A study on the relationships between some of the trace element levels of hair, nail, serum and urine in healthy subjects

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The distribution of trace elements, Cu, Mg, Zn and Fe levels and, intra- and inter- correlations among them were studied in hair, nail, blood and urine samples of healthy subjects. Specimens obtained from men, women and children were analyzed for trace element concentrations by using atomic absorption spectrophotometer. The mean values of these elements were found to be higher in the nails of children compared with those of men and women groups. However, hair values of these elements in men and women were higher than those of the children. Cu, Mg, Zn and Fe concentrations in hair, nail, blood and urine c," *nil three group express significant differences. Existence of positive correlations between elements in hair and in nail reflected steady turnover of these samples. It was found that hair levels of Fe and Zn in men and hair level of Cu in women exhibited significant parallelism with serum levels of these elements.*

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Key Words: Trace elements, Sex, Hair, Nail, Serum, Urine

Studies on trace elements have become intensified within the last two decades. Since the levels of trace elements in human tissues and fluids play important roles in health and disease conditions, studies about them have been attracting great interest (1,2).

Trace elements exist in very low concentrations in the body and consist of 0.01% of total body weight (3). These essential elements are as follows: Silicon, Vanadium, Chrome, Manganese, Iron, Cobalt, Hickel, Copper, Zinc, arsenic, Selenium, Molybdenum and lodine. In addition to these, due to onset of some disorders related to absence of Fluorine and Tin in the body, they have been also accepted as essential elements (4). In the case of insufficient intake of these trace elements with diet, besides the insignificant pathologic findings, also some pathologic results, that can threat the life, may appear.

Zinc, Iron, Magnesium and Selenium are important elements in the preservation of immune resistance (5). Zinc and copper are two important essential elements in growth an development (6-9). It was shown that copper is necessary for hemoglobin synthesis, for connective tissue development and for the normal function of central nervous system (10). Thus, in the absence of Cu, there become some disorders in connective tissue (11). Zinc deficiency affects protein synthesis negatively (12-15). Additionally, zinc also forms the prosthetic group of more than 80 metalloenzymes which play roles in many metabolic pathways. Similarly Mg is the cofactor of approximately 300 cellular enzymes. Therefore, irreversible disorders may appear in these two enzymes. Also in animal studies it was shown that ingestion of sufficient zinc with diet by the mother during gestation is essential for normal embryonic and foetal development. Zn insufficiency in prengnant rats causes many major and minor congenital malformations on live siblings (16,17). Also this insufficiency can increase the sensitivity of fetus against the effects of teratojenic substances (18).

Congenital malformations, caused by Zn insufficiency, are as follows; Intrauterine growth retardation, fetus with low brain weight and behavioral abnormalities in live sibling (19).

The metabolism of trace elements in the body is very complex and also interesting.

Absorption through gastrointestinal tract is performed at 3 phases:

1. Intraluminal phase

2. Translocation phase (passage through cell membrane or epithelium)

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3. Mobilisation phase (distribution to the cells or re-sequestration into the lumen).

Biovailability of ingested elements depends on the presence of some certain factors in food source such as phitate. Similarly, the content to tapwater changes from place to place and it gives different levels of supplement to the amount of mineral in the diet (20). Although the events in the intestinal epithelial cells are not known clearly in general, there are specific mechanisms for the two elements, zinc and iron. Metals, taken into the cell are bound to specific depot proteins like ferritin and metallothionein, respectively. The concentration of metallothionein in the organs can be increased by the intake of trace elements which specifically bind to it (4). Increased concentration of Zn in plasma increases the synthesis of metallothionein in intestinal cells and metallothionein becomes a good carrier for the absorbed Zn. When the plasma Zn decreases, the stimulus sent for the metallothionein synthesis becomes decreased and the absorbed Zn being available for transport is elevated (4).

The carrier molecules in blood for trace lements are the plasma proteins such as: Transferrin, ceruloplasmin, albumin,alpha-macroglobulin, transamanganin and the niceloplasmin. Additionally a certain part of the serum trace elements are carried in the form of aminoacid or little peptide complexes (21). Generally, binding capacity of carriers, having specific protein structure, is in unsaturated form. Such as transferrin carries only one third of its maximum capacity under normal conditions. This remaining empty capacity is thought to be functioning as buffer during exposure to excess metal. The more important protection mechanism than this buffering property, displays itself at the previous steps. This control mechanism can be established by absorbtion, or excretion or both of them.

This kind of control of trace elements, taken from outside, is important. This control is easier for the Fe and Zn, required to be taken in very little concentrations, this control is difficult and necessitates some specific measures. The main excretion way of trace elements is intestines and kidneys. Additionally, the loss of trace elements via skin and respiration is also important in hot climates (4).

There are many factors affecting the serum trace element levels, it has been shown that there was a decrease in plasma Zn concentraitons during normal gestation (22,23). The over exposure of indivuduals to some elements due to environmental conditions or specifically absence of these trace elements in that region are the important etiopathologic factors affecting the serum element levels (2). Diet protocols, geographical differences, age and sex also affect the serum elements. Additionally, serum Zn is 16% higher than plasma Zn (24).

The materials which will be used in the study of trace element balance of the body should have some properties. In this regard , serum is not an appropriate material. Because, it is known that different foods taken daily or within few hours change the serum element levels significantly. Therefore, researchers have concentrated their studies on hair and nail in recent years. In the last decade, the use of hair as a biopsy material for the expression of body trace element state or for the research of exposure degree of people exposed to toxic metals in industrial regions has become spreaded (20,25-27).

The superiorities of hair and nail to serum as follows (3,28).

— The amount of trace elements in plasma and serum is very low. But their concentrations are relatively higher in hair and this facillitates the analysis (29).

— When the data gathered from the serum, this data do not represent the body trace element state approximately.

— The analysis of scalp hair can reliably be used for the retrospective study of body trace elements state. It is not affected by daily alterations.

— Transportation and preservation of hair and nail are easy (30).

— In contrast to blood, chemically hair and nail are homogen and inert materials.

— To obtain the nail and hair is easier than the blood and other tissues. It does not have trauma and pain complication.

— 42 elements are determined in normal nail (31). This shows that the nail can be a useful tissues for the detection of intake and excretion of trace elements through the body.

— The slow metabolic turn-over of the nail is important because it provides information about the metals accumulated during long period of time (32).

MATERIALS AND METHODS

Blood, hair, nail and urine specimens were taken from 20 men, 10 women and 5 children of totally 35 healthy-volunteer individuals. The average ages and the ranges of ages of these individuals are as follows.

	Men	Women	Children
X±SD	26.4±6.9	24.0±6.2	4.8±1.5
Age Range	27-40	19-37	3-7

For collection of specimen, it was strictly noticed that these individuals must have been in Ankara for tha last six months. At the time of specimen collection and a few months ago, volunteers had not taken any oral or parenteral drugs, including vitamin preparations.

HAIR: Hair from the occipital region was obtained by a stainless steel scissors and the bottom segments of these specimens were used in analysis (the last 2-3 cm).

NAIL: Handfinger nail was obtained by using a stainless stell nail-scissors.

Both of specimens were passed through a distiled water priorly and then bidistiled water after making them waited for 3-4 hours in Aceton-Hexane (1/2, v/v) solution. They were dried in 70 °C etuve and then they were weighde.

BLOOD: After averagely 15 hours of fasting period, without having breakfast, the blood specimens of individuals were obtined from the veins of the forearm by using disposable plastic injectors. Since the disposable injectors were not deionised, they were waited in 10% H_2SO_4 for 24 hours, and after shaking with bidistiled water for few times, they were dried (33).

Blood, taken without anticoagulant use, were centrifuged after waiting for a while and then they were put to deionised tubes by separating.

URINE: Synchronisely with the blood, the mid part of the first wire of the day was taken (approximately 20cc).

All the specimens were put in to separate deionised tubes and they were waited in perchloric acid/nitric acid (1/5, v/v) compound for a while (34) and they were burned with a 250 °C beck fire in an isolated medium. This procedure was continued until the organic matrix was completely destroyed. The inorganic portion dissolved in a certain amount of tridistiled water was put into deionised tubes and after covering the mouths of tubes with paraffin, they were kept at 4°C. Then the values of Zn, Mg, Fe and Cu were determined with atomic absorption spectrophotometer (Model 1200 Varian Techtron) (35).

Results were expressed as pg/ml in urine and blood and as pg/g (ppm) in nail and hair. Every measurement was repeated three times and the avarage was taken. As a statistical analysis: average±SD values were calculated, intra -and inter- correlation analyses were done and the significance of the results was evaluated with student-t test.

RESULTS

The average levels of trace elements in nail, hair, serum and urine according to the groups are shown in Table 1. According to this Table; the averages of 4 trace elements present in the nails of the children are higher than the values present in the adult males (for

Table 1. A,B,C,D; For men, women and children and ingrouping the determined values of nail, hair, serum and urine trace elements (Mean±SD)

A (Non grouped) (n-35)	Nail (ppm)	Hair (ppm)	Serum (jig/ml)	Urine (pg/ml)
Fe	111.01131.3	79.46±94.68	0.619±0.52	0.104±0.15
Cu	13.7±26.0	16.52±19.38	1.79012.34	0.130±0.20
Zn	218.4±162.2	422.0±311.0	0.806±0.53	0.182±0.13
Mg	500.1 ±301.6	626.0±448.0	49.43+30.8	113.5±58.03
B (Women) n-20				
Fe	52.66±60.1	103.21±117.7	0.578±0.49	0.053±0.07
Cu	6.05±13.0	19.51 ±20.23	2.20±2.26	0.24±0.31
Zn	234.1 ±147.0	376.97±235.2	0.937±0.50	0.181 ±0.15
Mg	518.2±332.0	644.9±525.8	44.21 ±1.20	104.25±52.9
C (Men) n-10				
Fe	138.5±122.0	54.8±46.9	0.591 ±0.33	0.183±0.18
Cu	38.76±73.3	16.36±20.7	0.78±0.56	0.05±0.11
Zn	117.3±72.5	451.3±226.8	0.311 ±0.31	0.14±0.10
Mg	428.2±256.4	624.7±367.0	49.0±33.8	93.34±63.6
D (Children) n-5				
Fe	424.8±79.8	40.0±40.2	–	0.157±0.26
Cu	58.3±82.4	3.32±6.6	–	0.065±0.13
Zn	486.8±18.6	168.8±37.5	–	0.265±0.12
Mg	664.1 ±256.97	550.0±364.6	–	233.1 ±96.7

Fe **p**<0.0005, for Cu p<0.0005, for Zn p<0.0005, for **Mg p**<0.1). Same situation is also valid for the women (for Fe **p**<0.0005, for Cu p<0.25, for Zn p<0.0005, for **Mg p**<0.05) (Table 4).

But the hair levels of those 4 element are higher in both men and women than the levels in children. While this difference was found to be significant between adult male and child (for Fe p<0.0005, for Cu p<0.0005, for Zn p<0.0005, for Mg p<0.25), it was nod found to be significant between adult female and child (for Fe p<0.1, for Cu p<0.1, for Zn p<0.01, for **Mg p**<0.25). Since we could not obtain blood specimens from the children, we could not perform men-children and women-children comparisons.

Average urine levels express differences:

When the men-children averages are taken in to account; the average values of Zn, Fe and Mg in children were found to be significantly higher than the adult male values (respectively p<0.05, p<0.05 and p<0.0005).

When the adult women-children averages are taken in to account; we could not find a significant difference for the Cu and Fe elements (p<0.40). Zn amount in children Is approximately two times higher than the women have (p<0.05). Mg amount in children is approximately two and half times higher than the women have (p<0.0005).

When male-female averages are compared:

Fe and Cu levels in nail are significantly higher in women than men (respectively p<0.0005, p<0.025). While Zn average values in men are two times higher than the women have (p<0.01), no significant difference between Mg values was found.

No significant difference was found for the elements in the hair between the men and women.

While no significant difference could be found for the averages of Fe and Mg between men and women, the average values of Zn and Cu are 2-3 times higher in men than the women have (respectively p<0.05 and **p**<0.0005).

No difference was found between Mg values in urine (p<0.25). Average Cu value in men is approximately 4 times higher than the women have (p<0.025). Again Zn is higher in men (p<0.05). In contrast to this, Fe level in women is approximately 3-4 times higher than the men have (p<0.0005).

Intra-correlation analyses are shown in Table 2. According to this Table;

Nail: In men there are positive correlations between Fe-Zn, Fe-Mg, Cu-Zn, Zn-Mg. In women there are positive correlations between Fe-Zn, Fe-Mg, Cu-Mg and Zn-Mg.

Hair: Positive correlations in men between Fe-Mg, Cu-Zn, Cu-Mg, Zn-Mg were determined. But there are negative correlations between Fe-Cu, Fe-Zn.

Blood: In men between Fe-Mg, Cu-Zn, Zn-Mg and in women between Fe-Cu, Fe-Zn, Fe-Mg, Cu-Zn, Cu-Mg and Zn-Mg there are positive correlations.

Urine: In men, between Fe-Zn and Cu-Mg and in women between Fe-Zn, Fe-Mg and Zn-Mg there are positive correlations. In women there are negtaive correlations between Fe-Cu, Cu-Zn and Cu-Mg.

Intra-correlation analyses are given in Table 3. According to Table the correlation of elements values of inter-parameters are as follows;

Between nail and hair: In men Fe, Zn an Mg express positive correlations. In women Fe, Cu and Mg express positive correlations but Zn expresses negative correlations.

Between nail and urine: In men Fe, Zn and Mg express positive correlations but there are negative correlations In Cu. In women Fe, Cu and Mg express positive and Zn expresses negative correlations.

Between serum and hair: In men Fe and Zn exress positive and Mg expresses negative correlations. In women Fe, Zn, Mg express negative correlation.

Between serum and urine: In men Fe and Zn express positive and Cu and Mg express negative correlations. In women Fe, Zn, Mg express positive correlation but there is negative correlation in Cu.

Table **2.** The intra-correlation analysis between the elements in men and women (r). n.c: no correlation (r<0.1)

A -(Men) n-20	Fe-Cu	Fe-Zn	Fe-Mg	Cu-Zn	Cu-Mg	Zn-Mg
NAIL	n.c.	0.39	0.8	0.25	n.c.	0.4
HAIR	n.c.	n.c.	0.23	0.78	0.43	0.46
SERUM	n.c.	n.c.	0.64	0.17	n.c.	0.41
URINE	n.c.	0.77	n.c.	n.c.	0.31	n.c.
B-(Women) n-10						
NAIL	n.c.	0.24	0.89	n.c.	0.27	0.32
HAIR	-0.26	-0.23	0.46	0.34	n.c.	0.45
SERUM	0.35	0.98	0.97	0.37	0.53	0.94
URINE	-0.37	0.61	0.86	-0.34	-0.11	0.38

Table 3. The inter-correlation analysis between the specimens in men and women (r)

A-(Men) n-20	Nail-Serum	Nail-Hair	Nair-Urine	Serum-Hair	Serum-Urine	Hair-Urine
Fe	0.3	0.8	0.98	0.27	0.5	0.84
Cu	n.c.	n.c.	-0.19	n.c.	-0.21	n.c.
Zn	n.c.	0.18	0.51	0.46	0.39	0.36
Mg	0.28	0.25	0.19	-0.11	-0.14	0.28
B-(Women) n-10						
Fe	-0.51	0.65	0.15	-0.33	0.92	n.c.
Cu	0.54	0.37	0.18	0.74	-0.12	0.55
Zn	-0.53	-0.23	-0.26	-0.44	0.97	-0.52
Mg	-0.69	0.26	0.44	-0.62	0.62	-0.2

Table **4.** The statistical evaluation results between the elements (p values)

Men/	Fe	Cu	Zn	Mg
Nail-Hair	p<0.10	p<0.01	p<0.1	p<0.1
Serum-Unine	p<0.0005	p<0.0005	p<0.0005	p<0.0005
Women/				
Nail-Hair	p<0.25	p<0.1	p<0.0005	p<0.1
Serum-Unine	p<0.0005	p<0.0005	p<0.05	p<0.025
Women-men/	Nail	Hair	Serum	Urine
Fe	p<0.0005	p<0.1	p<0.4	p<0.0005
Cu	p<0.025	p<0.25	p<0.05	p<0.025
Zn	p<0.01	p<0.1	p<0.0005	p<0.05
Mg	p<0.1	p<0.4	p<0.025	p<0.25
Women-children/				
Fe	p<0.0005	p<0.1	–	p<0.4
Cu	p<0.25	p<0.1		p<0.4
Zn	p<0.0C05	p<0.01	–	p<0.025
Mg	p<0.05	p<0.25	–	p<0.0005
Men-children/				
Fe	p<0.0005	p<0.0005	–	p<0.05
Cu	p<0.0005	p<0.0005	. –	p<0.1
Zn	p<0.0005	p<0.0005	. –	p<0.05
Mg	p<0.0005	p<0.25	–	p<0.0005

Between hair and urine: In men, there are positive correlations in Fe, Cu and Mg. In women, Zn and Mg exprès negative, Cu expresses positive correlations.

DISCUSSION

When our results are compared with *\ho* values in the literature, it was seen that some of our results are relatively high. There are many reasons for this. In the determinations of hair, nail, serum and urine minorai levels, different methods such as neutron activation analysis, atomic absorption spectrophotometer and flame emission spectrophotometer have been used. The differences between the results originate from the different determination techniques as well as the differences in the preparation of specimen to the analysis may also cause different results. In their study Hilderband et al have determined that the concentrations of minerals in the hair depended on the length of the hair (34). In the hair taken from the different areas of the head and in the different parts of the hair (according to the proximity and distance to the deep part of the nail) the element concentrations have been found to be different (2,36).

According to the researches of Petering et al, it has been determined that during childhood in males Zn and Cu concentrations were high and relative decrease occured after physiologic adolescence. This increase-decrease point begins around the age of 12 (25). In women while the Cu content increases with the age, Zn content decreases. According to our results, the averages of nail Cu and Zn of children are higher than both adult male and adult female levels.

Although the serum Zn level of women were found to be higher than the men have, this difference was seen to be statistically Insignificant (20). According to our results, serum Zn level in men is higher than the women (respectively 0.937±0.50 ug/ml and 0.311±0.31 ug/ml, p<0.0005). Also in the same study hair Zn level of women has been found to be significantly higher than the men (respectively 208ppm and 176ppm). Our results are also inaccordance with this study (woman 451.3ppm, man 376ppm).

Klevay (37) has determined that there is a decrease in hair Zn at the first decade of life. After 20 years of age hair Zn level again has increased. We did not study according to age groups but we found high values of Zn in adult male and female and low levels of Zn in children.

Klevay (37,38) has not found correlation between plama Zn and hair Zn but he has found positive correlation between plasma Zn and hair Zn in males between the ages of 6-10, in pregnant women, and in lactating women over the age of 20. But in our study we found positive correlations between serum-hair Zn (r-0.459) and between serum-hair Fe in males (r-0.269). In the same specimens we found negative correlation for Mg and no correlation for Cu. Inter-correlations between hair and serum in women express a little difference: Zn, Fe and Mg express negative correlations (respectively r--0.438, r--0.327 and r--0.620). For the Cu, we found positive correlation which is in inaccordance with the results of Klevay et al (r-0.736).

Under the light of these data, it can be said that Fe and Zn values in the males' hair can show the state of serum to an extent. Same thing can not be said for the other elements. In women, only the hair Cu can express serum Cu to an extent, but it is not valid for Fe, Zn and Mg.

Serum Cu value in women were found to be significantly higher than the men have. Also hair Cu level in women is 2 times higher than the value in men, but this difference is not significant (20). In our results while the serum u is 0.78 ug/ml in women, it was found 2.20 ug/ml in men. There is approximately 3 fold difference from the hair Cu level point of view, but no difference between male and female could be found.

In a study done by Deeming and associates, no significant difference has been found in Fe and Mg levels between male and female serum and hair (20). Our results confirm this hypothesis.

As a result, as it is seen in intra-correlation analyses, hair and nail having a stable element balance express a significant positive correlation between these elements. In hair and nail, it was observed that elements did not affect each other in a negative manner. Also same things can be said for nail and hair in the women. But blood and urine -as it was mentioned previously- express a very complex element schedule. In women, between all serum elements positive correlations were determined.

As it seen, in male, female and children groups, Fe, Cu, Zn and Mg concentrations in hair, nail, serum and urine express significant differences. Relation between elements also express differences due to the groups and the materials used. For this reason, while trace element analyses are performed, these conditions should be taken in to account, and the results should be evaluated on the basis of these mentioned above. More importantly, it should be avoid to make a conclusion by using only one material. If information about the trace element balance of the body is wanted, analyses should be done with as many as possible material and the results should be evaluated by considering factors such as age, sex and environmental factors.

Sağlıklı şahısların saç, tırnak, serum ve idrarlarındaki bazı eser elementlerin seviyeleri arasındaki ilişkinin araştırılması

Bu çalışmada sağlıklı insanların saç, tırnak, kan ve idrarlarındaki Cu, Mg, Zn ve Fe gibi eser elementlerin dağılımı ve bu elementler arasındaki intra -ve inter- korelasyonlar araştırılmıştır. Bu amaçla kadın, erkek ve çocuklardan alınan örnekler atomik absorbsiyon spektrofotometresi kullanılarak eser element yönünden incelenmiştir. Çocukların tırnağında bulunan bu 4 eser elementin ortalama değerleri yetişkin erkek ve kadındaki değerlerden daha yüksek bulunmuştur. Buna karşılık aynı elementlerin saç değerleri hem kadın hem de erkekte çocuktaki değerlerden daha yüksektir. Her üç grubun saç, tırnak, serum ve idrarlarındaki Fe, Zn, Mg ve Cu konsantrasyonları anlamlı farklılıklar arzetmektedir. Ayrıca saç ve tırnakta elementler arasında pozitif korelasyonların bulunması bu iki materyalin dengeli ve sabit turnoverini göstermektedir. Elde edilen sonuçlara göre erkeklerin saç Fe ve Zn değerleri ile kadınların saç Cu değerleri, aynı elementlerin serum değerleri ile büyük bir paralellik göstermektedir.

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