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Association of cognitive function with nutritional zinc status in adolescent female students

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Summary. *Background and Objective:* Zinc deficiency has been recognized as a crucial public health issue, especially among younger adults in developing countries. Therefore, the main objective of this study was to assess the correlation of serum zinc concentrations and dietary zinc intake with cognitive function scores in high school female students. *Methods:* A sample of 100 students participated in this cross-sectional study. Each participant completed a 3 day 24-h food recall questionnaire to assess the daily zinc intake. We assessed serum zinc status using flame atomic absorption spectrometry technique. Cognitive function was obtained by summing the scores of five tests including Raven's Standard Progressive Matrices test (RPM) on non-verbal intelligence, Benton visual retention test, Wechsler memory scale, Bonnardel accuracy test and letter eliminating test. *Results:* The mean of zinc intake and zinc serum level were 9.49±2.08 mg/day, 105.51±31µg/dL respectively. The mean scores on Raven's RPM, Benton's, Wechsler's, Bonnardel's and letter eliminating tests were 91.44±11.8, 6.8±1.8, 84.64±11.2, 46±6 and 44.84±5.2 respectively. None of the participants reached the ceiling performance. IQ and Memory tests were strongly correlated ($p \leq 0.001$). After adjusting the basic variables, strong positive correlations between serum, but not dietary zinc levels, and Raven's, memory, Bonnardel's and letter eliminating scores ($p<0.001$ for all) were detected. Memory scores were marginally correlated with serum zinc concentrations ($p=0.05$). *Conclusion:* Our findings indicate that serum zinc levels are positively correlated with various aspects of brain cognitive function in young female students.

Key words: cognition, zinc status, nutrition, nutritional assessment, female students

Introduction

Cognition, in general, refers to the processes such as attention, language production, perception, judgment, memory, concept formation, and problem solving. Various environmental factors at different stages of life might alter the cognitive ability; among these factors the role of nutrition seems crucial. Neurochemistry is affected by nutrition status and dietary patterns in three ways. First, the ingested food alters the availability of the needed precursors in neurotransmitter synthesis. Second, obtained minerals and vitamins from foods are used as required co-factors/co-enzymes in enzymes which synthesize neurotransmitters. Third, dietary fats influence the composition of myelin sheath and cell membranes, which in turn, might alter the neuronal function (1). Among micronutrients, zinc is of highest importance due to its role in metabolic processes and several functions, such as vision, taste perception, cognition, cell production, growth and immunity (2). In fact, zinc is needed for brain development and central nervous system function (3). Over three hundred enzymes, known as zinc metalloenzymes, depend on zinc for normal neuronal function (4). The latter form of zinc may modulate responses in receptors for various neurotransmitters, both excitatory and inhibitory including the N-methyl-D-aspartate (NMDA) and -amino butyric acid (GABA) receptors (5). Zinc is essential for neurogenesis, neuronal migration and synaptogenesis; hence its deficiency could interfere with neurotransmission and subsequent neuropsychological behavior (6). Almost 80% of the zinc in the brain is within protein structures such as metalloproteins, coactive and structural proteins. The remaining zinc in the brain is histochemically reactive (7). At the present, the measures of plasma or serum zinc are considered practical and widely used as indicators of zinc deficiency (8). According to the Southern Ethiopia study (9), zinc deficiency was known as a factor in impaired cognition in pregnant women. Maylor et al also showed better performance in cognitive tests in younger adults following zinc supplementation in comparison with older adults. (10) However, due to the conflicting available results, the role of zinc in cognitive function is yet to be cleared. Due to inadequate zinc intake among the approximately 50% of population all over the world (11), zinc deficiency has also been recognized as a crucial public health issue especially in developing countries. Younger adults are more prone to zinc deficiency considering the increased nutritional demands for rapid growth and inappropriate eating habits (12-14). In marginally nourished children, impaired cognitive functions are among the initial signs of zinc deficiency. Lethargy and reduced learning ability have also been reported in zinc deficient animal models (15). Zinc deficiency is among the utmost important factors leading to the burden of disease in developing countries with higher rank of mortality. Furthermore, due to the specific physiological and psychological status, young women are at risk of zinc deficiency and its complications (16, 17).

In the present study, we aimed to investigate the correlations of serum zinc concentration and dietary zinc intake with cognitive function scores in high school female students.

Methods

The study design and subjects

This was a cross-sectional study and the data from a representative random sample of high school age girls (15-20 yrs) living in Izeh, a city of Khouzestan province, south west Iran, were collected. A detailed explanation about the research and consent form was given to the students and their parents. The subjects gave informed consent and their anonymity was preserved. A total random sample of 100 apparently healthy students (as one-fourth of all high school students) was registered. Exclusion criteria consisted of being on medications for any chronic disease (mental and congenital disorders and any other chronic diseases), taking any kind of antioxidant, ,vitamin/mineral supplements and having any abnormalities in hematology, liver, renal, and thyroid function confirmed by the screening laboratory reports.

Data collection

A one week intensive training on the questionnaires, cognitive tests and anthropometric measurements was given to research assistants prior to data collection. The questionnaires and the cognitive tests were pre-tested in 10 students who were not entered into the study. Mean daily intake of zinc was calculated from the 24-h recall questionnaire (3 nonconsecutive days: 2 weekdays and 1 weekend day) using Nut Survey Software System (18).

Through a face to face interview, each student was asked about her intake of food items consumed at breakfast, lunch, dinner and snacks using standard cups and spoons. The recipes for food items were also recorded. Portion size was obtained by average actual weights of a single serving of each food item from their households. Dietary zinc intake was assessed according to the Dietary Reference Intakes (19).

Demographic and Socioeconomic data

Baseline data were collected as part of a general history, including age, weight, height, breakfast and snacks consumption, economic status, daily physical activity and sleep patterns (Table 1). We adapted the socioeconomic status (SES) questions from a modified Ethiopia Demographic and Health Survey 2005

Table 1- Basic characteristics of the study participants *

Variable	Zinc deficient subjects (n=17)	Normal zinc subjects (n=83) #	p-value
Age (y)	17.92±1.21	17.53±1.03	0.265
Height (cm)	158±5.44	159±4.91	0.890
Weight (kg)	55.9±12.14	53.4±10.61	0.471
BMI (kg/m²)	22.38±4.43	20.99±4.11	0.521
zinc intake (mg/day)	7.61±1.19	9.88±2.03	0.113
Physical activity level (min/day)			0.406
- Less than 10	4(23.5)	11(13.3)	
- 10 to 20	8(47.1)	31(37.3)	
- More than 20	5(29.4)	41(49.4)	
Sleeping Patterns (hrs/day)			0 .151
- Less than 4	0	3(3.6)	
- 4 to 6	0	11(13.3)	
- 6 to 8	10(58.8)	35(42.2)	
- More than 8	7(41.2)	34(41)	
Monthly income			0.097
- Low	11(64.7)	50(60.2)	
- Middle	3(17.6)	26(31.3)	
- High	3(17.7)	7(8.4)	
Having breakfast			0.717
- Always	7(41.2)	28(33.7)	
- Seldom	8(47.1)	43(51.8)	
- Never	2(11.8)	12(14.5)	
Having snacks			0.341
- Always	6(35.3)	33(39.8)	
- Seldom	2(11.8)	46(55.4)	
- Never	9(52.9)	4(4.8)	
Having red Meats			0.064
- Daily	1(5.9)	2(2.4)	
- Several times/week	7(41.6)	33(39.8)	
- Once a week	1(5.9)	15(18.1)	
- Monthly	8(47.1)	33(39.8)	
Having white Meats			0.054
Daily	0	4(4.8)	
Several times a week	9(52.9)	57(68.7)	
Once a week	5(29.4)	18(21.7)	
Monthly	3(17.6)	4(4.8)	

**Values are mean±SD, otherwise indicated, # Based on serum zinc concentrations below 70 µg/dL. [8]*

report (20). SES was assessed using a combination of parental education levels and family income level.

Anthropometric measurement

Student's weight was measured by a digital scale (Seca, Hamburg, Germany) and recorded to the nearest 100 grams. Students wore light clothing, while wearing no shoes and heavy outer wear (e.g. sweaters) before measuring weight. Height was measured using a non-stretched stadiometer to the nearest 0.1 cm.

Sample collections

A venous blood sample (5 mL) was collected from each participant in sitting position after 8 to 10-h overnight fast (between 8:00 am to 10:00 am). An experienced laboratory technician did the blood sampling using hygienic techniques. Serum samples were separated by centrifuging at 2000 rpm for 15 minutes using Centrifuge 5810 R (Eppendorf, Hamburg, Germany). Serum samples were then stored at -4 °C for subsequent analyses of zinc status. Serum zinc levels were measured using an atomic absorption spectrometer (Chemtech Analytical Instruments Ltd.; UK) (21).

Cognitive tests

Raven's Standard Progressive Matrices (RPM)

This is a nonverbal test of performance that measures a person's ability to form perceptual relations and reasons by analogy in research settings and to form comparisons (i.e. IQ measure). The test is made up of 5 sets of diagrammatic puzzles exhibiting serial change in 2 dimensions simultaneously. Each puzzle has a missing part that the person has to find it among the options provided (22). The Persian form of this test has been validated in the research of Nissi et al. on 244 girl students (2006), the coefficient of reliability of the test (0.8) was calculated using Cronbach's method(23). Also this test was correlated with Wechsler Memory Scale (WMS); with correlation coefficient of 0.74(24).

Benton visual retention test

This is a recall of 10 complex and unfamiliar geometrical figures. There are 10 designs on 10 cards, each design is exposed for 10 seconds and the subject is asked to reproduce the design immediately by his/her mind (25) This test had a concurrent validity of 0.62 and spearman brown reliability coefficient of 0.97 in domestic samples(26).

The Wechsler Memory Scale (WMS)

This is a neuropsychological test designed to measure different memory functions. WMS-IV is made up of seven subtests: spatial addition, symbol span, design memory, general cognitive screener, logical memory, verbal paired associates, and visual reproduction. A person›s performance is reported as five Index Scores: auditory memory, visual memory, visual working memory, immediate memory, and delayed memory (27). The Persian form of this test had a concurrent validity with Raven's Standard Progressive Matrices (RPM) with correlation coefficient of 0.74. Also, the acceptable Cronbach's alpha between 0.83 to 88 has been reported for this test (24).

Bonnardel's Letter eliminating test

In the Bonnardel's letter eliminating test, participants are asked to mark the most repeated numbers within two minutes among 468 numbers from 0 to 9 which are typed down on a paper. The latter test is used to estimate the attention score. The test-retest reliability of the instrument was evaluated with a two-week interval in Persian high school students randomly selected and Pearson Correlation Coefficient of 0.95 0 was obtained(28).

Bonnardel's accuracy test

This is considered as an eliminating test, which is used to calculate accuracy score in individuals. In this test, the participant is asked to find the same pattern as a given sample within the tiny colored circles (25). This test had an acceptable concurrent validity and test-retest reliability with coefficients of at least 0.90 in studies with Persian girl samples (29).

All subjects completed test series mentioned above and scored. Cognitive function was then calculated from the sum of the scores of five tests mentioned above.

Statistical analysis

We exported the data to SPSS 18 (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.) and analyzed them using selected descriptive and analytical statistical measures. Pierson's r was used to show the correlation between dietary zinc intake and serum zinc levels. General linear method (GLM) was used for analysis of variance to determine the correlation between serum zinc levels and cognitive function scores.

The study design was approved by the Ethical Committee of Ahvaz Jundishapur University of Medical Sciences under the register number of U-90276.

Results

General characteristics

General characteristics of participants, including age, weight, height, breakfast and snack consumption, economic status, daily physical activity and sleep pattern are shown in table 1. The age (Mean ± SD) of the participants was 17±0.06 years. Participants are shown as zinc-deficient and normal zinc status groups.

Socioeconomic status

According to the SES classification, ten percent of families had high economic status while 29% and 61% of students were classified as middle and low income, respectively.

Dietary assessment

Mean daily intake of zinc was classified according to reference values in which daily zinc intake less than 7.3 mg/day in 14-18 years old females were regarded as zinc deficiency (19).

Data on having breakfast, snacks, and red and white meat within a month are also indicated in table 1.

Anthropometric measurements

Anthropometric characteristic of the students are shown in table 1. No significant differences were seen between the zinc deficient and normal subjects regarding these variables.

Cognitive Tests

Table 2 indicates the scores of participants on each cognitive test. Accordingly, The Mean ± SD scores on the tests measuring Raven's Progressive Matrices (IQ), visualization (Benton's), memory (Wechsler's), attention and accuracy test (Bonnardel's) and letter eliminating test were 91.44±11.8, 6.8±1.8, 84.64±11.2, 46±6 and 44.84±5.2 respectively. A significant correlation between serum zinc concentrations and its dietary intake was detected. (r=0.52, $p<0.001$, Fig 1).

There was a significant positive relation between serum zinc levels and cognitive function (expressed as the sum of scores of the five tests) , after adjusting for age, height, weight, family economic status, physical activity and the intake of breakfast, snacks and meats. Using GLM model, the effectiveness of each 10 µg/dL increase in serum zinc levels on each cognitive test score has been indicated in Table 3. For each 10 µg/dL increase in serum zinc concentrations, the scores of Raven's, Bonnardel's, letter eliminating and cognitive function were increased by 0.6, 0.1, 0.2, 0.2 and 0.92, respectively ($p<0.001$ for all). Wechsler's memory scores were marginally correlated to serum zinc concentrations (p=0.05). The scores of Benton's test were not correlated to serum zinc levels.

No significant relation was found between dietary zinc intake and different cognitive tests.

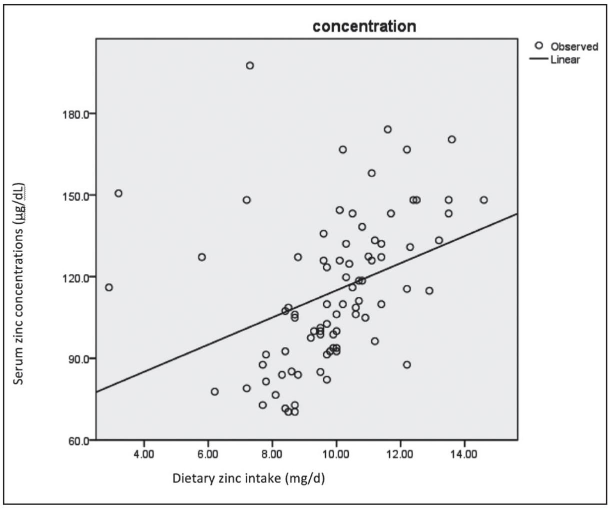

Figure 1. Correlation between dietary zinc intakes (mg/ day) and its serum concentrations (µg/dL) (r=0.515; p<0.001)

Table 2- Scores of students' cognitive performance

Cognitive tests	Mean ±SD	Minimum score	Maximum score
Ravens's Total (IQ scales)	91.4 ± 11.4	64	120
Benton's (Visual retention)	6.8 ± 1.8	4	10
Wechsler's (Memory scales)	84.6 ±11.2	55	110
Letter eliminating test	44.8±5.2	9.1	51.0
Cognitive function (Sum of total scores)	272.3±27.0	204.2	334.1

Discussion

Cognition and mood are suggested to be affected by nutritional status (30, 31). “Zinc has the second highest concentration of all transition metals after iron in the brain” (16). Within the micronutrient deficiencies, zinc deficiency is one of the most encountered deficiency usually seen in developing countries (1, 15, 32, 33). Zinc can only be obtained through the diet and is most available from red meat and seafoods. Low soil zinc content has also been reported to aggravate maternal zinc deficiency (34). Zinc deficiency has been associated with neurological dysfunction and human brain pathology (35-38). Measures of various psychological variables such as mood, perceived stress and cognitive status were related to lower plasma zinc concentrations, especially in areas with low zinc intakes and a limited variety of foods containing zinc (39). There have been no published studies on the serum zinc status and cognitive function of female adolescents, especially in Iran, historically known as a zinc deficient area. According to the studies, geographic conditions such as humid, hot climate induce endogenous losses through perspiration and exfoliation of the skin (40).

In the present study, a significant relation between dietary zinc intake and serum zinc concentration was detected (Fig. 1). However, no significant relation was detected between dietary zinc intake and cognitive function. There was a positive correlation between serum zinc levels and cognitive function scores, this correlation was defined as 2.5 times increase in total cognitive function scores for each 10 µg/dL increase in serum zinc levels (p <0.001). Moreover, each 10 µg/dl increase in plasma zinc levels leaded to 1.8 times increase in Raven's test scores, identified as nonverbal IQ scale, 1.1 times increase in Wechsler's (memory) test scores, 1.2 times increase in both Bonnardel's test and letter eliminating scores all were statistically significant (Table 3).

However, the effectiveness of increase in serum zinc levels on Wechslers' scores was marginally significant (p=0.05). No significant correlation was detected between Benton's test scores and serum zinc levels. Our findings were in consistence with the results of

Table 3- General linear model (GLM) coefficient for increasing each 10 µg/dL serum zinc concentrations and cognitive function scores in female students [1]

			%95 confidence interval		
Cognitive tests	**ß** [2]	**SD Error**	**Lower**	**Upper**	***p***
Ravens's test					
Increase in score	0.60	0.0049	0.050	0.070	<0.001
Odd ratio	1.8	1.00	1.05	1.07	<0.001
Wechsler's test					
Increase in score	0.1	0.0049	0.17	0.19	0.050
Odd ratio	1.1	1.00	0.54	1.2	0.050
Benton's test					
Increase in score	0.000	0.0049	-0.009	0.010	0.964
Odd ratio	0.0	1.00	-1.00	1.01	0.964
Bonnardel's test					
Increase in score	0.2	0.0049	0.011	0.030	<0.001
Odd ratio	1.2	1.00	1.01	1.03	<0.001
Letter eliminating					
Increase in score	0.2	0.0049	0.030	16.83	<0.001
Odd ratio	1.2	1.00			<0.001
Cognitive function[3]					
Increase in total scores	0.92	0.0050	0.083	0.102	<0.001
Odd ratio	2.5	1.00	1.08	1.10	<0.001

1. Analysis was done after adjustment for age, height, weight, BMI, sleeping patterns, physical activity, breakfast and snack consumption, family economic status, red and white meat consumption

2. Improved cognitive function scores for each 10 µg/dL enhancement in serum zinc concentrations. 3. The sum of the scores of five tests including; Raven's Standard Progressive Matrices test (RPM), Benton visual retention test, Wechsler memory scale, Bonnardel accuracy test and letter eliminating test.

Stoecker et al study, in which the correlation between different micronutrients with cognitive function were assessed in pregnant women in southern Ethiopia(9). The latter findings might provide more evidence of the impacts of zinc deficiency on cognitive function in developing countries, where mild to moderate zinc deficiency is common (41). However, Maylor and colleagues in the ZENITH study on healthy middle aged and older adults indicated no consistent association between zinc intake, urine or blood levels of zinc and cognition at the baseline (10).

To our best knowledge, this study is the first work conducted to assess serum zinc status and its relation to cognitive function of adolescent females. Considering the limitations of the previous studies and in order to modulate the impact of confounding factors such as socioeconomic factors, we obtained the complete information regarding the SES from participants and then adjusted them in our data analysis. As a strength point of current study, each cognitive test was done separately on a particular day. Besides, the surrounding environment was made prepared enough, while administering cognitive function tests. Moreover, considering the side effects of hunger on cognitive performance, we were assured that the participants had their meal at least 30 minutes before commencing the tests. At this point, randomized controlled trials, at different ages and physiological conditions are required in order to see the extent of the effects of zinc status on cognitive status.

Limitations

Data are collected on a single point in time; hence readers should be aware of the predictive limitations of this cross-sectional study. Furthermore, causality cannot be concluded from this study.

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