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The Role of Serum Levels of Zinc, Copper, Magnesium and Iron in Alopecia Areata

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Abstract

Background: Alopecia areata (AA) is a recurrent, non-scarring type of hair loss. Although its etiopathogenesis is not fully understood, imbalance of some trace elements in the blood has been suggested as a possible contributory cause. *Objective:* To evaluate the role of serum levels of zinc, copper, magnesium and iron in the etiopathogenesis of AA. *Methodology:* This case control study was carried out in 40 patients with AA and 40 healthy volunteers who served as controls. Clinical assessment was performed for every patient. A 5 ml venous blood sample was collected from every patient and control, centrifuged and sent for quantitative detection of serum levels of zinc, copper, magnesium and iron by atomic absorption spectrometer. *Results:* The mean value of serum zinc was significantly lower, and the mean values of serum copper and magnesiumwere significantly higherin AA patients than the control group ($p<0.001$). The serum copper/zinc ratio was significantly higher in patientscompared with the control group ($p<0.001$). The mean value of serum iron was higher in AA patients compared to the controls; but this finding was not statistically significant ($p=0.632$). Further, the serum level of zinc was found to be significantly affected by patients' sex, age and duration of disease. *Conclusion:* Elevated serum copper and depressed serum zinc levels could be one of the contributing factors of AA. Decreased serum zinc level may additionally have a role in prolongation of the disease course. The role of magnesium levels need further elucidation. Serum iron levels do not seem to contribute to the pathogenesis of AA.

Keywords: Alopecia Areata; Atomic Absorption Spectrometer; Copper; Iron; Magnesium; Zinc.

Introduction

Alopecia areata (AA) is a non-scarring, autoimmune, inflammatory disease characterized by loss of scalp and/or body hair, the scalp being the most commonly affected site [1]. The etiology of AA is still unknown, although both genetic and environmental factors are believed to contribute towards the immune dysregulation. T cell-mediated inflammation is a central component to the autoimmune process of AA. Such inflammation likely requires the interplay of the effect of multiple cytokines, and there is evidence that the helper T-cell type 1 cytokines might be central to the pathophysiology of this disease[2-4].

Hair is composed of proteins, lipids, water and small amounts of trace elements [5]. There are many trace elements in the body, which directly or indirectly participate in metabolism and play key role in its modulation. Approximately 25% of the enzymes of the human body require metals for their activation to participate in vital metabolic functions of the system [6]. Lack of some elements in the blood and hair has been suggested as a cause for alopecia areata by earlier workers. In studies evaluating the role of serum levels of trace elements in the etiopathogenesis of different kinds of alopecias, results have been contradictory [7]. In one study, no meaningful difference was found in the serum levels of zinc (Zn), copper (Cu), magnesium (Mg) and iron (Fe) in patients with alopecia areata compared to healthy volunteers [8]. Bruske and Salfeld statistically compared theblood and serum levels of Zn, Mg and Cu in patients with many dermatological disorders including AA with healthy people, and

found statistically significant difference only in Mg levels [9]. In the case-control Indian study by Bhat et al, serum Zn levels were significantly decreased in AA patients whose disease was extensive, prolonged, and resistant to treatment, whereas serum Cuand Mg levels showed insignificant elevation compared to controls [10].

The present study was conducted to evaluate the possible role of Zn, Cu, Mg and Fe in AA by estimating serum levels of these trace elements in AA patients and comparing them with apparently healthy subjects (controls).

Methodology

This case control study was carried out on 40 adult patients (age > 18 years) with patchy AA affecting the scalp and 40 healthy volunteers who served as controls. All subjects were recruited from *Kasr El Aini* Dermatology outpatient clinic and National Research Center Dermatology outpatient clinic over a period of 14 months. Only those AA patients were included who had stopped any oral or topical treatment for AA at least 3 months prior to sample taking. The other exclusion criteria were: (a) diseases that may affect trace elements level such as chronic diarrhea, (b) cases with alopecia universalis or totalis, (c) and pregnant and lactating patients. The study was carried in accordance with the Helsinki Declaration of biomedical ethics [11]. Informed consent was obtained from each participant. Every patient was subjected to history taking including personal data, history of the present illness and clinical examination to determine the anatomical sites and the extent of hair loss according to the Severity of Alopecia Tool (SALT score) [12]. A 5 ml venous blood sample was collected from every patient and control, centrifuged and sent for quantitative detection of serum levels of Zn, Cu, Mg and Fe by atomic absorption spectrometry.

Determination of Zinc, Copper, Magnesium and Iron by Atomic Absorption Spectrometer

Trace element determination of Zn, Cu, Mg and Fe were carried out using *Varian SpectrAA 220 Flame Atomic Absorption Spectrometer*. The spectral lines used for determination were 213.9 nm for Zn, 324.7 nm for Cu, 285.2 mm for Mg, and 248.3 nm for Fe. The standard sources for the investigated elements are hollow cathode discharge lamps made by Cathodeon, England. Serum samples were diluted five times with double distilled water just before sample measurement. The calibration graphs used for the mof the easurementinvestigated elements were constructed applying normal aqueous standards according to the instrument manual [13]. Serum concentrations of Zn, Cu, Mg and Fe were calculated from the corresponding calibration curves.

Statistical Analysis

The collected data was statistically analyzed using Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 16 for Microsoft Windows. Data was statistically described in terms of range, mean ± standard deviation (± SD), frequencies (number of cases) and percentages when appropriate. Numeric data was compared using independent t-test. Correlation was done using Pearson's correlation coefficient test for numeric data and chi square test for non-numeric data. A probability value (p value) less than 0.05 was considered statistically significant.

Results

The study group comprised 40 patients with AA affecting the scalp. The age of patients ranged from 18 to 52 years (mean age = 29.55±8.2 years). Of the 40 patients, eight (20%) were females and 32 (80%) were males. In the control group of 40 healthy age- and se-matched volunteers, the age ranged from 18 to 50 years (mean age = 31.8±7.4 years) and there were [12 (30%) females and 28 (70%) males. The mean duration of AA was 15.3±20.7 months, ranging from 0.5-120 months. The extent of lesion ranged from 5-70% with a mean of 24.6±20.1%. Previous (before 3 months of inclusion) topical or intralesional steroid treatment was received by 32 (80%) patients.

Serum trace element levels in patients and controls are summarized in Table 1, and Figures 1 and 2. The mean value of Zn in AA patients was significantly lower than that in the control group ($p< 0.001$). The mean value of Cu and Mg in AA patients was significantly higher than that in the control group ($p< 0.001$). Patients' Cu/Zn ratio was significantly higher than that of the control group ($p<0.001$). The mean value of Fe in AA patients was higher than the control group but statistically insignificant ($p=0.632$).

When correlating trace elements level with patients' age, duration of the disease and extent of lesions a positive significant correlation was found with patient age for Zn levels, while a negative

significant correlation was found with the duration of disease for Zn and Mg levels (Table 2).

Serum levels of Cu, Fe and Mg were not found to be significantly affected by patients' sex (p=0.168, 0.489, 0.130 for Cu, Fe and Mg respectively) or previous treatment (p=0.567, 0.477, 0.475 for Cu , Fe and Mg respectively). While the level of Zn was significantly affected by patients' sex (it was found higher in females (p=0.001)), it was not affected by previous topical treatment for AA (p=0.304).

Table 1: Summary of Zinc, Copper, Iron and Magnesium levels in Alopecia Areata patients and controls groups

Level of trace element		Patient group (n=40)	Control group (n=40)	P value
Zinc (µg/dl)	Range Mean ± SD	44.1-73.2 61.485 ± 8.776	50.2-155.5 106.865 ± 31.460	< 0.001*
Copper (µg/dl)	Range Mean ± SD	137.8-257.0 196.875 ± 28.915	66.8-179.4 115.045 ± 30.597	< 0.001*
Cu/Zn ratio	Range Mean ± SD	2.3-4.9 3.274±0.724	0.5-2.2 1.186±0.491	< 0.001*
Iron (µg/dl)	Range Mean ± SD	99.7-149.4 128.120 ±16.338	94.7-162.6 126.285 ± 17.740	0.632
Magnesium (mg/dl)	Range Mean ± SD	1.8-3.6 2.725± 0.478	1.0-2.7 2.030 ± 0.398	< 0.001*

** P value < 0.05 is statistically significant.

Table 2: Correlation between trace elements level with age, duration and extent of lesion in Alopecia Areata patients

	Zinc level		Copper level		Iron level		Magnesium	
	(r) value	p value	(r) value	p value	(r) value	P value	(r) value	P value
Age	0.308	005.0*	-0.068	0.547	0.095	0.401	-0.099	0.382
Duration of disease	-0.0473	002.0*	0.092	0.571	0.203	0.210	-0.373	018.0*
Extent of lesion (%)	-0.32	0.16	-0.011	0.96	0.06	0.77	0.11	0.64

** P value < 0.05 is statistically significant

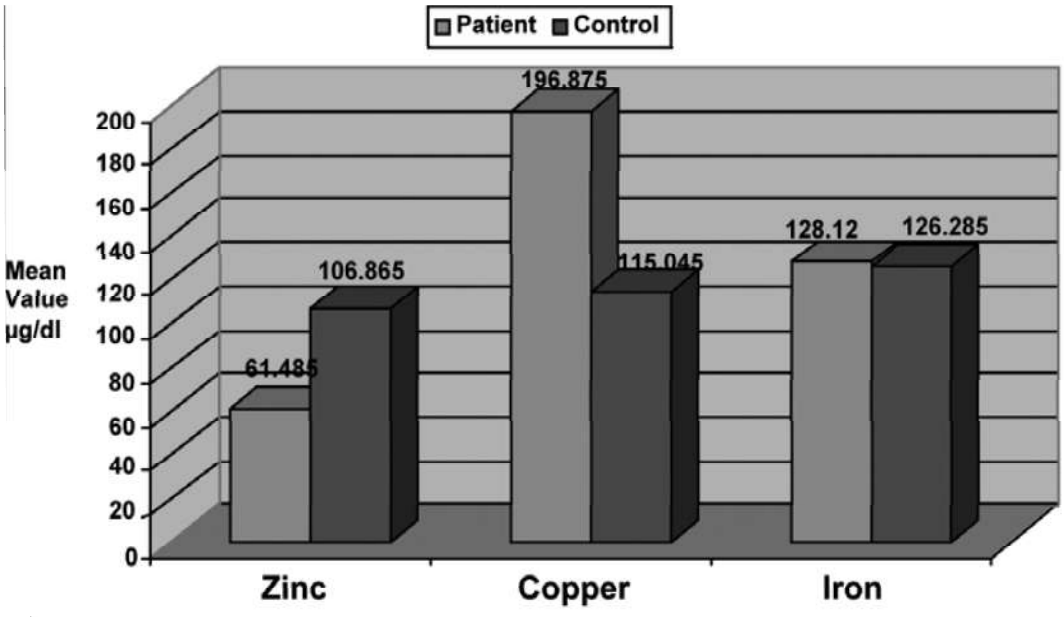

Fig. 1: Summary of Zinc, Copper and Iron levels in Alopecia Areata patients and controls

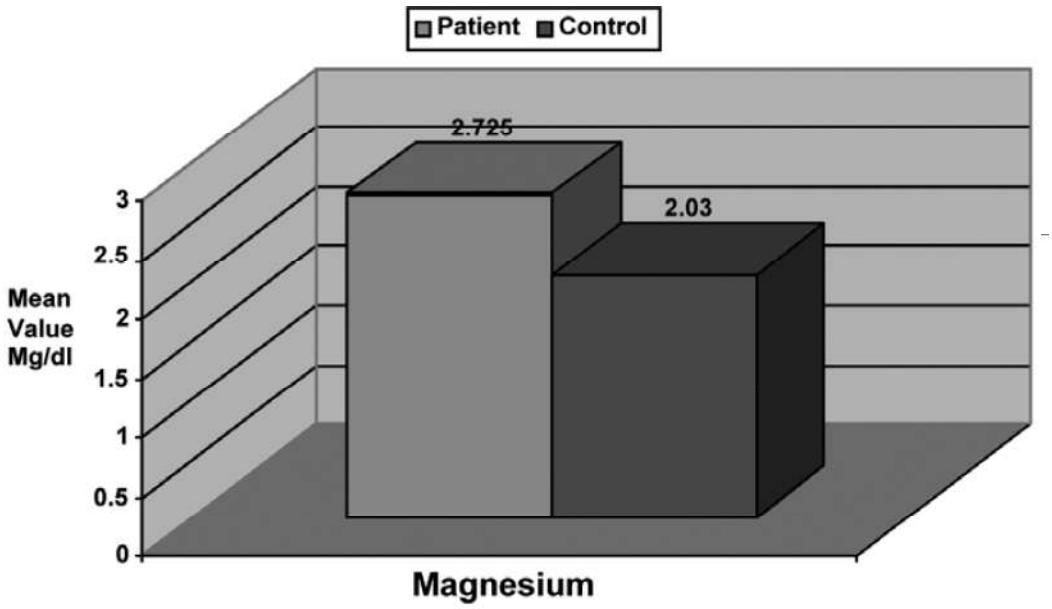

Fig. 2: Summary of Magnesium levels in Alopecia Areata patients and controls

Discussion

The present study showed a significant decline in serum Zn level in patients suffering from AA ($p<0.001$), a fact that is in agreement with the results of various other previous investigators [7,10,14-16]. The decrease in plasma Zn content has been attributed to reductions in intake or absorption in small intestine, increased urinary losses, and to a possible redistribution from plasma to tissues. It is noteworthy that tissues with high cellular turnover (e.g. skin) are characteristically affected by Zn deficiency calling the attention to the possibility that some dermatological manifestations, such as AA, may be attributed to Zn deficiency [17]. As a vital cofactor of various metalloenzymes, zinc has considerable effects on nearly all aspects of human metabolism including the skin. So, zinc deficiency induced by relatively higher loads of heavy metals can lead to AA besides other factors [18]. Zn is required for enzyme activities necessary for cell division, cell growth and wound healin and also plays an important role in maintaining the immune balance of the body. Immunologic defects of T-cell function are typical in Zn deficiency [16]. Experimentally, suboptimal intake of Zn has rapid adverse effects on the immune system of humans including T cell mediated responses [19]. The strong impact of Zn homeostasis on T-lymphocytes has been well demonstrated; Zn deprivation is known to result in the impairment of their development, polarization into effector cells and functional ineffectiveness [20]. Some authors have stated that Zn deficiency increases TNF-α levels, the cytokine central to the auto-immune destruction of the hair follicle [21]. Therefore, the presence of a significant reduction in Zn level of alopecic patients may lead to the dysimmune profile of those patients. A significant negative correlation was found between serum Zn levels and the duration of disease ($p<0.001$), a concurrent result of the study by Bhat *et al* [10]. Thus, it can be concluded that decreased serum Zn levels could be a factor leading to prolonged AA disease duration.

Oral Zn supplementation has been reported to stimulate both T and B-cell activity [22] and improve the immune system in the elderly [23].Based on the results of these studies including ours, Zn supplementation may be an effective adjuvant therapy for AA patients who have a low serum zinc level and/or are treatment-refractory [24].

A positive correlation was found between Zn level and age ($p=0.005$). Levels of Zn were significantly higher in females, but not affected by previous topical treatment. This suggests that serum Zn levels are variable in relation to age and gender. Gender variation may be explained by the less number of females involved in this study. Favier and Ruffieuxfailed to find such variation [25].

Patients of the present study also showed a significant increase in serum Cu levels than in controls group ($p<0.001$), an observation reported by some other investigators as well [14]. While Bhat *et al* reported insignificantly higher serum levels of Cu in AA patients [10], the serum levels of Cu in AA

patients have also been reported lower than normal [7,15].

Increases in serum Cu content occur in various inflammatory and connective tissue disorders [17]. In a study from Iraq, a significant increase in basal malondialdehyde (MDA) level, a strong biomarker of lipid peroxidation, and a significant decrease in glutathione (GSH) level, a major antioxidant, was reported in erythrocytes of AA patients compared to their normal controls suggesting the role of oxidative stress in the pathogenesis of alopecia areata [26]. Based on those findings, it was suggested that Cu could be a potential inducer of LDL oxidation and high ceruloplasmin levels have pro-oxidant properties [14].

One of the most common trace-metal imbalances is elevated copper and depressed zinc (the optimal plasma or serum ratio of Cu/Zn is 0.70 - 1.00). The ratio of copper to zinc is clinically more important than the concentration of either of these trace metals [27].

In our study, the patients' Cu/Zn ratio was significantly higher than that of control group. The present finding may therefore suggest the presence of an inflammatory condition in patients with alopecia areata. It is possible that the observed changes in Zn and Cu plasma levels reflect the presence of an imbalance in those trace metals metabolism in alopecia areata. The consequences of this imbalance are unknown at present. These changes, however, are reflected in the Cu/Zn ratio, which was increased in AA patients compared with controls. Since Zn is an essential component of superoxide dismutase (SOD), a critical cytoplasmic antioxidant enzyme, the deficiency of zinc could induce an increase in tissue oxidative damage [14]. Further, Zn deficiency is associated with an increase of Cu and Fe due to the antagonistic relationships between these metals [28].

In the present study, the mean value of Mg in AA patients was significantly higher than the controls ($p < 0.001$). Similar result was obtained by Bruske and Salfeld [9]. Insignificant elevation in serum Mg levels in AA has been reported by other investigators [10,29]. However, the exact mechanism of high serum magnesium level contributing to the pathogenesis of AA remains to be explored.

In this study, the serum iron levels were found to be mildly (statistically insignificant) elevated in the AA patients compared to controls. The results of many studies are in accordance with our resultsof no apparent association between AA and iron deficiency. The iron status of 32 AA patients in UK was investigated and it was found that the prevalence of iron deficiency is not significantly increased in patients with AA [30]. In another study, low levels of serum iron were reported in AA patients, but with no statistical significance [31]. Esfandiarpour *et al* reported higher mean level of serum iron and ferritin in AA patients compared to control subjects, but the difference was insignificant. They stated that the prevalence of Fe deficiency is not increased in patients with AA [32]. On the other hand, ferritin has been accepted as a novel marker for autoimmunity and elevated levels of ferritin in autoimmune disorders have been reported [33].

Conclusion

In conclusion,depressed zinc and elevated copper levels in serum constitute the most common trace-metal imbalances in AA patients and could be contributory to its pathogenesis. Also, decreased serum Zn level might be considered as a potential risk factor leading to prolonged disease course. Serum iron level does not seem to contribute to the pathogenesis of alopecia areata. The role of serum magnesium level in AA needs further research. We recommend the performance of evaluative studies on disease outcome on patients with AA after receiving oral Zn supplementation. Meanwhile, we advocate institution of empirical oral Zn supplementation as an adjuvant therapy in all cases of AA.

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