, Vishnu Bhat B, Benet BD, *et al*. Efficacy of zinc supplementation on serum calprotectin, inflammatory cytokines and outcome in neonatal sepsis - a randomized controlled trial. *J Matern Fetal Neonatal Med* 2017;30:1627–31.
13. EPIDAT 4.2. Análisis epidemiológico de datos [Internet]. A Coruña Servizo Galego de Saúde [Accessed cited 2019 July 31].
14. Fenton TR. A new growth chart for preterm babies: Babson and Benda's chart updated with recent data and a new format. *BMC Pediatr* 2003;3:13.
15. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;163:1723–9.
16. Wulf K, Wilhelm A, Spielmann M, *et al*. Frequency of symptomatic zinc deficiency in very low birth weight infants. *Klin Padiatr* 2013;225:13–17.
17. Brown KH, Rivera JA, Bhutta Z, *et al*. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food and nutrition bulletin* 2004.
18. Hambidge KM, Krebs NF. Upper limits of zinc, copper and manganese in infant formulas. *J Nutr* 1989;119(Suppl 12)–1861–4.
19. Sandström B, Almgren A, Kivistö B, *et al*. Effect of protein level and protein source on zinc absorption in humans. *J Nutr* 1989;119:48–53.
20. Oz H. Nutrients, infectious and inflammatory diseases. *Nutrients* 2017;9:1085.
21. ClinicalTrial.gov [Internet]. Bethesda (MD): National Library of Medicine(US). 2000 Feb 29-. Identifier NCT03532555, Enteral zinc to improve grow in infants at risk of bronchopulmonary dysplasia, 2018. Available: http://clinicaltrials.gov/ct2/show/NTC03532555 [Accessed 10 Aug 2019].
22. Loui A, Raab A, Obladen M, *et al*. Nutritional zinc balance in extremely low-birth-weight infants. *J Pediatr Gastroenterol Nutr* 2001;32:438–42.