



Zinc serum levels and their association with vitamin A deficiency in preschool children

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Abstract

Objectives: To identify the prevalence of zinc deficiency in a population with high prevalence of vitamin A deficiency; to verify whether zinc deficiency is associated with vitamin A deficiency in the population studied; to verify risk factors for zinc deficiency (sex, age, diarrhea and fever).

Method: Cross-sectional study of 182 healthy children aged ≥ 24 months and < 72 months. Peripheral blood samples were obtained from fasting children to determine zinc serum levels. Information about presence of diarrhea and/or fever during the 15 days preceding the study was also obtained. Vitamin A deficiency was identified by a serum 30-day dose-response test (+S30DR).

Results: Of the children studied, 0.5% (1/182) presented zinc serum levels $< 65 \mu\text{g/dL}$; however, 74.7% (136/182) of them had vitamin A deficiency. Zinc serum levels were not correlated with retinol serum levels. Zinc serum levels were not changed by previous diarrhea and/or fever. There was no difference in zinc levels between boys and girls. Children aged between ≥ 48 and < 60 months tended to have lower zinc serum levels than children of other ages.

Conclusion: Zinc deficiency prevalence was low and did not represent a risk factor for vitamin A deficiency. Children aged between ≥ 48 and < 60 months tended to have lower zinc serum levels than children of other ages. Zinc serum levels were not changed by previous diarrhea and/or fever.

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Introduction

Over the last few years, micronutrient deficiency has been gaining importance as a public health problem compared to macronutrient deficiency (proteins, carbohydrates and fats), attracting the attention of health professional and authorities all over the world. Most of these deficiencies are subclinical,

this phenomenon has been called as "hidden hunger". Particularly in relation to zinc, various studies of intervention with supplementation have been performed in populations with previous disease.^{1,2} However, probably due to technical difficulties to obtain a reliable biologic marker to this micronutrient, few works have studied the prevalence of deficiency in healthy populations, in Brazil and around the world. In a

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national study, Villalpando et al.³ found 25.3% low zinc serum levels in Mexican children below 12 years; in Brazil, in a study with healthy children aged 1-5 years, Silva et al.⁴ found low zinc serum levels in all children.

However, vitamin A is one of the most studied micronutrient. Nowadays, there may be 127 million preschool children with vitamin A deficiency and 4.4 million with xerophthalmia living – in its overwhelming majority – in developing countries.⁵ In Brazil, specifically in Ribeirão Preto (SP), Ferraz et al.,⁶ studying preschool healthy children attended at a health care center, found 74.5% of children with low hepatic reserves of vitamin A.

Zinc is a trace element with numerous functions in the human organism. It is important for the appropriate functioning of metabolism, growth, immunological system and an essential component of various metalloenzymes in the organism participating in the synthesis of other enzymes, especially in the liver.^{7,8} It is possible that, due to high growth rates, zinc requirements may be higher in males and infants/young children, especially in those with low birthweight, which classifies these groups as high risk of zinc deficiency.⁹ Regarding vitamin A metabolism, zinc is particularly important in the process of retinol-binding protein synthesis and consequently in the hepatic mobilization of retinol.^{10,11} Thus, zinc deficiency may contribute to vitamin A deficiency even in the presence of adequate hepatic reserves of the vitamin. Animal studies have shown that zinc deficiency can interfere with intestinal retinol absorption.¹²

Human and animal studies have also shown the effect of zinc on the biological functions of vitamin A in the organism.¹¹ Studies in children have showed the synergic effect from both zinc and vitamin A supplementation on dark adaptation tests,¹³ improvement of conjunctival impression cytology (CIC),¹⁴ increase of retinol serum levels¹⁴ and decrease in prevalence of diarrhea and dysentery.¹⁵

Zinc deficiency can have serious consequences for children's health. The deleterious effects of zinc deficiency can be perceived by reduction of morbidity after zinc supplementation in groups with deficiency. A reduced duration and severity of episodes of diarrhea and of pneumonia with a consequent reduction of mortality have been observed in several studies.^{1,2,16} On the other hand, some works have shown decrease in zinc serum levels during inflammatory processes, as it occurs in infections, a phenomenon that can influence the interpretation of studies about prevalence of deficiency of this micronutrient.¹⁷

In turn, vitamin A deficiency, even in its subclinical form, causes increased child morbidity and mortality due to increased number of cases of respiratory infection and to increased severity of cases of diarrhea. In extreme cases, vitamin A deficiency may lead to blindness due to irreversible loss of the cornea.¹⁸

As extensively reported literature, many children in developing countries^{3,19,20} have simultaneous deficiency of various micronutrients. Thus, we may infer that when a child presents deficiency of a micronutrient it will also be at risk to have other concomitant deficiencies. The objective of the present study was to determine the prevalence of zinc deficiency, as well as to verify its possible association with vitamin A deficiency in a population of children with high prevalence of this vitamin as observed in previous papers.^{6,20} This work also tried to study the influence of possible risk factors for zinc deficiency, such as sex, age and episodes of diarrhea and fever, which were used in this study as markers of inflammation.

Methods

This was a cross-sectional study performed during a 1 year-period (from September 1999 to September 2000). All children aged ≥ 2 years and < 6 years that attended the periodic return of the Child Health Program were invited to participate in the study, independent of previous clinical conditions. After written parental consent, the children were asked to come to the unit after a 6-8-hour fast, when the blood sample was obtained by venipuncture for both zinc and retinol determination. Blood samples were not obtained from children with diarrhea and/or febrile episodes at the time of collection. Each child participated in the study only once.

Serum zinc was determined by atomic absorption spectrophotometry. A blood sample of approximately 1 mL for zinc determination was drawn from a peripheral vein into a Vacutainer[®] tube for trace elements (blue cap) containing sodium heparin as an anticoagulant. After collection, the tubes were sealed, stored in a refrigerator for 3 to 4 hours and centrifuged at 2,500 rpm (1,300 g) for 10 minutes for plasma and cell element separation. After centrifugation, serum (approximately 500-600 μ L) was separated from blood with the aid of a micropipet and transferred to Eppendorf tubes and a 200 μ L aliquot was removed with a micropipet and mixed with 800 μ L deionized water in polyethylene tubes.

Two "standard solutions" were prepared for spectrophotometer calibration, one containing 1 μ g/mL zinc (200 μ L of the "stock solution" with 1,000 μ g Zn/mL in 1% HCl in 200 mL deionized water) and the other containing 2 μ g/mL zinc (400 μ L of the "stock solution" with 1,000 μ g Zn/mL in 1% HCl in 200 mL deionized water). After calibration of the spectrophotometer with the "standard solutions," the samples were examined in the apparatus, which emitted a flame at 213.9 nm wavelength with a hollow zinc cathode, thus measuring the concentration of zinc in the sample. Serum values < 65 μ g/dL were considered to indicate zinc deficiency.

The 30-day dose-response test (+S30DR) was used to identify vitamin A deficiency. Details of this method for identification of children with vitamin A deficiency can be found in previous papers,^{6,20} as well as other information about the

characteristics of children with and without vitamin A deficiency on this population, which is summarized in Table 1.

Laboratory analysis of serum retinol was performed by high-performance liquid chromatography (HPLC).

Those responsible for each child were asked whether the child had presented an episode of fever (axillary temperature > 37.0 °C measured using a thermometer) and/or diarrhea (three or more loose stools or any number of loose stools containing visible blood in a 24-h period) during the 15 days preceding enrollment in the study.

Children were measured and weighed at enrollment in the study and the data obtained were used to obtain the z-scores of weight/age (w/a), weight/height (w/h) and height/age (h/a) ratios and compared to the growth curves of the National Center for Health Statistics (NCHS).²¹

Student's *t* test was used to determine a possible association of zinc deficiency with vitamin A deficiency. Mann-Whitney test was applied to determine the possible influence of inflammation (fever and/or diarrhea during the 15 days preceding child enrollment in the study) on zinc serum levels and to determine the difference in zinc levels between genders. Bonferroni post-test was used to determine the difference in mean zinc serum levels between the various age ranges studied. Spearman correlation coefficient was applied to determine the relationship between zinc serum levels and age. Finally, Pearson correlation coefficient was used to determine the correlation between zinc serum levels and retinol serum levels.

The level of significance was set at 0.05 (5%) in all analyses.

The study was approved by the Ethics Committee of HCFMRP-USP (Process HCRP n° 4016/99).

Results

A total of 188 children were studied to determine prevalence of vitamin A deficiency by the +S30DR test; of these, 74.5% (140/188) had tests indicative of vitamin A deficiency and no child had xerophthalmia.⁶ Samples for serum zinc determination were obtained from 182 children, while this determination could not be performed in the remaining six children due to insufficient material. Of the 182 children whose zinc serum levels were measured, 74.7% (136/182) presented vitamin A deficiency. Only one (0.5%; 1/182) child showed low zinc serum level (40 µg/dL); this child also had vitamin A deficiency but was not malnourished according to the anthropometric measurements.

Student's *t* test did not show a significant difference in mean ± SD zinc serum levels between children with and without vitamin A deficiency, which were 119.9±24.4 and 115.0±22.4 µg%, respectively (*p* = 0.23). Also, there was no correlation between retinol serum levels and zinc serum levels (*r* = -0.12).

Overall mean zinc serum level was 118.7 µg/dL and the first quartile, median and third quartile values were 103.0, 116.4 and 133 µg/dL, respectively. Range was 40-212 µg/dL.

Of the 182 children whose zinc serum levels were measured, 56.6% (103/182) were boys and 43.4% (79/182) were girls. Mean ± SD serum zinc values were 119.0±22.6 µg/dL for boys and 118.2±26.0 µg/dL for girls, with no statistically significant difference between genders (*p* = 0.37).

We observed that 31.9% (58/182) of the children presented episodes of fever and/or diarrhea during the 15 days preceding enrollment in the study. Although mean zinc serum levels were lower among children with episodes of fever and/or diarrhea than among children with no such episodes

Table 1 - Characteristics of children with and without vitamin A deficiency aged ≥ 24 to < 72 months attended at Centro Médico Social e Comunitário de Vila Lobato (Ribeirão Preto, Brazil, 2000)

Characteristic studied (n)	Without vitamin A deficiency (n = 48)	With vitamin A deficiency (n = 140)
Age (mean in months)	44.0	43.8*
Sex		
Male (n = 105)	21.9% (23/105)	78.1% (82/105)*
Female (n = 83)	30.1% (25/83)	69.9% (58/83)
Prevalence of malnutrition (n)	6.2% (3/48)	3.6% (5/140)*
mean <i>per capita</i> income (US\$)	126.4	111.3*
Frequency of episodes of fever and/or diarrhea (n)	29.2% (14/48)	33.6% (46/140)*
Prevalence of iron deficiency (n)	27.3% (12/44)	38.5% (52/135)*

*Nonsignificant.

(115.6 and 120.1 µg/dL, respectively), difference between groups was not statistically significant ($p = 0.18$).

Although there was no association between age and zinc serum levels ($r = -0.06$), when the age ranges were stratified into 12-month intervals, children aged ≥ 48 to < 60 months were found to have lower mean zinc levels than children of the remaining age ranges (Table 2). This difference was statistically significant regarding children aged ≥ 24 to < 36 months ($p = 0.02$; Bonferroni post-test); as to the remaining age ranges, although the difference was not statistically significant, zinc serum levels tended to be lower in the ≥ 48 to < 60 month age range ($p = 0.07$).

Seven children in the total sample presented some degree of malnutrition. Three had < -2 z-scores of NCHS reference values²¹ for the w/h ratio (wasting), two for the w/a ratio (underweight), one for the h/a and w/a ratio (stunting/underweight), and one for the w/h and w/a ratios (wasting/underweight).

Discussion

In contrast to some studies that detected a high prevalence of zinc deficiency in communities with high prevalence of other micronutrient deficiencies,^{3,4,22,23} in the present study only 0.5% (1/182) of the children had low zinc serum levels, despite a high prevalence of vitamin A deficiency and also of iron deficiency, as reported in a previous study by our group.^{6,20} Thus, in this population, zinc deficiency cannot be considered, in principle, as a risk factor for vitamin A deficiency, which is also corroborated by the fact that mean zinc serum levels did not differ between children with and without vitamin A deficiency. However, the lack of a reliable biological marker for zinc deficiency may mask the real role of this trace element in the genesis of vitamin A deficiency in this population, whereas, as it occurs in other micronutrients, due to a strict homeostatic control, zinc serum levels can be found within normal limits even in individuals with low body stores of this element; however, despite these limitations, zinc serum levels are still the most widely used biomarker for zinc status

of the organism, since other biomarkers also showed limitations in addition to present major technical difficulties in its execution.^{24,25} Moreover, it should be stressed that other studies based only on zinc serum levels to study the association of zinc deficiency with vitamin A deficiency often detected an association between the two conditions, especially in malnourished children with deficiency of both micronutrients.¹¹

Comparing the values of zinc serum levels of our population with other studies, we found that our levels were higher than the levels observed by Donângelo & Azevedo²⁶ in a study of 103 low-income Brazilian children aged 3 months to 6 years; in that study, the authors found that mean zinc serum levels of children without malnutrition was 98.3 µg/dL (SD \pm 15.7). Furthermore, in another Brazilian study with children from low-income families in the metropolitan area of Rio de Janeiro aged 1-5 years, Borges et al.²³ observed higher means of zinc serum levels than our study (137 µg dL).

Despite such Brazilian papers, as this present study, having analyzed children from low-income families, zinc serum levels in the non-malnourished group were closely similar or higher to those observed in Canadian children aged 1-5 years,²⁷ which it presented values of 67.3 and 118.3 µg/dL for the 2.5 and 97.5 percentiles, respectively. On the other hand, a study with children aged 6-13 years from poor rural communities of Thailand observed mean zinc serum levels of 62.7 µg/dL.²⁸ It is difficult to explain the observation of higher serum zinc values in our population, especially if we consider the high prevalence of both vitamin A and iron deficiency^{6,20} among children studied, since zinc-rich foods (animal source) are usually also rich in both iron and vitamin A; achievement of dietary surveys (not performing them is a limitation of this study) could help answer this question, whereas ingestion of zinc-rich foods could be below recommended values and, therefore zinc serum levels observed in this study would only correspond to the levels found in strict homeostatic control of the organism, a phenomenon that was previously observed in experimental studies with human beings.²⁴

Although Thurlow et al.²⁸ have observed that male sex represented a risk factor for zinc deficiency, in our study, as

Table 2 - Mean zinc serum levels in children aged ≥ 24 to < 72 months, stratified into 12-month intervals attended at Centro Médico Social e Comunitário de Vila Lobato (Ribeirão Preto, Brazil, 2000)

Age (n)	Mean zinc serum levels (µg/dL) (SD)
≥ 24 to < 36 months (61)	122.75* (26.9)
≥ 36 to < 48 months (44)	121.9 [†] (22.8)
≥ 48 to < 60 months (40)	108.8* [†] (19.3)
≥ 60 to < 72 months (37)	118.8 [†] (21.5)

SD = standard deviation.

* Bonferroni post-test; $p = 0.02$.

[†] Bonferroni post-test; $p = 0.07$.

well as in the study by Donangelo & Azevedo,²⁶ no sex differences in zinc serum levels were detected in the children studied.

A recent national survey in Mexico³ revealed that prevalence of zinc deficiency was higher in children younger than 2 years and decreased with age. In the present study, we observed that mean zinc serum levels of children aged ≥ 48 to < 60 months were lower than those observed in the other age ranges (Table 2); however, even though these mean levels tended to be significantly lower, they were still within normal limits. In addition, the only child with a zinc serum level below normal limits was in the age range of ≥ 48 to < 60 months, a fact that may have influenced mean value, since this was an extreme value. Once again, dietary surveys may help explain this phenomenon.

Although some studies in animals²⁹ and humans³⁰ have shown a positive correlation between serum retinol and zinc levels, this was not observed in the present study. This result may be explained by the fact that in our study the prevalence of low zinc levels was very small, whereas the populations had deficiency of both zinc and vitamin A in the studies in which this association was detected.

As reported in some studies, presence of an inflammatory process may alter zinc homeostasis, usually reducing zinc serum levels.¹⁷ Borges et al.²³ observed significant lower zinc serum levels in children that had reported diarrhea episodes 30 days before admission to the study than the children without that report. In our study, however, presence of diarrhea and/or fever (used here as markers of inflammation) during the 15 days preceding the child's admission to the study did not cause significant changes in zinc serum levels. This was probably due to the fact that, at the time of collection, all children studied were healthy, with no observable infectious and/or inflammatory processes. In addition, the survey regarding presence of fever and/or previous episodes of diarrhea was conducted using an open interview depending on more accurate recall by parents, which is not always possible and which may cause some bias in data collection.

In this population, only 3.8% (7/182) of the children were malnourished, that it helps to explain the low prevalence of zinc deficiency detected in the children studied, considering that in Brazilian studies^{4,23,26} with high prevalence of zinc deficiency, there was also high frequency of malnutrition.

However, in our study, high prevalence of vitamin A deficiency and iron deficiency was observed.^{6,20} Thus, further studies involving dietary surveys, as well as the development of more adequate biomarkers for the detection of zinc deficiency would help explain the interrelations of the various micronutrients not only in the present population, but also in other communities. This information is necessary for an adequate combat of micronutrient deficiencies all over the world, reducing the socioeconomic burden of this endemic problem in developing countries.

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