THE SERUM ZINC AND COPPER LEVELS OF HEALTHY PEOPLE LIVING IN KAHRAMANMARAŞ

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ABSTRACT
In this study, the serum copper(Cu) and zinc(Zn) levels of healthy subjects living in Kahramanmaraş were determined. After overnight fasting, blood samples were collected from 95 male and 115 female subjects and the serum samples were analyzed using atomic absorption spectrophotometry (AAS). The data were evaluated according to gender and age groups. The average values were found to be 95.99±1.25 µg/dL for Zn and 123.14±1.63 µg/dL for Cu. The Zn values did not show a significant change with either age or gender while Cu levels were observed to be significantly higher in women than men ($p<0.001$). It also markedly increases with age. The values observed were within the normal limits reported in literature and it was concluded that there was no Zn or Cu deficiency in our region.

Key Words: Trace elements, Zn, Cu.

KAHRAMANMARAŞ'TA YAŞAYAN İNSANLARDA SERUM ÇİNKO VE BAKIR DÜZEYLERİ

ÖZET
Bu çalışmada Kahramanmaraş'ta yaşayan insanlarda serum bakır(Cu) ve çinko(Zn) düzeyleri saptandı. 95 erkek ve 115 kadından bir gece açlıktan sonra kan örnekleri toplandı ve serumlar Atomik Absorpsiyon Spektrofotometresi ile analiz edildi. Veriler cinsiyet ve yaş gruplarına göre değerlendirildi. Ortalama değerler Zn için 95.99±1.25 µg/dL ve Cu için 123.14±1.63 µg/dL olarak bulundu. Cu değerleri kadınlarda erkeklerden belirgin olarak daha yüksekken($p<0.001$), Zn değerleri yaş ve cinsiyete göre belirgin bir farklılık göstermedi. Bakır değerleri yaşla da önemli bir artış gösterdi . Elde edilen değerlerin literatürde belirtilen normal aralıkta olduğu ve bölgemizde bakır ve çinko eksikliği olmadığı sonucuna varıldı.

Anahtar Sözcükler: Eser elementler, çinko, bakır.

INTRODUCTION

The physiological and pathological significance of the trace elements in human body and their use in diagnostic and curing therapies have been subjected to extensive interest (1,2). In order to use trace elements in diagnosis and treatment it is necessary to know their normal serum values. These normal values however are effected by so many factors such as nutritional intake, age, gender and aquatic and soil conditions of the region. The environmental pollution related to the rapid industrialization has also a marked effect on the level of trace elements (3).

Among the trace elements Zn and Cu are extensively investigated due to the fact that they actively take place in so many biological systems (4). Zn is accepted as an essential growth factor. It has been reported that the amount of Zn taken with dietary intake is insufficient and Zn deficiency prevails all over the world (5). After Prasad et al. diagnosed Zn deficiency in adolescent males had growth defects and hypogonadism in Egypt and Iran in 1960, this element gained an increased importance in biochemical research (6).

The most abundant trace element in human body after iron is Zn. A normal adult has 2-3 g Zn in his body . The normal levels reported for serum are 65-115 μg/dL or 10-17 μmol/L (5-7). Zn is the fundamental element of many enzymes and has many biological functions. Human body is known to have more than 70 enzymes such as carbonic anhydrase, tymidine kinase, alkaline phosphatase, DNA and RNA polimerases, carboxypeptidase, alcohol dehydrogenase, lactate dehydrogenase etc. containing Zn (5,6).

The daily dietary Zn intake is 3-5 mg for babies, 10 mg for children, 15 mg for adults, 20 mg for pregnant women and 25 mg for women on breast feeding (5,7). The main sources of Zn are animal originated food such as meat, milk, seafood and egg. Zn present in wheat and vegetables is not sufficiently bioavailable due to its low solubility and digestibility (7,8).

Cu is one of the essential elements. It is an important structural part of many important enzymes of the metabolism such as ceruloplasmin, superoxide dismutase, cytochrome oxidase, lysyl oxidase, uricase, dopamine β-hydroxylase, ascorbate oxidase etc. A normal adult body contains 80-100 mg Cu (7,9). The daily dietary Cu intake is 0.4-0.7 mg for babies, 0.7-2.5 mg for children and 1.5-3.0 mg for adults (10). Liver, meat, fish and legumes are good Cu sources. In a normal diet a person gets 1.5-4 mg of Cu per day which is more than enough for the daily need (7).

Cu deficiency leads to skeletal disorders, anemia, demyelination and degeneration in nerves. An excessive Cu intake on the other hand causes a phenomena called Wilson's disease where there is an excessive Cu accumulation in liver and brain (6,7).

This study concerns the determination of Zn and Cu serum levels of the healthy people living in Kahramanmaraş region.

MATERIALS AND METHODS

Reagents

All the reagents were of analytical grade and obtained from Merck Co.(Germany). Deionized water was used for the preparation of all the solutions and at all other stages of analysis.

Standard Solutions

The glycerol was used for preparation of the standard solutions to approximate the viscosity characteristics of the diluted samples (11). The glassware was first washed with 2M HNO_3 and then rinsed thoroughly with deionised water. Standard solutions were stored in acid washed polyethylene dropper bottles. Glycerol Diluent(5%): 50 mL of reagent grade glycerol was diluted to 1000 mL with deionized water. Glycerol Diluent(10%):100 mL of reagent grade glycerol was diluted to 1000 mL with deionized water.

Stock Zn Standard Solution: 1.0000 g of pure Zn metal was dissolved in 100 mL of ten fold diluted HCl and was diluted to 1 L with deionized water.

Working Standars: 1 mL of 1 g/L Zn standard was diluted to 100 mL with a 5% (V/V) glycerol solution. Similarly, 1, 1.5, 2, 3, and 4 mL aliquots of this intermediate stock were diluted to 100 mL with the same glycerol solution.The standards 10, 15, 20, 30 and 40 μg Zn/dL, correspond to serum Zn concentrations of 50, 75, 100, 150 and 200 μg/dL, respectively (11).

Stock Cu Standard Solution: 1.0000 g of pure Cu metal was dissolved in 100 mL of ten fold diluted HNO_3 and was diluted to 1 L with deionized water.

Working Standards: 1 mL of 1 g/L Cu standard was diluted to 100 mL with a 10% (V/V) glycerol solution. Similarly, 2.5, 5, 7.5, 10 and 15 mL aliquots of this intermediate stock were diluted to 100 mL with the same glycerol solution.The standards 25, 50, 75, 100, and 150 μg Cu/dL, correspond to serum Cu concentrations of 50, 100, 150 200 and 300 μg/dL, respectively (11).

Apparatus

All the analyses were made by a flame atomic absorption spectrophotometer, AAS (Perkin-Elmer 107). The working conditions at the AAS are shown in Table 1.

Sampling Procedure

Sample Collection

This study was carried out upon 115 females and 95 males having average age of 34.28 years (ranging 15 to 75 years) living in Kahramanmaraş. The subjects were chosen among volunteers having no visible health problems. The pregnant women or the ones using oral contraceptives were excluded since it is known that these factors effect the serum Zn and Cu levels appreciably (12-14).

Following overnight fasting, 5 mL of venous blood was taken from each subject with sterile syringes without using any anticoagulant and the sera were separated after being centrifuged at a rate of 3000 rpm for ten minutes. Great care was taken not to cause the hemolysis of the blood samples and the ones which had already been hemolysed were discarded. The serum samples were put in trace element free, disposable, capped polypropylene tubes (113118, LP, Italiana) and kept in a deep freeze at -20 ^{0}C until they were used.

Analyses of Zn and Cu in the AAS

Serum specimens were diluted five fold for Zn and two fold for Cu analyses with deionized water. Then, the diluted samples and standards were aspirated directly into the AAS flame. The concentrations of Zn and Cu were calculated against Zn and Cu standards which were prepared in the diluent glycerol matrix. Also, the glycerol solution, 5% and 10%, were used as blank for analyses of Zn and Cu, respectively. The readings were taken in duplicate and the average value was given.

Statistical Analysis

The data analysis was carried out using variance analysis (F test) and subgroup averages were compared by Duncan test. Results are expressed as the mean±SEM(Standart Error of Means). SAS programme was used for statistical evaluation (15).

Table 1- The working conditions at the AAS.

	Cu	Zn
Wavelength (nm)	324.8	213.6
Slit width(nm)	0.7	0.7
Hallow Kathode Lamp (mA)	15	10
Flame	Air: Acetylen	Air: Acetylen

RESULTS

Table 2- Serum Zn And Cu Values of Healthy Subjects.

	Zn (μg/dL) X ± Sx	Cu (μg/dL) X ± Sx
Females (115)	94.13±1.68	127.97±2.30
Males (95)	98.23±1.86	117.29±2.03
Total (210)	95.99±1.25	123.14±1.63
F	2.63	16.32
P	>0.05	<0.001

The average values are given as mean±SEM

As seen from the table 2 the average Zn and Cu values in the healthy people in our region were found to be 95.99±1.25 and 123.14±1.63 μg/dL respectively. These values are in the same range with those reported in literature demonstrating that there is no Zn or Cu deficiency in healthy people of our region (6,7).

The average Zn values in males and females were found as 98.23±1.86 and 94.13±1.68 μg/dL respectively. However the difference was not significant ($p>0.05$). On the other hand the Cu values in females (127.97±2.30 μg/dL) were significantly higher than the corresponding values in males (117.29±2.03μg/dL) and the difference was statistically significant ($p<0.001$). The serum Zn and Cu levels according to the age groups were presented in Table 3.

Table 3- Average Serum Zn And Cu Levels According To Age

Age group	N	Zn(μg/dL) X ± Sx	Cu(μg/dL) X ± Sx
15-24 years	76	91.18±2.08 a	121.93±2.23 ab
25-34 years	54	98.56±2.46 a	118.24±2.65 b
35-44 years	25	98.32±3.41 a	123.24±3.89 ab
45-54 years	25	100.76±3.49 a	126.16±3.89 ab
55-64 years	17	94.53±5.02 a	133.88±4.72 a

The difference between the groups having the same letter is insignificant and the difference between the groups with different letters is significant ($p<0.05$).

The serum levels of Cu and Zn in healthy males and females are tabulated in Tables 4 and 5 respectively.

Table 4 shows that there is not a significant difference between the age groups for Zn levels and there is a statistically insignificant increase in Cu levels in healthy males ($p>0.05$) . As seen from table 5, the Zn value is the lowest in the first and highest in the last group (64+). However the difference were not found to be significant ($p>0.05$). Cu also shows an increasing trend by age but it is statistically insignificant as well ($p>0.05$).

Table 4- The Serum Zn And Cu Levels In Healthy Males .

Age group	N	Zn(μg/dL) X ± Sx	Cu(μg/dL) X ± Sx
15-24 years	23	94.43±3.87 a	113.78±3.86 a
25-34 years	32	100.16±3.28 a	113.41±3.27 a
35-44 years	14	96.79±4.96 a	117.79±4.94 a
45-54 years	12	105.42±5.36 a	121.17±5.34 a
55-64 years	7	94.29±7.01 a	127.14±6.99 a
64+ years	7	96.43±7.01 a	129.14±6.99 a

The average values are given as mean±SEM. a; $p>0.05$

Table 5- The Serum Zn And Cu Levels In Healthy Females.

Age group	N	Zn(μg/dL) x ± sx	Cu(μg/dL) x ± sx
15-24 years	53	89.77±2.45 a	125.47±2.65 a
25-34 years	22	96.23±3.80 a	125.27±4.11 a
35-44 years	11	100.27±5.37 a	130.18±5.82 a
45-54 years	13	96.46±4.94 a	130.77±5.35 a
55-64 years	10	94.70±5.63 a	138.60±6.10 a
64+ years	6	107.67±7.27 a	132.00±7.88 a

The average values are given as mean ± SEM. a; $p > 0.05$

DISCUSSION

This study concerns with the determination of serum Zn and Cu levels of 95 male and 115 female subjects in Kahramanmaraş region using AAS. The Zn values did not show a significant change with either age or gender while Cu levels were observed to be significantly higher in women than men. It also markedly increases with age.

Tuncel et al. found an average Zn value of 117.29 μg/dL in their study which they carried out with a group of 117 people in Erzurum region (16). They also reported that the Cu value was 114 μg/dL for females and 109 μg/dL for males. Their Zn value was a little bit higher and Cu value was a little bit lower than the corresponding values for our region. The Zn and Cu values determined by McMaster et al.(17) in Northern Ireland were 12.7 and 20.1 μM in females and 13.2 and 17.9 μM in males.

It is observed that serum Zn and Cu levels vary from region to region. Undoubtedly the methods and the apparatus employed in the measurement have quite a big impact on the results. Nevertheless the Cu and Zn values obtained in our study are within the normal limits and there is no case of Cu or Zn deficiency in our region in healthy population.

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