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## The Status of Trace and Toxic Elements in Biological Samples (Scalp Hair) of Skin-Disease Patients and Normal Subjects

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**Background:** Direct association of trace and macro-elements in relation to human disease has been observed in many research studies. In many cases, an alteration in the metabolism of these minerals has been demonstrated.

**Methods:** In this investigation, the hair levels of the trace elements zinc (Zn), copper (Cu), chromium (Cr), iron (Fe), nickel (Ni) and the toxic elements lead (Pb) and cadmium (Cd) were determined in 110 subjects (57 skin-diseased subjects and 53 controls). Samples were analyzed using atomic absorption spectrophotometric methods.

**Results:** Analysis of hair samples revealed significantly lower levels of zinc, iron and copper in skin-diseased patients compared with normal controls, and significantly higher levels of chromium and nickel and of toxic elements, lead and cadmium. The same pattern was observed in males and females and in both age groups studied (6-15 and 16-30 years).

**Conclusions:** These data can guide clinicians and other professionals investigating deficiencies in essential trace metals and excessive levels of toxic metals in biological samples.

**Key Words:** Trace elements, toxic elements, skin disease, scalp hair, atomic absorption spectrophotometer

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### Cilt Hastaları ve Sağlıklı Kişilerin Saç Örneklerinde Eser ve Toksik Element Düzeyleri

**Giriş ve Amaç:** Eser ve makro-elementlerle hastalıklar arası ilişki birçok çalışmada rapor edilmiştir. Birçok olguda bu minerallerin metabolizmasında da bozukluklar gözlenmiştir. Bu çalışmada cilt hastalığı olan kişilerde ve sağlıklı kontrollerde eser element ve toksik element düzeylerinin saptanması amaçlanmıştır.

**Yöntem ve Gereç:** 57'si cilt hastalığı olan 53'üde sağlıklı normal kişilerden oluşan toplam 110 kişinin saç örneklerinde çinko (Zn), bakır (Cu), krom (Cr), demir (Fe), nikel (Ni) gibi eser elementler ve kurşun (Pb) ve kadmiyum (Cd) gibi toksik elementlerin düzeyleri araştırılmıştır. Örnekler atomik absorpsiyon spektrofotometre ile analiz edilmiştir.

**Bulgular:** Cilt hastalığı olan hastaların saç örneklerinde yapılan incelemede, sağlıklı kontrollere oranla çinko, bakır ve demir düzeyleri anlamlı oranda düşük iken, krom ve nikel düzeyleri ile kurşun ve kadmiyum gibi toksik elementlerin düzeyleri ise anlamlı derecede artmış olarak bulundu. Aynı benzerlik kadın ve erkekler ve çalışılan yaş grupları arasında da mevcuttu (6-15 yaş ve 16-30 yaş).

**Sonuç:** Bu bulgular, biyolojik örneklerden ölçülen toksik metal ve esansiyel eser element düzeylerinin hem klinisyenlere hem de profesyonel araştırmacılara bir rehber olabileceğini göstermektedir.

**Anahtar Sözcükler:** Eser element, toksik element, cilt hastalıkları, saç, atomik absorpsiyon spektrofotometre

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### Introduction

Metals and their compounds have been used since ancient times for their therapeutic as well as cosmetic effects on the skin. The numerous enzymes activities exhibited by the skin are a reflection of the metabolic role of that organ. The unique process of keratinization and melanin formation is enzyme- dependent and therefore could be influenced by trace metal deficiencies or excesses (1) .

Zinc (Zn) is a trace mineral that is used in the treatment of a range of skin diseases including acne, boils, eczema, bedsores, general dermatitis, wound healing, herpes simplex, and skin ulcers, etc. Zinc is a cofactor for many enzymes required for healing

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damaged skin. It may contribute to maintaining healthy skin cells and be helpful in generating new skin after burns or injury. All body tissues contain zinc; in skin, it is five to six times more concentrated in the epidermis than the dermis. Topical zinc, in the form of divalent zinc ions, has been reported to provide antioxidant photoprotection for skin (2). Zinc deficiency is common in patients with Crohn's disease (CD), especially in those with skin lesions and growth retardation (3).

Iron (Fe) deficiency is probably the most frequent nutritional deficiency disorder in the world. A recent estimate based on World Health Organization (WHO) criteria indicated that around 600-700 million people worldwide have a marked iron deficiency anemia (4). Dermatopathic anemia has attracted the attention of clinicians because iron deficiency was found to be a metabolic consequence of skin diseases such as erythroderma, exfoliative dermatitis, psoriasis, eczema, and many others (5).

Copper (Cu) forms an important constituent of many metalloproteins and metalloenzymes of various organs and tissues. Copper is responsible for the metabolism of amino acids, tyrosine and for the production of melanin, the pigment of skin freckles (6-9).

Allergic dermatitis may be caused by persistent contact with hexavalent chromium (Cr). Hexavalent Cr is water soluble and thus can penetrate the skin (10, 11).

Lead (Pb) can inhibit Ca, Zn, Mn, Cu, and Fe, causing deficiencies. Intolerance to nickel (Ni) salts is considered in these patients the major factor in the development of skin disorders (12-14). Both black-Ni and cold-sealed aluminum were found to be unexpected causes of dermatitis due to Ni release. Sources of occupational Ni exposure are often missed by dermatologists, due to lack of knowledge. Occupational Ni exposure is, however, important to identify, and the dimethylglyoxime test is a helpful tool (15,16).

An excess of cadmium (Cd) accumulation is linked with similar problems to those associated with high Pb (17-19). With exposure to either a strong (cadmium) or weaker (zinc) inducer of metallothionein, Cu accumulation was increased in normal cells, while there was no change from the already elevated level of Cu accumulation in blotchy cells (20).

Trace mineral analysis is a test that measures the mineral content of hair. Mineral content of the hair

reflects the mineral status of the body tissues. If a mineral deficiency or excess exists in the hair, it usually indicates a mineral deficiency or excess within the body, or biounavailability.

Trace mineral patterns in hair are proving fruitful data, not only as a diagnostic procedure, but also in providing answers pertaining to treatment. It will become increasingly common for physicians to use hair analysis in the diagnosis and treatment of chronic degenerative disease.

## Experimental

### Materials and Methods

This study was conducted on a total of 57 Pakistani patients from Hyderabad, Sindh province, Pakistan. The control group consisted of 53 healthy individuals from the same location of the sampling campaign with no general complications and who were receiving no medication.

Scalp hair was cut close to the scalp in the suboccipital area of the head (about 1 to 2 cm) after ascertaining that no coloring agent had been used. The hair sample was washed twice, first with acetone and then with double-distilled water. Hair samples were analyzed with flame and flameless atomic absorption spectrophotometer (AAS) (Model 180-50, Hitachi, Japan) respectively.

### Instrument

A model 180-50 AAS (Hitachi Ltd., Tokyo, Japan) with burner and graphite furnace GA-3 was used for the measurement of Zn, Fe, Cu, Cr, Ni, Cd, and Pb. The Fe, Zn and Cu were determined by flame burner and Cr, Ni, Cd and Pb by graphite furnace.

### Reagents

All the chemicals used were of analytical grade supplied by Merck. Concentrated nitric acid and hydrogen peroxide were used for wet acid digestion method. Standard solutions of Zn, Fe, Cd, Cr, Pb, Cu and Ni of 1000 ppm were prepared from certified standards (Fluka kamika). The internal standards were prepared from metal salts obtained from Merck. CRM-397 (certified reference material of hair) was used.

### Sampling

The experimental group consisted of 57 patients (male = 32, female = 25) of two age groups (6-15 and

16-30) selected from occupants of urban populations of Hyderabad and Latifabad regions, Pakistan, on personal request, and a questionnaire was administered in order to collect details concerning physical data, ethnic origin, health, dietary habits and consent. The control 53 subjects (male = 30, female = 23) within the same age ranges were collected three times in a one-year period. A file containing complete information and all the demographical data on each contributor was made. From each subject (normal and patients), approximately 1.0 g scalp hair samples, which had not been dyed, bleached or straightened for three months, were collected from the back of the head, at the nape of the neck. The hair samples were cut one-inch to the scalp, using stainless steel scissors to avoid contamination by other heavy metals. The samples were stored in polyethylene bags at room temperature. All samples were collected with the consent of the donors.

#### Questionnaire employed in the sampling campaign

Subject No. ....

Family name .....

First name .....

Full address .....

Sex (Male)..... (Female).....

Age..... (Years)..... (Months)

Weight ..... (kg)

Height ..... (m) ..... (cm)

Health conditions (brief description):  
.....  
.....  
.....

Comments on food habits and lifestyle in general (brief description)  
.....  
.....  
.....

Specific remarks (e.g., type of shampoo normally used, frequency of application)  
.....  
.....  
.....

#### Washing

Before analysis, each individual hair sample was cut into approximately 0.5-cm-long pieces and mixed to allow a representative sub-sampling of the hair specimen. After cutting, each sample was washed four times with diluted Triton X-100; samples were then rinsed with distilled water. The samples were then rinsed with acetone and allowed to drain. This was followed by three rinses with de-ionized water and two rinses with acetone (22-24). The samples were then dried in an oven at  $75 \pm 5^{\circ}\text{C}$ . Dried samples were stored separately in polyethylene bags.

#### Consent

Before the start of this study, all control subjects and skin-diseased patients were informed about the aim of the study via the distributed questionnaire, and all agreed to participate and signed the form.

#### Sample preparation

Triplicate samples from each subject (10 mg each) were weighed in Pyrex flasks, The flasks were capped and then digested at  $60-70^{\circ}\text{C}$  for 1-2 h till semidried. The digests were then treated with 5 ml more nitric acid and a few drops of  $\text{H}_2\text{O}_2$ , heated on a hot plate at approximately  $80^{\circ}\text{C}$  until the color of the digestion solution became bright yellow, cooled and then diluted to a volume of 25 ml in volumetric flasks with 2N nitric acid. The certified hair sample was also digested in the same manner as described above. (25-27)

Blank and standard solutions were prepared in a similar acid matrix. All of these sample solutions and a series of standard solutions of Fe, Zn, Cu, Cr, Ni, Cd, and Pb were atomized in air-acetylene flame with a Hitachi 180-50 AAS. Results were calculated from the calibration curve obtained by statistical analysis of Concentration Vs Absorbance data for elements using fitting of straight line by least square method. The validity of the acid digestion method was checked by employing the certified values (the percentage recovery of all elements was 98-99%). Mean values for Zn differed by less than 1% from the certified values. The coefficient of variation was less than 2% for Zn. The color of the digesting mixture was light yellow, with a very low viscosity. We calculated the mean, standard deviation, p- value and F-value with the Minitab 13.2 version, a computer software statistical program and Excel program.

**Results**

Concentrations of elements in hair vary widely among individuals, thus a significantly large number of samples from a large population need to be analyzed and the results treated statistically for meaningful correlation. The results are shown in Table 1. The scalp hair samples analyzed were categorized according to age and gender.

The mean values of Fe were significantly lower in the scalp hair samples of both male and female skin-diseased patients than controls (mean Fe concentration in male patients (21.63 ± 0.31, 13.2 ± 1.10 µg/g) compared to control male subjects (p = 0.008, 0.000), and in female patients (20.89 ± 0.76, 15.65 ± 1.64 µg/g) compared to control female subjects (p = 0.000, 0.001) in 6-15 and 16-30 age groups, respectively). The mean concentrations of Zn in the scalp hair samples of control male and female subjects of both age groups were found

to be significantly higher (225 ± 11.4, 283 ± 9.12 and 247.7 ± 8.9, 219.7 ± 7.11 µg/g, respectively) vs. patients of both age groups (p = 0.009, 0.002 and 0.008, 0.000). These results also differed according to genders. The mean Cu concentrations in control male subjects were 17.29 ± 1.41 and 11.73 ± 0.65 vs. patient values of 11.29 ± 0.54 and 9.33 ± 0.36 µg/g in the age groups of 6-15 and 16-30 years, respectively. In females, the mean Cu concentration in patients was lower compared to controls (p = 0.001 and 0.001, respectively) in both age groups.

The mean concentration of Cr in the scalp hair samples of male skin-diseased patients, 8.14 ± 0.67 and 7.25 ± 0.27 µg/g, was higher than controls (p = 0.004 and 0.000) in the two age groups, respectively. The same pattern was observed in females (p = 0.002 and 0.000) in both age groups.

Table 1. Trace element concentrations in scalp hair samples of normal and skin-diseased subjects (µg/g).

Age groups	Male n=62			Female n= 48		
	Normal n= 30	Skin-diseased n=32	P-value n= 23	Normal n= 25	Skin-diseased	p-value
Iron						
6-15	26.17±1.09	21.63±0.31	0.008	24.19±1.70	20.89±0.76	0.000
16-30	26.89±1.21	13.2±1.10	0.000	30.15±1.14	15.65±1.64	0.001
Zinc						
6-15	225±11.4	121±4.96	0.009	247.7±8.9	121.4±4.96	0.008
16-30	283±9.12	170.5±9.8	0.002	219.7±7.11	125±8.68	0.000
Chromium						
6-15	3.81±0.49	8.14±0.67	0.004	3.8±0.48	7.98±0.54	0.002
16-30	3.7±0.33	7.25±0.27	0.000	4.00±0.31	6.22±0.41	0.000
Copper						
6-15	17.29±1.41	11.29±0.54	0.009	17.29±1.41	11.55±1.02	0.001
16-30	11.73±0.65	9.33±0.36	0.005	11.78±0.58	10.19±0.65	0.001
Nickel						
6-15	6.86±1.57	14.1±1.12	0.007	6.93±2.05	18.14±2.41	0.000
16-30	7.53±1.59	20.0±2.16	0.000	7.29±1.41	12.79±1.32	0.000
Cadmium						
6-15	2.49±0.69	3.35±0.24	0.000	1.69±0.81	4.19±0.29	0.001
16-30	1.92±0.14	2.76±0.22	0.001	1.34±0.035	1.60±0.18	0.001
Lead						
6-15	10.17±2.31	18.76±1.85	0.001	6.41±1.42	18.73±1.59	0.003
16-30	7.37±0.91	25.78±1.05	0.003	7.37±0.91	15.51±0.41	0.006

The concentrations of Ni in the scalp hair samples of male and female skin-diseased patients were found to be high ( $14.1 \pm 1.12$  and  $20.0 \pm 2.16$ ;  $18.14 \pm 2.41$  and  $12.79 \pm 1.32$   $\mu\text{g/g}$ ) vs. controls of both genders ( $6.86 \pm 1.57$  and  $7.53 \pm 1.59$ ;  $6.93 \pm 2.05$  and  $7.29 \pm 1.41$   $\mu\text{g/g}$ ) in both age groups, respectively. The mean level of Cd in the scalp hair samples of male controls ( $2.49 \pm 0.69$  and  $1.92 \pm 0.14$   $\mu\text{g/g}$ ) was lower than in skin-diseased patients ( $3.35 \pm 0.24$  and  $2.76 \pm 0.22$   $\mu\text{g/g}$ ) in both age groups, respectively, and the same pattern was observed in females ( $p = 0.001, 0.001$ ). The mean concentration of Pb in the scalp hair samples of male skin-diseased patients ( $18.76 \pm 1.85$  and  $25.78 \pm 1.05$   $\mu\text{g/g}$ ) was higher vs. controls ( $10.17 \pm 2.31$  and  $7.37 \pm 0.91$   $\mu\text{g/g}$ ) in both age groups, respectively. In females, the level of Pb in the scalp hair samples of skin diseased patients ( $18.73 \pm 1.59$  and  $15.51 \pm 0.41$   $\mu\text{g/g}$ ) was also higher vs. controls ( $6.41 \pm 1.42$  and  $7.37 \pm 0.91$   $\mu\text{g/g}$ ) in both age groups ( $p = 0.003$  and  $0.006$ ), respectively.

## Discussion

The study population consisted of 110 human subjects (both male and female) of two age groups: 6-15 years and 16-30 years. The results in Table 1 show that the total mean value of iron in the scalp hair samples of male skin-diseased patients is lower compared to the normal controls. Deficiency in nutritional iron represents a public health problem recognized throughout much of the world. The overall prevalence rate of patients with iron deficiency (ID) who need supplementary iron therapy ranges markedly from less than 10% to as high as 70% among various ethnic and socioeconomic groups. Dermatologically, the iron-deficit state can be a secondary condition or trigger a wide range of mucocutaneous alterations. Early appreciation of adverse cutaneous manifestations of ID seems to have commensurate significance not only in predicting the presence of undiagnosed ID, but also for providing specified avenues for rational therapeutic approaches to patients with ID. Previous studies have suggested that iron may be lost in accelerated turnover of the keratinocyte from scaling; currently, malabsorption of iron is accepted as accounting for dermatopathic anemia (28).

A significant difference was observed in the concentration of zinc in the scalp hair samples from male

and female skin disease subjects from both age groups ( $p$  value 0.000-0.009). Zinc deficiencies are quite common in people living in poor countries. People with skin disease from the urban areas of Hyderabad region mostly belong to those families who do not have a proper diet that provides enough nutrients and vitamins for maintaining good health. Dietary zinc deficiency is common in people living in poor countries. Phytate, a substance found in unleavened bread (pita, matzos, and some crackers) significantly reduces absorption of zinc, increasing the chance of zinc deficiency. Children suffering from the heredity disease acrodermatitis enteropathica, caused by an inability to absorb zinc from their food, suffer from hair loss and skin problems as well as diarrhea and defective growth and development. These symptoms disappear when they are given high doses of zinc supplementation. More commonly, zinc supplementation has been found to be helpful for eczema, acne and leg ulcers in the elderly (29). Zinc oxide is used typically as a sun block and to treat skin conditions such as chafing, diaper rash, and minor skin irritations (30). Zinc may also enter the body following the topical application of its oxide salt to damaged skin (31). The more damaged the skin, as in severe burn patients, the greater the expected zinc absorption. In the case of skin-diseased patients, vegetarian foods have high phytate, and its intake may result in reduced availability of iron and zinc for intestinal absorption (32).

It was observed that the level of copper is low in the scalp hair samples of skin-diseased subjects from both age groups in both genders. Signs of a copper deficiency include anemia, a decrease in certain white blood cells, skeletal demineralization, loss of hair color, and skin pallor. Children with copper deficiencies may experience ruptured blood vessels, central nervous system abnormalities, growth retardation and poor temperature regulations (33). Copper deficiency may occur in infants who are fed formula with low copper content, for example cow's milk, instead of breast-milk. It is also likely in pre-term babies, especially those with low birth weight, since they have not had the full opportunity to develop their stores in the liver (34). The faster growth rate they experience compared to full-term babies means that they require more copper but have a lower supply and thus may be deficient (35). A possible source of copper may be due to insecticidal sprays on soil-producing tobacco. It was determined in an investigation that beef and other meat contain the highest amount of copper and cadmium as compared to vegetarian food (36).

It was observed that the mean concentration of chromium is higher in the scalp hair samples of skin-diseased subjects from both age groups in both genders. The causes of allergic dermatitis include hexavalent chromium from cement, cobalt, nickel, rubber gloves or boots, epoxy resins, asphalt and coal tars, some sawdust, and poison ivy (37). The effect of ferrous sulfate is based on reducing water-soluble chromium VI to less soluble chromium III in wetted cement. The penetration of chromium VI into the epidermis is much higher than that of chromium III (38).

The high chromium concentration obtained may be attributed to the chromium content in the leaves of crops, especially in tobacco, which might get incorporated in the leaves from the soil (39).

In this study, the level of nickel was higher in the skin-diseased subjects of both age groups in both genders compared to normal subjects. Moreover, oral doses of nickel sulfate in water, which is well absorbed in the absence of food, in amounts as low as 0.6 mg produced a positive skin reaction in fasting individuals with nickel allergy (40).

Exposure of nickel is through non-occupational exposure to costume jewelry, metallic accessories worn by patients and other metal objects, e.g. coins and metal tools. Women are more prone to nickel allergy than men due to ear piercing and costume jewelry.

Although certain European countries have adopted legislative measures to limit nickel release in nickel-containing products, leading to a decreasing incidence of sensitization to nickel in their younger population (Germany (41), Denmark (42, 43), no such measures have yet been undertaken in Asian countries.

It was observed that the different pattern between females and males probably reflects the differences in occupational exposure more than an actual sex difference. It is well known that both nickel and chromium allergy are of mainly occupational origin in males, while in females, nickel allergy primarily derives from contact with nickel-releasing consumer items such as earrings (44,45).

Nickel allergy from work tools and metalworking were frequent causes in both sexes. It has been shown that nickel in a significant amount may be released from tools (46), and metalworkers are known to have an increased risk of nickel allergy (47,48).

A significantly higher value of cadmium was found in skin-diseased subjects in both genders from both age groups compared to normal subjects. Cadmium, which enters the environment from mining, industry, vehicles and household waters, binds strongly to soil particles or dissolves in water (49). Once taken up by fish, plants and animals, cadmium stays in the body for a long time (50). Humans are also affected by cadmium through smoking and consumption of foods and beverages. Rice is the main source of cadmium in rice-eating countries.

The level of lead is higher in the scalp hair samples of male and female skin-diseased subjects from both age groups as compared to the normal controls. Lead can cause severe health effects at a relatively low level of exposure. Human lead exposure is mainly through air and food. The presence of lead in fuels has contributed to much of the current human exposure (51). In most developed countries, the fuel content of lead has been controlled but it remains an issue of immediate consideration in developing countries, including Pakistan. Other sources of lead exposure include lead-based paints, lead pipelines in water supply systems and ceramics. Lead-based products, including paints and food containers, are not completely banned in Pakistan (52).

There are a number of factors contributing to the higher levels of cadmium and lead in congested areas like Hyderabad city. Hyderabad city represents a typical urban environment with heavy traffic load, high population density and industrial units. In addition, open burning of plastics and brick-making among other activities contribute to this higher level of toxic elements. Our study revealed that the levels of cadmium and lead in hair were higher in Hyderabad city, suggesting that dust containing these heavy metals is attached to hair samples due to a typical urban environment with heavy traffic load, high population density and industrial activities.

## Conclusion

Higher values of chromium, nickel, cadmium and lead were observed in the scalp hair samples from subjects with skin disease as compared to normal subjects from the same age groups. These samples also show a lower value of essential trace elements such as zinc, iron and copper as compared to normal subjects.

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