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Serum and Hair Levels of Zinc, Selenium, Iron, and Copper in Children with Iron-Deficiency Anemia

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ABSTRACT

In the present study, the serum and hair levels of zinc, selenium, and copper were determined in children with iron-deficiency anemia (IDA). A total of 52 anemic children aged 1–4 yr constituted the study group. Forty-six healthy children acted as controls. The copper and zinc levels were measured with an atomic absorption spectrophometer. Serum and hair selenium was determined by a spectroflourometric method. The serum zinc and selenium concentrations in the IDA group were found to be significantly lower and serum copper significantly higher than those in the controls ($p < 0.05$). Lower iron, zinc, and selenium concentrations ($p < 0.001$) but not copper were found in hair ($p > 0.05$).

Index Entries: Iron deficiency anemia; zinc; selenium; copper; hair.

INTRODUCTION

Iron, zinc, selenium, and copper are some of the inorganic elements that are necessary for normal growth and sustained biological activities *(1)*. Iron deficiency is probably the most common deficiency in children both in developing and industrialized nations *(2–6)*.

Prasad et al. first reported zinc deficiency in humans in 1963 *(7)*. The combined deficiencies of zinc and iron cause mental lethargy, hepatosplenomegaly, and growth retardation in geophagous children are referred

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to as "Prasad syndrome" in the literature. Other studies have shown that the serum zinc and copper levels were significantly altered in children with iron deficiency, in which zinc protoporfirin is produced instead of heme and is consequently increased in erythrocytes. Measuring zinc protoporfirin along with ferritin and hemoglobin can be used to assess the degree of iron deficiency *(8–10).*

Copper deficiency can also impair the absorption of iron because ceruloplasmin is involved in the oxidation of Fe(II) to Fe(III) *(11).* Selenium is an essential trace element that works as a cell membrane protector against lipid peroxidation *(12,13).* It is central to enzymes such as glutathione peroxidase (GSH-Px), an enzyme that converts hydrogen peroxide into water, thus preventing formation of the oxidized form of hemoglobin *(14,15).*

The purpose of this study was to determine the serum and hair zinc, selenium, and copper levels and its relation to hematological parameters in children with iron-deficiency anemia (IDA).

METHODS

Fifty-two anemic and 46 healthy children, all between 1 and 4 yr of age participated in this study. They were under medical care at a Health Policlinic in Elazığ, Turkey. All had been nursed by their mothers for at least 4 mo after birth. Children having hemoglobin concentrations under two standard deviations of the mean hemoglobin levels and ferritin values below 10 ng/mL and/or transferrin saturation below 12% were considered as anemic. Children who were malnourished or had chronic diarrhea or intestinal parasites were excluded from the study. Also excluded were children who had an infectious disease as recent as 2 wk before the study. Fasting blood samples were collected from all subjects between 8:00 and 10:00 AM. The serum was separated by centrifugation and stored at –30°C until needed for trace element analysis.

Hemoglobin, hematocrit, white blood cells, and red cell morphology (MCV, MCH, MCHC, and RDW, respectively), were determined with an automatic cell counter following standard clinical protocols. Serum ferritin levels were determined by the ACS 180 automated immunuassay method. The ratio of serum iron and total-iron-binding capacity (TIBC) or transferrin saturation (serum iron level/TIBC × 100) was also determined.

For the determination of iron, copper, zinc, and selenium in hair the samples were digested in acid using a domestic microwave oven in low-pressure Teflon bombs. The hair samples were washed twice with 1% Triton X-100 and deionized, doubly-distilled water (dd-water) and then dried at 60°C for 24 h. The dry samples were homogenized using an agate homogenizer and stored in polyethylene bottles until analysis. All plastic and glass containers were cleaned by overnight soaking in a 10% nitric acid solution and then rinsed with dd-water.

Table 1
Results of Hematologic Parameters in the Two Groups

	Iron deficiency anemia group	Control group	p
Hb (gr/dl)	9.6±1.1*	12.6±0.9	<0.05
Hct (%)	30.9±2.7	37.0±3.0	<0.05
MCV (μm^3)	63.3±7.2	78.2±5.0	<0.05
Ferritin (ng /ml)	5.8±3.0	33.5±20.0	<0.001
Transferrin saturation (%)	7.3±6.2	19.8±10.8	<0.001

* Mean ± standard deviation.

A sample portion weighing approx 0.5 g was placed into the Teflon vessel and treated with 2 mL concentrated HNO_3, 2 mL $HClO_4$, and 1 mL H_2O_2. The vessel was placed into the microwave oven and twice subjected to radiation for 2 min each at 10%, 25%, and 40% power followed by 5 min at 50% power. After cooling, the solution was transferred into a Teflon beaker, made up to 5 mL with dd-water and kept at 4°C until analysis *(16).*

Hair and serum concentrations of iron, copper, and zinc were determined in an ATI UNICAM 929 atomic absorption spectrophotometer using an air–acetylene flame with deuterium background correction *(17).* Serum and hair selenium was analyzed by Lalonde's flourimetric method using a Perkin-Elmer Model 100 spectrofluorometer *(18).*

The results are given as mean ± SD with 95% confidence intervals. The commercially available SPSS software was used for the statistical evaluation of the data using Student's *t*-test and correlation tests.

RESULTS

Among the 52 children with IDA, 31 (59%) were boys and 21 (41%) were girls. In the control group, there were 46 children, of whom 28 (60%) were boys and 18 (40%) girls. The ages of all children ranged from 1 to 4 yr. The mean age was 1.96 ± 0.76 yr in the IDA group and 2.32 ± 1.07 yr in the controls.

The hematological parameters of both groups are given in Table 1. Children with IDA had a statistically significant lower mean hemoglobin level (9.6 ± 1.1 g/dL) than those in the control group (12.6 ± 0.9 g/dL, $p < 0.05$). Also, serum iron, TIBC, and ferritin values in the anemic children were significantly lower than the healthy controls ($p < 0.001$).

The serum iron concentration was 5.9 ± 3.9 μmol/L in the IDA group and 12.9 ± 5.1 μmol/L in the control group ($p < 0.001$). Serum zinc was 16.0

Table 2
Serum Levels of Trace Elements

Trace element	Iron deficiency anemia group (n=52)	Control group (n=46)	p
Iron (μmol/L)			
Mean ±SD	5.9±3.9	12.9±5.1	<0.001
95% Confidence interval	(4.8-6.9)	(11.9-13.9)	
Zinc (μmol/L)			
Mean ±SD	16.0±5.2	19.1±5.9	<0.05
95% Confidence interval	(16.0-17.4)	(18.0-19.7)	
Copper (μmol/L)			
Mean ±SD	25.1±5.8	21.9±3.8	<0.05
95% Confidence interval	(24.8-26.4)	(21.9-23.1)	
Serum selenium (μmol/L)			
Mean ± SD	0.44±0.10	0.73±0.22	<0.001
95% Confidence interval	(0.42-0.45)	(0.70-0.76)	

± 5.2 μmol/L and 19.1 ± 5.9 μmol/L, respectively ($p < 0.05$). The mean serum copper in the IDA and control groups was 25.1 ± 5.8 μmol/L and 21.9 ± 3.8 μmol/L, respectively ($p < 0.05$). The mean serum selenium concentrations were also significantly lower in the IDA group (0.44 ± 0.10 μmol/L vs 0.73 ± 0.22 μmol/L in the controls; $p < 0.001$). The serum iron, zinc, selenium, and copper levels are shown in Table 2.

The iron, zinc, selenium, and copper levels in hair are shown in Table 3. In hair, the mean iron, zinc, copper, and selenium concentrations were also lower in the IDA group. The difference was significant for Fe, Zn, and Se ($p < 0.001$) but not for Cu ($p > 0.05$).

A statistically significant correlation between the mean serum copper, selenium, and zinc concentrations could not be found; nor could it be found between the measured hematological parameters and the mean serum element concentrations.

DISCUSSION

Iron deficiency is the most common nutritional deficiency among children in the world and it is also the most common reason for childhood anemia *(5,20,21)*. IDA is most common between 6 and 24 mo of age *(20)*. The children included in this study were within this age group. The sex and mean age of both groups were well matched, with no significant difference of their ages ($p > 0.05$).

The mean serum zinc concentration was significantly lower in the IDA group than in the controls ($p < 0.05$). This might indicate that in addi-

Table 3
Hair Levels of Trace Elements

Trace element	Iron deficiency anemia group (n=52)	Control group (n=46)	p
Iron (microg/g)	17.76 ± 3.45*	43.49 ± 6.23	<0.001
Zinc (microg/g)	59.24 ± 9.67	104.14 ± 8.59	<0.001
Selenium (microg/g)	0.61 ± 0.12	1.02 ± 0.14	<0.001
Copper (microg/g)	9.37 ± 1.83	11.59 ± 2.30	NS**

* Mean±standard deviation.
** Not significant.

tion to the iron deficiency, a nutritional deficiency of zinc is possible in IDA. Alternatively, in iron deficiency, zinc protoporfirin production is increased, resulting in a replacement of iron by zinc, leading to lower serum Zn values *(10,22,23).* Similar results have been reported in Russian adult subjects *(24)* and in children from Manisa, Turkey *(9).*

The mean serum copper concentration was higher in the IDA group than in the control group, and the difference was statistically significant ($p < 0.05$). This might be the result of the fact that copper is used insufficiently and accumulates excessively in tissues during iron deficiency. In experimental studies with iron deficiency, changes of some trace elements levels in tissue and increases of the level of some trace elements like copper have been observed *(25,26).* Results of a study conducted by Ece et al. among children were also similar *(9).* On the contrary, in humans with severe copper deficiency, little or no copper-binding ceruloplasmin was found in serum and tissues because of the absence of active ferroxidase, leading to iron accumulation in the liver *(11).*

The mean serum selenium concentration was significantly lower in the IDA group ($p < 0.001$). In another study from Turkey, Yetgin et al. *(27)* found a mean serum selenium concentration of 0.80 ± 0.14 μmol/L in IDA children aged 6 mo to 16 yr vs age-matched controls having 0.95 ± 0.16 μmol/L ($p < 0.001$). McAnulty et al., however, reported that the serum selenium concentration of a low iron stores group were not significantly different from those of the controls *(28).* Results of previous studies on IDA showed decreased levels of GSH-Px activity, which might be useful for evaluating the nutritional selenium status *(27,29,30).* Although GSH-Px is not an iron-containing metalloprotein, its activity has been found decreased in erythrocytes of patients with IDA, suggesting that selenium deficiency might accompany iron deficiency. Moriarty et al. found that serum selenium and GSH-Px levels are decreased in rats with IDA *(31).*

The mean iron, zinc, copper, and selenium concentrations in hair were lower in the IDA cases. Previous studies showed that the concentrations of

zinc and copper in hair were lower in persons with iron deficiency *(32,33).* Hac et al. *(34)* found that low serum selenium might accompany low selenium in hair. There was a direct relationship between the serum and hair concentrations of iron, selenium, and zinc, but no relationship was found between the increased serum and decreased hair copper concentrations. This observation is consistent with previous studies that also showed no relationship between the serum and hair copper levels *(35,36).*

Our results show that the serum and hair zinc, copper, and selenium concentrations were altered in children with IDA. The differences observed might be attributed to relations between iron and different elements at the absorption, transportation, and storage areas *(26).* We suggest that the zinc, selenium, and copper status of children with IDA should be taken into account before and after iron supplementation therapy.

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