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Serum Copper and Zinc levels in Vitiligo Patients

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ABSTRACT

Background: vitiligo is a common, acquired, discoloration of the skin, characterized by well circumscribed, ivory or chalky white macules and patches. Researchers suggested that vitiligo may arise from autoimmune (AI), genetic, oxidative stress or neural causes. Zinc (Zn) and copper (Cu) are trace elements that are required in minutely small doses. The unique process of keratinization and melanin formation is enzyme-dependent and therefore could be influenced by trace elements deficiencies or excesses as trace elements are involved in enzymatic activities and immunologic reactions. **Aim of work:** this study aimed to detect the levels and roles of serum Zn and Cu in the pathogenesis of vitiligo. **Patients and methods**: our study included 50 vitiligo patients and 50 apparently healthy controls. Age of study groups ranged from 15 to 60 years and both sexes. Serum Zn and Cu levels were measured in each study group. **Results:** serum Zn levels were statistically significant lower in both the studied groups, but in vitiligo group they were much lower than the control group. Serum Cu levels were statistically insignificant higher in vitiligo group than the control group. Conclusion: there is a relationship between vitiligo and serum Zn. Further studies are needed to obtain better knowledge about effect of the trace elements in vitiligo patients.

Keywords: vitiligo, serum Zn, serum Cu.

INTRODUCTION

Vitiligo vulgaris is defined as an idiopathic, acquired type of leukoderma manifested by depigmentation of the epidermis resulting from destruction of melanocytes [(1)]. Researchers suggested that vitiligo may arise from autoimmune (AI), genetic, oxidative stress (OS) or neural causes [(2)]. It is believed that AI etiology is the most plausible factor [(3)].

The global incidence of vitiligo is less than 1% [(4)], with some populations averaging between 2-3% and as high as 16% [(5)]. Vitiligo is classified into segmental vitiligo (SV) and non-segmental vitiligo (NSV). NSV is the most common type. There is no cure for vitiligo, but several treatment options are available [(6)]. Conventional treatments for vitiligo included photochemotherapy (psoralen plus ultra-violet A) (PUVA), phototherapy (UVB), vitamin D3 analogues, topical corticosteroids (TCS), topical immunomodulators, excimer laser and surgery. These treatment options have limited success [(7)]. The best evidence is for applied steroids and the combination of UV light in combination with creams [(8)].

Zinc (Zn) and copper (Cu) are two of the trace elements that found in small amounts in the body [(9)]. Zn and Cu are involved in many homeostatic mechanisms of the body, such as specific immunity, inflammation and oxidative stress (OS) [(10)]. Decreased serum Zn and Cu levels have been reported in vitiligo by some investigators [(11)], while others contradicted these findings [(11)].

Aim of study: this study aimed to detect the possible changes in the metabolism of serum Zn and Cu levels in vitiligo patients and their relation to the etiopathogenesis of vitiligo.

PATIENTS AND METHODS

Patients

The present study was conducted on 50 vitiligo patients and on 50 healthy controls. Serum Zn and Cu levels was measured in both groups. Patients were selected from the attendants of outpatient clinic, Department of Dermatology, STDs and Andrology, Faculty of Medicine, Fayoum University and ETSA Govermental Hospital in the period from 1/3/2015 to 1/10/2015. Written consent was obtained from every individual. Approval was obtained from the Ethical Committee of Human Rights in Research of Fayoum University before study initiation.

Inclusion criteria

Vitiligo patients
All types of vitiligo
Both sexes
Age from 15 to 60 years

Exclusion criteria

Presence of leukoderma secondary to other causes.

History of other obvious skin diseases. Undergoing treatment with zinc or any history of zinc intake for 6 weeks before this study. Suffering from any other systemic diseases such as: hepatic cirrhosis, viral hepatitis, neoplastic condition, myocardial infarction, steatorrhea, or renal failure, pregnancy or consumption of oral contraceptive

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pills and GIT troubles (like dyspepsia, diarrhea…. etc.)

METHODS

Serum Zn and Cu levels was determined by using Zn and Cu colorimetric method for "*in vitro*" determination of Zn in serum, plasma or urine and Cu in serum or plasma provided from Quimica Clinica Aplicada S.A, Spain.

Collection and preparation of serum samples

A volume of 3 ml of venous blood was collected from the cases and the control group in special sterile tubes and centrifuged for 10 min at 3000 rpm. The supernatant serum was transferred to a separate sterile tube (Eppendorf tubes) and kept at -20°C in the deep freezer until analysis.

Principle of the assay for serum Zn by method of Johnsen and Eliasson [12] and serum Cu by method of Abe *et al* . [13]

Reference values

Serum Zn 60-110µg/dl

Serum Cu Men 70-140 µg/dl

Female 80-155 µg/dl

Statistical Analysis

Data were collected and coded to facilitate data manipulation and double entered into Microsoft access and data analysis was performed by using Statistical Package for Social Sciences (SPSS) software windows (2007).

Simple descriptive analysis in the form of numbers and percentages for qualitative data and arithmetic means as central tendency measurement, standard deviations (SD) as measure of dispersion for quantitative parametric data and inferential statistic test.

For quantitative parametric data

Simple one way ANOVA test was used to compare more than two independent groups of the quantitative data. Student t-test was used to compare measures of two independent groups of quantitative data. Pearson correlation coefficients were also processed. The level **p≤ 0.05** was considered the cut-off value for significance.[14]

The study was approved by the Ethics Board of Ain Shams University.

RESULTS

The present study was conducted on 50 vitiligo patients and on 50 healthy controls. Serum Zn and Cu levels were measured in both groups. Patients were selected from the attendants of outpatient clinic, Department of Dermatology, STDs and Andrology, Faculty of Medicine, Fayoum University and Etsa Govermental Hospital. Sex distribution of the vitiligo group showed that there were 33 females (66%), 17 males (34%), while in the control healthy group there were 36 females (72%) and 14 males (28%).

The age of both studied groups ranged from 15 to 60 years old, with a mean±SD of 36.74±8.17 in vitiligo group and with mean±SD of 31.28±6.49 in the control group **(Table 1).**

Table 1: age distribution among the different studied groups

		N of individuals	Mean	SD
Age	Patients	50	36.74	8.17
	Controls	50	31.28	6.49

Mean±SD of serum Zn levels in vitiligo group age between 15 to 29 year (n=19) were (31.32±7.83). Mean±SD of serum Zn levels in vitiligo group age between 30 to 39 year (n=7) were (34.14±8.30). Mean±SD of serum Zn levels in vitiligo group age between 40 to 49 year (n=12) were (39.92±9.71).

Mean±SD of serum Zn levels in vitiligo group age between 50 to 60 year (n=12) were (45.42±10.22). There was no statistically significant difference with p-value > 0.05 between serum Zn levels and age of vitiligo group **(Table 2).**

Table 2: relation between serum Zn levels and age of the vitiligo group

	Age	N of patients	Mean	SD	p-value	Sig.
Serum Zn in µg/dl	15-29 y	19	31.32	7.83	0.33	**NS**
	30-39 y	7	34.14	8.30		
	40-49 y	12	39.92	9.71		
	50-60 y	12	45.42	10.22		

Mean±SD of serum Cu levels in vitiligo group age between 15 to 29 year were (156.53±37.67). Mean±SD of serum Cu levels in vitiligo group age between 30 to 39 year were (119±25.60). Mean±SD Serum Cu levels in vitiligo group age between 40 to 49 year were (117.33±72.96). Mean±SD of serum Cu levels in vitiligo group age between 50 to 60 y were (155.50±33.18). There was no statistically significant difference with p-value > 0.05 between serum Cu levels and age of vitiligo group **(Table 3).**

Table 3: relation between serum Cu levels and age of the vitiligo group

	Age	Mean	SD	p-value	Sig.
Serum Cu in µg/dl	15-29 y	156.53	37.67	0.19	**NS**
	30-39 y	119	25.60		
	40-49 y	117.33	27.96		
	50-60 y	155.50	33.18		

Mean±SD of VASI in vitiligo group age between 15 to 29 year were (1.78±0.42). Mean±SD of VASI in vitiligo group age between 30 to 39 year were (9.25±2.09). Mean±SD of VASI in vitiligo group age between 40 to 49 year were (1.29±0.34). Mean±SD of VASI in vitiligo group age between 50 to 60 year were (1.67±0.39). There was a statistically significant difference with p-value = 0.05 between VASI and age of vitiligo group **(Table 4).**

Table 4: relation between VASI and age of the vitiligo group

	Age	Mean	SD	p-value	Sig.
VASI	15-29 y	1.78	0.42	0.05	**S**
	30-39 y	9.25	2.09		
	40-49 y	1.29	0.34		
	50-60 y	1.67	0.39		

Mean±SD of serum Zn levels in vitiligo group were (37.16±9.16), while in the control group were (50.49±11.02). There was a statistically significant difference with p-value < 0.05 between serum Zn in vtiligo group and controls group **(Table 5).**

Table 5: relations between serum Zn levels in the different studied groups

		Mean	SD	p-value	Sig.
Serum Zn in µg/dl	Patients	37.16	9.16	0.003	**HS**
	Controls	50.49	11.02		

Mean±SD of serum Cu levels in vitiligo group were (141.62±32.56) while in the control group were (128.38±29.03). There was no statistically significant difference with p-value > 0.05 between serum Cu levels and different study group **(Table 6).**

Table 6: relations between serum Cu levels in the different studied groups

		Mean	SD	P-value	Sig.
Serum Cu in µg/dl	Patients	141.62	32.56	0.21	**NS**
	Controls	128.38	29.03		

Mean ±SD of serum Zn levels in vitiligo males patients were (55.12±10.44) and in females were (27.91±5.33). There was a statistically significant difference with p-value > 0.05 between serum Zn levels and sex distribution with higher levels in vitiligo males patients **(Table 7).**

Table 7: relations between serum Zn levels and sex distribution in the vitiligo group

		Mean	SD	P-value	Sig.
Serum Zn in µg/dl	Male	55.12	10.44	0.0001	**HS**
	Female	27.91	5.33		

Mean±SD of serum Zn levels in the control males group were (60.29±13.02) and in females were (47.31±9.34)**.** There was no statistically significant difference with p-value > 0.05 between serum Zn levels and sex distribution with higher levels in control males group **(Table 8).**

Table 8: relation between serum Zn levels and sex distribution in the control group

		Mean	SD	P-value	Sig.
Serum Zn in µg/dl	Male	60.29	13.02	0.07	**NS**
	Female	47.31	9.34		

Mean ±SD of serum Cu levels in vitiligo males patients were (143.47±31.32) and in females were (140.67±29.07). There was no statistically significant difference with p-value > 0.05 between serum Cu levels and sex distribution in vitiligo group **(Table 9)**.

Table 9: relations between serum Cu levels and sex distribution in the vitiligo group

	Mean	SD	P-value	Sig.

Serum Cu in µg/dl	Male	143.47	31.32	0.87	**NS**
	Female	140.67	29.07		

Mean±SD of serum Cu levels in control males group were (119.43±28.17) and in females were (131.86±30.47). There was no statistically significant difference with p-value > 0.05 between serum Cu levels and sex distribution in control group **(Table 10).**

Table 10: relations between serum Cu levels and sex distribution in the control group

		Mean	SD	P-value	Sig.
Serum Cu in µg/dl	Male	119.43	28.17	0.36	**NS**
	Female	131.86	30.47		

Mean ±SD of serum Zn levels in active vitiligo patients (new lesions appeared within last 6 months) (N: 41) were (37.78±7.76) and 9 patients had stable vitiligo (no new lesions appeared within last 6 months) were (34.33±5.69)**.** There was no statistically significant difference with p-value > 0.05 between serum Zn levels and vitiligo disease activity **(Table 11).**

Table 11: relation between serum Zn levels and vitiligo disease activity

		N	Mean	SD	P-value	Sig.
Serum Zn in µg/dl	active	41	37.78	7.76	0.66	**NS**
	stable	9	34.33	5.69		

Mean ±SD of serum Cu levels in active vitiligo patients were (137.27±32.52) and in stable vitiligo patients were (161.44±35.70). There was no statistically significant difference with p-value > 0.05 between serum Cu levels and vitiligo disease activity **(Table 12).**

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Table 12: relation between serum Cu levels and the disease activity in the vitiligo group

		Mean	SD	P-value	Sig.
Serum Cu in µg/dl	active	137.27	32.52	0.28	**NS**
	stable	161.44	35.7		

Among the vitiligo group the vitiligo disease duration was less than 1 year in 6 patients in which mean ±SD of serum Zn levels were (30.67±5.46). The disease duration between 1 to 10 years in 30 patients in which mean ±SD of serum Zn levels were (39.83±7.64). The disease duration between 10 to 20 years in 9 patients in which mean ±SD of serum Zn levels were (32.56±6.30). The disease duration more than 20 years in 5 patients in which mean ±SD of serum Zn levels were (37.20±9.12). There was no statistically significant difference with p-value > 0.05 between serum Zn levels and vitiligo disease duration **(Table 13).**

Table 13: Relation between serum Zn levels and the vitiligo disease duration

		N of patients	Mean	SD	P-value	Sig.
Serum Zn in µg/dl	<1 y	6	30.67	5.46	0.71	**NS**
	1-10 y	30	39.83	7.64		
	10-20 y	9	32.56	6.3		
	> 20 y	5	37.2	9.12		

Mean ±SD of serum Cu levels in vitiligo patients whom period of disease was less than 1 year were (187.67±44.63). The disease duration between 1 to 10 years, mean ±SD of serum Cu levels were (143.30±30.82). The disease duration between 10 to 20 years, mean ±SD of serum Cu levels were (124.56±28.24). The disease duration more than 20 years, mean ±SD of serum Cu levels were (107±15.19). There was no statistically significant difference with p-value > 0,05 between serum Cu levels and vitiligo disease duration **(Table 14).**

Table 14: relation between serum Cu levels and vitiligo disease duration

		Mean	SD	P-value	Sig.
Serum Cu in µg/dl	<1 y	187.67	44.63	0.11	**NS**
	1-10 y	143.3	30.82		
	10-20 y	124.56	28.24		
	>20 y	107	15.19		

Mean±SD of VASI in vitiligo patients whom duration of disease was less than 1 year were (1.57±0.26). The disease duration between 1 to 10 years, mean ±SD of VASI were (5.11±0.92). The disease duration between 10 to 20 years, mean ±SD of VASI were (1.83±0.40). The disease duration more than 20 years, mean ±SD of VASI were (2.24±0.49). There was a statistically significant difference with p-value < 0.05 between VASI and the vitiligo disease duration with high scale with disease duration between 1 to 10 years **(Table 15).**

Table 15: relation between VASI and the vitiligo disease duration

		Mean	SD	p-value	Sig.
VASI	<1 year	1.57	0.26	0.01	**S**
	1-10 years	5.11	0.92		
	10-20 years	1.83	0.4		
	>20 years	2.24	0.49		

This study showed that there was a statistically significant positive correlation with p-value < 0.05 between VASI and age and between VASI and the vitiligo disease duration **(Table 16).**

Table 16: correlation between VASI, age and disease duration among the vitiligo study group

	VASI		
	r	**P-value**	**Sig.**
Age	0.34	0.016	**S**
Period of disease	0.44	0.002	**HS**

Also, this study showed a statistically significant negative correlation between serum Cu and the vitiligo disease duration with p-value > 0.05 **(Table 17).**

Table 17: correlation between Serum Cu and the vitiligo disease duration

	Serum Cu in µg/dl		
	r	p-value	Sig.
Period of disease	-0.27	0.06	**NS**

DISCUSSION

Vitiligo is an acquired skin disease characterized by white areas of the skin. The disease may affect individuals of both sexes and is mostly characterized by loss of melanocytes [(15)]. The etiology of vitiligo and the causes of melanocyte death are not clear. At least three pathogenic mechanisms immunological, neural and biochemical [(16)] have been suggested, but none can completely explain the disease. Some findings showed that OS may be an important phenomenon in the pathophysiology of vitiligo [(17)]. Zn is one of the important trace elements related to health and disease [(18)]. Zn in combination with other micronutrients such as Cu, cobalt, nickel, iron, manganese and Ca^{++} [(19)] plays an important role in the process of melanogenesis [(20)]. The present study was designed to assess serum Zn and Cu levels in vitiligo patients and correlate it with age, sex, disease duration, activity and compare it with their levels in healthy control individuals to understand their roles in pathogenesis of vitiligo.

Our study included 50 patients with vitiligo of both sexes: 33 females (60%) and 17 males (34%). Fifty volunteers were included as a control group. Age in vitiligo group ranged between 15 to 60 years with a mean ± SD of (36.74±8.17) years and in control group ranged from 15 to 60 years with a mean ± SD of (31.28±6.49) years.

Our data revealed that serum Zn levels was significantly low in vitiligo patients with a mean ± SD of (37.16±9.16) compared to the controls, with a mean ± SD (50.49±11.02), these results are consistent with results of **Zeng *et al*** [(21)], whose researches showed that serum **Zn** levels were also significantly lower in vitiligo patients than in healthy controls.

On the contrary, **Basha *et al.*** [(22)] showed that the mean Zn level in both groups was found to be within the normal reference range, but in vitiligo patients the mean **Zn** levels was observed to be statistically significantly higher than that of controls.

The present study also measured serum **Cu** and found insignificantly higher levels in vitilgo group with mean ± SD of (141.62±32.56) than the controls, with a mean ± SD of (128.38±29.03). In the presence of Zn deficiency, absorption of Cu is enhanced [(23)]. As a result, reduced serum Zn levels was accompanied by elevated serum Cu levels [(24)].

Also, **Helmy *et al.*** [(25)] showed that Cu levels were significantly higher in active vitiligo patients compared to the controls.

Melanins are colloidal pigments and have a high affinity for metal ions; therefore, Cu and Zn are found in high levels in pigmented tissues involved in melanin synthesis. As melanocytes degenerate in vitiligo patients, less Cu and Zn are utilized for the melanin synthesis, which consequently raise levels of Cu and Zn in serum in vitiligo patients [(26)].

On the contrary, **Wang** [(27)], reported no statistically significant Cu level change between the vitiligo patients and the control group. No significant alteration in serum Zn and Cu levels in vitiligo patients possibly supports other theories such as AI theory.

Also, **Madhavi *et al.*** [(28)], presented significant decrease of Cu level in vitiligo patients.

Cu and Zn are antioxidants involved in destruction of free radicals and potential anti-apoptotic factors for protecting cell proteins from oxidation. Furthermore, Cu and Zn may play roles

in stimulating cell-mediated immunity responses, synthesizing and releasing of melanocyte stimulating hormone, which are also important in melanogenesis [19]. So, decreased serum Zn and Cu levels can cause vitiligo.

Our study showed more vitiligo females patients, which is similar to other studies such as **Akay *et al.*** [29], which explained by more awareness of the women to cosmetic disfigurement and therefore more likely to seek treatment. However, **Majumder** [30] showed both sexes were equally affected.

Basha *et al.* [22] reported that there was no statistically significant difference of the serum Zn level and sex distribution. Conversely, our study showed that serum Zn levels were significantly higher in vitiligo males patients than vitiligo females. Also, there was a tendency to be a statistically significant higher in male control group than female control group. Our study showed that there was a statistically significant difference between VASI and age of vitiligo group and between VASI and disease duration. Also, statistically significant was a positive correlation between VASI and age. Also, this study found a tendency to be statistically significant negative correlation between serum Cu levels and vitiligo disease duration.

Our study found that serum Cu was insignificantly higher in vitilgo group compared to the control group. There was no statistically significant difference between serum Zn and Cu levels and age of vitiligo group. There was no statistically significant difference between serum Zn and Cu levels and vitiligo disease activity. There was no statistically significant difference between serum Cu levels and sex distribution in the vitiligo group and in the control group. There was no statistically significant difference between serum Zn and Cu levels and the vitiligo disease duration.

CONCLUSION

In our study serum Zn levels were lower in the different studied groups, but in the vitiligo group it was much lower and insignificantly higher serum Cu levels in the vitilgo group compared to the control group. So serum Zn and Cu may have an effect on the vitiligo disease as Zn in combination with other micronutrients such as Cu, cobalt, nickel, iron, manganese and Ca^{++} plays an important role in the process of melanogensis.

RECOMMENDATIONS

1- Further investigation are needed on larger number of patients and divide them into further classifications of the disease (acrofacial,vitiligo vulgaris... etc) to obtain better knowledge of the effect of these trace elements.
2- Treatment with Zn supplements can be tried in these patients to see the outcome. A survey in Fayoum City populations is needed to diagnose Zn deficiency.
3- Measurement of the serum Cu level may be a helpful test in the diagnosis of Zn deficiency.
4- Further studies are needed to recognize the role of Zn and Cu and other trace elements in vitiligo etiopathogenesis.
5- Further studies are needed to recognize the role of Zn and Cu and other trace elements in other skin diseases.

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