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Serum Copper, Zinc and Copper/Zinc Ratio and their Relationship to Age and Growth Status in Yemeni Adolescent Girls

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تقييم مستويات النحاس والزنك ونسبة النحاس إلى الزنك في مصل المراهقات اليمنيات ودراسة العلاقة بين مستويات هذه الأملاح في المصل ومقاييس النمو

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الملخص: الهدف: تعتبر هذه أول دراسة في اليمن تعني بقياس مستويات النحاس والزنك في مصل المراهقات اليمنيات وتهدف إلى تقييم العلاقة بين مستويات هذه الأملاح في المصل وبين الفئات العمرية المختلفة والمقاييس الجسدية عند البنات. **الطريقة:** بدأ العمل في أبريل 2007 حيث تم إجراء التحاليل المخبرية في مديريه الوحدة في العاصمة صنعاء. تم اختيار 114 مراهقة باستخدام العينة الطبقية المنهجية في مدرسه اختيرت عشوائيا من بين مدارس البنات الحكومية الموجودة في تلك المديرية. تم اخذ عينات دم لإجراء الفحوصات المخبريه اللازمة. كما تم قياس أطوال وأوزان البنات. **النتائج:** أظهرت النتائج إن متوسط ± انحراف معياري مستوى النحاس والزنك ونسبة النحاس / الزنك في المصل عند كل البنات هو **كالآتي:** 3.31±17.47مكرومول/لتر، 1.04±12.24 مكرومول/لتر ، 0.31±1.44 على التوالي. كان معدل انتشار نقص النحاس 4.4% بينما كان معدل نقص النحاس 2.6%. من جهة أخرى كانت مستويات الزنك هامشية في 96.5% من البنات ، ومعدل انتشار انخفاض الزنك 3.5%. كانت مستويات النحاس عالية بصورة معتدة إحصائيا (p=0.007) بينما مستويات الزنك منخفضة بصورة معتدة إحصائيا (p=0.003) في الفئة العمرية من 10-12 سنة عن غيرها من الفئات العمرية. كانت العلاقة بين الطول ومستويات النحاس سلبية (p=0.01) والعلاقة بين الطول ومستويات الزنك إيجابية (p=0.008). **الخلاصة:** أظهرت الدراسة أن مستوى الزنك منخفض لدى البنات اليمنيات ، وأن لدى الفئة العمرية من 10 – 12 سنة أدنى مستوى من بقية الفئات العمرية. هذا ينذر بالتأثيرات السلبية على الصحة خاصة إذا ما علمنا أن هناك زيادة في حاجة الجسم للزنك خلال فترة المراهقة .

مفتاح الكلمات: نحاس ، زنك ، نسبه النحاس/ الزنك ، النمو ، البنات المراهقات ، اليمن.

ABSTRACT ***Objectives:*** As no previous study has evaluated copper and zinc status in adolescent Yemeni girls, the purpose of this study was to measure their serum levels of zinc and copper and to examine the relationship between the serum levels of these two trace minerals with age, and anthropometric parameters. ***Methods:*** The study was conducted in April 2007 in Alwehda district in the municipality of Sana'a, Yemen. One hundred and fourteen adolescent girls were selected using systematic stratified sampling from a representative school which was randomly selected. Anthropometric indices were measured and blood samples were collected for biochemical analysis. ***Results:*** The mean ±SD for copper, zinc, and copper/zinc ratio for the entire cohort were 17.47±3.31 µmol/L, 12.24±1.04 µmol/L, and 1.44±0.31, respectively. The prevalence of hypocuprimea was 4.4% and hypercuprimea was 2.6%. The levels of zinc were marginal in 96.5% of the girls and the prevalence of hypozincimea was 3.5%. The levels of copper were significantly higher (p = 0.007) and the levels of zinc were significantly lower (p = 0.003) in the 10-12 yrs girls than in other age groups. Height showed significant negative correlation with the levels of copper (p = 0.01) and significant positive correlation with the levels of zinc (p = 0.008). ***Conclusion:*** The results revealed that the Yemeni girls had marginal serum zinc levels, and 10-12 yrs girls had significantly lower zinc levels than other age groups. This provides a warning of consequent negative health effects since the physiological requirements for zinc are high in adolescence.

Key words: Copper; Zinc; Copper/zinc ratio; Growth; Adolescent girls; Yemen.

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Advances in Knowledge

- This is the first study in Yemen assessing the serum levels of zinc and copper in adolescent Yemeni girls.
- The Yemeni girls had adequate serum levels of copper, but marginal serum levels of zinc.
- A significant correlation was found between the levels of the two trace minerals in relation to height and age categories.

Applications to Patient Care

- The reported results call for more attention to the girls' dietary zinc intakes because inadequate diet during adolescence can substantially delay sexual maturation and growth.
- Special care should be given to girls aged 10-12 years because the results show a significant increase in the levels of copper and a significant decrease in the levels of zinc compared to other age categories. This is critical in this stage of life during which a growth spurt begins.
- Routine screening for these trace minerals should be undertaken by paediatricians in Yemen.

Adolescence is a period of intense physical growth and evidence from supplementation trials suggests that marginal zinc status may be common in adolescents and limit skeletal growth.[1] Zinc and copper are essential trace elements involved in adolescent growth and development. [2] Both copper and zinc have diverse physiological roles and the interaction between them was considered to be mutually antagonistic.

Zinc is required for the optimum function of as many as 300 enzymes.[3] These metalloenzymes are involved in the metabolism of proteins, nucleic acids carbohydrates and lipids. They also influence gene transcription.[4] Therefore, zinc is vital for growth and development,[5] sexual maturation and reproduction,[6] dark vision adaptation, olfactory and gustatory activity,[7] insulin storage and release,[8, 9] and for a variety of host immune defense,[10] among other things. Zinc deficiency can result in growth retardation, immune dysfunction, increased incidence of infections, hypogonadism, oligospermia, anorexia, diarrhoea, weight loss, delayed wound healing, fetal neural tube defects, increased risk of abortion, alopecia, decreased ethanol clearance, mental lethargy and skin changes.[11-13] Zinc deficiency is usually nutritional, but can also be associated with malabsorption, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses

Copper is necessary for growth development and maintenance of bone, connective tissue, the brain, the heart, and many other body organs. It is involved in the formation of red blood cells, the absorption and utilisation of iron, and the synthesis and release of life-sustaining protein and enzymes. Copper stimulates the immune system to fight infections, [14-17]repairs tissues and promotes healing. Copper also helps to neutralize 'free-radicals' which can cause severe damage to cells.[18] Deficiency in humans is rare, but it does occur under certain circumstances such as high intake of zinc or iron, increased requirement induced by rapid growth, and increased copper losses or decreased copper intake.[19] In recent years, nutritionists have been more concerned about copper toxicity than copper deficiency. Some experts believe that elevated copper levels, especially when zinc levels are also low, may be a contributing factor in many medical conditions including schizophrenia, hypertension, stuttering, autism, fatigue, muscle and joint pain, headaches, childhood hyperactivity, depression, insomnia, senility, and premenstrual syndrome.[20, 21]

Studies have shown that zinc-induced alteration in other essential metals, especially copper, [22-25] is responsible for the production of biological effects. A decreased plasma zinc level and increased plasma copper level have been reported in pregnancy, acute infections, malignancy, cardiovascular disease, renal disease, schizophrenia, and certain endocrine diseases such as acromegaly and Addison's disease. Therefore, the copper/zinc ratio is clinically more important than the concentration of each metal separately.

As shown above, it is important to assess the serum levels of copper and zinc, particularly in adolescents. The enhanced growth during adolescence makes the

Table 1: ***Descriptive data of the general characteristics of the Yemeni adolescent girls in the Alwehda district sample***

		Number of girls (n)	Percent (%)
Age (years)			
	10-12	34	30.4
	13-15	38	33.9
	16-19	40	35.7
	Total	112[a]	100.0
Stature-for-age percentile			
	< 3rd	21	19.3
	3rd	10	9.1
	5th- 10th	44	40.4
	25th- 75th*	33	30.3
	> 75th	1	0.9
	Total	110[b]	100.0
BMI-for-age percentile			
	<5th	16	14.8
	5th- <85th *	85	78.8
	85th- <95th	4	3.7
	>= 95th	3	2.8
	Total	109[c]	100.0
Copper (µmol/L)			
Entire cohort	<12.56	5	4.4
	12.56-29.83**	106	93.0
	>24.34	3	2.6
	Total	114	100.0
10-12yrs	<12.56	0	0
	12.56-29.8*	34	100.0
	>29.83	0	0
	Total	34	100.0
13-19yrs	<12.56	5	6.4
	12.56-24.34*	72	92.3
	>24.34	1	1.3
	Total	78[a]	100.0
Zinc (µmol/L)			
	<10.7	4	3.5
	10.7-22.9*	110	96.5
	>22.9	0	0
	Total	114	100.0

*International reference range
**Extended normal reference range to include pre-adolescence reference range
[a]Two girls did not know their exact date of birth
[b]Five girls did not respond when they were called for weight measurement.
[c]Four girls were absent during height measurement

Table 2: ***The mean values and ranges of copper, zinc, and copper/zinc and zinc/copper ratios for the entire cohort (n = 114) of Yemeni adolescent girls in Alwehda district***

	Mean	Std. Deviation	Minimum	Maximum
Copper (µmol/L)	17.47	3.31	9.02	29.96
Zinc (µmol/L)	12.24	1.04	10.26	16.44
Copper/zinc	1.44	0.31	0.65	2.67
Zinc/copper	0.73	0.15	0.37	1.53

requirement of these minerals of paramount importance. The present study was designed to determine the serum levels of copper and zinc in a randomly selected sample of Yemeni adolescent girls and to study associations between these minerals and some anthropometric indices.

METHODS

The study area was the Alwehda district, one of ten districts that exist in the capital city of the Republic of Yemen, Sana'a. The study subjects consisted of 114 apparently healthy girls aged 10-19 years. They were students in grades 4th-12th in a representative public school, randomly selected from a total of 5 public schools for girls that exist in that area. Twenty girls were chosen from each class using the school records by systematic sampling technique. From the 180 students selected, girls who were not in the age range of 10-19 years old were excluded. Girls with normal blood chemistry and haematology were included. Girls using vitamins and minerals supplementations and girls suffering from chronic diseases were excluded. The purpose of the study was explained to the school administration and the parents of the students, and girls who did not obtain parental written consent were also excluded. Finally, 114 apparently healthy adolescent school girls were enrolled in this study. The study was approved by the Research and Ethics Committee at the Sana'a University and the ethical clearance was obtained before the study was started.

A face to face interview was conducted to record information regarding age, class level, health problems, any supplements or medications used, and menarcheal status. Anthropometric measurements were taken in school. The students were asked to remove heavy clothing and shoes. Weight was taking in kilograms using and electronic scale and height was measured using a standiometer. Height and weight measurements were compared to the international reference values of the National Center for Health Statistics/Center for Disease Control and prevention (NCHS/CDC). Body mass index (BMI) was calculated by dividing the weight (kg) by square of height (m^2). Under weight was defined as the CDC BMI-for-age percentile <5. Overweight was defined as BMI-for-age percentile ≥95 and those which fell between 85th and <95th percentiles were considered to be at risk of overweight. Stunting was defined as the CDC stature-for-age <3rd percentile, short stature <5th percentile, and long stature >75th percentile.[26]

In April 2007, the girls were taken to Althawra General Hospital by bus in the early morning in groups of 15-30 girls each day for 5 days. Fasting blood samples (approx. 5ml) were collected in the morning between 9 and 11am at the laboratory department. Blood samples were drawn by venipuncture into vacutainer tubes and the samples were immediately centrifuged, after clotting, in patches of five clotted blood samples. The serum obtained was kept in freeze at -20 C until it was analysed 4 months later for serum copper and zinc. The metals zinc and copper were analysed by the direct colorimetric method using kits from Quimica Clinica Aplicada S.A. and analysed by the Hitachi 912 analyser. The colour reagent for copper was 3,5-DiBr-PAESA stain in acid solution. Copper is released from the ceruloplasmin protein and reduced; the cuprous ion forms a coloured complex with the stain and is measured photometrically at 582nm. The zinc ions of the sample produce a red coloured complex with 2-(5-Brom-2-pridylazo)-5-[-N-propyle-N-(3-sulfopropyl) amino]-phenol in alkaline solution, and the colour intensity was measured at 560nm. The normal reference values for the instruments for women were 80-155 µg/dl for copper and 68-115 µg/dl for zinc. The values obtained in µg/dl were converted to the international unit (µmol/L) using conversion factors (x 0.157 and x 0.153 for copper and zinc respectively).

Table 3: ***The copper, zinc and cu/zn ratio of the Yemeni adolescent girls in Alwehda district according to the age category***

	Age (years)	N	Mean	SD	Minimum	Maximum	*p*-value [a]
Copper (μmol/L)	10-12	34	18.66	3.51	14.16	29.96	
	13-15	38	17.65	3.30	9.02	26.48	
	16-19	40	16.34	2.87	11.71	23.83	
	Total	112[b]	17.49	3.33	9.02	29.96	
							0.002
Zinc (μmol/L)	10-12	34	11.80	0.78	10.26	13.85	
	13-15	38	12.61	1.19	11.06	16.44	
	16-19	40	12.25	0.96	10.54	15.07	
	Total	112[b]	12.24	1.05	10.26	16.44	
							0.094
Copper/zinc Ratio	10-12	34	1.59	0.34	1.10	2.67	
	13-15	38	1.41	0.29	0.65	2.22	
	16-19	40	1.34	0.24	0.97	1.81	
	Total	112[b]	1.44	0.30	0.65	2.67	
							<0.001
Zinc/copper Ratio	10-12	34	0.65	.12	.37	.91	
	13-15	38	0.74	.17	.45	1.53	
	16-19	40	0.77	.13	.55	1.04	
	Total	112[b]	0.72	.15	.37	1.53	
							0.001

[a]Based on Pearson's correlation analysis test
[b]Two girls were not included because they did not know their exact date of birth

Data were analysed using the Statistical Package for the Social Science (SPSS), Version 15. A Kolmogorov-Smirov test for normality was performed and the means and standard deviations and prevalence were obtained by descriptive statistics. Data were analysed using the one way analysis of variance (ANOVA) followed by Tukey's test to assess differences of continuous variable between two or more groups.

Pearson's r coefficient was used to assess the correlation between two continuous variables. Statistical significance was assigned for *p* values less than 0.05.

RESULTS

The study subjects consist of 114 apparently healthy Yemeni adolescent girls. The mean age was 14.42yrs ± 2.71 and ranged from 10-19 years. The general characteristics of the girls according to age, anthropometric measurements, copper, and zinc concentrations are presented in Table 1.

Table 1 shows the percentage of girls with abnormal characteristics as compared to the international reference values. The results revealed that 96.5% of the girls had marginal serum levels of zinc and 68.8% of the girls were below the normal stature-for-age percentiles, of which 19.3% were stunted. Because the copper international normal reference range for pre-adolescent girls aged 10-12yrs was different from that for the older adolescent girls, separate descriptive data for copper were added in Table 1, and the normal reference value for the entire cohort was extended to cover both ranges. The mean levels and the ranges for copper, zinc, copper/zinc ratio, and zinc/copper ratio for the girls residing in Alwehda district are shown in Table 2.

For the entire cohort, the mean value for serum copper was normal (17.47 ± 3.31 μmol/L; 111.32μg/dL), and the mean value for serum zinc was at the lower edge of the normal value (12.24 ± 1.04μmol/L; 80.01 μg/dL), while the mean value for serum copper/zinc ratio was 1.44 ± 0.31 ranging from 0.65 to 2.67.

COPPER, ZINC, AND COPPER/ZINC RATIO

Table 4: ***Correlation (Pearson's r) between copper, zinc, cu/zn and anthropometric indices in the Yemeni adolescent girls.***

Anthropometric indices	Copper		Zinc		Copper/zinc Ratio	
	r	*p*-value	r	*p*-value	R	*p*-value
Height	-0.245(*)	0.010	0.250(**)	0.008	-0.325(**)	<0.001
Weight	-0.072	NS	0.167	NS	-0.143	NS
BMI-for-age percentile	0.001	NS	0.093	NS	-0.050	NS
Stature-for-age percentile	-0.032	NS	0.030	NS	-0.038	NS

*Correlation is significant at the 0.01 level (2-tailed).
**Correlation is significant at the 0.05 level (2-tailed).

levels in association with age categories

When the girls were divided into three age categories, there was a statistically significant negative correlation between the age and the levels of copper (r = –0.284, *p* = 0.002), and the levels of copper tended to decrease with increasing age. Further analysis showed that copper levels were significantly lower in the 16-19 year old girls than the 10-12 year olds (*p* = 0.007) and there were no significant differences in the levels of copper between the 13-15 year olds as compared to the 10-12 year olds (*p* = 0.383) and the 16-19 year olds (*p* = 0.179).

Bivariate correlation analysis showed no significant correlation between the levels of zinc and the age of the girls (r = 0.159, *p* = 0.094). Further investigations with one-way analysis of variance (ANOVA) revealed statistically significant differences in the levels of zinc between the 10-12 year old girls and the 13-15 year olds (0.003), but no significant differences were found among the other age groups.

Analysis with one-way ANOVA also indicated statistically significant differences in the levels of copper/zinc between 10-12 year olds and the 13-15 year old girl (*p* = 0.024) and between the 10-12 year olds and the 16-19 year old girls (0.001). Copper/zinc ratios tended to decrease with increasing age. The mean values for copper, zinc, copper/zinc, and zinc/copper ratio according to the age category are shown in Table 3.

copper, zinc, and copper/zinc levels in association with anthropometric variables

A statistically significant negative correlation was found between the levels of copper and the height of the girls (r = -0.245, *p* = 0.010), and there was a statistically significant positive correlation between the serum levels of zinc and the height of the girls (r = 0.250, *p* = 0.008). No significant correlation was found between the levels of zinc or copper and the other anthropometric variables [Table 4].

Despite the findings that copper and zinc levels did not correlate significantly with stature-for-age percentiles, the results revealed a decreasing trend in the levels of copper and an increasing trend in the levels of zinc with increasing stature-for-age percentiles [Table 5]. Short girls had the lowest levels of zinc and the highest levels of copper compared to normal and tall

Table 5: ***Mean values for serum copper, zinc and copper/zinc ratio of the Yemeni adolescent girls in Alwehda district according to stature-for-age category***

Stature-for-age percentile category	Mean		
	Copper (μmol/L)	Zinc (μmol/L)	Copper/zinc Ratio
<25th (Short)	17.61	12.22	1.45
25th-75th (Normal)	17.47	12.26	1.43
>75th (Tall)	16.33	12.53	1.30
Total	17.56	12.24	1.44

Table 6: ***Mean values for serum copper, zinc and copper/zinc ratio of the Yemeni adolescent girls in Alwehda district according to BMI-for-age category***

BM-for-age percentile category	Mean		
	Copper (µmol/L)	Zinc (µmol/L)	Copper/zinc Ratio
<5th (underweight)	17.92	12.27	1.47
5th-<85th (normal)	17.39	12.22	1.43
85th-<95th (at risk of overweight)	18.62	12.38	1.51
>=95th (overweight)	18.50	12.39	1.48
Total	17.55	12.24	1.44

girls.

Furthermore, girls who were above the normal BMI-for-age percentile reference range had higher serum levels of both copper and zinc compared to girls who were below the normal reference range [Table 6], although, this trend was not statistically significant.

copper zinc interaction

There was a negative association between the levels of the copper and zinc, However, this was not statistically significant (r = – 0.018, p = 0.849).

DISCUSSION

Copper and zinc are essential trace elements involved in adolescent growth and development. They have diverse physiological roles and both are particularly related to linear physical growth. Nevertheless, copper and zinc status have not been assessed in Yemeni adolescents. The first purpose of this study was to measure the levels of zinc and copper in the serum of randomly selected Yemeni adolescent girls, and the second purpose was to study the association between the levels of copper and zinc in relation to age and anthropometric indices.

The results revealed that Yemeni adolescent girls had normal serum levels of copper (17.48µmol/L; 111.32µg/dL), but marginal serum levels of zinc (12.24µmol/L; 80.01µg/dL), as compared to the international reference ranges. Comparisons with other populations showed that the obtained mean values for copper and zinc were lower than those reported for the Kuwaiti population (24.9µmol/L; 158.6µg/dl, and 15.5µmol/L; 101.31µg/dl for copper and zinc respectively) [27] and were close to those reported for the Greek population (18.13µmol/L, 115.46 µg/dl; and 11.79µmol/L, 77.11 µg/dl for copper and zinc respectively).[28]

The results also revealed that 96.5% of the girls had marginal serum levels of zinc, which is critical at this stage of life, when the requirements for zinc are high to meet the demands for increased physical growth. These results demanded nutritional assessments because common causes of low zinc levels are usually nutritional due to inadequate dietary zinc intakes or a diet high in fibre and phytate which reduces zinc absorption.

A high percentage (68.8%) of the girls was found to be below the normal stature-for-age percentile of which approximately 20% were stunted. These findings call for studying the effect of low serum zinc levels on some anthropometric indices, since zinc was particularly related to physical growth impairment. When relationships between the levels of zinc and the height and stature-for-age percentiles were investigated, this study revealed a statistically significant effect of zinc on the height of the girls. In fact, girls who were short had the lowest zinc levels and the highest copper levels and the levels of zinc were increasing with increased stature-for-age percentiles. However, this trend was not significant probably because the study sample size was not large enough to show any existing significance.

The relationship between the levels of copper and zinc and the age of the girls was also investigated and results showed a statistically significant correlation between them. The levels of zinc were significantly lower and the levels of copper were significantly higher in girls aged 10-12 years than the other age categories. This might cause some concerns since the adolescent growth spurt begins in girls at age 10 or 11 and reaches its peak at age 12. This intensive growth period (between 10-12 years) is characterised by a dramatic increase in height and an immense demand for zinc which can exhaust body zinc. The low zinc levels can substantially delay sexual maturation and growth and, therefore, more care should be given to the Yemeni girls in this age group.

The mean value for the copper/zinc ratio was 1.44, ranging from 0.65 to 2.67 and the results revealed that copper/zinc ratio tended to be significantly higher in girls at the pre-menarcheal age (10-12 years), than in the older post-menarcheal girls (13-19 years). These findings were in accordance with what was previously reported.[27, 29] The mean age for the first onset of menarche in the studied girls was found to be 13 years, and puberty could affect the Cu/Zn imbalance. Copper levels are sensitive to estrogen levels which increase during puberty while zinc is depleted by the rapid cell divisions during growth at puberty.[29]

CONCLUSION

A high percentage of marginal zinc levels is considered alarming in adolescence age, especially for girls aged 10-12 years, a period where the growth spurt demands high levels of zinc. There are some concerns, at the present time, about the consequences of marginal zinc status on the health and the linear physical growth of the studied girls. In the near future, there should be some concerns about the health of these girls as pregnant women and the health of their offspring since, traditionally, a high percentage of girls get married after they finish high school, if not before.

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