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LOWER SERUM LEVELS OF SELENIUM, COPPER, AND ZINC ARE RELATED TO NEUROMOTOR IMPAIRMENTS IN CHILDREN WITH KONZO

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Abstract

We assessed the relationship between key trace elements and neurocognitive and motor impairments observed in konzo, a motor neuron disease associated with cassava cyanogenic exposure in nutritionally challenged African children. Serum concentrations of iron, copper, zinc, selenium, and neurotoxic lead, mercury, manganese, cadmium, and cobalt were measured in 123 konzo children (mean age 8.53 years) and 87 non-konzo children (mean age 9.07 years) using inductively coupled plasma mass spectrometry (ICPMS). Concentrations of trace elements were compared and related to performance scores on the Kaufman Assessment Battery for Children, 2nd edition (KABC-II) for cognition and Bruininks-Oseretsky Test, 2nd edition (BOT-2) for motor proficiency. Children with konzo had low levels of selenium, copper, and zinc relative to controls. Selenium concentration significantly correlated with serum 8,12-iso-iPF_{2α}-VI isoprostane (spearman $r = 0.75$, $p < 0.01$) and BOT-2 scores ($r = 0.31$, $p = 0.00$) in children with konzo. Elemental deficiency was not associated with poor cognition. Mean (SD) urinary levels of thiocyanate were 388.03 (221.75) $\mu\text{mol/l}$ in non-konzo compared to 518.59 (354.19) $\mu\text{mol/l}$ in

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konzo children ($p < 0.01$). Motor deficits associated with konzo may possibly be driven by the combined effects of cyanide toxicity and Se deficiency on prooxidant mechanisms. Strategies to prevent konzo may include dietary supplementation with trace elements, preferentially, those with antioxidant and cyanide-scavenging properties.

1. INTRODUCTION

Epidemiological studies consistently show an association between poor nutrition, chronic dietary reliance on poorly processed bitter cassava, a staple for millions of people under the tropics, and outbreaks of a distinct motor neuron disease known as konzo (1–6). A recent study indicates that children from konzo-affected areas may also present with poor cognition (7). Whether the motor and cognition deficits associated with the disease share the same mechanisms has yet to be elucidated to better inform policy and strategies for the prevention of the cassava-associated neurological disease. We recently found a significant correlation between serum levels of 8,12-iso-iPF₂ α isoprostane, a well-established marker of oxidative damage, and poor cognition in children affected by konzo (8). Our first and plausible explanation was that oxidative damage was induced by cyanide poisoning through the ingestion of poorly processed bitter cassava. Alternative explanations include but not limited to nutritional deficiencies and/or yet to be uncovered exposures to neurodevelopmental toxicants (8). While permissive limits of potentially neurotoxic elements in blood and levels of essential elements seem to be established, little is known about elemental toxicity threats which may vary under conditions of co-morbid conditions common to most of the developing countries. For example, deficiencies in select essential elements may increase the toxicity of lead. Co-exposures may influence the levels of essential and/or potentially toxic elements in blood and modify their respective kinetic and biological (toxicity) behaviors (9).

A recent study raised concerns over exposures to toxic elements in the Democratic Republic of Congo (DRC) (10). However, studies on the human health effects resulting from such exposures, within the context of malnutrition, are still lacking. In this study, we sought to determine the relative contribution of essential versus potentially neurotoxic elements to the neurocognitive impairments observed in children with konzo.

2. SUBJECTS AND METHODS

2.1. Subjects

A detailed description of the study population including children with konzo ($N = 123$) or without the disease ($N = 87$; gender and tentatively age-matched to those with konzo) has been provided in a previous publication by our study group (7). Children with the diagnosis of konzo had to fulfil the WHO criteria for the disease i.e. a visible symmetric spastic abnormality of gait while walking or running; a history of onset of less than 1 week followed by a non-progressive course in a formerly healthy person; and bilaterally exaggerated knee or ankle jerks without signs of disease of the spine (11). The severity of the disease was graded according to the same criteria (11). Of the 123 konzo children included the present analysis [median age 9 years (Inter Quartile Range, IQR: 7–11 years)], 91 were in the mild stage (able to walk with no support) of the disease, 13 in the moderate stage (need a stick to walk), and 18 in the severe stage (unable to walk) of the disease. The

median age of non-konzo children 8 years (IQR: 12-7). Children with history of illness that may affect the CNS (e.g., cerebral malaria, HIV-I/II, or HTLV-I/II infections) were excluded.

Ethics statement—Informed consent and child assent were obtained verbally by investigators who were fluent in Lingala and/or Kikongo, the local spoken languages. Parents who allowed their children to participate in the study were then asked to sign the consent forms that were kept for records at the study office. Ethical approval of research activities including informed consent and assent procedures was obtained from the Oregon Health & Science University (OHSU) Institutional Review Board FWA00000161 and from the Ministry of Health of the DRC.

2.2. Measures of cognition and motor performances

All study participants were subjected to neuropsychological testing using the Kaufman Assessment Battery for Children, 2nd edition (KABC-II) for cognition and the Bruininks/Oseretsky Test, 2nd Edition (BOT-2) measure for motor proficiency as previously described (7). Both the KABC-II and BOT-2 composite scores are used as outcomes measures in the present study, along with the disease status and severity according the WHO criteria for the definition of konzo (11).

2.3. Ascertainment of cyanide exposure and oxidative damage

Interviews with members of a technical team from the Ministry of Health revealed that shortcuts in cassava processing times were common in Kahemba. The analysis of flour samples from 18 consenting households revealed cyanide concentrations ranging from 30 to 200 ppm, well-above the 10 ppm safe limit proposed by the World Health Organization (Joint FAO/OMS report on food contaminants, Rotterdam, 2009). Urinary concentrations of thiocyanate have been used as a marker of cassava cyanide poisoning. Serum levels of isoprostanes were used as markers of oxidative damage. Detailed data on these markers have been provided in our previous publications but partially included in the present analysis as needed (8).

2.4. Inductively coupled plasma mass spectrometry for elemental analysis

Blood was collected through venipuncture in Vacutainer tubes with no anticoagulants and kept at room temperature for approximately 2 hours. Thereafter, specimens were centrifuged at 15,000 rpm for 15 min, and the serum aliquoted in cryotubes and flash-frozen in liquid nitrogen. Specimens were then shipped to Kinshasa, the capital city of DRC, and stored at -80°C until shipment to OHSU on dry ice for elemental analyses using inductively coupled plasma mass spectrometry (ICP-MS). The analysis was carried out in the Elemental Analysis Core at OHSU using an Agilent 7700x equipped with an ASX 250 autosampler. The system was operated at a radio frequency power of 1550 W, an argon plasma gas flow rate of 15 l/min, and Ar carrier gas flow rate of 1.08 l/min. Manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), and zinc (Zn) were measured in kinetic energy discrimination (KED) mode using He gas (4.2 ml/min); selenium (Se) was measured in collision cell mode using H_2 gas (3.5 ml/min); cadmium (Cd), mercury (Hg), and lead (Pb) were measured in no-gas mode. For measurements, 21 $\times$ dilutions of samples were prepared in 1% HNO_3 .

(trace metal grade, Fisher Scientific) in acid-treated 15 ml conical tubes (via incubation in 1% HNO₃, trace metal grade, Fisher Scientific) for at least 24 hrs. Data were quantified using a 10-point calibration curve (0, 0.25, 0.5, 1, 2, 5, 10, 50, 100, 1000 ppb with external standards for Mn, Fe, Co, Cu, Zn, Se, Cd, Hg, and Pb (Common Elements Mix 1 Multi-Element Aqueous Standard; Selenium Single Aqueous Standard; Mercury Single Aqueous Standard; and Lead Single Aqueous Standard, all VHG Labs) in 1% HNO₃. For each sample, data were acquired in triplicate and averaged. An internal standard (Internal Standard Multi-Element Mix 3, VHG Labs) introduced with the sample was used to correct for serum instabilities, and frequent measurements of a 2.5 ppb (parts per billion) all-analyte solution as well as a blank (containing 1% HNO₃ only) were used as quality control and to determine the coefficient of variance. To assess recovery rates of elements and probe background contamination from containers, certified NIST (National Institutes of Standards and Technology) standard reference material controls were treated, prepared, and analyzed by the same method as the samples (bovine and porcine serum, 1598a; trace metals in water, 1643e).

2.5. Statistical analyses

Measures of central tendency were compared using ANOVA or Mann-Whitney or Kruskal-Wallis tests across the study groups. Spearman correlation was used to assess correlations between the global KABC-II and BOT-2 scores and serum concentrations of trace elements. Conditional logistic regression analysis was used to evaluate the association between the disease, stunted growth (height-for-age Z-score ≤ -2), and levels of trace elements. All statistical analyses were run on Stata (version 11.2) with a significance level set at $p = 0.05$.

3. RESULTS

3.1. Overall neurocognitive performances and biochemical profiles

Children with konzo had a mean (SD) BOT-2 motor proficiency score of 24.86 (6.00) relative to 35.32 (7.54) in children with no konzo, and a mean KABC-II score of 58.51 (8.16) relative to 61.40 (9.08) in those with no konzo ($p = 0.02$ for all comparisons). Urinary levels of thiocyanate ranged from 34.40 to 1,032.00 or 17.20 to 1,720.00 $\mu\text{mol/l}$ in non-konzo or konzo children, respectively; with a mean (SD) of 388.03 (221.75) in non-konzo versus 518.59 (354.19) $\mu\text{mol/l}$ in konzo children ($p < 0.01$). One hundred fifty-three (153) children had stunted growth whereas 57 (of whom 17 had konzo) appeared to be nutritionally normal. Of the 153 children with stunting, 106 (69.69%) had konzo while only 17 (29.8%) from the non-stunting group had the disease ($p < 0.01$).

Serum concentrations of Fe, Se, Cu, and Zn were found at levels far below those reported for South African children (9). Trace elements with potential neurotoxic properties Pb, Hg, Mn, Cd, and Co were detected at levels sometimes significantly different between males and females for Pb, Mn, and Cd (Tables 1a and 1b). Of these elements, only Se had concentrations that appeared to be associated with the nutritional status. Mean (SD) of Se in stunted children was 25.45 (16.02) relative to 41.13 (25.59) ppb in non-stunted children ($p < 0.01$).

3.2. Trace elements by disease status and severity

Children with konzo had lower mean levels of Se, Cu, and Zn relative to those with no konzo. In general, severely affected children had mean and median serum concentrations lower than those moderately or mildly affected by the disease. Concentration in Zn followed a similar trend except that no significant difference was seen between those mildly affected by the disease relative to those with no konzo (Table 2 and Figures 1, 2, and 3). The changes in mean serum concentrations of Fe remained insignificant with respect to the disease status and severity. No conclusive pattern was seen in elements with neurotoxic potential though children mildly affected by konzo had a mean (SD) concentration Hg of 1.36 (0.61) ppb compared to 1.11 (0.40) ppb in non-konzo children ($p = 0.02$).

3.3. Association between serum levels of trace elements, nutritional status, and neurocognition profiles

Conditional regression analysis revealed that serum concentrations of Se, or Cu, or Zn were significantly associated with konzo ($p < 0.05$ for all association models). However, no significant association was seen after adjusting for nutritional (stunting) status. The interaction between stunting and serum levels of trace elements was non-significant. Stunting was, however, significantly associated with konzo (odds ratio: 6.1; 95% CI: 2.80 – 13.25) even after adjusting for serum levels of trace elements. Within the konzo group, serum concentrations of Se, Cu, and Zn, significantly correlated with the BOT-2 score for motor proficiency; with correlation coefficients (p-value) of 0.31 (0.00), 0.19 (0.04), and 0.24 (0.01) for Se, Cu, and Zn; respectively. Serum levels of these essential elements showed no correlation with the KBC-II score for cognitive performance. No correlation was seen between concentrations of Hg and BOT-2 or KABC-II scores. Only serum concentrations of Se showed a positive correlation (Spearman coefficient $r = 0.75$, $p < 0.01$) with levels of 8,12-iso-iPF_{2α}-VI isoprostane.

4. DISCUSSION

We report, for the first time, low serum levels of Se, Cu, and Zn in proportion to motor deficits in children with konzo. Low levels of these trace elements may be explained by low dietary intake in the context of chronic malnutrition. Concomitant changes in serum levels of the aforementioned elements and perhaps several other trace elements explored in our study group may also reflect the interplay between individual elemental metabolic pathways (12). We also showed stunting is associated with konzo, a finding that is consistent with the literature on this specific neglected tropical disease (13). Deficiency in Se, Cu, or Zn appeared, however, not associated with the disease status after adjusting for nutritional status. The interaction nutrition and element also appeared insignificant in the different association models. However, within the konzo group, concentrations of Se, Cu, and Zn significantly correlated with the extent of motor deficits. Of specific interest to the pathogenesis of motor deficits was the finding that Se concentrations, in particular, correlated with both greater motor impairment and the higher levels of serum 8,12-iso-iPF_{2α}-VI isoprostane, a well-established marker of oxidative damage (8, 14–16). The later association is consistent with numerous findings that suggest that Se and its derivative compounds including selenoproteins may play important roles in redox and

neurodegenerative mechanisms (17, 18). Whether the observed motor deficits are mechanistically caused by oxidative damage has yet to be established.

While stunting appears to be a clear risk factor for konzo, deficiency in Se, Cu, or Zn appear to play a role in the extent of motor deficits associated with the disease. The association between levels of Se, extent of motor deficits, and levels of oxidative damage is appealing. It is possible that Se deficiency is an aggravating factor for the type of motor deficits seen in konzo. However, reverse causation remains possible as children severely affected by konzo may be prone to severe malnutrition and hence, elemental deficiency and oxidative stress. In both instances, nutritional supplementation with Se or its antioxidant derivatives may be required to establish proper functioning of children affected by the disease. Cognitive impairment seen in children of this study could conceivably be explained by a deficiency and/or excess in trace elements. No deficit in the essential elements was found to be correlated with cognition performances. Despite the detection of neurotoxic elements such Hg in our study sample, levels of all potentially neurotoxic trace elements did not correlate with the cognitive performance scores. We previously showed that cognition performance scores negatively correlated with serum levels of oxidant marker 8,12-iso-iPF_{2α}-VI isoprostane (8). In light of the present findings, we suggest that the toxicity of cyanide itself be retained to explain the levels of oxidative damage associated with poor cognition. However, other types of nutritional deficiencies e.g. vitamin deficiencies have yet to be ruled out. Further mechanistic insights into neurocognitive deficits may also be gained through the analysis of elemental fractions of specific biological relevance rather than total element content as measured by ICP-MS.

Lack of reference values for DRC rural children is another limitation of the present analysis. A control group of non-economically disadvantaged children would have provided better reference values for this study. Such a reference group would also allow us to determine whether neurocognition profiles in the presumably healthy controls could also be associated with deficiency in essential elements that still appeared to be low compared to values observed in other populations of children (9). However, in practice, defining “normal values” is a difficult task that would require considerations of age and gender differences, dietary habits, and numerous other complex biological interactions. Despite the aforementioned limitation, our study does retain a level of internal validity that rests on a consistent pattern of elemental deficiency with respect to the severity of the disease konzo and biologically sound links to oxidative damage, in particular, for selenium. The role of Cu and/or Zn deficiencies as potential risk factors for higher motor deficits in konzo still needs further exploration. In our studies, the observed values may just reflect elemental dyshomeostasis concurrent to the disease process.

In sum, we have shown that deficiency in essential trace elements notably Se is associated with greater motor impairment in children with konzo. It is likely that Se deficiency contributes to the pathogenesis of konzo through mechanisms that are responsible for oxidative damage. Interventional trials to prevent konzo may benefit from a combination of dietary (element) supplementation and processing methods to remove cyanogenic compounds from cassava prior to human consumption. Exploring the potential for selenium

derivatives to act as antioxidants and cyanide scavengers would be of great research interest, high translational impact, and public health relevance (19).

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Highlights

- Selenium deficiency is a risk factor for motor impairments in konzo
- Dual effects of cyanide and low selenium may mediate oxidative damage in konzo
- Selenoproteins with antioxidant and cyanide-scavenging properties may help prevent konzo

Figure 1.

Median serum concentrations of Se appeared to be lower in severely affected children konzo children (all $p < 0.05$).

Figure 2.

Median serum concentrations of Cu appeared to be lower in severely affected children (all $p < 0.05$) except for mild konzo relative to non-konzo children ($p > 0.05$).

Figure 3.

Median serum concentrations of Zn also appeared to be lower in severely affected children (all $p < 0.05$) except for the mildly affected group relative to the non-affected children ($p > 0.05$).

Table 1a

Gender-specific serum concentrations (ppb) of essential trace elements in children of the Kahemba District, Democratic Republic of Congo.

Essential elements Fe, Se, Cu, and Zn are found in serum at concentrations that do not differ between boys and the girls.

Elements	Males (N = 107)	Females (N= 88)	* P values
Fe			
Mean (SD)	1275.49 (885.60)	1127.05 (795.37)	0.22
Median (IQR)	1038.63 (810.33 – 14.91.42)	916.79 (600.87 – 1442.00)	
Se			
Mean (SD)	31.21 (21.33)	27.89 (18.87)	0.25
Median(IQR)	24.96 (16.12 – 39.02)	24.20 (15.10 – 34.92)	
Cu			
Mean (SD)	1051.98 (458.65)	978.01 (384.08)	0.22
Median (IQR)	949.34 (714.95 – 1295.13)	909.38 (731.69 – 1197.48)	
Zn			
Mean (SD)	701.37 (284.80)	678.73 (245.17)	0.55
Median (IQR)	665.21 (474.86 – 899.57)	652.55 (490.82 – 843.44)	

N = number of subjects; SD = standard deviation; IQR = Inter quartile range;

* P-value based on *t*-test for the comparison of means.

Gender-specific serum concentrations (ppb) of potentially toxic trace elements in children of the Kahemba District, Democratic Republic of Congo. Potentially neurotoxic Pb, Hg, Mn, and Cd are found at levels that differ between boys with girls with most elements at higher serum concentrations in boys relative to girls.

Table 1b

Elements	Males	N	Females	N	* P values
Pb					
Mean (SD)	1.23 (3.00)	80	0.56 (0.83)	65	0.08
Median (IQR)	0.52 (0.27 –0.81)		0.37 (0.19 –0.58)		
Hg					
Mean (SD)	1.27 (0.54)	107	1.20 (0.52)	88	0.37
Median (IQR)	1.19 (0.91 –1.54)		1.07 (0.85 –1.56)		
Mn					
Mean (SD)	4.04 (15.28)	88	1.16 (1.06)	73	0.05
Median (IQR)	1.07 (0.67 –1.47)		1.01 (0.58 –1.33)		
Cd					
Mean (SD)	0.08 (0.04)	9	0.14 (0.05)	7	0.02
Median (IQR)	0.08 (0.07 –0.08)		0.11 (0.11 –0.19)		
Co					
Mean (SD)	1.17 (4.41)	63	0.26 (0.42)	59	0.11
Median (IQR)	0.18 (0.11 –0.30)		0.18 (0.11 –0.25)		

N = number of subjects; SD = standard deviation; IQR = Inter quartile range;

* P-value based on t-test for the comparison of means.

Table 2

Mean serum concentrations (ppb) of essential trace elements by the severity of konzo according to the WHO definition criteria of the disease.

Serum concentrations Se, Cu, and Zn tend to differ according to the severity of konzo. More severely affected children tend to have lower levels of these essential elements. No difference was seen, however, in mean serum concentrations of Zn between those with mild konzo relative to the non-konzo children.

Elements	Non-konzo	Mild Konzo	Moderate Konzo	Severe Konzo
Fe				
Mean (SD)	1153.40 (565.48)	1295.59 (999.64)	1003.80 (415.66)	1171.76 (1267.72)
Median (IQR)	1015.33 (763.98 – 1464.77)	1034.23 (672.23 – 1525.87)	883.89 (656.75 – 1241.14)	643.16 (461.63 – 1191.57)
Se				
Mean (SD)	*35.39 (22.94)	*28.96 (17.99)	21.78 (11.68)	*12.39 (6.37)
Median (IQR)	31.38 (19.58 – 43.96)	24.13 (16.47 – 37.28)	17.57 (13.97 – 32.89)	12.26 (6.93 – 16.12)
Cu				
Mean (SD)	*1106.73 (448.19)	*1024.11 (419.28)	863.08 (279.27)	*685.73 (240.92)
Median (IQR)	985.13 (786.27 – 1277.19)	952.77 (712.35 – 1323.71)	886.36 (610.32 – 1140.40)	671.15 (525.12 – 738.09)
Zn				
Mean (SD)	*705.66 (235.74)	*735.49 (287.43)	607.40 (230.48)	*457.71 (202.87)
Median (IQR)	708.03 (504.95 – 863.58)	687.36 (507.19 – 899.57)	606.02 (400.69 – 811.41)	403.76 (340.52 – 540.75)

* ANOVA p-value 0.01 for between-groups differences.