

## FOOD AND NUTRITION SURVEYS

# Linkages of biomarkers of zinc with cognitive performance and taste acuity in adolescent girls

Shashi A. Chiplonkar<sup>1</sup> and Rama Kawade<sup>2</sup>

<sup>1</sup>Hirabai Cowasji Jehangir Medical Research Institute, Pune, Maharashtra, India and <sup>2</sup>Health Genesis, Pune, Maharashtra, India

### Abstract

A cross-sectional study ( $n = 403$ ) was conducted to examine the relationship of plasma zinc (PZ) and erythrocyte zinc (EZ) levels with cognitive performance and taste acuity for salt in Indian adolescent girls. PZ, EZ and hemoglobin were estimated in schoolgirls (10–16 years). Cognitive performance was assessed by simple-reaction-time (SRT), recognition-reaction-time (RRT), visual-memory, Raven's Progressive Matrices (RPM) test. Taste acuity was determined by recognition-thresholds-for-salt (RTS) using 10 different salt concentrations. Low PZ ( $<0.7$  mg/l) and EZ ( $<8$  µg/g of packed cells) were observed in 72% and 23.6% of girls, respectively. PZ and EZ were negatively associated with SRT ( $r = -0.41, -0.34$ ), RRT ( $r = -0.49, -0.4$ ), and positively with Memory ( $r = 0.43, 0.34$ ) and RPM ( $r = 0.39, 0.31$ ;  $p < 0.05$ ) and remained significant after adjusting for socio-demographic factors and hemoglobin. RTS was impaired in 18.3% girls and significantly correlated with EZ ( $r = -0.31, p < 0.05$ ). Zinc deficiency in adolescent girls was associated with poor cognition and taste function implying need for improving their dietary zinc intakes.

### Keywords

Cognitive function, hemoglobin, Indian adolescent girls, reaction time, taste acuity, zinc status

### History

Received 19 May 2013  
Revised 30 November 2013  
Accepted 31 December 2013  
Published online 4 February 2014

### Introduction

Zinc plays a crucial role as a cofactor of a host of enzymes for optimal metabolism and body functions (International Zinc Nutrition Consultative Group, 2004). Zinc deficiency has serious health consequences, particularly in children, contributing to impairments in brain function and physical growth (Gibson, 2006). Mild to moderate zinc deficiency may induce a number of functional abnormalities, such as impaired cognitive performance, reduced taste acuity or loss of appetite in children (Benton, 2008; Black, 2003; Cavan et al., 1993). As zinc deficiency affects the health of children in developing and developed countries, it has become a veritable public health concern.

Based on the estimated prevalence of inadequate zinc intakes, the global prevalence of zinc deficiency is ranging from 4% to 73% in different countries (International Zinc Nutrition Consultative Group, 2004). Although severe zinc deficiency is rare, the incidence of mild to moderate deficiency is common throughout the world. Dietary intakes of Indian adolescents are inadequate in most of the micronutrients including zinc (Srihari et al., 2007; Tupe & Chiplonkar, 2010). Indians are lacto-vegetarians and animal source of zinc is meager in their diets causing low intakes of bioavailable zinc (Chakravarty & Sinha, 2002). However, its consequence on their health is scarcely documented (Vazir et al., 2006). Moreover, Indian girls, particularly from low or middle socio-economic class are vulnerable to nutritional deficiencies due to their secondary social status, early

marriage and gender bias in familial food distribution (Puri et al., 2008). Recent studies have indicated low zinc and micronutrient status of adolescent girls from urban India; however, its association with functional indices of zinc are scarcely reported (Vazir et al., 2006; Tupe & Chiplonkar, 2009).

Although all nutrients are required for body functioning, the deficiency of some micro-nutrients like iron, zinc influence the cognition and behavior of children (Benton, 2008) with majority of such studies reported in infants and young children. Cognitive function was found to be associated with zinc status in pregnant women (Stoecker et al., 2009), and zinc supplementation improved memory in young and older adults (Maylor et al., 2006). As zinc is closely related to growth, examining the association of zinc status and cognitive development in adolescence is of vital importance.

Other than cognition, zinc deficiency can potentially lead to impaired taste acuity (Pluhator et al., 1996). Taste perception was found to be associated with the levels of serum zinc and erythrocytes zinc in older Europeans (Stewart-Knox et al., 2005) and adults (McDaid et al., 2007). Hypozincemic women with plasma Zn  $<700$  ng/ml had decreased thresholds of electrical stimulation for gustatory nerves (Yokoi et al., 2003). Studies in young children and women have demonstrated the link of recognition for salt taste threshold with zinc status (Cavan et al., 1993; Ishida et al., 1985). However, suboptimal zinc status and its relation with taste acuity has been scarcely studied in adolescent girls (Gibson et al., 2002; Kawade, 2012).

Therefore, the aim of this study was to investigate the association of zinc status with cognition and taste perception in Indian adolescent girls. The objectives were to assess relationships between plasma and erythrocyte zinc (EZ) levels with (i) taste acuity for salt and (ii) cognitive performance using a battery of tests in apparently healthy Indian adolescent girls.

## Methods

### Subjects

A cross-sectional study was carried out in school girls from Pune, India. Based on the SD of plasma zinc (PZ) in females (International Zinc Nutrition Consultative Group, 2004), a sample size of 330 was estimated for the power of the study to be 0.9. A physician conducted clinical examination to assess health status of the girls. Current health complaints if any were recorded.

### Exclusion criteria

Girls who were currently suffering or those who had suffered in the previous fortnight from any illness such as fever, respiratory or gastro-intestinal infections or those under medical treatment, or taking multi-vitamin mineral supplements were excluded. Two schools were randomly selected from a list of 14 girls' secondary schools in Pune city, India. From the school attendance records of the selected schools, a random sample of 403 apparently healthy schoolgirls in the age of 10–16 years were recruited for the study using computer-generated random number sequence. The study protocol was reviewed and approved by the local ethics committee under the chairmanship of a retired professor of the International Institute of Population Sciences, Mumbai, for Zensar Foundation of India. All the parents gave their written informed consent and the girls gave their assent to voluntarily participate in the study.

### Cognitive tests

A battery of four tests was used to assess cognitive performance of the girls. Simple Reaction Time (SRT) and Recognition Reaction Time (RRT) were measured using a visual reaction time apparatus (Anand Agencies, Pune, India). Raven's Standard Progressive Matrices (RPM), a non-verbal test of performance (Raven et al., 2004) was calculated as percentage score for each girl from her raw score using the reference percentile age norms for Indian adolescent girls (Deshpande & Ojha, 2002). A short-term visual Memory test was carried out adopted from the subtest of Post Graduate Institute (PGI) memory scale for Indian children and adolescents (Kohli et al., 1998). These tests were conducted under the supervision of a psychologist in a classroom on different days within a week's time of conducting the health examination. The nature and content of the test was explained to the girls and demonstrations were given before actually conducting the test.

### Taste acuity

Taste acuity was assessed by determining recognition thresholds for salt (RTS) using the modified method of Gibson et al. (1989). Ten salt solutions were prepared using 0.75–25.0 mmol/l of analytical grade sodium chloride in distilled, de-ionized water. All solutions were prepared freshly and were at room temperature during administration. The test was performed in a separate room on each girl in the morning after an overnight fast. For the test, plain distilled water was given before any test solution was administered. Different salt concentrations were given to each girl in increasing order until, at a particular concentration; the girl could identify the taste. The reliability of this test was assessed on a subsample of 15 age-matched girls (intraclass correlation coefficient,  $r = 0.72$ ,  $p < 0.05$ ).

### Biomarkers of zinc and iron

Venous blood samples were collected under fasting condition at 7.30 am in EDTA-coated sterile bulbs. PZ was estimated by the wet digestion method using three acid mixture (HNO<sub>3</sub>:HClO<sub>4</sub>:H<sub>2</sub>SO<sub>4</sub> in 3:2:1 proportion) and deionised distilled water as per

the manual of National Institute of Nutrition, India (Raghuramulu et al., 2003) using atomic absorption spectrometer (AAS) (Perkin Elmer Model 3110, Waltham, MA) with a specific cathode lamp. Erythrocyte (RBC) zinc was measured as per the method by Kenney et al. (1984). Weighed aliquots of red cells (0.5 to 1 g) were digested with the similar acid mixture and diluted with deionized water to estimate zinc by AAS. Hemoglobin was estimated using Automated Cell counter (ActDiff II, Beckman Coulter, Fullerton, CA). To ensure accuracy, reference serum sample (RANDOX Laboratories, Mumbai, India) was used as a standard for each batch of blood estimations.

### Socio-demographic observations

Using standard questionnaire (International Institute for Population Sciences, 2006), demographic characteristics of the girls, such as family size, education and occupation of mother and father were recorded from their parents.

### Statistical methods

All statistical analyses were carried out using SPSS 16.0 for Windows (SPSS Inc, Chicago, IL). The data were checked for normality by a one-sample Kolmogorov–Smirnov test. Pearson's or Spearman's correlation coefficients were computed between hemoglobin, plasma or RBC zinc status and four cognitive scores, i.e. simple and recognition reaction time, visual memory, RPM and also with recognition threshold of salt taste acuity considering the normality of continuous variables. Partial correlations were computed to explore the association of zinc status with functional indices after adjusting for socio-demographic factors and hemoglobin. Statistical level of significance was set at 0.05. For the type I probability between 0.05 and 0.1, the significance was considered as marginal.

## Results

Mean age of the girls ( $n = 403$ ) was  $12.1 \pm 1.0$  years (Table 1). Around 72.9% girls had normal height for age ( $Z$  score  $> -1$ ) and BMI for age ( $-2 < Z$  score  $< +2$ ) as compared to Indian reference standards (Khadilkar et al., 2009). Majority of the mothers (91%) and fathers (86%) had education up to high school. Family size of 32% girls was  $\leq 4$  members and 68% girls had more than 5 members in the family. Among fathers, 86.9% were salaried employees and mothers of 37.8% girls were working in industry or as domestic servant.

Mean plasma levels of zinc were low (Table 1) and 72% girls were below the normal cutoff ( $< 0.7$  mg/l) (International Zinc Nutrition Consultative Group, 2004; Yokoi et al., 2003). Mean RBC zinc was low ( $< 8$   $\mu$ g/g of packed cells) in 23.6% girls (Kenney et al., 1984). Prevalence of anemia (hemoglobin  $< 12$  g/dl) was 26.1%.

### Cognitive performance of the girls

SRT of 60% girls was higher than 300 ms, which is the average SRT in normal children (Solso & Kimberly, 2001). Average RRT was found higher than SRT (Table 2) as RRT has complex stimulus (two stimuli in RRT versus one stimulus in SRT) eliciting a slower reaction time (Kosinski, 2010). Majority of the girls (71.5%) had their RPM percentile below 50th percentiles of Indian norms (Deshpande & Ojha, 2002). Visual memory score was low ( $< 50$ ) in 50% of the girls.

Table 2 describes mean ( $\pm$ SD) cognitive scores across quartiles of plasma and RBC zinc levels. There is a gradual decrease in mean SRT and RRT, and a gradual increase in mean RPM and Memory with increasing quartile of plasma (RBC) zinc levels. Pearson correlation coefficients of PZ with SRT and RRT

Table 1. Characteristics of the study population.

Parameter	Age group (year)			
	10–12 ( <i>n</i> = 137)	12–14 ( <i>n</i> = 240)	14–16 ( <i>n</i> = 26)	All ( <i>n</i> = 403)
Age (year)	11.2 ± 0.4	12.4 ± 0.5	14.6 ± 0.7	12.1 ± 1.0
Height (cm)	141.0 ± 8.0	144.5 ± 7.6	148.4 ± 8.5	143.2 ± 8.1
Weight (kg)	29.9 ± 6.6	32.7 ± 6.5	35.7 ± 6.2	31.9 ± 6.7
BMI (kg/m <sup>2</sup> )	14.9 ± 2.5	15.7 ± 2.7	16.2 ± 2.5	15.5 ± 2.6
Hb (g/dl)	12.4 ± 1.2	12.5 ± 1.3	12.3 ± 1.3	12.4 ± 1.3
Plasma zinc (mg/l)	0.63 ± 0.18	0.64 ± 0.26	0.59 ± 0.19	0.63 ± 0.25
Erythrocyte zinc (µg/g of packed cells) <sup>a</sup>	10.5 ± 4.2	11.7 ± 3.7	10.5 ± 5.7	11.2 ± 4.1

Values are expressed as Mean ± SD.

<sup>a</sup>On a sub sample of 127 girls.

Table 2. Cognitive scores of the girls across quartiles of plasma zinc and RBC zinc level.

Parameter	Quartiles of plasma zinc (mg/l)				<i>r</i> <sup>a</sup>	<i>r</i> <sup>b</sup>
	Q1 (≤0.47)	Q2 (0.48–0.60)	Q3 (0.61–0.71)	Q4 (≥0.72)		
SRT (ms)	357 ± 76	333 ± 81	315 ± 62	302 ± 50	−0.41*	−0.39*
RRT (ms)	440 ± 80	433 ± 87	391 ± 65	390 ± 52	−0.49*	−0.44*
RPM (percentile)†	23 (9)	30 (11)	35 (18)	37 (17)	0.43*	0.45*
Memory (%)†	48 (16)	53 (16)	55 (22)	58 (17)	0.39*	0.41*
Parameter	Quartile of RBC zinc (µg/g of packed cells)				<i>r</i> <sup>a</sup>	<i>r</i> <sup>b</sup>
	Q1 (≤ 8.2)	Q2 (8.2–10.7)	Q3 (10.7–13.4)	Q4 (≥ 13.4)		
SRT (millisecond)	346 ± 75	337 ± 65	332 ± 67	314 ± 51	−0.34*	−0.30*
RRT (millisecond)	419 ± 79	414 ± 54	408 ± 81	394 ± 68	−0.40*	−0.38*
RPM (percentile)†	24 (10)	29 (16)	33 (20)	38 (25)	0.34*	0.33*
Memory (%)†	48 (18)	52(16)	56 (20)	57 (15)	0.31*	0.29*

Values are expressed as mean ± SD. \**p* < 0.05.

SRT, simple reaction time; RRT, recognition reaction time; RPM, ravens progressive matrices; †, RPM, Memory and taste acuity threshold were not normally distributed. Hence median and interquartile range is given for them instead of mean and SD.

<sup>a</sup>Pearson's correlation coefficient between plasma zinc or RBC zinc level and other parameters.

<sup>b</sup>Partial correlations of plasma zinc or RBC zinc level after controlling for significant socio-demographic factors (i.e. age, family size, mother's education and occupation).

were negative and statistically significant (*p* < 0.05) (Table 2). Significant positive Spearman's rank correlations were observed for Memory and RPM with PZ (*p* < 0.05). Similarly, RBC zinc levels were negatively correlated with SRT and RRT (*p* < 0.05) and positively correlated with Memory and RPM (*p* < 0.05) (Table 2). Partial correlations of SRT, RRT, Memory and SPM with PZ also remained significant when adjusted for age, family size, parents' education and occupation (*p* < 0.05). Partial correlations of RBC zinc with cognitive parameters also persisted after controlling for the socio-demographic parameters.

Partial correlations of PZ, with SRT (*r* = −0.24), RRT (*r* = −0.21), Memory (*r* = 0.19), and RPM (*r* = 0.19) remained significant (*p* < 0.05) after adjusting for hemoglobin status. Similarly, partial correlations of RBC zinc with SRT (*r* = −0.22), RRT (*r* = −0.18), Memory (*r* = 0.18), and RPM (*r* = 0.12) were also significant (*p* < 0.05). Correlations of hemoglobin with SRT (*r* = −0.12), RRT (*r* = −0.10), Memory (*r* = 0.17) and RPM (*r* = 0.08) were not statistically significant (*p* > 0.1).

### Taste acuity of girls

Figure 1 represents the percent distribution of girls for recognition threshold for salt (RTS) over 10 solutions of different salt concentrations. Median RTS was 5 mmol/l, and first and third quartiles were 5 and 7.5 mmol/l, respectively (Figure 1). No observation was reported for lowest (0.75 mmol/l) and highest (25.0 mmol/l) salt concentrations. Girls with lower

RTS (≤2.5 mmol/l) were 18.3%; those with high RTS (≥7.50 mmol/l) were 45.2%.

Mean (±SE) plasma and RBC zinc levels showed a decreasing trend with increasing salt concentrations (Figure 2). The differences in mean plasma and RBC levels between the lowest and highest salt concentration were significant (*p* < 0.05). Correlation of RTS with PZ was marginally significant (*r* = −0.12, *p* < 0.1) but RTS was significantly correlated with RBC zinc (*r* = −0.31, *p* < 0.05).

### Discussion

Our study demonstrated significant association of plasma and RBC zinc status with the cognitive measures, i.e. reaction time, visual memory and RPM in Indian adolescent girls. Though not zinc status, early life under nutrition is reported to affect audiovisual reaction time to a visual stimulus in Indian adolescents (Agarwal et al., 1998). Our results indicated that higher the zinc status better will be visual memory scores. A higher average memory score (99) was observed in hostel-resident Indian children (6–15 year) (Vazir et al., 2006) as compared to the present study group which might be because of the zinc adequacy in their diet. These hostel children when supplemented with a multiple micronutrient beverage showed better attention-concentration but no improvement in memory. This might be the result of high memory score and zinc adequacy at baseline. In adults zinc supplementation resulted in beneficial effect on spatial working memory but a detrimental effect was seen on attention



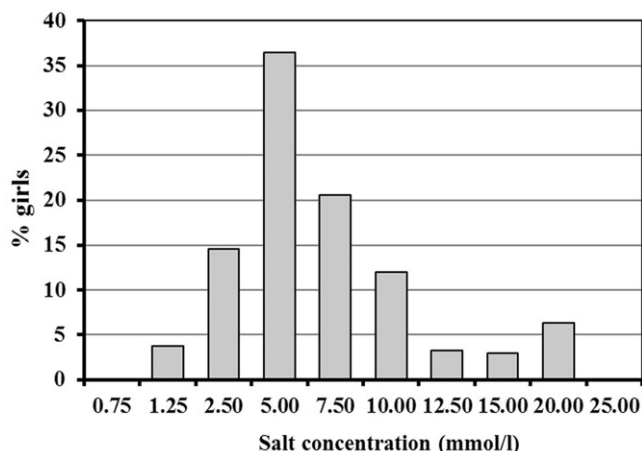


Figure 1. Percent distribution of the girls for recognition threshold for salt.

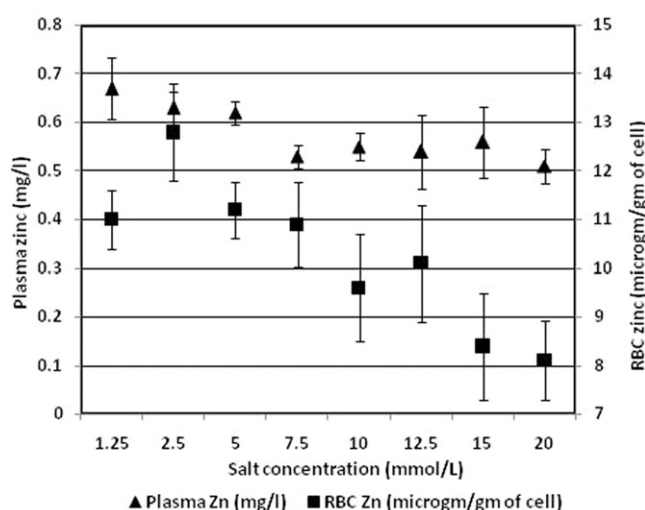


Figure 2. Average plasma and erythrocyte zinc levels at different taste acuity thresholds in girls.

(Maylor et al., 2006). Similarly, zinc deficiency was found to affect cognition in pregnant women (Stoecker et al., 2009).

Certain other micronutrients like iron, iodine, vitamin B12 have been identified as important for brain function in various life stages like infancy or preschool years (Benton, 2008). Multiple micronutrient supplementation with or without zinc in healthy schoolchildren (5–16 years) have shown marginal increase in fluid intelligence but not with crystallized intelligence, and improvement in visual recall and Raven's colored Progressive Matrices test scores (Eilander et al., 2010; Manger et al., 2008; Nga et al., 2011). These results indicate that the interactions among different micronutrients may have variable effect on the cognition. However, the risk of interactions varies with dose and duration of supplementation and baseline micronutrient status of participants (Hettiarachchi et al., 2008; Sandstrom, 2001). Thus, it is necessary to understand the role of specific nutrients like zinc and interactive effects among multiple micronutrients especially in growing age.

Mechanisms linking zinc to cognitive development and function are still unclear. However, certain neurons of the hippocampus are rich in zinc (Frederickson & Danscher, 1990), and fluid cognitive deficits and impaired working memory have been associated with the hippocampus (Blair, 2006) that has a role in spatial memory and has been suggested to be sensitive to zinc

deficiency (Takeda, 2001). Our results support the role of zinc in memory and cognition.

Our study girls exhibited median threshold for salt taste recognition to be 5 mmol/l. The median RTS reported in 7-year-old periurban Guatemalan children (Cavan et al., 1993) was (7.5 mmol/l) higher than the study group. This may be because of low zinc status of the study girls. Less sensitivity for salt taste has been reported in Nigerian children (9–17 years) (Okoro et al., 1998). Taste acuity for salt in our girls was significantly correlated with EZ but weakly correlated with PZ. Similar finding has been reported in older Europeans (Stewart-Knox et al., 2005) where in higher serum zinc was found to be associated with sour but not salt, sweet or bitter tastes and EZ was associated with salt taste. Further association of low zinc status with higher median RTS for salt has been reported by studies in 7-year-old children (Cavan et al., 1993). Negative association of PZ with rate of correct discrimination of salt concentration was found in college-aged women (Ishida et al., 1985) and higher dietary zinc intake was associated with better taste acuity for salt in females (McDaid et al., 2007; Noh et al., 2012).

In conclusion, studies showing putative biological mechanism linking poor zinc status with lower cognitive performance and higher threshold for salt taste especially in childhood are scarce. Present study demonstrated the adverse effects of zinc deficiency on cognition and taste perception. The study population of adolescent girls with low zinc status showed lower cognitive performance and higher threshold for salt taste. Therefore, measures need to be taken to improve the micronutrient status especially zinc nutrition of Indian adolescent girls.

The present study was carried out in girls; however, similar study needs to be undertaken in boys since sex differences (McDaid et al., 2007) in taste acuity have been observed. Secondly responses for different tastes such as bitter, sweet may vary with zinc status which was not examined in the present study. Further we used plasma and EZ as biomarkers of zinc status. Several indices of zinc status such as erythrocyte membrane zinc, zinc metalloenzymes, zinc binding proteins such as metallothionein and other genetic markers are reported (Gibson et al., 2008). However, no unique biomarker of zinc status is recommended so far, especially in community settings (Gibson et al., 2008). For the simplicity of the expression, plasma and EZ are used in the present study. Another limitation of the study is that we did not measure other micronutrient status except iron for their role in cognition. Interactions of other micronutrients with zinc and in turn on the cognition may be examined by further research.

## Acknowledgements

Authors thank Director, Agharkar Research Institute, Pune for providing necessary facilities for this work.

## Declaration of interest

This study was partially funded by Zensar Foundation, Pune, India. The authors declare that there is no potential conflict of interest or disclosures regarding the research work in the article.

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