Dermatology Practical & Conceptual

Serum Copper and Zinc Levels Among Iranian Vitiligo Patients

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Key words: vitiligo, trace elements, zinc, copper

Citation: Khoshdel Z, Gholijani N, Niknam M, Rahmani N, Hemmati-Dinarvand M, Naghibalhossaini F. Serum Copper and Zinc levels among Iranian vitiligo patients. *Dermatol Pract Concept.* 2022;12(4):e2022140. DOI: https://doi.org/10.5826/dpc.1204a140

Accepted: January 2, 2022; **Published:** October 2022

Funding: This research was supported by the Vice Chancellor for Research, Shiraz University of Medical Sciences through Grant Number 98-01-17-19678.

Competing interests: None.

Authorship: All authors have contributed significantly to this publication.

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ABSTRACT

Introduction: Vitiligo is a chronic skin disease, which its etiopathogenesis is not fully understood. Numerous studies have suggested that oxidative stress may play a role in the pathophysiology of vitiligo. There are controversial reports as to the changes of serum trace elements, copper (Cu) and zinc (Zn) levels in vitiligo patients.

Objectives: We evaluated the alterations in the level of serum Cu and Zn among a group of Iranian vitiligo patients.

Methods: The levels of serum Cu and Zn were compared between 117 vitiligo patients and 137 healthy controls using atomic absorption spectrophotometry.

Results: The mean Cu and Zn levels in the cases (113.57 ± 59.43 and 95.01 ± 58.95 µg/dl, respectively) were significantly lower than those of the controls (138.90 ± 38.14 and 121.83 ± 33.80 µg/dl, respectively) (P = 0.00). We also observed significantly lower serum Cu and Zn concentrations in young (< 50 years) than the elderly (≥ 50 years) patients (P = 0.00). The mean Cu and Zn levels in the patients with generalized vitiligo (111.63±54.18 and 93.11±59.33 µg/dl, respectively) were significantly lower than patients with localized vitiligo (120.74 ±71.64 and 98.69±58.63 µg/dl, respectively) and those in the control (P = 0.00). The serum Cu/Zn ratio obtained in the young and male patients was higher than those in their matched controls (P = 0.01).

Conclusions: The current study has shown that the disturbance of serum Cu and Zn levels is associated with vitiligo, and may play an important role in the disease development of Iranian patients.

Introduction

Vitiligo is a multifactorial hypo-melanotic disorder characterized by the appearance of white spots of different sizes and shapes on the skin. The disease affects 0.1% to 8.8% of the world's population, with a higher prevalence among pigmented racial groups [1,2]. The loss of functional melanocytes has been detected histochemically in the skin as well mucous membranes, hair, and the retina of vitiligo patients [3]. Many different factors, including genetics, oxidative stress, environment, metabolic abnormalities, autoimmunity mediated by autoreactive CD8+ T lymphocytes, and interferon-γ CXCL10 cytokine signaling pathway may be involved in the pathogenesis of the disease [4-6]. The exact mechanisms and interactions of these factors in the etiology of vitiligo are unclear. Previous studies have suggested that oxidant/antioxidant imbalance and oxidative stress may also play important roles in the pathogenesis of vitiligo [7,8]. Antioxidant deficiency and the accumulation of reactive oxygen species in the epidermal lesions of vitiligo patients have been also reported. Epidermal lesions of vitiliginous skin show decreased catalase activity and increased H_2O_2 and nitric oxide levels [8,9]. Previous studies have indicated that both localized and generalized vitiligo were associated with low total antioxidant and high total oxidant status, as compared with healthy controls [10].

Oxidative stress inhibits melanin production by interfering with tyrosinase activity in the epidermis, thereby exerting the cytotoxicity of melanocytes [11]. Melanin, a dark biopigment found in the skin has a high affinity for binding and sequestering zinc, copper, and other metal ions [12]. Zn and Cu trace elements are required as cofactors by tyrosinase, and many other metalloenzymes involved in melanin biogenesis [13,14]. They are also essential cofactors for the activity of superoxide dismutase, an antioxidant enzyme that protects skin against reactive oxygen species [15]. Although these trace elements are essential for human health, the imbalance of the Cu/Zn ratio suggested being associated with many human diseases [16,17]. Zinc may play a role in the etiology of vitiligo through Zn-α2-Glycoprotein (ZAG), a major plasma protein involved in melanocyte growth and differentiation. Studies have shown that ZAG deficiency is involved in the pathogenesis of vitiligo through the shedding and loss of melanocytes [18]. The relationship between changes in the levels of Cu and Zn trace elements in tissues or serum and the pathogenesis of vitiligo is controversial. Some studies have shown that oral supplementation or topical treatment of Cu and Zn may improve the clinical symptoms of vitiligo [19,20]. Both increased and decreased serum levels of these trace elements in vitiligo patients have been reported [19,21-23]. In a study of 50 Sudanese vitiligo patients, a significant increase in the mean serum concentration of Cu level was observed [24]. It has been suggested that melanocytes degeneration and lower melanin biosynthesis in vitiligo patients may result in increased serum Cu and Zn levels. Hence, the high serum levels of these elements in vitiligo are the result of the disease rather than its underlying cause [25]. Several other studies have reported significantly decreased serum levels of Cu and/or Zn among vitiligo patients [21,26-29].

Objectives

In view of this controversy, the purpose of the present study was to investigate the changes in serum Cu and Zn concentrations among Iranian vitiligo patients.

Methods

Experiment Groups

One hundred and seventeen sera were obtained from the previously collected serum samples of vitiligo patients (48 men and 69 women) from the biobank of the Autoimmune Diseases Research Center of Shiraz University of Medical Sciences (SUMS). The control group consisted of 137 healthy blood donors (62 males and 75 females) with no signs of skin or systemic disease. According to the dermatology and Wood lamp examination, 84% of patients had generalized/universal and 16% of localized/segmental stable vitiligo. The University ethics committee approved the protocol for this study (Approval Number: IR.SUMS.REC.1398.629), and the informed consent was obtained from all study participants.

Preparation of the Samples and Analytical Methods

The methods for the preparation of samples and their analysis have been described previously [16]. Briefly, blood samples were collected from participants, and allowed to clot, and serum was separated and stored in plastic tubes at -80 °C until analysis. The concentrations of Cu and Zn in the samples were measured using a flame atomic absorption spectrometer (PerkinElmer model analyst 300). Stock standard solutions of Zn and Cu were prepared by 1:1 dilution of either trace element (Merck) solutions (2 mg/ml in HCl) with deionized water. The stock solutions of Zn or Cu were diluted with glycerol (5% or 10%, respectively) and used as working solutions to calibrate the instrument. The absorbance of Cu and Zn was read at 324.7 and 213.9 nm, respectively. The concentrations of trace elements in samples were calculated using PerkinElmer AA WinLab software.

Statistical Analysis

The statistical analyses of data were performed using the SPSS software package (version 16; SPSS Inc.). The results were

expressed as mean ± standard deviation (SD). Differences between cases and controls were tested by Kruskal–Wallis test, and the influence of sex and age on the serum levels of trace elements was evaluated using the Mann–Whitney U-test. The Spearman rank correlation test was used to analyze the correlation between quantitative variables. A P equal or lower than 0.05 was considered statistically significant.

Results

A total of 117 subjects with vitiligo and 137 healthy volunteers were included in this study. Tables 1 and 2 show the demographic characteristics and serum levels of Cu and Zn in patients with vitiligo and the control group. Ninety-seven patients had generalized vitiligo and 20 had localized vitiligo (Table 2). The skin phototypes were type III (70%) and type IV (30%). The duration of the disease ranged from 1-11 years, with a mean of 9.57± 9.25 years.

In this study, the serum Cu level in patients ranged from 6.11 to 289.36 µg/dl (mean: 113.57 ± 59.43 µg/dl) and that for the control group was 71.21 to 231 µg/dl (mean: 138.90 ± 38.14 µg/dl). Serum zinc concentrations in patients with vitiligo ranged from 6.15 to 273 µg/dl (mean: 95.01 ± 58.95 µg/dl) and that for the control group was 32.64 to 190.20 µg/dl (mean: 121.83 ± 33.80 µg/dl). Statistical analysis showed that compared with healthy controls, there were significant differences in serum copper and zinc levels in patients with vitiligo (P = 0.00) (Table 1).

The age of patients ranged from 19 to 66 years old (Mean: 37.64 ± 13.01 years) and that of control 26 to 60 years old (Mean: 47.19 ± 8.03 years). In terms of the prevalence of disease, the highest rate was in the 25 to 28 year age group (17%). The median age of vitiligo patients was about 34 years. Since the serum levels of Zn and Cu have been reported to increase by age [30], we divided the subjects into two groups of young (< 50 years) and old (≥ 50 years) groups. We found significantly lower serum Cu and Zn concentration among young patients (107.12 ± 61.18 and 82.47 ± 47.40 µg/dl, respectively) than the elderly group (122.53 ±56.30 and 114.37 ± 67.27 µg/dl, respectively) (P =0.00) (Table 1). In the case-control comparison of two age groups, we found that the serum copper concentrations of cases were lower than those in the respective control group (P ≤ 0.05). The mean serum Zn levels of young cases were also lower than the corresponding value in the respective controls (P = 0.00) while there was no significant difference between the mean serum Zn concentrations of the elderly subjects and controls (P = 0.535). Although, in the case-case comparison of two age groups, a higher Cu/Zn ratio was found in the young cases than elderly patients (1.96 ± 2.09 vs 1.64 ± 1.67), the difference didn't reach the level of significance (P = 0.406). There was no significant difference in serum copper and zinc levels between

Table 1. Serum copper and zinc levels among vitiligo patients

Group	Cu (µg/dl) Median	Cu (µg/dl) Mean ± SD	P[a]	P[b]	Zn (µg/dl) Median	Zn (µg/dl) Mean ± SD	P[a]	P[b]	[Cu]/[Zn]	P[a]	P[b]
Cases (N) Total (117)	103.41	113.57 ± 59.43	0.000		76.17	95.01 ± 58.95	0.00		1.81 ± 1.90	0.488	
Age <50 Y (68) ≥50 Y (49)	95.07 128.67	107.12 ± 61.18 122.53 ± 56.30	0.000 0.050	0.003	72.01 101.06	82.47 ± 47.40 114.37 ± 67.27	0.00 0.535	0.000	1.96 ± 2.09 1.64 ± 1.67	0.018 0.161	0.406
Sex Male (48) Female (69)	108.94 110.68	114.65 ± 63.88 112.95 ± 56.32	0.015 0.00	0.992	76.34 75.09	99.12 ± 62.10 92.25 ± 56.83	0.000 0.000	0.429	1.67 ± 1.48 1.90 ± 2.15	0.011 0.087	0.559
Controls, (N) Total (137)	138.58	138.90 ± 38.14			118.42	121.83 ± 33.80			1.28 ± 0.56		
Age <50 Y (71) ≥50 Y (66)	138.97 138.74	143.82 ± 30.73 138.70 ± 31.59		0.338	122.14 117.02	124.80 ± 33.05 118.84 ± 34.59		0.321	1.24 ± 0.43 1.32 ± 0.66		0.001
Sex Male (62) Female (75)	137.08 142.38	135.46 ± 25.13 146.22 ± 34.76		0.044	126.10 111.40	126.87 ± 29.28 112.53 ± 33.78		0.113	1.14 ± 0.3 1.39 ± 0.69		0.008

Cu = copper; SD = standard deviation; Y = years; Zn = zinc.
[a] values for case–control comparisons from Kruskal–Wallis or Mann–Whitney U test where appropriate
[b] values for case–case or control-control comparisons from Kruskal–Wallis or Mann–Whitney U test where appropriate

male and female patients. However, in male and female cases, the average serum copper and zinc levels were significantly lower than their matched control group (P<0.02) (Table 1). The patients with generalized vitiligo had significantly lower serum Cu levels (111.63±54.18 µg/dl) than patients with localized vitiligo (120.74 ±71.64 µg/dl) (P≤0.04) and healthy controls (138.98±28.12 µg/dl) (P=0.00). The mean serum Zn level of the patients with the generalized vitiligo (93.11±59.33 µg/dl) also differed significantly with those with the localized vitiligo (98.69±58.63 µg/dl) and the control group (P = 0.00). We found significantly lower serum Zn, but higher Cu/Zn ratio in patients with skin type IV (86. 16±60.62 µg/dl and 2.45±2.75, respectively) than in patients with skin type III (99.24± 59.91 µg/dl and 1.48± 1.16, respectively) (P < 0.02, Table 2). However, there was no significant difference between the mean serum Cu level of these patients (117.04± 59.95 µg/dl) and that of patients with type III skin (112.67±59.66 µg/dl) (P > 0.05). The patients with a history of other autoimmune diseases had a higher serum Cu, Zn, and Cu/Zn ratio than patients without a history of autoimmune disease (P < 0.01) (Table 2). We divided the patients into two groups of early-onset (the first onset before the age 50) and late-onset (the first onset after the age 50). Patients with an early-onset of vitiligo had lower serum Cu (107.12 ± 61.17 µg/dl, P = 0.035) and Zn (83.06 ± 49.89 µg/dl, P = 0.001) than those in patients with late onset (122.53 ±56.30 and 112.81± 67.19 µg/dl, respectively) (Table 2). In patients with early-onset vitiligo, the male subjects had significantly higher serum Zn, but a lower Cu/Zn ratio than female subjects (P < 0.01) (Table 2). Among the patients with the late-onset vitiligo, the serum copper concentration and the copper-zinc ratio

Table 2. Selected clinical features of vitiligo patients and serum Cu and Zn levels

Group	Cu (µg/dl) Median	Cu (µg/dl) Mean ± SD	P[a]	Zn (µg/dl) Median	Zn (µg/dl) Mean ± SD	P[a]	[Cu]/[Zn]	p[a]
Early Onset < 50 (59.83%)	95.07	107.12 ± 61.17	0.035	72.56	83.06 ± 49.89	0.001	1.96 ± 1.44	0.164
Late Onset >50 (40.17%)	138.37	122.53 ±56.30		100.23	112.81± 67.19		1.72 ± 2.13	
Early-Onset (<50 y)								
Male (23.9%)	98.41	110.78± 62.80	0.189	76.92	89.70±45.13	0.008	1.73± 1.61	0.00
Female (35.8%)	90.04	116.54±54.15		68.96	78.63±52.90		1.77± 2.00	
Late-Onset (>50 Y)								
Male (17.1%)	131.69	119.65±57.57	0.017	88.58	113.54±78.26	0.629	1.49 ± 1.11	0.005
Female (24.7%)	128.67	124.52±56.34		100.23	112.27± 59.25		2.54±2.79	
Clinical type								
Generalized (82.9%)	101.96	111.63±54.18	0.04	73.01	93.11±59.33	0.006	1.88±2.02	0.078
Localized (17.09%)	104.72	120.74 ±71.64		110.50	98.69±58.63		1.40±1.24	
Skin phototype								
Skin type I and II (0%)								
Skin type III (69.93%)	103.41	112.67±59.66	0.113	84.68	99.24± 59.91	0.015	1.48± 1.16	0.00
Skin type IV (29.97%)	103.61	117.04± 59.95		67.32	86. 16±60.62		2.45±2.75	
History of other auto immune disease								
Yes (6.84%)	136.36	143.99±62.61	0.001	74.48	98.29±68.92	0.008	2.08±1.33	0.00
No (93.16%)	98.15	111.34±58.89		77.08	94.71± 58.59		1.79± 1.96	
Smoking status								
Smokers (16.23%)	99.86	106.93 ± 57.08	0.023	74.03	99.67 ± 59.49	0.05	1.71± 1.63	0.273
Non-smokers (83.76%)	103.51	115.45 ± 60.20		76.25	94.34± 59.50		1.83±1.95	

Cu = copper; SD = standard deviation; Y = years; Zn = zinc.
P[a], case –case comparison.

Table 3. Spearman correlation coefficient (r) and P value between serum Cu, Zn, age of onset and gender in patients

Correlation between	r (P value)
Serum Cu and serum Zn	0.144 (0.252)
Serum Cu and age of onset Early onset	0.251 (0.039)
Late onset	0.042 (0.774)
Serum Zn and age of onset Early onset	0.157 (0.362)
Late onset	0.189 (0.175)
Serum Cu or Zn and skin type Type III Type IV	0.207 (0.097) 0.071(0.688)
Serum Cu or Zn and clinical type of vitiligo Generalized Localized	0.022 (0.844) 0.453 (0.105)
Serum Cu or Zn and clinical type of vitiligo Segmental Non-segmental	0.20 (0.045) 0.062 (0.666)
Serum Zn and the duration of disease	-0.018 (0.862)
Serum Cu and the duration of disease	0.256 (0.010)
Serum Zn in male and the duration of disease	-0.186 (0.252)
Serum Zn in female and the duration of disease	0.058 (0.662)
Serum Cu in male and the duration of disease	0.112 (0.448)
Serum Cu in female and the duration of disease	0.225 (0.034)

Cu = copper; Zn = zinc.

of female patients was higher than those in male patients (P < 0.02). In these cases, the serum levels of Cu were significantly lower (P = 0.023), but Zn levels were higher in smokers than those in non-smoker patients (P = 0.05) (Table 2).

We also evaluated the association between serum Cu and Zn levels and the patients' clinical data. The serum Cu levels showed a positive correlation with the early age of the disease onset (r = 0.251, P = 0.039). We found a significant positive correlation between serum Cu concentration and duration of disease (Spearman correlation test, r = 0.256, P = 0.01). However, the correlation coefficient only reached statistical significance in females (r = 0.225, P = 0.034), but not in male patients (Table 3). We also observed a positive correlation between serum Cu and Zn levels and segmental Vitiligo (r = 0.20, P = 0.045). There was no correlation between the levels of two serum trace elements and other parameters including skin and clinical types of the disease (Table 3).

Conclusions

Numerous studies have shown a role for the oxidant–antioxidant imbalance and accumulation of ROS in the skin lesion of vitiligo patients [7,10,20]. Cu and Zn are necessary cofactors for as many as forty metallo-enzymes involved in the skin pigmentation process. These two trace elements are essential cofactors of tyrosinase and superoxide dismutase, two enzymes that are widespread in the human skin and are specifically involved in skin pigmentation and melanocytes protection against free radicals, respectively [13,15,27]. There are conflicting reports on the relationship between serum Cu and Zn concentrations and vitiligo. Some authors have reported that compared with healthy controls, the serum Zn, but not Cu levels in vitiligo patients have undergone significant changes [7,28,31]. A recent case-control study of 100 vitiligo patients from India reported decreased serum Zn, but increased Cu levels as compared with healthy individuals [32]. Arora et al found no significant difference between serum Zn levels of vitiligo patients and the healthy control group [22]. A meta-analysis of 16 studies conducted to compare the serum Cu and Zn concentrations among 891 vitiligo patients and 1682 healthy controls, demonstrated that the serum Cu and Zn concentrations were significantly lower in the cases than controls (P < 0.0001) [27]. The controversy about the changes of serum Cu and Zn levels in patients with vitiligo prompted us to carry out this study to investigate the relationship between the serum levels of

these trace elements and the pathogenesis of vitiligo among a group of Iranian patients.

In our study, the median serum Cu and Zn levels in both the localized (104.72 and 110.50 µg/dl, respectively) and generalized vitiligo patients (101.96 and 73.01 µg/dl, respectively) were significantly less than those in the control group (138.58 and 118.42 µg/dl, respectively). Compared with the control group, the mean serum levels of Cu and Zn in the patients group were also significantly decreased (P = 0.00) (Table 1). The mean serum concentrations of both trace elements were significantly lower in male and female vitiligo patients, as compared to their healthy counterparts (Table 1). Our results are consistent with the findings of Zeng et al. who reported a significant reduction in serum concentrations of both Cu and Zn in Chinese vitiligo patients [27]. Since both the increased and decreased levels of trace elements can affect the activity of antioxidant enzymes and oxidative stress, a U-shaped relation between zinc and copper status and optimal health condition has been proposed [33]. Thus, the conflicting reports regarding the changes in the level of these trace elements in vitiligo might be true findings. Otherwise, the discordance could be linked to the sample size, racial, or environmental factors such as diet variety.

In our study, the mean concentration of serum Cu in smoker patients (106.93 ± 57.08 µg/dl) was lower, but the mean Zn levels (99.67 ± 59.49 µg/dl) was higher than those in non-smoker patients (115.45 ± 60.20 µg/dl and 94.34± 59.50 µg/dl, respectively) (P ≤ 0.05). It has been suggested that there is a close relation between Cu/Zn ratio and systemic oxidative state and the balance between serum Cu and Zn is clinically more important than their concentrations in serum [17,34]. Wacewicz et al found a significantly higher Cu/Zn ratio in the serum of vitiligo patients as compared to healthy controls [35]. A previous study on 151 cases of three groups of skin diseases including skin cancer, inflammatory, and non-inflammatory diseases reported that the serum Cu/Zn ratio among examined patients was higher than the control group and reflected the clinical severity in each disease group [36]. We also found a higher Cu/Zn ratio among vitiligo patients (1.81 ± 1.90) than healthy controls (1.28 ± 0.573), but the difference was not statically significant (P = 0.49, Table 1). We observed a higher serum Cu/Zn ratio in young (1.96 ± 2.09) and male cases (1.67 ± 1.48), as compared to their healthy counterparts (1.24 ± 0.438 and 1.11 ± 0.358, respectively) (P = 0.01).

A linear regression analysis showed positive correlations between serum Cu and the early age of onset (r =0.251, P = 0.039) and the segmental type of the disease (r =0.2, P = 0.045) (Table 3). A significant positive correlation was also observed between serum Cu concentrations and the duration of disease, especially in the female patients (r=0.225, P = 0.034). No significant correlation was observed between serum Cu and Zn levels and other parameters in either patients or the control group. Some previous studies detected no correlation between serum Cu and Zn levels and the disease subgroups [37,38]. But, others reported a negative correlation between serum levels of Zn and the generalized vitiligo, age, and duration of disease [28,31].

In a study of tissue copper in vitiligo patients, the mean lesion (1.3 µg/g) and non-lesion (1.4 µg/g) Cu levels were non-significantly lower than tissue Cu levels of the control group (1.9 µg/g) [39]. Melanin has a high affinity for the sequestration of metal ions, and pigmented tissues contain a significant amount of metal cations including Cu and Zn [12]. By binding and sequestering metal ions, melanosomes are protected from oxidative damage. The serum concentrations of Zn and Cu have been reported to increase with age [30,40,41]. In our study, the elderly patients (≥ 50 years) had significantly higher serum Cu and Zn concentrations than young (< 50 years) patients (P = 0.00) (Table 1). In the case-control comparison, both the elderly and young patients had lower serum Cu levels than their respective healthy controls (P ≤ 0.05). The young patients also had significantly lower serum Zn levels than the respective control group, but no significant difference was found in terms of serum Zn concentration between elderly patients and their respective elderly control group (P > 0.05). A relationship between vitiligo and the incidence of other autoimmune disorders has been suggested [42,43]. In our study, 6.8% of vitiligo patients had a history of other autoimmune diseases including rheumatoid arthritis, type 1 diabetes mellitus, and hypothyroidism. Several previous studies have suggested an association between Zn and Cu deficiency and hypopigmentation of the skin and hair, immune defects, and autoimmune diseases like type 1 diabetes, rheumatoid arthritis, multiple sclerosis, systemic Lupus erythematosus, celiac disease, Hashimoto thyroiditis, and juvenile idiopathic arthritis [44,45].

In the current study, the median and mean serum Cu and Zn concentrations in the generalized form of vitiligo were less than those in the localized form (P ≤ 0.05). Mirnezami et al also reported significantly lower zinc levels in the generalized vitiligo than the localized form [28].

In conclusion, our results are consistent with prior study findings of decreased and imbalanced levels of serum Cu and Zn in vitiligo patients. The main drawback of this study was the relatively small sample size that limited the statistical power to perform analyses across all subgroups of patients. Future studies with a larger sample size should be conducted to understand the etiopathogenic roles and the potential therapeutic benefits of Cu and Zn supplementation for vitiligo patients.

Acknowledgements

This research was supported by the Vice Chancellor for Research, Shiraz University of Medical Sciences through Grant Number 98-01-17-19678.

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