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The prevalence of wheezing and its association with serum zinc concentration in children and adolescents in Brazil

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ABSTRACT

Objective: To assess the influence of zinc serum status on the prevalence of wheezing in a sample of children and adolescents in Northeastern Brazil.
Research methods and procedures: This is a cross-sectional study which included 592 students of 6–12 years old, from the public elementary schools of São Francisco do Conde, Bahia, Northeastern Brazil. Report of wheezing in the past 12 months was collected using a questionnaire of the International Study of Asthma and Allergies in Childhood Program (ISAAC) phase III, adapted to Portuguese. The determination of serum Zn levels was performed using a flame atomic absorption spectrometer. Data on anthropometric status, level of physical activity, pubertal development and socioeconomic information, for each participant were obtained. Multivariate logistic regression analyses were used to assess the associations of interest.
Results: Of the students, 8.6% (95% CI 6.30–10.9) reported having wheezing. The mean (SD) serum zinc level was 114 (22.9 μg/dL). The results of the multiple logistic regression analysis showed, after adjustments, positive and significant association between low serum zinc levels and wheezing. Students categorized as being below the median for serum Zn concentration presented an almost 1.9-fold increase in the wheezing prevalence ratio (OR = 1.9; 95% CI 1.03–3.53).
Conclusion: The main findings of this study suggest that the level of zinc may influence the risk of wheezing in late childhood on the study population.

Introduction

Asthma is a multifactorial illness; its causes are still not completely understood, and there is no consensus on its etiology. A large body of research emphasises the role of environmental pollution, genetic and psychosocial factors furthermore of changing diet and/or nutrient status [1–6]. Numerous studies have suggested that low intake of dietary antioxidants (vitamin E, vitamin C, carotenoids, polyphenols, selenium and zinc) [7–9], a reduction in n-3 PUFAs (polyunsaturated fatty acids) and an increase in n-6 PUFAs [8,10,11] may be associated with the development of asthma and other allergic disorders and may be an important contributing risk factor for the increase in asthma incidence over the last three decades [7]. Zinc (Zn) is an important antioxidant element obtained from food. Zinc is an essential micronutrient for the proper functioning of more than 100 enzymes involved in human metabolism that promote protein folding and helping the regulation of gene expression [12]. Furthermore, zinc plays a central role in modulating the immune system; it is essential for cellular function of the immune response acting as an antioxidant microelement. The literature on zinc as a potent antioxidant is vast, but the role played by zinc in airway inflammation remains unclear [12–14]. Studies have examined abnormal distributions of trace minerals, including zinc, some of them have reported the effect of the lower zinc status in asthma/wheezing [15–18]. On the other hand, some studies have reported discrepant results [19,20] or even an inverse association, as demonstrated by Urushidate et al. [21].

Overall, the body of evidence from these studies is methodologically poor but weakly suggestive of a possible effectiveness of zinc

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in preventing asthma/wheezing [22]. Therefore, studies examining the role of this element in the etiology of wheezing and/or asthma are necessary before any conclusion can be drawn. In this work, our aim was to investigate the influence of zinc serum status on the prevalence of wheezing in a sample of children and adolescents from Northeastern Brazil.

Materials and methods

Study design/population/sampling

A cross-sectional design was used to study 6- to 12-year-old children living in São Francisco do Conde (SFC), a municipality located in the metropolitan region of Salvador, Northeast Brazil. This study is part of another large investigation about understanding risk factors for asthma/wheezing [23]. From 1187 students enrolled, the majority came from rural area (779 students) and urban (408 students) from the SFC. Due to the limitation of funding and logistic condition, we choose to select at random half (50%) of these students from each area to participate in this study. However, the number of students who appeared in the collecting point of blood sample measured was 592. Then, 125/204 students from urban area (about 60%) and 467/390 students from rural area (about 120%) were enrolled.

Outcome variables

The prevalence of asthma and rhinitis symptoms was determined using a validated International Study of Asthma and Allergies in Childhood (ISAAC) phase III questionnaire adapted and translated into Portuguese [24]. For this study, wheezing was defined based on the following two questions: (1) "Has your child ever experienced asthma or wheezing in his/her lifetime?"; and (2) "In the last 12 months, has your child experienced wheezing?" The children whose parents answered yes to both questions or who answered yes to only the second question were considered cases for this study.

Determination of zinc serum levels

Laboratory investigations: Venous blood samples were collected from subjects in the morning, before food intake. Serum samples were aliquoted in Eppendorf tubes and stored at −20 °C until the zinc levels were measured. The determination of serum Zn levels was performed using a flame atomic absorption spectrometer (Varian AA240). The calibration graphs were constructed using normal aqueous standards (NIST-USA). All tubes used for blood collection and serum storage were free of trace microelements.

Confounding variables

Anthropometric status

Each participant's weight was obtained using a Master® portable digital scale and a Leicester Height Measure® portable stadiometer (Seca, Hamburg, Germany). The measurements were performed in duplicate using the techniques of Lohman et al. [25]. Body mass index (BMI) was used for the diagnosis of anthropometric status, adopting the percentiles for age and gender proposed by the World Health Organization [26]: underweight (<3rd percentile); normal weight (≥3rd percentile and <85th percentile, category reference); overweight (≥85th percentile and <97th percentile); and obese (≥97th percentile). For this analysis, the overweight and obese categories were combined. Therefore, children with excess BMI were situated in or above the 85th percentile.

Level of physical activity

To evaluate the frequency of physical activity, we used the International Physical Activity Questionnaire (IPAQ), which assesses physical activity for leisure, transport, work and domestic purposes in the past week [27]. This information allows one to estimate the weekly time spent engaged in physical activities. For this study, the final score was dichotomized using a cutoff of 300 min/week of moderate or vigorous physical activity [28]. Children with ≥300 min of activity per week were considered active (reference category), and children with <300 min per week were classified as inactive.

Pubertal development

The evaluation of the stages of sexual maturity was based on the characteristics of breast and pubic hair in girls and genital and pubic hair in boys. Based on this staging, the adolescents were grouped into pre-pubescent (reference category) and pubescent according to the categories described by Marshall and Tanner [29,30]. The identification of these stages was achieved by self-description with the help of portraits provided by the interviewers.

Other variables

The variables used in this study as confounders were as follows: sex (male, female reference category); age (<10 years, ≥10 years reference category); education of caregiver (≤4th grade, 5th grade ≥ reference category); household location (urban, rural reference category); per capita income (as minimum salary (MS); <1 SM, ≥1 SM reference category); number of people living in the household (>3, ≤3 reference category); and the presence of smokers in the household (no reference category, yes).

Ethical issues

Ethical approval was provided by the Ethical Committee of the School of Nutrition, the Federal University of Bahia, Brazil, under registration number 27-09/CEPNUT. Written informed consent detailing all procedures to be performed on the subjects was signed by a parent or legal guardian of each child.

Statistical analysis

For processing and construction of the database, we used Epi Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA). The data were entered in duplicate after reviewing the questionnaires and correcting for errors in data collection. The characteristics of the population were identified by descriptive analysis using categorized prevalence data.

The magnitude of the associations between wheezing and zinc status (based on median cutoff) was expressed as odds ratio (OR) with 95% confidence intervals (95% CI). Statistical analyses used two-tailed tests and a significance level of 5%. The logistic multivariate analysis used to study the association between zinc status and wheezing was adjusted for age, sex, the education of caregivers, per capita income, the number of people living in the household, the presence of smokers in the household, body mass index (BMI), pubertal development and physical activity. The choice of variables for modeling was based on knowledge gathered from pre-existing literature [31] and reports on this population [5,23].

Statistical analyses were performed using the Statistical Package for Social Sciences – SPSS, v. 13.0.

Table 1
Characteristics of caregivers, children, and adolescents aged 6 to 12 years enrolled in public schools in São Francisco do Conde, Bahia, Brazil, 2010.

Variables	Population number		Wheezing within last 12 months		p value
	n	%	n	%	
Gender					
Male	303	51.2	26	8.6	0.98
Female	289	48.8	25	8.7	
Age (years)					
6 to <10	270	45.6	20	7.4	0.34
≥10 to <13	322	54.4	31	9.6	
Per capita income[a]					
>1 MS	497	84.0	39	7.8	0.13
≤1 MS	95	16.0	12	12.6	
Household location					
Urban	125	21.1	18	14.4	0.01
Rural	467	78.9	33	7.1	
Levels of physical activity					
Active	482	81.4	44	9.1	0.35
Inactive	110	18.6	7	6.4	
Smokers in the household					
Yes	467	78.9	41	8.8	0.78
No	125	21.1	10	8.0	
Anthropometric status					
Malnourished	67	11.3	6	9.0	0.96
Eutrophic	439	74.2	37	8.4	
Overweight/obese	86	14.5	8	9.3	
Pubertal development					
Pre-pubescent	370	62.5	32	8.6	0.97
Pubescent	222	37.5	19	8.6	
Serum zinc level					
≥Median	306	51.7	21	6.9	0.12
<Median	286	48.3	30	10.5	

[a] Measured in Brazilian minimum salary (BSM; value in 2010; BRL510.00 is equivalent to US$290.7).

Results

The final study sample consisted of 592 students of both sexes, aged 6–12 years. In order to guarantee an effective analysis, children were included in the study only if the data for the variables that were to be analyzed were completed. With the exception of the place of the household location ($p < 0.001$), should be highlighted that there were no statistically significant differences in anthropometric or demographic characteristics between students who did not have blood sample collected and those were collected. There was a slightly higher percentage of male students (51.2%) and students older than 10 years (54.4%). Other characteristics are presented in Table 1.

It was determined that 8.6% (95% CI 6.30–10.9) of the study participants had symptoms of asthma (wheezing in the last 12 months). The prevalence of asthma symptoms was higher among families who lived in urban households (urban = 14.4%; rural: 7.1%, $p = 0.01$). Statistically significant associations were not observed between symptoms of asthma prevalence and the other variables investigated (Table 1).

A prevalence of serum zinc (<70 μg/dL) deficiency of 1.5% (95% CI 0.8–2.4) was found. The mean (SD) serum zinc level was 114.0 (22.9 μg/dL). Household locations also influenced serum Zn concentrations; the highest mean levels were found in urban households [rural household mean 112.1 (22.0 μg/dL), urban household mean 120.5 (24.5 μg/dL); $p < 0.001$)].

Table 2 shows the data analyzed using two multivariate unconditional logistic regression; one with only household location as confounding factor (OR = 1.8; 95% CI 1.03–3.2) and other using gender, age, per capita income, household location, level of physical activity, presence of smokers in the household and anthropometric status and pubertal development as adjusted variables (OR = 1.9; 95% CI 1.03–3.53). There were no significant interactions between household location and below the median for serum Zn concentration (household_Zn concentration $p = 0.37$).

Discussion

The prevalence of asthma symptoms recorded among school children in SFC (8.6%) is lower than the prevalence reported in other regions of Brazil [32]. A possible explanation for this discrepancy is that most of the studies on asthma in Brazil have been carried out in large cities, whereas the present study was performed in a small town with a peripheral rural area nearby the downtown. The prevalence of asthma was lower in the rural area. This protection might originate from individual, behavioral, and lifestyle factors such as rural location, early childhood infections, pet ownership and exposure to farm animals [33]. The percentage of school children with plasma zinc levels below the cut-off points for deficiency (<70 μg/dL) was 1.5%. In Brazil, data on prevalent zinc deficiency are scarce; however, some published studies have shown low levels of plasma zinc and hair zinc in at-risk populations, mainly children, varying from 0.5% to 15% [34–36]. Some studies reported significantly lower zinc values in children from urban locations [37] whereas we found the opposite; according to other studies [38,39].

The purpose of this study was to investigate the influence of zinc serum status on the prevalence of wheezing. The results of the multiple logistic regression analysis adjusted for confounding variables showed that subjects with serum zinc levels below the median presented an almost 1.9-fold increase in the wheezing prevalence. The potential confounding variables used in the analysis have been identified in several previous studies as associated with both zinc deficiency and asthma [23,31,39,40]. The results of the present study reinforce the findings of a limited number of studies concerning the association of wheezing/asthma with serum Zn concentration, demonstrating some evidence of asthma symptoms caused by zinc deficiency. In these reports, asthmatic/wheezier individuals presented lower levels of plasma [15,16,41], hair [42] and nail zinc [18] than healthy individuals. The reports cited above as well as our study suggest that zinc may play an important role in the occurrence of wheezing/asthma.

Although zinc is toxic to human beings, it is an important microelement for our organism. Because it is not stored, daily intake is necessary. It is obtained from several food sources such as red meat, dairy products, sea food, cereal and nuts [43]. It is found in the blood and intracellular environment and is involved in several mechanisms that maintain the homeostasis of the human organism including the immune cell pathways that perhaps justify this finding [44]. Several families of proteins are involved in the uptake and cell efflux of zinc, where gene polymorphism and deficiency of these proteins are associated with a variety of diseases [45]. Some of the Zn transport proteins are found in the lungs, such as ZnT4, a protein that is abundant in the airway compartments [44]. Zinc is a potent anti-oxidant element and oxidative stress caused by internal (by-product of aerobic respiration), and inhaled environmental toxins – such as allergens, cigarette smoke and pollutants – play a central role in asthma pathogenesis [46]. The reduced daily consumption of antioxidants is positively correlated with an increased risk of asthma [7–9,47]. Some of the anti-oxidative effects of Zn are a result of the stabilization of membrane lipids and sulphydryls [48], blockage of nitric oxide production [49] as well as co-enzyme of the major anti-oxidant enzyme CuZnSOD. Larsen et al. [50] elucidated the role of this enzyme in the lungs in a study that demonstrated wild mice were more susceptible to allergen-induced hypersensitivity than transgenic mice presenting high levels of this enzyme. Zinc is highly present in goblet cells and binds to mucins – a carbohydrate-rich protein family – that are secreted by these cells and excreted to the lumen of lower airways. These proteins trap

Table 2
Crude and adjusted odds ratios (OR) of the association between zinc status and wheezing among children and adolescents enrolled in public schools in São Francisco do Conde, Bahia, Brazil, 2010.

Variables	Crude OR (95% CI)	Adjusted OR (95% CI)[a]	Adjusted OR (95% CI)[b]
Serum zinc level			
Serum zinc level (≥median)	1	1	1
Serum zinc level (<median)	1.59 (0.89; 2.85)	**1.80 (1.03; 3.2)**	**1.90 (1.03; 3.5)**

Boldface numbers are statistically significant.
[a] Model_1: Adjusted for household location.
[b] Model_2: Adjusted for gender, age, per capita income, household location, level of physical activity, presence of smokers in the household, anthropometric status and pubertal development.

harmful molecules found in this milieu, and together are expelled by the ciliated epithelial cells to the high airways as mucous, which is then ingested or leaves the body through the sputum [51,52].

Zinc is also an important element in the maintenance of the optimal activity of the immune system. Low Zn serum levels found in children with recurrent wheezing/asthma suggest that zinc deficiency affects the regulation of T-cell lymphocytes, which may play some part in the development of allergies [53,54]. It has been demonstrated that mild Zn deficiency affects the immune system, causing an imbalance between Th1 and Th2 functions, and reduces the production of interleukin (IL)-2, interferon (IFN)-gamma, and tumor necrosis factor (TNF)-alpha. As Zn deficiency shifts the immune system into a Th2 pro-inflammatory response and induces the production of several proinflammatory cytokines, it may increase inflammation in persons with asthma [55].

Note that this study has limitations inherent to cross-sectional studies, particularly when simultaneously estimating the relationships between the variables, response, and exposure, as well as not taking into consideration the temporality between events. Thus, this study design did not allow us to establish a cause-and-effect relationship. The changes in trace element status may be the effect of the chronic disease state and therefore may not associate with the disease pathogenesis. Cohort studies and experimental studies are desirable in clarifying a possible causality role of microelements, including zinc deficiency in atopy and asthma, and their mechanisms of action upon different phenotypes of asthma. A possible limitation in our study was a larger number of children without blood sample collected in the urban area indicating that the random selection was not perfect. Also, it is important to highlight that none data about children's healthy status was collected (other health problems than wheezing), as well as dietary supplements intake and use of medicines (which can modify zinc status). In this study, a report of wheezing in the past 12 months was used as the outcome, as in other population-based studies. Whereas the lack of asthma diagnosis by a physician and medication usage could be interpreted as a limitation of the study, the report of symptoms (wheezing) is less dependent on access to and quality of health services. However, the ISAAC questionnaire has shown good specificity and sensibility for use in worldwide multicenter surveys [56]. We emphasize the importance of conducting further modeling approaches that could potentially measure factors that predispose children to both zinc deficiency and wheezing and that were not included in other studies.

Conclusion

The main findings of this cross-sectional study suggest that the level of zinc may influence the risk of wheezing in late childhood. A zinc-rich diet, which is thought to increase antioxidant activity and immune system functions, may be useful in the prevention and treatment of airway inflammation.

Authors' contribution

Rita de Cássia Ribeiro-Silva participated in the study design, data collection, interpretation of results and writing of the manuscript. Luce Alves da Silva and Luis Fernandes Pereira Santos participated in the field work, laboratory analysis and reviewed the manuscript. Rosemeire Leovigildo Fiaccone designed the work and reviewed the manuscript. Mauricio L. Barreto participated in the interpretation of results and revision of the manuscript. Neuza Maria Alcantara-Neves participated in the study design and revision of the manuscript.

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Conflicts of interest

The authors declare no conflicts of interest.

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