

## ORIGINAL ARTICLE

## Evaluation of Copper and Zinc Disturbances in the Patients of Alopecia

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## ABSTRACT

**Objective:** To evaluate serum copper and zinc levels in patients with different types of alopecia compared to healthy controls, and to assess the potential relationship between trace element deficiencies and specific alopecia types.

**Study Design:** Descriptive cross-sectional study.

**Place and Duration of Study:** Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from 1<sup>st</sup> June 2021 to 30<sup>th</sup> April 2023.

**Materials and Methods:** A total of 250 patients categorized into five groups; Group-1 male pattern hair loss, Group-2 female pattern hair loss, Group-3 alopecia areata, Group-4 telogen effluvium, and Group 5 healthy individuals were enrolled. Serum zinc and copper levels were tested by using atomic absorption spectrophotometer and results were compared with the help of SPSS by applying one-way ANOVA and post hoc test. The  $p$  value  $\leq 0.05$  was considered statistically significant.

**Results:** In Group-3, the mean serum copper was  $15.14 \pm 3.26$  followed by Group-4 ( $14.99 \pm 2.23$ ), Group-2 ( $14.11 \pm 3.83$ ), Group-1 ( $9.92 \pm 2.23$ ), and the control group was  $14.97 \pm 3.6$ . As in Group-2, the mean serum zinc was  $12.93 \pm 2.97$  followed by Group-4 ( $11.13 \pm 3.50$ ), Group-3 ( $10.23 \pm 2.91$ ), and Group-1 ( $8.70 \pm 2.08$ ), and the control group was  $14.01 \pm 1.82$ . Serum zinc had a statistically significant difference ( $p$  value  $\leq 0.05$ ) between all groups of alopecia. Whereas statistically significant low copper levels were observed only in male pattern hair loss with  $p$  value  $\leq 0.001$ .

**Conclusion:** Low zinc levels are related to all types of alopecia. Whereas low copper levels are observed only in male pattern alopecia. Hence zinc supplementation can be used to get some beneficial effects in all types of alopecia. Whereas patients with male pattern alopecia should also be evaluated for serum copper levels and supplementation is required only if they have low copper levels.

**Key Words:** Alopecia, Serum Copper, Serum Zinc, Trace Element.

## Introduction

Alopecia is a disorder marked by hair loss from the body areas where hair is normally found especially head. The distressing illness lowers sufferers' self-esteem, impacting them mentally and socially. The disorders can be caused by a variety of factors, including stress, inheritance, hormones, diet, certain illnesses, and some drugs such as those used to treat cancer.<sup>1</sup> Nutrition and food can be used to treat hair

loss, and this is an active field of research.<sup>2</sup> The FDA has approved just two serendipitous medications (minoxidil and finasteride) for the treatment of alopecia.<sup>3</sup>

There are several forms of alopecia, among them the most frequent are androgenic alopecia, Telogen effluvium, and alopecia areata.<sup>4</sup> Androgenic alopecia is a prevalent form of baldness that can affect both men and women. Males are mainly impacted by it as opposed to females, primarily due to the higher production of testosterone, which is a male sex hormone responsible for triggering the condition.<sup>5</sup>

Alopecia areata is a common localized non-scarring, inflammatory, autoimmune hair loss disease that occurs on any skin-bearing hair. Autoimmune, genetic, and environmental factors are considered to be the possible etiologies of Alopecia areata. Alopecia areata is a disorder characterized by a focally, organ-specific autoimmune disorder.<sup>6</sup> The

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prevalence of Alopecia areata in female individuals is 0.252%–0.271% and in male individuals is 0.145%–0.171%.<sup>7</sup> Telogen effluvium is a condition where hair loss occurs due to hair follicles being pushed prematurely into the dormant telogen stage, without causing any permanent scarring. The disease is widespread among the elderly, persons who are physically and emotionally disturbed, and those who have thyroid or additional hormonal imbalances.<sup>8</sup>

Zinc (Zn) and copper (Cu) which are found in trace quantities in the human body are considered to play a significant role in maintaining the metalloenzyme processes of the body. Cu plays a significant role in the body by assisting in the production of melanin and facilitating collagen cross-linking through its involvement in tyrosinase and lysyl oxidase processes. Zn plays a part in various cellular functions and metabolic pathways. Zn has a very significant role as a trace metal in the human body as it controls many functional activities in the hair follicles. It also speeds up hair follicle regrowth and is a strong inhibitor of hair follicle regression. Some patients having alopecia show significant deficiency of Zn and other trace elements.<sup>9</sup> These trace metals are thought to be engaged in many forms of hair loss. However, this statement that Zn and Cu are the reasons for pathogenesis in hair loss in patients of alopecia is still unresolved and requires further consideration.

To diagnose at the early onset, it is important to conduct routine examinations such as an anemia panel, complete hemogram, erythrocyte sedimentation rate, serum calcium, thyroid function tests, serum proteins, etc to determine proper precautionary measures.<sup>10</sup> In the current study, we evaluate the Zn and Cu serum levels in patients having alopecia. Therefore, assessment of serum Zn levels in patients with alopecia appears to be helpful as a marker of severity, disease duration, and resistance to therapies.<sup>11</sup>

## Materials and Methods

This cross-sectional study was conducted at the Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi after taking approval from the Institutional Review Board (IRB) of AFIP vide Ref number FC-CHP-29/READ-IRB/21/657. After a thorough literature search, we calculated a sample

size of 8 via the WHO calculator, keeping the margin of error at 5%, a confidence level at 95%, and an alopecia prevalence at 0.252% (0.271% in females and 0.145% - 0.171% in males).<sup>6</sup> Sampling was done using a purposive sampling technique at the Dermatology Department, CMH, Rawalpindi. A maximum number of available participants (250) during the study period were recruited.

Participants from 19 – 60 years, without considering any economical status, were enrolled in this study. To find the serum Zn and Cu concentrations for different types of hair loss, the participants were divided into 5 groups comprised of patients with male pattern hair loss (MPHL) as Group 1, female pattern hair loss (FPHL) as Group 2, alopecia areata (AA) as Group 3, telogen effluvium (TE) as Group 4, and healthy individuals (control group) as Group 5. Case and control groups were matched for age, gender, Zn level, and Cu level. Informed consent was taken about the study and all the patients participated voluntarily. The normal reference interval of Zn is 12 to 18  $\mu\text{mol/L}$ , and Cu is 10-23  $\mu\text{mol/L}$  in our hospital.

Venous blood samples (5mL) were collected from alopecia patients and healthy controls in yellow top gel tubes without any anticoagulant. This serum sample was used to measure trace elements. For Zn analysis, 0.5 mL of serum was mixed with 4.5 mL of deionized water in a test tube. Cu analysis involved adding 0.5 mL of serum to a test tube with concentrated nitric acid ( $\text{HNO}_3$ ), heating it in a thermoreactor at 150°C for 15 minutes, cooling it, and then diluting it with 4.5mL of deionized water to make the total volume of 5mL, and at last injecting the mixture in a new plain tube through a filter. The filtered samples are ready to run on a flame atomizer (atomic absorption spectrophotometer). They are then analyzed using a flame atomic absorption spectrophotometer (Agilent Technologies 200 series AA). Quality control and calibration standards were prepared similarly.

Healthy individuals of 19-60 years with a history of hair loss and normal people without any underlying disease as controls were included in the evaluation study. Individuals taking supplements containing Zn and Cu, individuals already under treatment for hair loss with systemic illnesses diabetes mellitus (DM), trace metal disorders, chronic liver disease (CLD), hypertension (HTN), chronic kidney disease (CKD),

and taking any medication including chelating agents were excluded from the study design.

Statistical Package for Social Sciences (SPSS) version 25.0 was used for data analysis. Mean and standard deviation (SD) were calculated for continuous variables and frequency and percentage were calculated for categorical variables. Shapiro-Wilk test was performed to check the distribution of data. One-way ANOVA and posthoc analysis were for comparing serum Zn and Cu level in hair loss patients (male and female), telogen effluvium patients, alopecia areata patients, And The Control Group. A  $P$  Value  $\leq 0.05$  Was Considered Significant.

## Results

A total of 250 patients were included in the study, out of the total, 125 (50.0%) patients were males and 125 (50.0%) were females. The mean age of participants was  $31.63 \pm 7.25$  years from 19 to 60 years. The mean ages of the patient's group and control group were  $33.72 \pm 7.40$  and  $31.13 \pm 6.23$  years, respectively. The detail of the demographic variables of all groups is shown in Table-I.

Normal reference interval of serum Zn is 12 to 18  $\mu\text{mol/L}$ . Low mean serum Zn levels were observed in group 4 ( $9.88 \pm 2.47$   $\mu\text{mol/L}$ ), group 3 ( $10.23 \pm 2.91$   $\mu\text{mol/L}$ ) and group 1 ( $8.70 \pm 2.08$   $\mu\text{mol/L}$ ). Mean serum Zn levels in group 2 were at the borderline ( $12.38 \pm 2.97$   $\mu\text{mol/L}$ ) whereas mean serum Zn levels in control group were within normal reference interval ( $14.01 \pm 1.82$   $\mu\text{mol/L}$ ). There was a significant difference in mean serum Zn levels among all the groups ( $p$  value  $< 0.05$ ) shown in Table II. Post hoc analysis showed significant difference in mean serum Cu levels between group 1 and control group ( $p$  value  $< 0.05$ ) where as there was no significant difference in mean serum Cu levels between controls and other groups ( $p$  value  $> 0.05$ ). However a significant difference was found in mean serum Zn levels between control group and all four groups of alopecia ( $p$  value  $< 0.05$ ) shown in Table II..

**Table I: Demographic Variables of the Patients (n=250)**

Variables		MPHL* (n=50)	FPHL* (n=50)	AA* (n=50)	TE* (n=50)	Control Group (n=50)
Gender	Male	50 (100.0%)	0 (0%)	25 (50.0%)	25 (50.0%)	25 (50.0%)
	Female	0 (0%)	50 (100.0%)	25 (50.0%)	25 (50.0%)	25 (50.0%)
Age (Mean $\pm$ SD)		31.64 $\pm$ 7.44	33.34 $\pm$ 8.26	30.90 $\pm$ 6.70	32.12 $\pm$ 6.58	31.12 $\pm$ 6.08

\*Male Pattern Hair Loss: MPHL, \*Female Pattern Hair Loss:FPHL, \*Alopecia Areata:AA, \*Telogen Effluvium:TE.

**Table II: Comparison of Mean Serum Cu and Zn levels within Groups Based on Post Hoc Analysis**

Variables	Groups	Mean $\pm$ SD	p-Value
Copper $\mu\text{mol/L}$	Male Pattern Hair loss	8.74 $\pm$ 2.97	0.001*
	Control Group	15.52 $\pm$ 4.05	
	Female Pattern Hair loss	14.11 $\pm$ 3.83	0.410
	Control Group	15.52 $\pm$ 4.05	
	Alopecia Areata	15.14 $\pm$ 3.62	0.990
	Control Group	15.52 $\pm$ 4.05	
	Telogen Effluvium	13.45 $\pm$ 5.29	0.078
	Control Group	15.52 $\pm$ 4.05	
Zinc $\mu\text{mol/L}$	Male Pattern Hair loss	8.70 $\pm$ 2.08	< 0.001*
	Control Group	14.01 $\pm$ 1.82	
	Female Pattern Hair loss	12.38 $\pm$ 2.97	0.011*
	Control Group	14.01 $\pm$ 1.82	
	Alopecia Areata	10.23 $\pm$ 2.91	< 0.001*
	Control Group	14.01 $\pm$ 1.82	
	Telogen Effluvium	9.88 $\pm$ 2.47	< 0.001*
	Control Group	14.01 $\pm$ 1.82	

\*The  $p$  value 0.05 was considered statistically significant

## Discussion

Alopecia areata (AA) is an autoimmune disorder that specifically affects hair follicles in the anagen phase. This condition follows an unpredictable pattern of chronic relapses which result in transient non-scarring hair loss.<sup>12</sup> Trace elements such as Zn and Cu play a crucial role as cofactors in multiple enzymes and exhibit significant functional activities within hair follicles. They play a great role in the healthy growth and development of hair.<sup>13</sup> However, many studies suggest that the imbalance of these elements is the main cause of AA.<sup>14</sup>

In the current study, 250 patients were recruited; 125 (50.0%) patients were males and 125 (50.0%) were females with a mean age of  $31.63 \pm 7.25$ . The current study states that the serum Zn had a statistically prominent difference in all groups while only the serum Cu in MPHL had a statistically significantly different value from the control group. Serum Cu in the rest of the groups was almost similar to that of the control group.

In comparison to the present study Kil MS *et al.*,<sup>15</sup> showed that hair loss patients have a mean Zn value of  $84.33 \pm 22.88$ , which was significantly lower than their control group ( $97.94 \pm 21.05$   $\mu\text{g/dl}$ ) whereas the mean value of Cu was  $96.44 \pm 22.62$ , which wasn't significantly different ( $p=0.975$ ).

In another study conducted by Farah HS *et al.*,<sup>2</sup> Zn concentration in patients was  $110.3 \pm 17.8$  and in the control group was  $109.5 \pm 15.3$  in the age group of 17-32. In contrast to the present study, no significant changes in the mean serum Zn value of the case and control group were observed as the population belonged to young age and was in good health. The defects, which impair Zn absorption rates were seen in older ages.

In a study by Yavuz *et al.*,<sup>16</sup> 40 subjects with chronic telogen effluvium, and 30 healthy females as controls were recruited. In contrast to the present study, no significant differences in Zn serum levels were noticed in patients than in the controls. However, the patients showed a significant increase in Manganese (Mn), Cobalt (Co), Palladium (Pb), Iron (Fe), Magnesium (Mg), Cadmium (Cd), and Cu ( $p < 0.05$ ) levels. This study is a complete antipode to the present study. Also contrary to the present study, this study only recruited women in their control group.

Gowda D *et al.*,<sup>17</sup> indicated Zn deficiency in 11.76% of MPHL patients and 8.3% of FPHL patients which was almost similar to the present study. This study also stated that Cu deficiency was seen in 29.41% and 31.48% of MPHL and TE patients, respectively, however, in the present study a significant copper deficiency was only seen in MPHL patients. Cheung EJ *et al.*,<sup>18</sup> showed that 115 subjects were diagnosed with TE (acute and chronic), and had similar results of 9.6% Zn deficiency to the present study. Another similar study conducted by Chen S *et al.*,<sup>19</sup> showed a 21.4% reduction in Zn content and a 42.1% reduction in Cu levels and observed that 11.76% of MPHL patients had Zn deficiency and 29.41% of MPHL patients had copper deficiency which was almost similar to the present study.

According to a study by Bhat YG *et al.*,<sup>20</sup> the majority of the patients were in the third decade of life like this study. All patients with prolonged duration showed decreased Zn levels. Whereas the difference in serum Cu levels was insignificant. This study showed an increased incidence of male pattern hair loss than female pattern hair loss (34:16), comparable to the present study.

### Limitations

We did not consider severe alopecia areata cases like total and universalis because there were no hair samples. Further multicenter studies with more

patient series are needed to deepen our understanding of the effects of trace elements.

### Conclusion

The results showed that low levels of Zn are related to all four types of alopecia. Whereas low Cu levels are observed only in male pattern alopecia. Hence Zn supplementation can be used to get some beneficial effects in all types of alopecia. Whereas patients with male pattern alopecia should also be evaluated for serum Cu levels and supplementation is required only if they have low Cu levels.

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**CONFLICT OF INTEREST**

Authors declared no conflicts of Interest.

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**DATA SHARING STATMENT**

The data that support the findings of this study are available from the corresponding author upon request.

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