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Nutritional and Zinc Levels in Serum and Hair of University Students in Nigde, Turkey

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This research has been designed for the determination of the nutritional status of university students, in particular reference to dietary zinc amount taken. The aim was to find out reflection of dietary zinc to that of serum and hair zinc contents. Forty volunteers' students of Nigde University (24 females and 16 males) participated in the first step of research. Three years later, 17 females and 10 males from this group participated for the second stage of research. Students' nutritional status was evaluated according to their food consumption and physical examination. After analyzing the collected blood and hair samples, the mineral status was also evaluated. At the two steps of research no one had any serious mineral and other nutrients deficiencies. Only one female student having mineral deficiency at the first step of work recovered after the treatment. Regression analysis showed that there was a statistically significant correlation between dietary zinc concentration and hair and serum mineral levels ($r_{diet\text{-}hair} = 0.500$, $n = 40$, $p < 0.01$, $r_{diet\text{-}serum} = 0.470$, $n = 40$, $p < 0.01$).

Key Words: Hair zinc, Serum zinc, Atomic absorption spectrometry, Food consumption.

INTRODUCTION

Zinc is an essential trace element for microorganisms, plants, animals and human. It is a constituent of enzymes involved in most major metabolic pathways. Zinc participates in reactions which involve either the synthesis or degradation of carbohydrates, lipids, proteins and nucleic acids. Zinc is distributed throughout the body with the highest concentrations in the liver, pancreas, kidney and voluntary muscles. Other tissues with high concentrations are eye, prostate, spermatozoa, skin, hair and nails. Relatively large amounts of zinc are deposited in bones, but these stores do not move into rapid equilibrium with the rest of the organism[1-3]. The most prominent sign of zinc deficiency in animals and man are loss of appetite,

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failure grow, skin changes, impaired regeneration of wounds, hypogeusia (decreased taste acuity), mental retardation, hepatosplenomegaly, iron and copper deficiency[2,3]. Zinc deficiency has been demonstrated in man in several circumstances. A clinical syndrome characterized by small stature, hypogonadism, mild anaemia and low plasma Zn occurs in older children and adolescents in poor peasant communities in Iran and elsewhere in the Middle East where the staple diet is unleavened bread, *tanok*. After giving supplements of 120 mg zinc sulphate (410 μmol or 27 mg Zn) daily for several months, puberty developed and growth rates were accelerated. The zinc intake is low and its absorption is impaired by phytate in the unleavened bread. This syndrome occurs in girls as well as youths[4]. Nutritional zinc deficiency develops when diets are either low in zinc or contain high concentration of certain components that complex zinc, thereby making it less bioavailable. Deficiency also occurs when absorption is reduced due to any mucous membrane alteration in intestinal like pathological condition[1,2]. The zinc deficiency problem in Turkey has been reported in some workers[5-7]. Geophagia is prevalent and a common cause of iron and zinc deficiencies in adolescents[8]. The diet of Turkish especially villagers mainly consists of wheat bread rich in phytate, which is known to decrease the availability of iron and zinc[1,2,4,8,9]. Zinc responsive growth failure has also been reported in young children from middle-class homes in the USA, in Denver, Colorado. The children had hypogeusia, poor appetite and hair zinc under 1.1 μmol/g (70 μg/g). Dietary histories showed that they had been eating little meat. The children improved markedly with daily supplements of 0.4-0.8 mg zinc/kg[4]. Some searches have shown that students at universities in general do not have enough knowledge about healthy nutrition rules and/or they also do not consume, enough food because of their low incomes. Insufficient and unbalanced diets can be related to some nutrients deficiency including zinc deficiency[4,10]. For the assessment of zinc nutrition status, hair and serum are employed as biopsy materials[11]. The aim of the present study is to investigate nutritional status and dietary, serum and hair zinc contents of University students in Nigde, Turkey.

EXPERIMENTAL

Forty volunteers' students of Nigde University aged 18-23 years of old (24 females and 16 males) participated in the first step of research. Three years later, 17 females and 10 males from this group participated for the second stage of research. A Physician from Nigde State Hospital examined all the students' health who joined at the first and second steps of research.

Anthropometrics measurements: Anthropometrics measurements were applied for body weight and height by using inflexible measure and sensitive balance, respectively. These values were used for calculation of the body mass index (BMI) as follows:

$$BMI = \text{Body weight (kg)}/\text{height}^2 \text{ (m)}$$

Evaluation of body sculptures was used in WHO standard[12].

Daily energy and nutrient intakes: Food consumption method was applied for the determination of daily energy and nutrient intakes. All the meals were weighed and recorded before eating. This application continued for three consecutive days as two-week days and one weekend day[13]. The food contents of meals were taken from Standard Meal Book[14,15]. The amount of nutrients, in consumed foods were calculated by using Bestuk computer programme[16]. The basal metabolic rates (BMR) of subjects were calculated from following equations[12]:

for females: {[13.3 × W [body weight (kg)] + 334 × H [height (m)] + 35]} kcal/d or [(55.6 W + 1397.4 H + 146)] kJ/d, and

for males: [(15.4 × W - 27 × H + 717)] kcal/d or [(64.4 W-113.0 H + 3000)] kJ/d.

Energy requirements were calculated by using standard factors for medium activity from following equations:

for females: 1.64 × BMR and

for males: 1.78 × BMR[12].

Daily energy intakes were calculated from amounts of protein, carbohydrate and fat by multiplying Atwater factors[17]. Daily dietary intake of energy and nutrients were compared using following equation:

$$[(X^1\text{-}A)/(SD^1/n^{½})]$$

where A is recommended daily allowances (RDA)[18].

Sample collection: The blood samples (10 mL) were collected after overnight starvation from the antecubital vein. After 2 h, serums were separated by centrifugation at 3000 rpm and then were kept in stopped tubes at -20ºC until assayed. Hair samples (after making sure that the hair was not dyed or chemically treated) were cut from the sub-occipital region within the first two cm from the scalp, collected in individual paper bags and stored in a desiccator in the dark at room temperature until required. Serum and hair zinc levels were determined by flame atomic absorption spectrophotometer (Shimadzu AA-6501) using the wet-digestion method, as described elsewhere[6,11,19]. The biochemical parameters were determined according to Nigde State Hospital Biochemistry Department's methods' and standards. Student t-test and regression analysis (Pearson's test) were used for statistical analysis[20].

RESULTS AND DISCUSSION

According to WHO BMI classification, majority of subjects were in standards, except 1 female and 4 males, these five students were found to be mild over weight[12] (Table-1a). Almost similar results were obtained for

the subjects who joined the second step of research. During these three years period no statistically differences between their previous and present anthropometrics measurements have been observed (Table-1b).

TABLE-1a
ANTHROPOMETRICS VALUES OF THE SUBJECTS (n = 40)

Anthropometry	Female (n = 24)		Male (n = 16)	
	X^1	SD^1	X^1	SD^1
Age (year)	18.5	1.0	20.0	1.2
Height (cm)	160.0	0.1	170.0	0.1
Weight (kg)	59.2	7.0	67.3	12.6
BMI	23.0	2.3	22.9	3.5

X^1: Arithmetic mean. SD^1: Standard deviation

TABLE-1b
ANTHROPOMETRICS VALUES OF THE SUBJECTS (n = 27)

Anthr-opometry	First step				Second step				Statistical analysis	
	Female (n = 17)		Male (n = 10)		Female (n = 17)		Male (n = 10)			
	X^1	SD^1	X^1	SD^1	X^1	SD^1	X^1	SD^1	Female	Male
Age (Year)	19.9	1.6	19.1	1.0	22.9	1.6	22.1	1.0	ns	ns
Height (cm)	161.5	6.4	171.9	5.2	161.6	6.3	172.4	5.2	ns	ns
Weight (kg)	57.2	6.7	72.6	13.5	55.9	5.3	76.1	15.9	ns	ns
BMI	22.1	2.8	24.5	3.8	21.5	2.4	25.4	3.8	ns	ns

X^1: Arithmetic mean, SD^1: Standard deviation, ns: non significant

Laboratory test for assessing zinc is classified into two groups as determination of zinc in body fluids and tissue and enzymes involving mineral. Useful tests in the first group are determination of zinc in plasma or serum, hair, urine and others[21]. Table-2a represents serum and hair zinc values of subjects. Even though the mean values of serum and hair mineral levels were higher than normal value that were 50 µg/100 mL for serum and 70 µg/g for hair[9,19], only one subject had lower hair zinc level. This person also had some mineral deficiency symptoms such as diverse forms of skin lesions. It is known that zinc deficiency could cause some skin lesions including acrodermatitis enteropathica and immunological abnormalities[22]. The ranges of mineral serum and hair values are wide. One research done among pre-school children showed this wide range as serum 106 ± 16 µg/dL and hair 152 ± 44 ppm[6]. It has been reported that zinc levels in human hair is influenced by sex, age and hair pigmentation[6,23].

There were still no statistically significant differences ($p > 0.05$) between the two studied groups as regards hair and serum zinc concentrations. However, serum and hair zinc levels of the subjects joined to the second step of research, were not found to be increased after three years (Table-2b). This might be due to some kinds of stress such as exams and unsuitable living conditions of students. However, at this stage none of the subjects showed any mineral deficiency as well.

TABLE-2a
SERUM AND HAIR ZINC LEVELS OF SUBJECTS (n = 40)

Sample	Female (n = 24)		Male (n = 16)	
	X^1	SD^1	X^1	SD^1
Serum (μg/100 mL)	120.3	30.2	131.3	32.6
Hair (μg/g)	122.0	38.1	134.0	47.4

TABLE-2b
SERUM AND HAIR ZINC LEVELS OF SUBJECTS (n = 27)

Anthr-opometry	First step				Second step				Statistical analysis	
	Female (n = 17)		Male (n = 17)		Female (n = 17)		Male (n = 10)			
	X^1	SD^1	X^1	SD^1	X^1	SD^1	X^1	SD^1	Female	Male
Serum (μg/100 mL)	125.3	25.6	116.0	25.6	119.0	16.1	103.9	13.3	ns	ns
Hair (μg/g)	127.3	43.0	117.2	43.0	124.5	23.2	115.6	21.8	ns	ns

According to physicians examination all subjects (except one) had not any clinical health problems and these findings were also confirmed by the biochemical parameters which were found to be in normal levels (Table-3). A subject with skin disorders was zinc supplemented and educated about nutrition. After treatment all the symptoms were disappeared. Chronic zinc deficiency is an important disorder in sickle cell disease which is seen Mediterranean countries including Turkey[24,25]. It has not came across any haematological and mineral disorders in this research.

Daily dietary intake of energy and nutrients of the subjects and statistical results obtained dietary intakings compared with RDA were showed in Table-4. Total energy was obtained as 11.7 % from proteins, 24.9 % from fats and 63.4 % from carbohydrates for female students, 14.7 % from proteins, 28.7 % from fats and 56.6 % from carbohydrates for male students. However, the dietary energy (except one male amount), B_1, B_2, niacin, vitamin C and E, calcium and zinc of subjects were found to be

TABLE-3
SERUM BIOCHEMICAL PARAMETERS OF SUBJECTS (n = 40)

Biochemical parameters	Female (n = 24)		Male (n = 16)		Normal value
	X^1	SD^1	X^1	SD^1	
Leukocytes (K/μL)	6.4	1.3	7.0	2.0	4-10
Erythrocytes (M/μL)	4.7	0.2	5.3	0.5	4-6
Hemotocrite (%)	38.7	2.7	44.2	2.7	35-50
Hemoglobine (g/dL)	13.6	1.1	15.6	1.1	11-17
Total cholesterol (mg/dL)	156.0	22.0	157.0	16.0	120-220
Triglycerides (mg/dL)	86.0	41.0	91.0	41.0	35-135

statistically lower than recommended daily allowance values (Table-4)[18]. On the other hand, dietary vitamin A was higher than RDA value. This might be due to calculation of vitamin A together with β-carotene, which are plant sources pro-vitamin. While intaking of iron was statistically lower among female, but male iron consumption was in normal range. The high RDA level of iron for women (18 mg/day) in Turkey is due to high birth rate. Only one female student out of 24 had low level of haemoglobin and three of them had low level of hematocrite. Calcium consumption of both sexes was statistically lower than RDA. It is well known that calcium is most abundant in body and can be found in bones, teeth, muscle and serum and has a lot of important functions. It is also well known that calcium intaken regularly during life span prevents several chronic diseases such as osteoporoses and teeth decay might develop in the future[26]. Dietary zinc intake of female was lower than RDA but male consumption amount was in normal range. The average zinc content of mixed diets consumed by the American adults has been reported as between 10 and 15 mg. However, recent study measuring the zinc content of 20 free living adults over a period of 6 d detected an average intake of only 8.6 mg/day, ranging from 6 to 12.4 mg. These findings emphasize the need for careful dietary planning if the RDA of 15 mg/day is to be met[21]. It was clear that consumption of nutrients of these subjects was not in RDA range but there were no clinical signs of deficiency. Therefore it could be said that they were either having quality foods during by staying their family in summer period or they had some sub-clinical sign of deficiency. Moreover, the other explanation of this situation was that the fruit and vegetables consumption were quite high in Turkey[27,28]. They cause to drop pH level which is suitable condition for mineral absorption[2].

The results obtained from regression analysis are given in Table-5. There were statistically significant correlations between energy intake and some dietary nutrients: Ca, Fe, Cu, B_1, B_2, niacin, vegetable protein and

TABLE-4
DAILY INTAKE ENERGY AND NUTRIENTS OF THE SUBJECTS (n = 40)

Energy and Nutrients	Female (n = 24)		Male (n = 16)		Recommended[18] daily allowances		Statistical analysis			
							Female		Male	
	X[1]	SD[1]	X[1]	SD[1]	Female	Male	t	p	t	p
Energy (Kcal)	2021	383	2797	648	2260	2860	-3.06	0.01	-0.39	ns
Total Protein (g)	59	23	83	24	43-66 (1g/kg)	57-71 (1g/kg)	–	–	–	–
(Animal Protein)	22	10	26	6	–	–	–	–	–	–
Total Fat (g)	56	29	72	24	–	–	–	–	–	–
(Animal Fat)	22	16	23	7	–	–	–	–	–	–
Vit A (IU)	2640	2095	2547	916	1500	1500	2.66	0.05	4.50	0.01
B_1 (mg)	0.63	0.17	0.99	0.38	1.1	1.2	-13.42	0.01	-2.20	0.05
B_2 (mg)	0.85	0.24	0.99	0.23	1.1	1.3	-5.00	0.01	-5.40	0.01
Niacin (mg)	8.8	2.9	11.9	3.9	14	16	-8.66	0.01	-4.20	0.01
Vit.C (mg)	61.1	33.2	57.3	17.3	90	90	-4.25	0.01	-7.50	0.01
Vit.E (mg)	7.8	3.6	8.1	3.6	15	15	-9.90	0.01	-7.70	0.01
Ca (mg)	386	127	383	121	1000	1000	-23.70	0.01	-20.40	0.01
Fe (mg)	8.9	4.2	12.2	5.2	18	10	-10.70	0.01	1.69	ns
Cu (mg)	1.26	0.38	2.06	0.72	0.9	0.9	4.68	0.01	6.40	0.01
Zn (mg)	5.7	2.2	9.6	7.4	10	11	-9.56	0.01	-0.76	ns
Energy (%)										
Protein	11.7		14.7							
Fat	24.9		28.7							
Carbohydrate	63.4		56.6							

vegetable fat. Energy was also correlated well with haemoglobin and hematocrite. The examination of Table-5 shows that there is a significant correlation between serum and hair zinc concentrations ($r = 0.330$, $p < 0.05$). There were also statistically significant correlations between dietary zinc concentration and hair and serum zinc levels ($r_{diet\text{-}hair} = 0.500$, $p < 0.01$, $r_{diet\text{-}serum} = 0.470$ $p < 0.01$). Diet, hair and serum zinc levels generally agreed well with the levels reported in the literature for healthy subjects[6]. The consumption of legumes (dried bean, chic bean, broad bean, lentil), whole wheat flour, parboiled-pounded wheat that are rich sources of several minerals and B complex vitamins, are high all over Turkey. Poor zinc bioavailability ensures from non-heme iron intaking, related to the competitive absorption interaction of zinc and iron. The comparative interaction between iron and zinc is unlikely to be significant under ordinary dietary condition. The same interaction is also valid for copper absorption[29,30]. These correlations were found to be significant among our subjects. B complex vitamins should be taken together, because of their functions in metabolic pathways. The other interesting point was that significant reflection of animal fat, cholesterol calcium, iron and zinc consumption on BMI.

TABLE-5
CORRELATION BETWEEN ENERGY, NUTRIENTS, SOME BIOCHEMICAL PARAMETERS, SERUM AND HAIR ZINC LEVELS AS WELL AS BODY MASS INDEX (n = 40, r)

	Serum Zn	Hair Zn	Energy	Animal protein	Veget. protein	Animal Fat	Vegetable Fat	Leukocytes	Erythrocytes	Hemoglobine	Hematocrite	Cholesterol	Triglycerides	Calcium	Iron	Cupper	Zinc	Vit A	B_1	B_2	Niacin	Vit C	Vit E	Fiber	BMI
Serum Zn	1																								
Hair Zn	0.330*	1																							
Energy	0.080	-0.130	1																						
Animal protein	0.150	-0.030	0.170	1																					
Vegetable protein	0.100	0.050	0.870‡	0.040	1																				
Animal Fat	0.0004	-0.090	0.180	0.650‡	0.080	1																			
Vegetabe Fat	-0.003	-0.140	0.630‡	-0.220	0.550‡	-0.220	1																		
Leukocytes	-0.100	-0.050	-0.020	0.240	-0.100	0.110	-0.002	1																	
Erythrocytes	-0.100	0.020	0.280	0.050	0.320*	-0.010	0.220	0.300	1																
Hemoglobine	0.100	0.180	0.360*	0.050	0.380*	0.010	0.230	0.310*	0.520‡	1															
Hematocrite	0.100	0.150	0.370*	0.090	0.390*	0.010	0.240	0.290	0.590‡	0.980‡	1														
Cholesterol	-0.010	-0.020	0.250	0.260	0.210	0.240	0.040	0.140	0.060	0.040	0.050	1													
Triglycerides	-0.040	0.020	0.070	0.310	0.020	0.230	-0.130	0.330*	0.230	0.040	0.080	0.440†	1												
Calcium	0.004	-0.100	0.430†	0.380*	0.420†	0.650‡	0.140	0.060	-0.130	0.170	0.140	0.330	0.180	1											
Iron	0.100	0.040	0.690‡	0.280	0.730‡	0.560‡	0.400†	-0.060	0.190	0.260	0.280	0.300	0.110	0.700‡	1										
Cupper	0.200	0.100	0.740‡	0.230	0.780‡	0.090	0.460	0.002	0.360*	0.450†	0.490†	0.200	0.060	0.380*	0.630‡	1									
Zinc	0.470†	0.500†	0.170	0.160	0.250	0.140	-0.100	0.010	0.120	0.210	0.190	-0.050	-0.040	0.100	0.280	0.220	1								
Vit A	-0.110	0.100	0.160	0.150	0.350*	0.230	-0.100	-0.02	0.040	0.050	0.050	0.140	-0.110	0.230	0.290	0.180	0.030	1							
B_1	0.040	0.100	0.780‡	0.220	0.840‡	0.150	0.600‡	0.040	0.380*	0.390*	0.410†	0.180	0.070	0.460†	0.780‡	0.810‡	0.190	0.200	1						
B_2	0.100	-0.120	0.470†	0.240	0.410†	0.130	0.050	-0.130	0.070	0.190	0.200	0.120	-0.060	0.260	0.300	0.450†	0.060	0.180	0.370*	1					
Niacin	0.160	-0.010	0.480†	0.420†	0.510‡	0.110	0.39*	-0.02	0.220	0.190	0.260	0.110	0.040	0.100	0.510‡	0.530‡	0.150	0.220	0.600‡	0.230	1				
Vit C	-0.080	-0.020	0.030	-0.0002	0.140	-0.003	0.11	-0.130	-0.002	0.100	0.120	-0.170	0.340*	0.060	0.220	0.130	-0.060	0.640‡	0.220	0.150	0.410†	1			
Vit E	0.010	-0.230	0.120	-0.300	-0.001	-0.180	0.08	-0.030	0.600	0.020	0.005	0.240	-0.008	-0.120	-0.130	-0.040	-0.180	-0.040	-0.130	0.030	-0.380	-0.230	1		
Fiber	-0.190	-0.040	0.490†	0.060	0.540‡	0.110	0.49†	-0.100	0.190	0.030	0.080	0.040	-0.140	0.280	0.570‡	0.560‡	0.120	0.170	0.650‡	0.270	0.430†	0.240	-0.100	1	
BMI	-0.290	-0.200	0.260	0.250	0.230	0.480†	0.05	0.160	-0.080	0.030	0.050	0.340*	0.070	0.570‡	0.460†	0.330*	-0.080	0.110	0.290	0.110	0.100	-0.030	0.070	0.270	1

*p < 0.05, †p < 0.01, ‡p < 0.001

It has been reported that a positive association between physical development shown height-for-age percentiles and zinc concentrations in serum and hair both in female and male[31].

The further research is necessary to find out the correlation between the nutrient level and success of students.

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