Original Article

Stunting and zinc deficiency among primary school children in rural areas with low soil zinc concentrations in Jiangsu Province, China

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Objective: To assess stunting and zinc deficiency among primary school children in north rural area of Jiangsu Province with low soil zinc concentrations, eastern part of China. Methods: Two data collection rounds were conducted. In the first data collection round, 2268 primary school children aged 6-9 years were included by cluster sampling from three counties with low soil zinc concentrations. Anthropometric measures were assessed and stunting was defined as a z-score of height-for-age (HAZ) below -2 according to the WHO new Growth Standards in 2006. For the second data collection round, the county with the highest prevalence of stunting was selected. From this county, 297 children aged 6-9 years were recruited by cluster sampling. Anthropometric measures, serum and hair zinc, and haemoglobin were measured at this stage. Results: The total prevalence of stunting (HAZ < -2) and mild stunting (-2 ≤ HAZ< -1) was 4.7% and 22.8% respectively, and Huaián had the highest prevalence of stunting (8.1%) among the three counties. In Huaián County the prevalence of zinc deficiency based on serum zinc concentration, hair zinc concentration, and both was 0.7%, 15.2% and 15.3%, respectively, and 32.3% of subjects were anaemic. Boys had a higher prevalence of zinc deficiency than girls (19.1 vs. 10.5%, $p < 0.05$), whereas the prevalence of anaemia in boys was lower than that in girls (28.2 vs. 37.3%, $p = 0.07$). Conclusion: Stunting and zinc deficiency were not highly prevalent among primary school children in rural counties with low soil zinc concentrations of Jiangsu Province.

Key Words: zinc deficiency, stunting, anaemia, children, China

INTRODUCTION

Almost one-third of the agricultural soils of China are considered to be zinc deficient.[1] On such soils, plants grow insufficiently resulting in low yields and low zinc content of crops.[2] In addition to this, the application of fertilizer with nitrogen is widely used in China, which may inhibit zinc uptake by crops.[3] Soil-to-plant transfer, as a food chain pathway, is one of the key components for zinc intake by animals and then humans, and therefore affect zinc status.[4]

Rice is the staple food in the traditional Chinese diet and is usually consumed with vegetables and a small amount of animal-derived food. The dietary pattern changed quickly from 1992 to 2002 according to the National Nutrition and Health Survey, but the change was unbalanced between urban and rural inhabitants.[5] Low intake of animal source food leads to a low intake of important micronutrients, such as zinc, iron, vitamin A, and calcium.[6] Simultaneously, cereal foods contain a high amount of phytate and fiber, which has been shown to inhibit the absorption of zinc and iron. Zinc deficiency is known to be related to retarded growth, higher morbidity of infectious diseases, and higher mortality in children.[7-11] Health of children is especially sensitive to zinc deficiency, since they have relatively higher requirements.[12] Ma et al. reported that phytate concentrations are higher in plant-based foods, and zinc deficiency may be prevalent in some rural areas in China, both as a result of low soil zinc and dietary habits that reduce zinc absorption.[13]

From the Chinese Nutrition and Health Survey in 2002 it has been estimated that 14.0% of rural children aged 7-10 yrs were at risk of inadequate zinc intake.[14] However, no accurate index for zinc deficiency is available to assess zinc status adequately up till now. The WHO/UNICEF/IAEA/IZiNCG has recently developed recommendations on the evaluation of zinc status.[15] Their conclusion was

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that serum concentrations are usually used as indicators for zinc status in population studies.[15-17] Therefore, we have undertaken a cross-sectional study with two data collection rounds in 2002-2003, aiming to assess stunting and zinc status among primary school children living in rural areas with relatively low soil zinc content in Jiangsu Province, eastern China, using serum zinc as a biomarker.

MATERIALS AND METHODS

Study site

The first round of data collection was conducted in Dafeng, Taixing and Huaián, three counties of Jiangsu Province, in 2003. The sites were selected because soil zinc content in each of these counties (lowest in Taixing County with 58.2 mg/kg and highest in Huai'an County with 71.7 mg/kg) was lower than the national average level (100 mg/kg).[18] In addition, other considered criteria were: rice as staple food (rice is consumed at least twice per day); low average income (per capita income was 1502US in Dafeng, 1116 US in Taixing and 1004US in Huai'an, respectively); average 81.6% consumption of food from plant sources; main consumption of local produced food; supportive local health leaders; and experienced health workers. The three counties were all located north of Nanjing, which is the capital of Jiangsu Province. In the first data collection round, anthropometric measurements were done in all three counties. In the second data collection round, biochemical measurements were performed only in Huaian County, which had the highest prevalence of stunting.

Ethical approval

The study was approved by the ethical board of the Jiangsu Province Centre for Disease Control and Prevention. The nature of the study was also fully explained to the local education bureau, the school's principal and teachers, and to the parents of subjects.

Subjects

All school children in the three counties aged 6-9 years in grades 1-3 of primary schools and apparently healthy were eligible for the study. In the first data collection round, a total of 16 schools and 50 students from each school in each county (total n = 2,400) were selected by cluster sampling, assuming a design effect for cluster sampling of 1.75,[19] 10% non-response, and an expected prevalence of stunting of 31.4% based on the Chinese National Survey in 1992.[20] A year later, a total of 320 subjects from 16 primary schools with 20 students from each school in Huaián County were selected by cluster sampling for the second data collection round, assuming a design effect of 2, 10% non-response, and an expected prevalence of zinc deficiency of 19%.[21, 22] Demographic information, such as name, gender, birth date of subjects, school, class and grade, was then collected after sampling.

Anthropometry

Anthropometry was measured in both data collection rounds. Height of the subjects was measured to the nearest 0.1 cm using the All Plastic Height Measure (Leicester model), with subjects standing erect without shoes on the floorboard. Weight of the subjects was weighed to the nearest 100g on an electronic scale (Tanita Field work Scale, BWB-800), with subjects dressed in light clothes and without shoes. The instruments were provided by Chasmors Limited, London, UK. All measurements were done in the morning, according to the standardized procedures as described by WHO.[23]

Blood and hair collection

Non-fasting morning blood samples were taken from the vein in the antecubital fossa between 8am and 10am. Before collection of blood samples, the tubes were soaked in acid for 24 hrs, washed three times with deionised water, and dried in an incubator to avoid environmental zinc contamination. Drops of blood were kept for assessment of haemoglobin before separation of serum from blood. After clotting for 30-60 minutes, blood samples were centrifuged at 1500g for 10 minutes to separate serum, and aliquots for zinc analysis and other biochemical measurements were frozen at –20 °C and analyzed within one month.

Hair samples were cut with stainless steel scissors from the occipitonuchal region of the head, adjacent to the scalp. The proximal 3 cm of hair close to the scalp was taken for analysis, weighing approximately 2 g. Blood and hair samples for zinc analysis were collected according to the procedures as suggested by Brown et al[3] to avoid contamination.

Laboratory analysis

Serum and hair zinc were analyzed by flame atomic absorption spectrometry. All glassware used for analysis was soaked in acid for 24 hrs and rinsed with deionised water. Certified control sera were used for quality control. Haemoglobin was measured by the cyanmethemoglobin method, according to WHO recommendations.[24] Serum albumin was determined by an automated dye-binding method with bromocresol green.[25] C-reactive protein (CRP) were measured by immunoturbidmetric methods.[26] All measurements had inter- and intra-assay CVs < 10%.

Statistical analysis

Z-scores of height-for-age (HAZ), weight-for-age (WAZ) and BMI-for-height (BMIZ) were calculated using SPSS Macro provided by WHO website according to the WHO new Growth Standards.[27] Height-for-age, WAZ and BMIZ below –2 were defined as stunting, underweight and wasting respectively, and mild stunting was defined as HAZ above -2 and below -1.

Overweight and obesity were calculated according to WHO-NCHS BMI criteria for children below 7 years old. For children above 7 years old, overweight was defined as a BMI between the 85th and 95th percentile, whereas obesity was defined as the 95th percentile or higher according to Chinese age-sex-specific BMI criteria for children.[28]

Serum CRP concentration ≥10 mg/L was used as an indicator for inflammation. Subjects with inflammation were excluded from the data analysis. Zinc deficiency was defined in three ways:[29] 1) based on serum zinc concentration as recommended by WHO/UNICEF/IAEA/IZiNCG, with a cut-off of 65 μg/dL for morning non-fasting blood among children below 10 yrs, 2) based on hair zinc concentration with a cut-off of 70 μg/g.[3] 3) combined serum and hair zinc cut-offs. Anaemia was defined as haemoglobin below 115 g/L, according to the WHO criteria.[24]

Data were checked for normal distribution by using the Kolmogorov-Smirnov test of normality. Log transformation was done, if data were not normally distributed. Descriptive indices were expressed as mean (SD) or median for variables with normal or non-normal distribution, respectively. Differences in anthropometric and biochemical indices were analyzed by t-test, ANOVA or chi-square test. Linear regression model was fitted for serum zinc and hair zinc, haemoglobin, HAZ, by adjustment for age, gender, CRP and albumin. A p-value <0.05 was considered statistically significant. All analyses were done with SPSS version 11.5 (SPSS Inc., Chicago, IL, USA).

RESULTS

Prevalence of stunting in the first data collection round

A total of 2268 eligible subjects from the three counties were included in the first data collection round (Table 1). The response rates for Dafeng, Taixing and Huaián were 94.9%, 94.5% and 94.1%, respectively. The overall prevalence of stunting and mild stunting was 4.7% and 22.8% respectively, and no difference was shown in stunting or mild stunting between boys and girls. Stunting increased with age in both boys (p = 0.024) and girls (p = 0.003). The total prevalence of underweight and wasting was 7.1% and 5.5%, respectively. The overall prevalence of overweight and obesity was 3.7% and 1.4%, respectively. Boys showed significantly higher prevalence of overweight and obesity than girls (4.6% and 1.7%, vs. 2.5% and 1.1%, $p < 0.05$), but no difference was found among age groups and area. Huaián County had the highest prevalence of stunting, underweight and wasting respectively among the three counties (Table 2).

Zinc deficiency in the second data collection round

Three hundred and twenty subjects were recruited from the 16 schools in Huaián County, and 297 subjects completed the survey. General information of these subjects is shown in Table 3.

The prevalence of zinc deficiency based on serum zinc concentrations, hair zinc concentrations, and a combination of the two biomarkers was 0.7%, 15.2% and 15.3%, respectively, after exclusion of subjects with inflammation. C-reactive protein concentrations were low; only 2% had CRP concentrations higher than 10 mg/L. The prevalence of anaemia was 32.3% in these primary school children. Boys had significantly lower concentrations of serum and hair zinc than girls ($p < 0.05$), while girls had lower haemoglobin concentrations than boys ($p < 0.05$), as presented in Table 3. Boys had a higher prevalence of zinc deficiency based on hair zinc concentration than girls ($p < 0.05$), whereas the prevalence of anaemia in boys was lower than that in girls (p = 0.07) (Table 4).

Linear regression model of zinc status and haemoglobin concentrations, HAZ adjusted for age, gender, CRP and albumin are shown in Table 5. There was a positive relationship with between serum zinc and hair zinc (stan-

Table 1. General characteristics of school children in three Chinese counties[†‡]

	Dafeng	Taixing	Huaián	Total
Subjects	759	756	753	2268
Age (yrs)	7.8 ± 0.9	8.2 ± 0.9	8.1 ± 1.0	8.0 ± 0.1
Height (cm)	122.7 ±6.8	126.4 ±7.1	124.0 ±7.0	124.3 ± 7.1
Weight (kg)	22.7 ±4.0	24.2± 4.5	23.3 ± 4.0	23.4 ± 4.2
HAZ	-0.41±0.90	-0.24±0.90	-0.63±1.02	-0.43±0.95
WAZ	-0.62±0.96	-0.54±0.95	-0.76±1.04	-0.64±0.99
BMIZ	-0.56±0.94	-0.62±0.92	-0.56±0.94	-0.58±0.94

[†]HAZ, WAZ, and BMIZ are z-scores of height-for-age, weight-for-age, BMI-for-age, respectively, calculated according to the WHO new Growth Standards. Data are mean ± SD.
[‡]Age, height, weight among three counties was compared by ANOVA, with adjustment for age and sex, respectively. Values in the same row with difference superscript letters are significantly different.

Table 2. Prevalence of stunting, underweight, and wasting among primary school children in three Chinese counties[†‡ §]

	Dafeng	Taixing	Huaián	Total
Stunting (%)	3.6	2.4	8.1	4.7
Mild stunting (%)	22.1	18.5	27.8	22.8
Underweight (%)	6.3	5.2	9.8	7.1
Wasting (%)	5.5	5.7	5.3	5.5
Overweight	4.1	3.2	3.9	3.7
Obesity	1.7	1.2	1.3	1.4

[†]Data are presented in prevalence (%).
[‡]Stunting, underweight, wasting were defined as z-scores of height-for-age (HAZ), weight-for-age (WAZ) and BMI-for-age (BMIZ) below -2, and mild stunting was defined as height-for-age (HAZ) above -2 and below -1, according to the WHO new Growth Standards. Overweight and obesity were calculated according to WHO-NCHS BMI criteria for children below 7 years old. For children above 7 years old, overweight was defined as a BMI between the 85th and 95th percentile, whereas obesity was defined as the 95th percentile or higher according to Chinese age-sex-specific BMI criteria for children.
[§]Significant differences among study sites were shown in different superscripts in the same row, measured by chi-square test.

Table 3. Zinc status of Chinese primary school children in Huaián County†

Characteristics	Total	Boys	Girls
Age (yes)	8.2±1.0	8.2±1.0	8.2±0.9
Serum zinc (μg/dL) (n=294)	103.7	96.5	108.9
Hair zinc (μg/g) (n=286)	118.7±42.0	103.2±34.2	138.2±42.9
Haemoglobin (g/L) (n=297)	119.9±11.6	121.1±11.2	118.3±12.0
Albumin(n=294)	46.8±7.5	46.6±7.5	46.7±7.5
CRP(n=289)	1.1	1.1	1.1
HAZ	-0.49±1.27	-0.49±1.36	-0.50±1.17
HAZ<-2, %	11.1	12.3	9.7

†Data are presented as Mean ± SD or median. HAZ was height-for-age z-score calculated according to the WHO new Growth Standards. Significant differences among genders were shown in different superscripts in the same row, using t-test, Mann-Whitney test or chi-square test.

Table 4. Prevalence of inflammation, zinc deficiency and anaemia among Chinese school children in Huaián County†‡

Indices	Prevalence (%)		
	Total	Boys	girls
Inflammation (n=289)	2.0	1.9	2.3
Zinc deficiency			
Based on Serum zinc < 65μg/dL (n=281)	0.7	0.6	0.8
Based on Hair zinc < 70μg/g (n=286)	15.2	19.0	10.5
Based on combination of serum and hair Zinc (n=271)	15.3	19.1	10.5
Anaemia (n=297)	32.3	28.2	37.3

†Data are presented with prevalence (%). Significant differences among genders were shown in different superscripts in the same row, measured by chi-square test.
‡Inflammation was defined as CRP > 10mg/L. Zinc deficiency was defined based on serum and/or hair zinc respectively, after exclusion by inflammation. Anaemia was defined as haemoglobin < 115 g/L.

Table 5. Linear regression model predicting serum zinc, hair zinc, HAZ and haemoglobin†

Dependent variable	Adjusted R square	Independent variables	Standardized coefficients	sig.
Serum zinc	0.59	hair zinc	0.71	<0.001
		Haemoglobin	-0.03	0.37
		HAZ	0.02	0.66

†Serum zinc was log transformed. The model was fitted by linear regression and adjusted for age, gender, CRP and albumin.

dardized beta = 0.71, p < 0.001). No relationship was found between serum zinc, and Haemoglobin (stand. Beta = -0.03, p = 0.37), HAZ (standardized beta = -0.02, p = 0.66), respectively.

DISCUSSION

In our study, we found that both stunting and zinc deficiency were not highly prevalent among primary school children in rural areas with low soil zinc in Jiangsu Province. Moreover, we found that boys were more vulnerable to zinc deficiency than girls.

Stunting has been suggested as a functional indicator of population zinc status.[30] The prevalence of stunting in the present study (4.7%) was lower than the cut-off of 20% that would indicate elevated zinc deficiency,[15] although mild stunting was more prevalent. Older children were more likely to suffer from stunting. Prevalence of stunting in children of 0-5 years old has decreased from 31.9% in 1992 to 14.3% in 2000, mainly due to economic improvement since 1983 in China.[32] Shi et al [33] recently reported the prevalence of stunting to be 2.9% in students aged 12-14 yrs in Jiangsu Province, which is in range with our data. Stunting in school children varies tremendously among different countries: 4.4% of stunting was shown among Turkish school children aged 7-10 years,[33] whereas an average of 51% of stunting was reported among school children aged 6-17 years in five countries in rural Africa and Asia.[34] Stunting ranged from 2.9 to 40.2%, and mild stunting ranged from 31.4 to 75% among school children aged 8-11 years in South Africa.[35] Opposed to stunting, the prevalence of overweight and obesity among childhood in China has increased quickly from 1985 to 1992.[36] The prevalence of overweight and obesity in the present study were lower than those reported on the national level.

Up till now, many studies on zinc status and zinc supplementation have focused on preschool children or toddlers, whereas only a few have addressed school children. Thurlow et al reported a prevalence of low serum zinc concentration of 57% among school children aged 6-13 years in North-East Thailand.[25] In Mexican school-age children below 12 years, 19-24% of zinc deficiency based on serum zinc was reported,[37] whereas only 16% of zinc deficiency based on dietary zinc intake was found among children aged 5-14 years in New Zealand.[38] In China, Ma *et al.*[14] reported proportions of inadequate zinc intake to be 15.3% and 12.9% in age groups 4-6 years and 7-10 years, respectively, based on WHO recommendations. Wuehler *et al*.[39] estimated that 14.3% of the Chinese population were at risk of inadequate zinc intake by using national food balance data in food supplies. Until now there has been no estimation of zinc deficiency using a biomarker among school children in China. In our study we have used serum and/or hair zinc to estimate zinc deficiency. Our results show that a combination of serum and hair zinc would be in good agreement with the estimation from the dietary intake studies in China.

Although serum zinc has recently been suggested as a good biomarker for zinc deficiency, we found that zinc deficiency based on serum zinc was much lower, as compared to hair zinc Serum zinc level reflects short-term status, and hair zinc concentration reflects long-term zinc status. Serum zinc can be influenced by recent dietary intake, and has been shown repeatedly to be sensitive to changes in zinc intake in supplementation studies.[7, 29] However, zinc deficiency might take a long course to develop, and serum zinc may remain within the normal range in marginal zinc deficiency.[40] Another explanation for the low prevalence of zinc deficiency defined by serum zinc concentrations would be unexpected contamination, although we have taken all precautious measures following the current recommended procedures. In addition, cut-offs of serum zinc are estimated mostly from surveys in America, which might not apply in China. Defining zinc deficiency in China still remains an unresolved discussion.

The discrepancy of zinc deficiency between boys and girls was consistent with other reports.[41] Compared to girls, boys have a higher requirement for zinc to meet their higher growth rate, and a greater proportion of muscle per kilogram body weight. Muscle contains a higher content of zinc than fat.[3]

Previously, anaemia was suggested as an indicator of zinc deficiency because iron and zinc have a similar distribution in the food supply, similar food sources, and low bioavailability in cereals due to the presence of phytate. However, anaemia is no longer recommended as an indicator of zinc deficiency due to inconsistencies, as recently advised by WHO/UNICEF/IAEA/IZiNCG.[15] In our study, anaemia is highly prevalent among rural primary school children, and does not coincide with zinc deficiency based on serum zinc concentrations. This supports the current recommendation not to use anaemia as an indicator of zinc deficiency.

There were some limitations in our study. Firstly, we only collected blood samples in one county, which might not be representative for zinc deficiency in the wider Province. Secondly, although we had a good sampling framework and sample size in Huaián County, the sample size for blood collections turned out slightly smaller than what was planned. We based the sample size on a prevalence of zinc deficiency of 19%, whereas in fact the prevalence was lower. In addition, although we have provided a fairly comprehensive assessment of the risk of zinc deficiency in the selected population, we do not address the role of the diet in determining zinc status.

In conclusion, in our study, we did not find a high prevalence of zinc deficiency and stunting among primary school children in rural areas in Jiangsu Province despite the low soil zinc concentrations, which suggests that dietary pattern, interaction of micronutrients, or other factors may play more important roles than soil zinc concentrations only. Zinc deficiency was more prevalent in boys than girls. In addition, anaemia is highly prevalent in the area, which should receive more attention.

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AUTHOR DISCLOSURES

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Original Article

Stunting and zinc deficiency among primary school children in rural areas with low soil zinc concentrations in Jiangsu Province, China

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中国江苏省土壤缺锌地区农村小学生发育迟缓和锌缺乏情况调查

目的：了解苏北土壤缺锌地区农村小学生的生长迟缓和锌缺乏状况。方法：實施两次抽样调查。第一次抽样调查在 3 个土壤缺锌的縣區集群抽样了 2268 名 6-9 岁小学生，对他们进行了身高和体重的测量。按照 2006 年世界卫生组织最新的生长曲线，将年龄别身高（HAZ）评分小于-2 定义为生长迟缓。第二次抽样调查在生长迟缓发生率最高的縣區集群抽样 297 名 6-9 岁小学生，进行身体和体重的测量，并采样检测血锌和髮锌、血红蛋白等。结果：在三个苏北土壤缺锌地区小学生生长迟缓和轻度生长迟缓（-2 ≤ HAZ 评分 < -1）发生率分别为 4.7%和 22.8%，其中以淮安县的学生生长迟缓发生率为最高（8.1%）。在淮安县的第二次调查结果显示，以血清锌、髮锌和二者联合定义的锌缺乏率分别为 0.7%、15.2%和 15.3%，贫血患病率为 32.3%。男生锌缺乏率高于女生（19.1 vs. 10.5%，p<0.05），而贫血患病率低于女生(28.2 vs. 37.3%，p=0.07)。结论：尽管土壤锌相对较低，但这些地区农村小学生生长迟缓和锌缺乏未见高发生率。

关键词：锌缺乏，生长迟缓，贫血，儿童，中国