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Serum and Dietary Zinc and Copper in Iranian girls

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Running title: Relationship between serum and dietary zinc and copper in Iranian girls

Objective: Girls with micronutrient deficiencies may have impaired growth and development, and furthermore this deficiency may impact on their childbearing. We have investigated the relationship between serum zinc and copper concentrations, dietary zinc and copper intake and anthropometric and demographic parameters, and cardiovascular risk factors, in 408 girls living in northeastern Iran.

Methods: Serum zinc and copper concentrations were measured by flame atomic absorption (Varian AA240FS) and zinc and copper intake were assessed using a 3-day dietary record.

Results: There was a slight correlation between serum and dietary zinc intake (r=0.117, p=0.018). The correlation between serum and dietary copper approached significance (r = -0.094, p = 0.056). The mean serum zinc and copper concentrations were 14.61±2.71 μmol/l and 19.48±8.01 μmol/l respectively. Height, total cholesterol (TC) and low density lipid profile (LDL) were positively correlated with serum copper concentration. Subjects with high serum copper concentration (>150 mg/dl) were found to have a significantly higher fasting blood glucose (FBG) compared to subjects with normal, or low serum copper concentrations (p=0.019). Girls who were in the 5th percentile or grater for height were found to have higher serum copper concentrations than girls in other height categories.

1. Introduction

Zinc and copper are two important trace elements in human metabolism. They are cofactors for more than 100 enzymes, including those involved in the synthesis of connective tissue (collagen and elastin) [1-3]. Several studies have reported that the serum copper/zinc ratio is inversely correlated with blood pressure[4]. There is some evidence that an adequate intake of copper and zinc are associated with a lower risk of obesity in children and adolescents[5, 6]. Furthermore, serum zinc and copper concentrations have been shown to be independently associated with risk of cardiovascular disease (CVD)[7]. Girls have a growth spurt during adolescence, and during this period have a greater requirement for several nutrients[8, 9]. Many common chronic diseases have their origins in childhood [10-13]. Several studies have been conducted in Iran to assess zinc concentration in the soil and its levels in various agricultural products such as rice, and have shown that low micronutrient content of soil may explain the low zinc content in Iranian foods[14]. The prevalence of zinc deficiency is estimated to be about 10% in the Iranian population[15]. We therefore aimed to investigate the relationship between serum and dietary zinc and copper in adolescents because they would be the ideal age for starting treatment, and because it is recognized that adolescent girls with micronutrient deficiencies, may have impaired growth and development, and furthermore this deficiency may impact on their childbearing. This information may also be useful for setting nutritional policy such as food fortification or supplementation.

2. Material and methods

2.1 Subjects

A total of 408 healthy participants**,** aged 12-18 years old, were participate using a randomized clustering method and computer generated random numbers within different areas of Mashhad, northwestern Iran between January and April 2015. Written consent was obtained from participants and their parent after approval was given by the Ethics Committee of Mashhad University of Medical Sciences. As previously we reported the methodology of this study, the menstruation status and any disorder of subjects were obtained from questionnaire [16]. We excluded participants with any autho-immune disease, metabolic bone disease, cancer, hepatic or renal failure, cardiovascular disease, malabsorption or thyroid, parathyroid or adrenal disease. Girls who taking supplement, anti-obisity, anti-diabetic, anti-depressant, anti-inflammatory or hormone therapy within the last 6months were excluded.

2.2 Estimation of anthropometric measurements

Anthropometric and demographic data were collected by trained staff. Weight and height of each subject were measured using a standard instrument to an accuracy of 0.1 Kg and 0.1 cm respectively. A standard mercury sphygmomanometer calibrated by the Iranian institute of standard and industrial research was used to assess blood pressure, twice with a 30 minute interval for any participant while seated and rested. The average of two measurements was taken as the blood pressure.

2.3 Blood sample

After a 14 hour over-night fast, blood samples were collected in the early morning between 8-10 am and were stored at -80° C in Eppendorf acid washed tubes at the reference laboratory in Mashhad University of medical science until analysis in university's laboratory [16]. The blood samples were collected into heparinized and non-heparinized tube. Heparinized blood samples were analyzed for biochemical parameters by using standard kit (Pars Azmoon, Iran). Samples for fasting blood glucose (FBG) were collected into vacuum collection tubes containing fluoride. Lipid profile was measured by routine enzymatic methods and LDL cholesterol was calculated using the Friedewald formula. For trace elements analysis, serum samples were diluted with nitric acid at a ratio of 1:10. Flame atomic absorption (Varian AA240FS) with graphite furnace HGA 300, with an analytical wave length of 213.9 nm for zinc and 324 nm for copper was used to assess serum level of zinc and copper, a standard curve was constructed using a zinc and copper standard (Merc and Co. Pharmaceutical Company). The limit of detection were ? and ? for respectively.

2.4 Estimation of dietary intake of micronutrients.

Dietary micronutrients were estimated using a 3-day food record which is a standard technique used widely in research as well as in empirically based clinical practice to evaluate recent dietary intake [17]. Participants were instructed on how to complete the food record by trained staff, and included data for two weekdays and one weekend on the amount, type, preparation and time of food or beverage consumed by subjects. The dietitian provided instruction on completion of this record at baseline for girls and their parents with household measures. Each record was analyzed for micro and macronutrients using the Nutritionist IV software.

2.5 Statistical analysis

SPSS version 18(SPSS Inc. Chicago, IL, USA) was used for all statistical analyses. The normality of the data was assessed using the Kolmogorov-Smirnov test and then Pearson's correlation test was applied to determine significance of the correlation between trace elements status and intake of elements with anthropometric and demographic data. A t-test was used to determine difference between two set of data and variation between subgroups determined by ANOVA statistics. Moreover, Chi-squared test was applied to determine frequencies of trace elements with respect to low and normal and high value of trace elements in serum.

3. Results

3.1 demographic characteristics and trace element

Demographic and anthropometric data for all participants are shown in Table 1. The mean age of participants was 15.07±1.52 years. We did not find significant correlations between age or menstruation status ($p>0.05$) and serum, or dietary trace elements. For the whole cohort, dietary zinc and serum zinc were 7.91±3.15 mg/day and 14.61±2.71 µmol/l; also, dietary copper and serum copper were 1.53±0.86 mg/day and 19.48±8.01 µmol/l respectively. Zinc and copper deficiency were defined by a serum concentration < 8.3 µmol/l and < 10.2 µmol/l respectively. We found that 6.9% of subjects had a serum zinc level below < 8.3 µmol/l and 19.8% had a serum copper level below < 10.2 µmol/l.

A sufficient zinc status was defined by a serum Zn between 8.3-18.8 µmol/l and higher zinc status by a serum zinc > 18.8; 358 (87.7%) and 22 (5.4%) of the population sample were in the sufficient and high serum level categories respectively. Insufficient zinc and copper intake were defined as values <7 mg/day and <1.1 mg/day, a total of 51.7% and 68.1% of participants consume zinc and copper less than these particular level.

Nor were there significant correlations between serum concentration and intake of trace elements with weight and height. The CDC growth chart for children and teens of the same age and sex was used to categorize weight and height of subjects. Underweight was defined as a BMI for age and sex below 5^{th} percentile, normal between 5^{th} and 85^{th} percentile , overweight was defined as a BMI at or above 85^{th} percentile and below the 95^{th} percentile, obesity is defined as a BMI at or above the 95^{th} percentile. Also, height below the 5^{th} percentile considered as short stature and the 5^{th} percentile up to 95^{th} percentile as normal and tall subjects defined as a score above 95^{th} percentile. Among participants, who were in the 5^{th} percentile on the CDC growth charts a non-significant higher serum zinc and zinc intake compared to normal and overweight or obese subject was seen while participants were in the 5^{th} percentile with normal weight, had higher copper intake and serum copper value respectively Table 3. Moreover, participants who were taller were shown to have higher serum zinc and copper concentration, although these values were only significant for serum copper (p= 0.011). We did not observe any significant relationship between age and trace elements (Table 3).

3.2 relationships between CVD risk factors and trace element

Neither dietary, nor serum copper and zinc were significantly associated with CVD factors such as blood pressure and obesity although subjects who had serum LDL cholesterol >130 mg/dl and total cholesterol (TC) >200 mg/dl had significantly lower serum copper concentrations (Table 2). Individuals with a serum copper concentration >150 mg/dl were found to have a higher FBG compared to other subjects (p=0.019) (Table 4).

3.3 dietary assessment and trace elements status

Correlation between serum and dietary trace elements therewith energy, protein, carbohydrate, fat and fiber intakes shown in table number 5. There was significant positive correlation between serum zinc concentrations and energy (r=0.116, p=0.019), protein (r=0.114, p=0.022), carbohydrate (r=0.114, p=0.021) and fiber (r=0.14, p=0.005) while correlation between fat intake and serum zinc was not significant (r=.0.065, p=0.187). Moreover, bivariate correlation between dietary intakes of trace elements with macronutrients was investigated. Dietary zinc intake had significant positive correlation with carbohydrate (r=0.618, $p<0.001$) and fat (r=0.601, $p<0.001$) and dietary copper intake shown positive correlation with energy (r=0.635, $p<0.001$), protein (r=0.597, $p<0.001$), carbohydrate (r=0.481, $p<0.001$), fiber (r=0.481, $p<0.0001$) and fat (r=0.525, $p<0.001$) while serum copper concentration had negative significant correlation with energy (r=-0.147, p=0.003) and fat (r=-0.134, p=0.007) respectively.

Discussion

A high percentage of the population sample [114 (27.9%)] had serum copper values > 31.5 which was higher than our expectation. A possible source of higher copper exposure is via the drinking water by corrosion of pipes that are still used in disadvantaged regions. According to Esmaeili parameters of hardness, alkalinity, sulfate, chloride, sodium, and TDS exceeded the standard level in this area and the existence of minerals and ions in these areas leads to aggravating the corrosion process [18]. The dietary sources of copper include wheat and whole grain, legume, chocolate and nuts, are widely consumed by Iranian population. Further work is required to explore this. An extensive study on copper in Germany showed the serum copper level of one month to 18 years old subjects to be 20.4±4.9 µmol/l [19]. Another study investigated the role of copper in children with attention deficit hyperactivity disorder and healthy subjects that didn't show significant differences although mean serum copper in healthy control groups (16.9±2.6 µmol/l) was lower than our finding [20].The mean values for serum zinc and copper concentration among adolescent girls were in good agreement with previous studies in Iran [21-23].

In agreement with our finding a previous study has reported a prevalence of 7.9% zinc deficiency in children age 3-18 years old in the Iranian population [24].

4.2 Association between dietary intake and trace elements

We observed the slight correlation between serum and dietary values of zinc; there was no similar finding for copper. Although it has been suggested that serum zinc and copper concentrations are independent of dietary source [25], serum concentrations were predicted by dietary intake. In addition, interaction with other cations and the bioavailability and storage of these elements in the body are influenced by recent intake of zinc and copper. Subjects with higher serum copper concentration had lower energy and fat intake which revealed that inverse relationship between copper and lipid profile is not consequence of higher intake of fat or carbohydrate as main energy source and less protein consumption. Moreover, ANOVA tests didn't show significant difference between mean of energy, protein, carbohydrate, fiber and fat intakes among zinc and copper intervals (Table. 5).

4.3 Association between trace elements and age

We did not find any significant difference in trace element concentrations related to age. In contrast other studies have reported a positive correlation between serum zinc and copper concentration and age in adolescents [19] Although there is some evidence for a fall in serum copper concentration with increasing age in adolescents, this may be due to a shift in copper between extra and intracellular storage [19]. The fall in zinc and copper with age has been reported in some other studies [26]. In consist with our finding the Korea National health and Nutrition Examination Story found that serum zinc level decreased with increasing age in adults and serum zinc positively associated with fasting blood glucose [27]

3.4 Association between trace elements obesity and stature

The prevalence of obesity and overweight among adolescents varies between 10% and 30% globally with urbanization and industrialization being important factors for childhood obesity. The rise in the prevalence of obesity among adolescents is increasing in developing countries, including Iran, which was one of the top seven countries with highest proportion of childhood obesity and overweight in

1988 [28, 29]. The prevalence of obesity and overweight among Iranian female students were reported 6.3% and 18.4% in 2014 which has increased further over the past decade [24]. Many studies have indicated that obesity in childhood and adolescents leads to cardiovascular disease in later life (CVD) and other health consequence such as endocrine/ metabolic comorbidities [30]. Consistent with some previous reports, we did not find any significant relationship between serum zinc and copper or their dietary intake, with obesity [31, 32]. Although, several studies have previously reported a strong inverse relationship zinc status and obesity [10, 33]. In agreement with the finding of our study previous study in obese adolescents with clinical insulin resistance didn't find significant correlation with age, BMI or cardio metabolic markers [34].

In 1961 for the first time zinc deficiency was observed among Iranian population which associated with dwarfism and delayed sexual development [35]. Deficiency of copper is uncommon because it is abundant trace element, but copper deficiency is associated with high diet fructose consumption [36]. In agreement with our finding Latinen et al and Vanderkooy et al reported stature was not correlated with serum zinc in adolescents with subclinical condition and serum copper inversely associated with stature [23, 37] Band Greger and colleagues in 1978 did not find significant correlation between serum zinc and stature in adolescents girls [38] while several study found strong association between serum zinc and copper with height or weight [39]. Yazbeck et al compare dietary and plasma zinc in short stature and healthy children which observed no difference among case ad control [40]

4.5 Trace elements and lipid metabolism

Several studies have investigated the association between lipid profiles, as a CVDs risk factor, among adolescents [21]. Several enzymes participating in lipid metabolism require copper as a cofactor and copper deficiency has been reported to reduce the activity of cholesterol acyl transferase[41].

Laitinen and colleagues observed lower serum copper levels were associated with higher levels of cholesterol and triglyceride in adolescents [42]. However there is some evidence for there being no relationship between serum zinc and copper concentrations in hyperlipidemia; although zinc and copper supplementation was reported to improve some aspects of lipid metabolism [43]. An increased serum copper was observed in patients with severe coronary heart disease although it may be a consequence rather than cause [44].

Conclusion:

Serum zinc and copper of participants showed that the prevalence of zinc and copper deficiencies in this population in northeastern Iran is low compared to previous reports in other Iranian populations. There may be regional differences in zinc and copper status. We have found an inverse relationship between serum copper and serum TC and LDL in female adolescents. In respect to conflict result of trace element and multifactorial condition of stature no conclusions about stature can be declare and more investigation are required in this area.

Limitations

It should be noted that we assessed non-ceruloplasmin copper and non-albumin zinc in the serum while these trace elements status are strictly related to their carrier and total amounts of these elements in the body might have affected by some unknown factors.

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Table 1. Demographic, anthropometric and biochemical parameters of for all participants

Variables	Mean±SD/Median(IQR)
Age (y)	15.07±1.52
Weight (Kg)	54.55±11.86
Height (Cm)	158.18±6.26
BMI(kg/m^2)	21.76±4.24
SBP (mmHg)	101.6±12.6
DBP (mmHg)	67.66±10.09
FBG mmol/l)	4.86±0.58
Cholesterol (mmol/l)	4.2±0.71
Triglyceride (mmol/l)	0.85(0.66-1.14)
HDL (mmol/l)	0.95±04
LDL (mmol/l)	2.57±0.6
Zinc (µmol/l)	14.61±2.71
Dietary Zinc (mg/day)	7.91±3.15
Cu (µmol/l)	19.48±8.01
Dietary Cu (mg/day)	1.53±0.86

Values are expressed as mean±SD for variables with normal distribution, and median and interquartile range for Triglyceride as a non-normally distributed variable. Data are presented as mean (SD) or inter quartile range. Zinc (Zn), copper (Cu), FBG (fasting blood glucose), LDL (Low density lipoprotein), HDL (high density lipoprotein), BMI (body mass index), SBP (systolic blood pressure), DBP (Diastolic blood pressure)

Table 2. Serum Trace Elements and intake in regard to lipid profile and blood pressure

		Serum Zinc(µmol/L)	Serum Copper(µmol/L)	Zinc intake(mg/day)	Copper intake(mg/day)
Triglyceride (mg/dl)	< 1.7	14.63±2.7	19.65±8.11	7.72±3.24	1.52±0.9
	≥ 1.7	14.71±3.2	19.94±6.33	6.03±2.94	1.47±0.94
p-value		0.2	0.4	0.3	0.8
TC(mg/dl)	< 5.2	14.61±2.74	19.96±8.02	7.67±3.22	1.51±0.88
	≥ 5.2	14.87±7.76	16.09±7.6	8.81±3.26	1.6±1.03
p-value		0.9	0.03	0.7	0.5
LDL(mg/dl)	< 3.4	14.73±2.71	19.93±7.9	7.68±3.26	1.52±0.89
	≥ 3.4	14.59±2.5	16.84±7.48	8.77±2.9	1.42±0.9
p-value		0.6	0.017	0.8	0.4
HDL(mg/dl)	≥ 1.3	14.63±2.63	18.81±7.44	7.49±2.97	1.5±0.88
	< 1.3	14.7±2.7	20.12±8.44	7.8±3.33	1.52±0.91
p-value		0.8	0.4	0.3	0.8
SBP (mmHg)	< 130	14.64±2.7	19.54±8.09	7.69±3.18	1.53±0.9
	≥ 130	14.57±3.25	19.54±7.89	8.82±3.1	1.4±0.78
p-value		0.6	0.4	0.8	0.4
DBP (mmHg)	< 85	14.63±2. 7	19.54±8.15	7.67±3.18	1.52±0.9
	≥ 85	14.81±6.7	19.68±6.1	8.1±3.29	1.65±0.9
p-value		0.3	0.1	0.5	0.4

Data are presented as mean (SD). Student t-test was done to compares the means of two groups. Difference between groups was present ($p<0.05$).TC (Total cholesterol), LDL (Low density lipoprotein), HDL (high density lipoprotein), SBP (systolic blood pressure), DBP (Diastolic blood pressure).

Table3. Serum Trace Elements and intake in regard to anthropometric measurements

		Serum Zinc (µmol/L)	Serum Copper (µmol/L)	Zinc intake (mg/day)	Copper intake (mg/day)
Adiposity	Under weight (<5%)	15.6±3.55	17.27±6.67	8.35±2.66	1.76±0.97
	Normal (5-85%)	14.67±2.68	19.57±8.1	7.71±3.26	1.53±0.89
	Over weight (85-95%)	14.5±2.63	19.37±7.91	7.83±2.69	1.46±0.71
	Obese (>95%)	14.26±3.39	18.82±7.36	7.1±3.15	1.63±1.27
	p-value	0.55	0.57	0.7	0.8
Height	< 149 (< 5%)	14.51±2.55	14.95±4.42	8.43±3.02	1.88±1.21
	149-168 (5-95%)	14.62±2.76	19.55±8.11	7.89±3.14	1.52±0.8
	> 168 (> 95%)	15.41±1.96	22.215±7.7217[a]	8.17±3.72	1.61±1.41
	p-value	0.51	0.026	0.7	0.1
Age	12-14 (y)	14.72±2.69	19.31±7.76	8±3.06	1.56±0.76
	14-16 (y)	14.5±2.82	19.62±8.47	7.06±3.1	1.49±0.93
	> 16 (y)	14.8781±2.5777	19.32±7.62	7.24±3.27	1.53±0.98
	p-value	0.5	0.6	0.1	0.7

ANOVA test was conducted to compare the effect of age, height and adiposity on serum zinc and copper and their intakes. a: 5-95^{th} percentile versus 5^{th} percentile and 95^{th} percentile versus 5^{th} percentile. The CDC growth chart for children and teens of the same age and sex was used to measure underweight, normal, overweight and obese subjects. Underweight is defined as a BMI for age and sex below 5^{th} percentile, normal between 5^{th} and 85^{th} percentile , overweight is defined as a BMI at or above 85^{th} percentile and below the 95^{th} percentile, obesity is defined as a BMI at or above 95^{th} percentile

The CDC growth chart for children and teens of the same age and sex was used to measure height.

Table4. Serum Trace Elements in regard to cardiovascular risk factors

	Serum Zinc(µmol/L)			Serum Copper (µmol/L)		
	Low (< 8.3)	Normal (8.3-18.8)	High (> 18.8)	Low (< 10.2)	Normal (10.2-31.5)	High (> 31.5)
FBG (mg/dl)	5.15±0.68	4.85±0.54	5.04±0.74	4.94±0.89	4.85±0.56	4.88±0.54
		0.15			0.019	
Cholesterol (mg/dl)	4.32±1.02	4.02±0.7	4.26±0.82	4.45±0.7	4.2±0.7	3.92±0.7[a]
		0.8			0.011	
TG (mg/dl)	0.96(0.62-1.15)	0.85(0.66-1.14)	1(0.82-1.26)	0.87(0.67. -1.15)	0.87(0.66-1.15)	0.77(0.61-1.26)
		0.14			0.3	
HDL (mg/dl)	1.3±0.38	1.21±0.22	1.19±0.16	1.24±0.19	1.2±0.23	1.16±0.17
	0.7				0.5	
LDL (mg/dl)	2.82±0.55	2.54±0.6	2.58±0.68	2.76±0.62	2.58±0.59	2.33±0.55
		0.4			0.3	
SBP (mmHg)	100.54±17.15	99.43±23.61	100.1±26.52	100.68±14.64	101.37±12.41	99.77±13.12
		0.2			0.5	
DBP (mmHg)	64.22±9.32	67.37±9.91	65.9±11.41	66.47±9.44	67.03±10.78	68.13±9.52[a]
		0.5			0.022	

ANOVA test was conducted to compare serum trace elements status in regard to fasting blood glucose (FBG), cholesterol, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), systolic blood pressure (SBP) and diastolic blood pressure (DBP). The reference low and high end-point values were the 2.5th and 97.5th percentiles.

b: normal versus high serum elements concentration

Table 5. Dietary assessment and trace elements status

	Serum Zinc(µmol/L)			Serum Copper(µmol/L)		
	Low (< 8.3)	Normal (8.3-18.8)	High (> 18.8)	Low (< 10.2)	Normal (10.2-31.5)	High (> 31.5)
Energy (Kcal)	1783.23±380.85	1984.75±633.24	2109.32±519.24	1951.7±599.84	2012.89±601.94	1691.7±624.519
p-value	0.51			0.4		
Protein(g)	63.51±23.53	62.18±22.49	70.02±26.03	62.43±22.43	62.89±22.79	56.96±25.96
p-value	0.56					
Carbohydrate(g)	204.57±63.17	227.17±82.72	270.21±100.35	222.11±71.15	229.9±83.67	211.73±82.05
p-value	0.18			0.09		
Fiber(g)	13.77±3.32	20.92±10.19	24.35±15.53	21.23±9.6	20.73±10.25	21.69±11.87
p-value	0.066			0.24		
Total Fat(g)	84.68±22.37	99.13±41.02	90.76±43.32	96.46±32.47	100.02±39.67	85.88±57.77
p-value	0.18			0.16		

ANOVA test was conducted to compare serum trace elements status in regard to energy, protein, carbohydrate, fat and fiber. The reference low and high end-point values were the 2.5^{th} and 97.5^{th} percentiles.

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