Assessment of Zinc and Copper Contents in the Hair and Serum and Also Superoxide Dismutase, Glutathion Peroxidase and Malondi Aldehyde in Serum in Androgenetic Alopecia and Alopecia Areata

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Abstract: Alopecia areata (AA) is a recurrent nonscarring type of hair loss that can affect any hair-bearing area. Androgenetic alopecia (AGA) or male pattern hair loss is a very common condition that has a significant psychosocial impact for patients. There are claims that imbalance of trace elements may trigger the onset of alopecia. The aim of this research was to assess the level of zinc, copper contents in the hair and level of lipid peroxidation as super oxide dismutase, Glutathion peroxidase and malon di aldehyde in serum in androgenic and areata alpecia. The prospective study was conducted in department of Dermatology of Sina Hospital and Biochemistry department of Tabriz University of medical science. The study population consists of 27 patients with alopecia areata and 27 patients with androgenetic alopecia. 27 age and sex matched healthy control subjects were studied. The level of zinc, copper contents in the hair and serum and level of lipid peroxidation as super oxide dismutase (SOD), Glutathion peroxidase(GPX-Px) and malon di aldehyde (MDA) in serum in androgenetic, areata alpecia and healthy control subjects were measured in all samples. The collected data were statistically analyzed, using SPSS. The duration of hair loss varied between 1-240 month among patients with alopecia areata and 6-180 month among patients with androgenetic alopecia. The mean of hair zinc level in AA patients, AGA patients and controls were 98.33 µg/dl, 105.35 µg/dl and 129.52 µg/dl. The mean level of hair copper level in study and control groups were, 7.91, 7.25 and 10.34. The mean of serum level of SOD, MDA and GPX-Px in study and control groups were (1945.25, 1861.57 and 2296.77), (3.64, 3.49 and 1.62) and (129.11, 118.84 and 138.74). Zinc and copper contents of hair and serum were significantly lower among patients with alopecia areata and androgenetic alopecia compared to controls (P<0.05). The serum level of SOD, GPX-Px were significantly lower and level of MDA were higher among patients with alopecia areata and androgenetic alopecia compared to controls (P<0.05). These results suggest that low levels of zinc and copper of hair and serum and lipid peroxidation and alterations in the oxidant-antioxidant enzymatic system (SOD, GPX-Px) with high level of MDA at serum may play a role in the pathogenesis of AA and AGA.

Keywords: Alopecia; Zinc; Copper

1. Introduction
Alopecia is one of the most common problems amongst the youth and it is responsible for numerous psychosocial problems especially in the youth. The patients in whom the disease emerges in younger ages may experience more discomfort. Alopecia areata can be devastating in the people who care much about their appearance as they try to cover the hairless areas with long hairs. These patients gradually become reserved and experience psychological disorders (Ruiz-Doblado et al., 2003). Etiology of this disease is not yet known but autoimmune events are to be blamed. Regarding the pathophysiology of the disease it is said that T-cells are responsible for this autoimmune phenomenon and increase the incidence of probability in the people who are genetically susceptible. (Ruiz-Doblado et al., 2003, Garcia-Hernandez et al., 1999, Gulec et al., 2004, Gulec et al., 2005).

Androgenic alopecia (boldness in men) is also seen in the men who are genetically susceptible. Regarding the possible causes for areata and androgenic alopecia, diverse hypotheses have been introduced however major causes of these two diseases have not been discussed in any of these hypotheses. Finding the probable causes of this
disease could lead to making preventive and therapeutic decisions. Different hypotheses such as lack of some elements in the blood and hair have been discussed. On the other hand, some researchers have blamed the increase in antioxidant enzymes in the hair as an initiative factor of the disease. Our hypothesis is that the decrease in copper and zinc serum levels causes hair loss in these patients. The concentration of these elements in the hair is also of importance. Prior to our study, no similar study was previously done in Iran regarding measuring copper and zinc content in the hair. On the other hand regarding the existing articles, the hypothesis that the deficiency of these elements in the hair could cause and develop the disease was considered by the researcher and defined as one of the primary goals of the current study. The role of serum and hair fat peroxidation in the patients with alopecia has been discussed in previous studies. The serum levels of super oxide dismutase, glutathione peroxidase, malondialdehyde were compared in the patients and regular people. We hope that the present research could help us discover alopecia etiology so that we can provide preventive and therapeutic measures.

2. Material and Methods

The present study is a descriptive-analytic study in which 27 patients with areata alopecia and 27 patients with androgenic alopecia referred to dermatology clinic of Tabriz Sina Hospital were randomly chosen and their findings were compared with 27 normal people.

The referred patients with areata and androgenic alopecia were examined by physicians in their offices and selected using Hamilton’s classification. Patients with areata alopecia were chosen considering their clinical signs including clear patches without single or multiple scales in head and face in different age ranges.

Regarding the role of antioxidant enzymes, it could be said that in normal conditions there is equilibrium between oxidative destruction and anti-oxidative protective reactions in cells. Super oxide dismutase (SOD) and Glutathion peroxidase(GPX) have been proved to play roles in stress oxidative reactions and skin diseases.

Based on the results obtained from the studies carried out on total antioxidant SOD and GPX levels in patient and control groups, using the formula for defining sample volume, the sample number were calculated as 40 which was considered 50 after a 20% increase for each of the groups.

As previously mentioned, one of the objectives of this study was to measure copper and zinc levels of the hair. However in spite of the explanations of the researcher not all of the selected patients were eager to go to the laboratory due to the distance between the clinic and the laboratory. Therefore only patients with complete information are brought in the findings section.

As above mentioned, the groups of the patients with areata and androgenic alopecia consisted of 27 people each and to be compared with normal population, 27 normal people were also entered that study. It should be mentioned that these two groups and the control group were matched regarding age and gender. The researcher was present in the above mentioned clinics and offices and completed the provided questionnaires and then guided the patients for having the necessary tests done after offering them the necessary explanations. After obtaining written consent from the patients, almost 1 g (100 to 200 hairs) fallen hairs were collected and the patients were introduced to the laboratory.

The hair sample consists of the fallen hairs which have been collected by the patient and sent to the laboratory. Blood samples were also taken in the laboratory. The primary questionnaire consisted of questions regarding demographic information, marital status, pregnancy and delivery items, history of recent surgery or disease, physical or mental stresses and hair fall duration and severity.

Laboratory findings were also included in this questionnaire including serum levels of copper, zinc, SOD, malon di aldehyde (MDA) and GPX and also copper and zinc levels in the hair.

The patients having symptoms for areata and androgenic alopecia were interred the study.

Areata alopecia is defined as completely hair fall in a particular part of the scalp skin with a clear margin which is usually round. Androgenic alopecia is defined as unusual thinning of the hair in the frontotemporal region followed by circular hair fall in one part of the vertex.

Exclusion criteria from this study were as following: any factor interfering deteriorating or amending patient status such as extrinsic factors like the history of consuming zinc, multivitamin, medications (allopurinol, cytotoxic medications), history of radiotherapy, thyroid dysfunction, psychological stress, local trauma, severe weakening diseases such as tuberculosis, any accompanying disease affecting hair fall like hirsutism, diabetes and age limitations. On the other hand, other interfering factors such as gastrointestinal disorders, stress, diet… that can impair copper and zinc absorption were borne in mind and patients with above mentioned diseases were excluded from the study.

After collecting the samples, copper and zinc levels were measured using the following technique:
Hair samples should be collected from occipital region and 1 cm above the scalp skin. Each sample should be placed in separate plastic bags and stored in a desiccator in the dark. The amount of the metals in the hair is significantly influenced by external pollutants such as: dust, sweat, epidermal scaling, and especially detergents and cosmetics. Therefore it is essential for the samples to be washed and cleaned from any extrinsic pollutants so that we can have appropriate samples. Washing procedure is carried out in three steps:

1. Washing and shaking for 3 consequent times for 10 minutes in a shaker with a 3 to 1 ethyl ether-acetone solution to eliminate the fat layer around the hair.
2. Soaking and immersing along with one-hour shaking in an EDTA 5% solution to neutralize chemical elements on the surface of the hair.
3. Frequent washing with deionized water with high purity degree

After drying the hair samples, almost 0.25 grams of the washed hair is transferred to a tube filled with tetrafluoroethylene. Later we add HNO3 solution and oxygenated water with a 3 to 1 ratio. After 24 hours a mega-microwave mortar MLS1200 is used to dissolve the minerals in the hair samples as following:

Three minutes in 250 W.
Six minutes in 0 W.
Five minutes in 250 W.
Six minutes in 0 W.
Two minute in 450 W.
Two minute and 500 W.

After cooling the sample, we increase the volume of the samples to 10 ml using DW. All of the above mentioned steps in the procedures should be done in a clean room. (Mustafa and Ibrahim 1999)

After data being collected by the researcher, all data were interred SPSS software and analyzed. In this research, statistical and descriptive analyses, and statistical indices like mean, median and... and K2 and independent t-test were used to compare the means.

It should be mentioned that P less than 0.05 is considered to be meaningful.

3. Results

In our study 27 patients with alopecia areata and 27 patients with androgenic alopecia were compared with 27 healthy people. The mean age for the patients with alopecia areata was 66.27 ± 9.90, with a median of 25; the youngest patient was 2 and at the oldest was three years old. The mean age of the patients with androgenic alopecia was 88.28 ± 9.85 with a median of 28; they youngest was 17 and the oldest 62 years old. The mean age for the normal group was 27.11 ± 5.55 with a median of 47; the youngest patient was 15 and the oldest was 38 years old.

Independent-test revealed no meaningful difference between the mean age of the patients with alopecia areata or androgenic alopecia with normal people (P = 0.435).

In the group of patients with alopecia areata, there were 24 females (88.9%) and three males (11.1%) and in the group of patients with androgenic alopecia there were 21 females (77.8%) and six males (18.5%) (P = 0.546).

13 Females with alopecia areata and 13 females with androgenic alopecia were housewives.

7 people with alopecia areata and 5 people with androgenic alopecia were students. There were 13 single (48.14%) and 14 married (51.85%) people in the group of patients with androgenic alopecia. There were also 12 single (4.44%) and 15 married (55.55%) in the group of patients with androgenic alopecia.

16 females from the group of the patients with alopecia areata (59.3%) and nine people from patients with androgenic alopecia (39.1%) had no history of delivery. None of the patients with alopecia areata had an underlying disease. Only one patient with androgenic alopecia had a history of diabetes, hypertension and cardiac disease.

A background of emotional stress was found in 2 patients with alopecia areata that and 2 patients with androgenic alopecia. There was one case of family member death and divorce in the patients with alopecia areata. There was also a case of family death and examination stress in the patients with androgenic alopecia.

Nine people from the patients with alopecia areata (33.3%) had not been examined by a physician regarding their hair fall and not given any medication. 5 people (18.51%) of the patients with androgenic alopecia had not received any medication. There was no family history of hair fall in the patients with alopecia areata.

Family history was positive for six patients with androgenic alopecia. The result of K2 test revealed a meaningful relation between a positive family history and the incidence of androgenic alopecia in our study (P = 0.011).

The mean duration of hair fall in the patients with alopecia areata was 34.11 ± 58.33 months with a minimum of one month and a maximum of 240 months (20 years). The mean duration of hair fall was also 35.80 ± 51.48 months for the patients with androgenic alopecia with a minimum of six months and a maximum of 180 months (15 years).

The results of the independent t-test revealed that there was no meaningful difference between the
duration of hair fall before referring to the center between the two groups of the patients with alopecia areata and androgenic alopecia(P=0.914).

In our study the hair fall of almost 50 to 100 hairs in a day was considered as mild hair fall and more than 100 hairs a day as severe hair fall.

Based on the above mentioned definition, in the group of the patients with alopecia areata 11 people (40.74 %) had mild hair fall and 16 people (59.25 %) severe hair fall. In the group of the patients with androgenic alopecia 10 people had mild hair fall (37.03%) and 17 people (62.96%) severe hair fall (P=0.50).

The mean zinc level in the hair of the patients with alopecia areata was 98.33 ± 24.25 µg/dl, 105.35 ± 32.43 µg/dl in the patients with androgenic alopecia, and 129.51 ± 29.61 µg/dl in normal people.

Independent t-test revealed that there was a meaningful difference between the zinc content of the hair in the patients with alopecia areata (P=0.001), androgenic alopecia (P=0.005) and normal people.

The mean copper content of the hair in the patients with alopecia areata was 7.91 ± 2.72 µg/dl, in the patients with androgenic alopecia 7.25 ± 2.5 µg/dl, and in normal people 10.34 ± 2.3 µg/dl.

Independent t-test revealed that there was a meaningful difference between the copper content of the hair in the patients with alopecia areata (P=0.001), androgenic alopecia (P=0.005) and normal people.

The mean SOD serum level in the patients with alopecia areata was 129.11 ± 12.77, in the patients with androgenic alopecia 118.84 ± 8.09 µg/dl, and in normal people 138.74 ± 7.18 µg/dl.

Independent t-test revealed that there was a meaningful difference between the mean GPX-Px serum level in the patients with alopecia areata (P=0.001), androgenic alopecia (P<0.005) and normal people and lower serum levels can be observed in these patients.

4. Discussions

In different studies, the prevalence of alopecia areata has been reported to be 1 to 2%. The onset age is also varied in different studies ranging from 0 to 80 years. In a study Yasmeen et al reported that the prevalence of alopecia areata is more in males compared to females with a ratio of 36 to 16. The mean age of the patients in their study was 27.3 years and most patients were under 40 (Yasmeen et al., 2009).

This research was similar to the results of the study of Sharma et al. The age of the patients with alopecia has been reported to be 15 to 29 years in another study (Sharma et al., 1996).

In our study also the mean age of the patients with alopecia areata was 27.6 ±9.90 (between two and 53 years) and the mean age for the patients with alopecia androgenic was 28.88±9.85(between 17 to 62 year) which is in accordance with their results of other studies. In a study carried out on 736 people with alopecia areata, the male to female ratio was reported to be 1 to 1. In a similar study their ratio of females was a little higher. (Tobin 2006). In our study both in the group of the patients with androgenic alopecia and alopecia areata, considering the randomized sampling, the ratio of females was higher than males. Although this result is in contrary to the results obtained from the study of Yasmeen et al (2009)and other studies, cultural characteristics and authors insight should also be borne in mind. (Yasmeen et al., 2009).It is obvious that the ratio of the female to male in our study is not the prevalence ratio of the disease in both genders whereas it shows the referring ratio of females to males. Therefore it seems that men pay
less attention to their hair fall than females do and they consider it as the natural cycle of their lives or an incurable disease and avoid referring to physicians. This issue should also be studied more comprehensively.

Regarding the relation between psychological or physical stress and chronic psychological disease with alopecia incidence, expanded studies have been carried out. The studies revealed a high incidence of psychological disorders in these patients including anxiety, depression, phobia, paranoia and mood disorders which are more frequent in patients with alopecia areata compared to the normal people. These studies emphasize on recent stress such as the death of a family member, divorce, difficult exams, or severe physical stresses in the patients with alopecia (Garcia-Hernandez et al., 1999, Koo et al., 1994, Kalafi et al., 1993).

In the study of Van der Steen P, the possibility of having a positive family background in the patients with alopecia areata is mentioned to be 10 to 20% which is considered to be 1 to 7% in normal people. (Chantal and Harvey, 2009) A positive family history has been reported to be as a risk factor in the incidence of androgenic alopecia. In this study none of the patients with alopecia areata had a positive family history whereas 22.22% of the patients with androgenic alopecia had a positive family history. As it was mentioned, the relation between the positive family history and the type of alopecia was meaningful (P=0.011)

In different studies carried out on the relation between the etiologies of different types of alopecia so far, diverse results have been obtained regarding the relation between serum levels of copper and zinc and also copper and zinc contents of hair with alopecia.

In his research, AF Alexis mentions that zinc and iron are two major elements in the growth of hair. (Alexis 2004) Musaaloa Rauhama, unlike Alexis, found no meaningful difference in the serum levels of zinc, iron and copper in the patients with alopecia compared to the normal people. He reported that only in the universal alopecia the urine levels of copper was meaningfully lower (Mussalo-Rauhama et al., 1986)

In a study titled as “serum levels of elements in alopecia areata” Jasmeen J Bhat et al compared serum levels of copper and zinc in the patients with alopecia areata and normal people. In the study the mean serum copper level was reported as 78±7.45 in the patients and 88±7.8 in normal people. The mean serum copper levels also were reported as 114±17.5 in the patients and 117±17.02 in the normal people. The difference in copper serum level was meaningful in both groups (P<0.005) whereas the difference between serum copper levels was not meaningful (Yasmeen et al., 2009).

In this study like the similar studies, meaningful results were obtained comparing zinc serum levels in patients with alopecia areata and androgenic alopecia and normal people. Serum levels of this element was meaningfully lower than normal people in both groups (P=0.004 and P<0.005).

Regarding the copper serum level, a meaningful difference also was observed between the patients with areata and androgenic alopecia. The serum levels of this element also were meaningfully lower than normal people. Inspite of not being in accordance with the findings of Yasmeen et al., 2009, these findings are in accordance with most studies (Yasmeen et al., 2009).

For the first time in Iran, the copper and zinc contents of the hair of the patients with alopecia were compared to normal people. The results obtained from this study are in accordance with the similar studies and show meaningful lowers zinc contents in the hair of the patients with areata and androgenic alopecia compared to the normal people (P=0.007 and P<0.005). In a series of experiments, the copper contents of the hair of the patients with areata and androgenic alopecia were also meaningfully lower compared to normal people (P=0.001 and P<0.005).

Numerous studies have also been carried out regarding the relation between serum level of antioxidants and alopecia. In the research carried out in 2002 by Ecar et al, it was said that the level of super oxidase dismutase, glutathione peroxidase and malondialdehyde are higher in the scalp of the patients with alopecia areata compared to normal people. The number of the patients and normal people was 10 in this research. Experiments revealed significant differences in the enzyme levels of antioxidants (Akar et al., 2002). On the other hand other studies have shown higher levels of lipid peroxidase and antioxidant enzymes in the scalp of the patients with alopecia. In Mustafa Naziroglu study, blood levels of vitamin E., beta carotenes and the activity level of glutathione peroxidase in 37 patients with alopecia were compared with plasma and RBC levels in 34 normal people. In this study the activity level for glutathione peroxidase was meaningfully lower than normal people (Mustafa and Ibrahim 1999).

In Koca R study on the comparison of serum levels of antioxidants in the patients with alopecia and normal people following results were obtained: serum level of malondialdehyde was meaningfully higher in the patients compared to the normal people. The activity level of super oxidase dismutase enzyme
was also meaningfully lower compared to the normal people. This study suggests that the increase in peroxidation of the fats in the patients with areata alopecia is in relation with degrees in the activity of super oxidase enzyme and therefore fat peroxidation is a key factor in areata alopecia pathogenesis (Koca et al., 2005). In this study the serum levels of the enzymes (SOD, MDA, and GPX) were evaluated. As was mentioned in the results section, the serum levels of all three enzymes were meaningfully different from normal people. Serum level of SOD enzyme was meaningfully lower in the patients with areata and androgenic alopecia compared to normal people (P<0.005 and P<0.005). These findings are similar to the results obtained from the study of Koca et al., 2005 whereas Kara reported opposite results in his studies. Comparing serum levels of this enzyme in two groups with areata and androgenic alopecia also revealed meaningful differences. The serum levels of this enzyme were meaningfully lower in patients with androgenic alopecia (P=0.004).

Like the results obtained from Koca et al., 2005 study, our study also revealed a meaningful difference in the serum levels of MDA in patients with alopecia and normal people. The serum levels of this enzyme was meaningfully more in the patients with areata and androgenic alopecia compared to normal people (P<0.005 and P<0.005). No meaningful difference was found comparing the serum levels of this enzyme in two groups of areata and androgenic alopecia.

In our study, like the study of Mustafa Naziroglu, serum levels of glutathione peroxidase was lower in the patients with areata and androgenic alopecia compared to normal people (P=0.001 and P<0.005).

**Conclusion:**

The results of this study confirm the hypothesis that some elements in the blood and hair could affect hair loss and on the other hand the increase in the level of antioxidant enzymes in hair has been blamed as an initiating factor in these patients which our study also confirms this hypothesis.

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