Towards bio monitoring of toxic (lead) and essential elements in whole blood from 1- to72-month old children: a cross-sectional study

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Abstract

Objectives: Minerals such as zinc, copper, selenium, calcium, and magnesium are essential for normal human development and functioning of the body. They have been found to play important roles in immuno-physiologic functions. The study is to evaluate the distribution and correlation of nonessential (lead) and essential elements in whole blood from 1- to 72-month old children.

Methods: The cross-sectional study was performed in 1551 children. Six element concentrations, including copper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg), iron (Fe) and lead (Pb) in the blood were determined by atomic absorption spectrometry. Distributions and correlations of trace elements in different age groups were analyzed and compared. A Pearson correlation controlled for age and gender was used to assess the relationship of non essential (lead) and essential elements.

Results: Levels of copper and magnesium were 18.09 ± 4.42 μmol/L and 1.42 ± 0.12 mmol/L, respectively. 6.04% of all children showed copper levels below the normal threshold, the levels of Magnesium were stable in different age groups. Though the overall mean blood zinc and iron concentrations (61.19 ± 11.30 μmol/L and 8.24 ± 0.59 mmol/L, respectively) gradually increased with age and the overall deficiency levels (24.1% and 36.0%, respectively) decreased with age, zinc and iron deficiencies were still very stable. Controlling for gender and age, significant positive correlations were found when comparing copper to zinc, calcium, magnesium, and iron((r = 0.333, 0.241, 0.417, 0.314 ,p < 0.01); zinc to magnesium and iron (r = 0.440, 0.497p < 0.01); and magnesium to Calcium and iron(r = 0.349, 0.645, p < 0.01). The overall mean blood lead levels (41.16 ± 16.10) were relatively unstable among different age groups. The prevalence of lead intoxication in all children was 1.3% .Calcium levels decreased gradually with age, with an overall concentration of 1.78 ± 0.13 mmol/L.

Conclusion: Significant negative correlations were also noted between Pb and Zn, Fe (r = -0.179, -0.124.p < 0.01) .The importance of calcium deficiency and supplementation is well realized, but the severity of iron and zinc deficiency is not well recorded. The degree of lead intoxication in all the children studied was low; The established reference intervals for Cu, Zn, Ca and Mg provide an important guidance for the reasonable supplementation of essential elements during different age groups.

Keywords: prenatal biomonitoring, copper, zinc, calcium, magnesium, iron, lead

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Introduction

Calcium(Ca), iron(Fe), Magnesium (Mg) and zinc(Zn) are the predominant nutritional essential metals. Lead (Pb) exposure is the inherent accompaniment of economy development and is inevitable for human beings. Elevated blood lead level is confirmed as multi target toxicant with effects on the gastrointestinal, hematopoietic, cardiovascular, nervous, immune, reproductive and excretory system[1-8] Neurotoxicity is the most severe toxic effect of lead. Children are the most vulnerable and affected group to lead exposure. The developing nervous system is thought to be far more vulnerable to the toxic effects of lead than the mature brain[9]. Therefore, Pb poisoning is now recognized as a grave environmental health threat to children. Cu, Zn, Ca, Mg and Fe, which are important metal cofactors for many enzymes and proteins, are essential for health, as they play important role in human metabolism[10-11]. In humans, particularly children, with dietary intake of calcium, iron and zinc are associated with elevated blood lead level[12].

The relationships between these essential trace elements and Pb is less well understood. Deficiencies of nutritional essential metals can increase the hazard of lead exposure by enhancing absorption and toxicity of dietary lead[13]. The deficiency of zinc, iron, copper, and calcium increases the absorption and toxicity of lead by interfering with the biological and physiological functions of the body[14].Absorption of Pb appeared to be higher in children who have a lower dietary intake of Fe, Ca or Zn; thus, dietary insufficiencies may contribute to Pb absorption[15]. One study on the interaction of lead and some essential trace metals reported that lead was negatively correlated with calcium and iron[16]. Recent researches showed that lead can be influenced by the status of some essential trace metals in children[17].

In the present study, in order to understand the detailed situation of trace elements in children, we estimated whole blood Pb, Cu, Zn, Ca, Mg and Fe levels by an atomic absorption spectrometry of 1551 children aged 1- to 72-month in Nanjing, China. The associations between blood Pb levels and other nutritional elements were investigated to show the prevalence of Pb intoxication in children and to offer support for trace element supplementation as a means of combating poisoning by certain heavy metals.

Materials and methods

Characteristics of subjects

Healthy children age 1- to 72-month were recruited in the study from January 2011 to September 2012. About one third of them were selected randomly from kindergartens in six districts of Nanjing , and the rest consisted of children who recruited from the child health center of our hospital for physical examination.

Fig. 1 Map of the Study area

A total of 1554 subjects were included. Three subjects were excluded: one for leukemia ,one for Mediterranean anemia, one for congenital heart disease. A total of 1551 healthy children were recruited for the study. All the children and their parents were residents of Nanjing in the Jiangsu province. Inclusion criteria were the following::

(1) no history of mellitus, hypertension, cardiovascular disease, liver disease, metabolism system, thyroid diseases, nutritional deficiency diseases, or other diseases that could affect the concentrations of selected elements.

(2)no supplements (i.e., Cu, Zn, Ca, and Mg) have been given during the course of the childrens' life.
(3) no ongoing treatment affecting the concentrations of the selected elements.
(4) living in Nanjing, Jang su Province.

All participants were given informed consent. The investigation was carried out according to the principles of the declaration of Helsinki.
After disinfecting the skin, approximately 2 mls of blood were drawn from the vein into a sodium heparin vacuum blood collection tube. The blood (80 µL) was then added to a tube containing diluent solution.. Venous sampling is the preferred method for measuring blood Pb levels. The experimental protocol was reviewed and approved by committee for the experimental work of Nanjing maternity and child health care hospital affiliated to Nanjing medical university

Elemental analysis
Whole blood Pb levels were analyzed using an atomic absorption spectrometer (283.3 nm) equipped with a tungsten atomizer (BH2100, Bo hui, Beijing, China); Cu, Zn, Ca, Mg and Fe levels were analyzed by flame atomic absorption spectrometry (BH5100, Bo hui, Beijing, China) using hollow cathode lamps (324.7, 213.9, 422.7, 285.2 and 248.3 nm for Cu, Zn, Ca, Mg and Fe, respectively). To reduce the risk of contamination, only pre-tested, trace element-free material was used for sampling and storage. All analyses on whole blood were performed in the same laboratory. Quality controls were assured by analyzing certified reference materials from general administration of quality, inspection and quarantine of the People's Republic of China (GBW(E)090033, 090034, 090035 and 080211 for lead and cadmium, GBW(E)080915, 080916 and 080917 for copper, zinc, calcium, magnesium and iron). Reference values were as follows: Cu: 11.8– 39.3 µmol/L, Ca: 1.55–2.65 mmol/L, Mg: 1.12–2.06 mmol/L, Fe: 7.52– 11.82 mmol/L, Pb: 0–100 µg/L, and Zn: (0–12 months) 58–100 µmol/L; (12–24 months) 62–110 µmol/L; (24–48 months) 66–130 µmol/L; and (48–72 months) 76.5–140 µmol/L. "Deficiency" was defined as values below the normal threshold. Iron deficiency includes: iron depletion(ID), iron deficient erythropoiesis(IDE), iron deficiency anemia(IDA).
Intoxication levels are as follows: Pb: ≥100 µg/L, These reference values were based on the U.S. Centers for disease control criteria for Pb poisoning[19].

Statistics analysis
Statistical data wasanalyzed using the statistical package SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as the mean ± standard deviation (SD). A univariate analysis was performed with a t-test and chi-square test. For statistical comparison of Cu, Mg, Fe, Zn, Ca, Pb levels between multiple groups, analysis of variance was performed by a one-way (ANOVA). The correlations of toxic and essential elements were analyzed by a Pearson correlation controlled for age and gender. p-value < 0.05 was considered significant.

Results
A total of 1551 healthy children (male=835; female=716) from January 2011 to September 2012 were recruited for the study. The mean age of the children studied was 22.0 ±14.0 months, and 53.8% of the subjects were male. The mean level of Pb was 41.16 ± 16.10 µg/L.

Table 1.Comparison of nonessential (lead) and essential element levels in the blood from different age groups

Group (months)	Num	Pb (µg/L)	Cu (µmol/L)	Zinc (µmol/L)	Ca (mmol/L)	Fe (mmol/L)	Mg (mmol/L)
I (1–6)	342	44.22 ± 14.41 **a**	15.10 ± 3.42**a**	48.14 ± 8.71	1.84 ± 0.12	7.20 ± 0.51	1.27 ± 0.11**a**
II (7–12	443	43.17 ± 15.43	19.12 ± 4.25	58.17 ± 9.14**b**	1.82± 0.10	8.37 ± 0.54	1.44 ± 0.12
III (13–24)	325	40.51 ± 15.07	19.41 ± 5.14	64.11 ± 11.21**b**	1.80 ± 0.09	8.69 ± 0.62b**b**	1.47 ± 0.12
IV (25–48)	241	46.84 ± 21.30	18.49 ± 5.27	67.02 ± 14.30	1.74 ± 0.18**c**	8.81 ± 0.51	1.47 ± 0.13
V（49-72）	200	31.51± 14.20	18.37 ± 4.01	70.24 ± 13.15**b**	1.71 ± 0.18**c**	8.14 ± 0.74**b**	1.49 ± 0.13
Total	1551	41.16 ± 16.10	18.09 ± 4.42	61.19 ± 11.30	1.78 ± 0.13	8.24 ±0.59	1.42 ± 0.12

a Compared with Group<49 months ,P<0.05
b Compared with Group in the last previous, P<0.05
c Compared with Group<6 months, P<0.05

The prevalence of lead intoxication in all children was 1.3%. (Table 2). Compared with Pb concentration determined in subjects <49 months, the concentration of Group V Pb(31.51 ± 14.20 µg/L) was lower (p < 0.01).

The mean blood Cu level was 18.09 ± 4.42 µmol/L. Compared to the levels determined for the subjects >6 months, the mean blood concentration of Group I Cu (15.10 ± 3.42 µmol/L) was a significantly lower (p <0.05) (Table 1). Overall, 6.04% of the children had Cu levels below the normal threshold; 10.3% of children in Group A had below normal levels(Table 2).
The overall mean blood Zn concentration was 61.19 ± 11.30 µmol/L. Levels of Zn increased gradually with age, with significant differences present between the youngest (Group I: 48.14 ± 8.71µmol/L) and the oldest (Group V: 70.24 ± 13.15 µmol/L) children (p < 0.05) (Table 1). 24.1% of whose were Zn deficient, and the prevalence of Zn deficiency decreased with age from 41.1% to 13.3%; however, Zn deficiency was still very common. (Table 2).
The overall mean blood Ca concentration was 1.78 ± 0.13 mmol/L. The level of Ca gradually decreased with age, from 1.84 ± 0.12 mmol/L to 1.71 ± 0.18 mmol/L (Table 1). Overall, 6.06% of children had low serum calcium level, and the prevalence of Ca deficiency gradually increased with age from 3.8% to 11.4%. (Table 2).
The overall level of Mg was 1.42 ± 0.12 mmol/L. Group I had an Mg concentration of 1.27 ± 0.11mmol/L, which was significantly lower than the level determined for subjects >6 months (p <0.05) in whom Mg levels were stable (Table 1). No Mg deficiency was found in any of the age groups (Table 2).

The overall mean blood Fe level was 8.24 ±0.59 mmol/L. Fe levels gradually increased with age from7.20 ± 0.51 mmol/L to8.14 ± 0.74 mmol/L (Table 1). Overall, 36.6% of the children we studied showed Fe deficiency, Although the incidence of Fe deficiency decreased with age from 59.2% to22.4% gradually, Fe deficiency was still very common in children. (Table 2).

The percentages of children with blood Cu, Zn, Ca, Mg and Fe levels below the normal threshold or Pb levels above the normal threshold in the various study populations are shown in Table 2.

Table 2 Percentages of children above / below normal thresholds in the various study populations.

Group (months	Num	Pb>100 (µg/L)	Pb>70 (µg/L)	Cu<11.8 (µmol/L)	Zinc# (µmol/L)	Ca <1.55 (mmol/L)	Mg<1.12 (mmol/L)	Fe<7.52 (mmol/L)
I(1–6)	342	0.9%	3.4%	10.3%	41.1%	4%	0	59.2%
II (7–12)	443	0.8%	12.1%	4.0%	31.3%	4.9%	0	43.7%
III(13–24)	325	0.7%	16.3%	4.9%	21.2%	3.8%	0	31.9%
IV(25–48)	241	4.1%	20.0%	8.1%	13.3%	6.2%	0	22.8%
V (49–72)	200	0	15.0%	2.9%	13.8%	11.4%	0	22.4%
Total	1551	1.3%	13.36%	6.04%	24.1%	6.06%	0	36.0%

Zn#: (0–12 months) 58–100 µmol/L; (12–24 months) 62–110 µmol/L; (24–48 months) 66–130 µmol/L; and (48–72 months) 76.5–140 µmol/L

Element correlations
After controlled for age and gender ,we found that there were significant positive correlations between Zn and Mg, Fe (r = 0.440, 0.497p < 0.01), Cu and Zn, Ca, Mg, Fe (r = 0.333, 0.241, 0.417, 0.314 p <0.01),Ca and Mg (r = 0.349, p < 0.01) and Mg and Fe (r = 0.645, p < 0.01). Significant negative correlations were also noted between Pb and Zn Fe (r = -0.179, -0.124.p < 0.01) .
Correlations between trace elements and age were also significantly different. A negative correlation was noted between Pb, Ca and age (r = -0.397, - 0.267, p < 0.01). Positive correlations were observed between age and Zn , Fe, Mg, (0.438, 0.10, 0.152, p < 0.01) (Table 3) .

Table 3 Correlation relationships between nonessential (lead) and essential element concentrations and age.

	r	*P*
Pb	-0.397	0.000
Ca	-0.267	0.000
Zn	0.438	0.001
Fe	0.100	0.008
Mg	0.152	0.005

Discussion

The impact of lead toxicity on children is especially severe for their living behaviors . The data of this study show that the levels of Pb in different age groups was unstable .Based on the U.S. centers for disease control criteria for Pb poisoning[19]. The mean blood Pb level in children was lower than the values reported in Shandong in 2012[20] and in sub-Saharan African[21]. Nanjing is now a low epidemic risk area. However, in our study, approximately 13.36% of all the children had a blood Pb level ≥70 μg/L. Therefore, a careful inspection of childrens' homes for environment sources of Pb is important in protecting them from continual exposure. Lead is a ubiquitous, toxic metal. In general, young children are exposed to it through environmental dust and paint, paint chips from lead-based paint, water contaminated by lead (leaching from valves, fixtures), pottery and ceramics, toys, cosmetics, and etc[22]. Possible explanations for the aforementioned are rapid in urban construction and increased motor vehicles in recent years in Nanjing. On the other hand, children are the most susceptible population due to the following reasons: (1) It is highly absorbed in the air of children than of adults (2) children absorb more environmental lead because of their hand-mouthing living behaviors;

In our study, we also found that the highest intoxication rate of Pb was in Group IV, which was consistent with the report from Beijing and Chengdu[22-23]. Because of their special features like the ability to hand-to-mouth behavior, walk, oral exploratory habits etc. accounting for children in this age group, they are easily poisoned .Cu, Zn, Ca, Mg and Fe are the predominant nutritional essential metals. Our data showed that blood Zn levels gradually increased with age, which was consistent with previous reports[22]. The main reason for this is that diet and gastrointestinal digestion and absorption capacity improved with age, although these capacities are still not well developed in young children[24].. Therefore, the need for Zn supplementation in children's food requires greater attention. The levels of Fe also gradually increased with age, with a mean blood Fe concentration of 8.24 ±0.59 mmol/L, which is lower than the reference mean of 9.67 mmol/L. Fe deficiency was widespread in children. Overall Fe deficiency gradually decreased with age, averaging 36.6%, which is consistent with previous studies[22]. A high incidence of anemia is often found in this age group, the incidence of anemia reported by World Health Organization is 52% The obvious reason for anemia is Fe deficiency during this period. Ca levels gradually decreased with age, but deficiency of Ca was not common in our study, demonstrating that the importance of Ca and the supplementation is necessary, Our results indicated correlations between age and Ca, Zn, and Fe (- 0.267, -0.397,0.438 , respectively). Considering the importance of these nutritional essential metals, the supplementation of trace elements during growth is significative for children. One inadequacy of the study was that family socioeconomic and dietary information were not available. Further researches on supplementation of trace elements were needed.

Previous reports shows that deficiencies in nutritional essential metals can increase the hazards of Pb exposure by enhancing the absorption of dietary Pb[17]. Pb is absorbed mainly in the digestive and respiratory tracts and shares the same ion transport channel in the intestinal epithelium with Ca, Fe, Zn .Competitive inhibition can occur in the process of intestinal absorption, which will result in a lack of these elements in the body.

In our study, only a negative correlations was also noted between Pb and Fe and deficiencies of Zn and Fe were very common, which was consistent with previous studies in the literature[22-23]. . No significant correlations between Pb and Zn was noted. The interaction between toxic(lead) and essential elements should be carefully studied. The present study indicated that significant positive correlations existed between Cu and Zn, Cu and Ca, Cu and Mg /Cu and Fe. It was also significantly positively correlated to Zn /Mg and Zn /Fe, and Mg to Ca and Fe. These studies of the above have shown that the relationship between the trace elements is near.

Conclusion

In summary, the new reference values will be helpful in assessing the health. A balanced diet that includes more foods rich in Fe, Ca and Zn is needed to compensate for an inherent lack of nutrients for children .To raise national health levels, the lead and essential elements levels in children should be monitored and lead prevention should be prioritized in health care. Health care organizations should carry out a series of measures to reduce lead exposure. However, further long-term follow-up researches are still needed.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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Conflict of interest

The author(s) declare that they have no competing interests.

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