Original Research Article

Evaluation of the Serum Zinc Level in Some Pigmentary Skin Disorders

Abstract

- **Background**

Pigmentary disorders are a common presentation in dermatology and the appearance of either hypopigmentation or hyperpigmentation can be a major psychosocial problem to the patients. Melanin pigment is the most important substance determining human skin color. It produced by melanocyte cells during melanogenesis. Zinc in combination with other micronutrients has an essential function in melanogenesis process.

- **Aim of work**

The aim of the study was to assess serum zinc level in patients with pigmentary disorders compared to healthy control subjects.

- **Patients and Methods**

This study was carried out on a total of 189 patients with pigmentary disorders (96 with hypopigmented and 93 with hyperpigmented disorders) and 50 apparently healthy controls. Serum zinc level was measured in all groups using a Flame Atomic Absorption Spectrophotometer.

- **Results**

The mean serum zinc level was in the hypopigmented, hyperpigmented disorders patients were 71.87 ± 20.23 and 72.36 ± 18.69, respectively, while the mean serum zinc level in controls was 87.48 ± 31.50, with the serum zinc level in patients with hypopigmented disorders was significantly lower than in healthy controls ($P < 0.05$). The average zinc level in the three groups was found to be within the normal reference range.

- **Conclusion**

Serum zinc level was within normal range in both patients and control groups. On the other hand, serum zinc level in pigmentary disorders was lower than that of control group with a statistically significance difference only between hypo pigmented disorders and control group.

Key words: Serum Zinc, Pigmentary, skin.

Introduction

Pigmentary skin disorders are frequently encountered in primary care practice. Although they are occasionally benign and easily distinguishable according to appearance and site, it may be important to

perform a skin biopsy to exclude melanoma as well as its precursors [1]. Skin imparts color owing to the presence of pigment called melanin, and any dysfunction or disturbance results in various kinds of pigmentation defects, categorized as hypopigmentation or hyperpigmentation and which may develop with or without an altered number of melanocytes. [2,3,4]

Hyperpigmentation occurs owing to excessive melanin production, distribution, or transport. Post inflammatory hyperpigmentation, melasma and solar lentigines are frequent etiologies of pigment excess. Hypopigmentation occurs owing to a reduction of melanocytes or an incapability of the melanocytes to form melanin or properly transport melanosomes. Vitiligo, pityriasis alba, pityriasis versicolor, and post inflammatory hypopigmentation are frequent etiologies of pigment loss. [1]

Zinc (Zn) represents less than 0.005% of total body weight, and is present in all kinds of cells [5]. It is an essential trace element required for a large number of structural proteins, enzymatic processes and transcription factors [6]. Zn is essential for the cellular growth, development, and differentiation. Zn deficiency may lead to number of cutaneous disorders. [7,8]

Skin contains approximately 6% of total body zinc. Zn plays a major role in melanogenesis by virtue of its catalytic function in the biosynthesis of 5,6-dihydroxy indole derivatives and increasing its incorporation into the pigment polymer. It suppresses the activity of TYR and glutathione reductase in vitro, exhibit dopachrome tautomerase activities. In addition, it has agonistic effects on melanocortin receptor signaling.[9,10]

In this study we assess the serum zinc level in patients with pigmentary disorders compared to healthy control subjects to help us to understand the role of zinc in the pathogenesis of these disorders.

Patients and Methods

This study was carried out on a total of 189 patients suffering from different types of pigmentary disorders (96 patients for hypopigmented and 93 for hyperpigmented disorders) and 50 healthy control peoples. Cases were selected from the Dermatology outpatient clinic, Faculty of Medicine, Tanta University Hospital, during the period from January 2019 to January 2021. All cases were evaluated by a full history, physical examination, and routine laboratory investigations.

- **Inclusion criteria:** Patients with hypo and hyper pigmentary disorders and being willing to contribute to the study.

- **Exclusion criteria:** Cases who have a history of active malignancy, under immunosuppressive therapy, hepatic cirrhosis, kidney failure, DM, pregnancy, alcoholism, malabsorption disorders, physical disability, any neurologic disorder, other physical diseases, that may induce psychological distress, also cases who refuse to participate in the study or who under any treatment with Zn in the six weeks before diagnosis.
- **Sample Collection:** A volume of 8ml of venous blood was drawn from the cubital vein of the selected cases; 2ml was delivered into EDTA tube for CBC estimation. The rest was gathered in a plain tube, and was allowed to coagulate for 2 hours and following centrifugation at 5000 rpm for 10 min the serum was separated and the serum was divided into two parts. One part for estimation of blood glucose level, renal and liver functions, and the other part was used for estimation of zinc.
- **Measurement of Zinc:** Serum zinc estimation was performed by Colonimetric method. [(11)]

1-<u>Test principle</u>

Zn forms a red chelate complex with 2-(5-Bromo-2-pyridylazo)-5-(N-propyl – N – sulpho - propyl amino)-phenol. The increase of

absorbance of such complex could be calculated. It has a positive correlation with the level of overall Zn in the sample.

2-Technique

Chart 1: Reagents and samples were ready at 22C.

Pipette into test tubes	blank	Standard	Sample
Reagents	1000µl	1000µl	1000µl
Samples	----	----	50µl
Standards	----	50µl	----
Dist. water	50µl	----	----
They were mixed and incubated for 8 minutes at 25° c for 3 minutes at 37°c measure absorbance of the standard and the sample versus the reagent blank.			

3-Calculation

$$\text{Zinc (µg/dl)} = \frac{\text{AA Sample}}{\text{AA standard}} \times \text{conc Standard (µg/dl)}$$

4- Chart 2 Reference range

Serum/plasma		µg/dl
10-13 yrs.	male	78-98
	female	78-118
14-19 yrs.	male	65-118
	female	59-98
Adults		46-150

- **Statistical analysis:** SPSS software was used to do statistical analysis on the data (Version 19. SPSS Inc, United States). The student's t-test was used

to compare the continuous variables, while the c2 test was used to compare the categorical variables. The Pearson correlation analysis was used to assess the relationship between the scores on the various scales and other pertinent factors. A statistically significant p-value of 0.05 was considered. The data were presented in the form of means ± standard deviations.

Results

Regarding gender: The hypopigmented disorders group included 68 females (70.8%) and 21 males (29.2%), and the hyperpigmented disorders group included 82 females (88.2%) and 11 males (11.8%) while the control group included 30 females (60%) and 20 males (40%). The mean age of hypopigmented disorders patients was 30.46 ± 14.68 years, and that of hyperpigmented disorders was 35.72 ± 10.73 years, while the mean age of control subjects was 35.02 ± 12.32 years. There was no significance difference between studied groups regarding sex and age. **Table (1)**

Out of 96 hypopigmented disorders patients there were 65 vitiligo patients (67.7%), 19 hypopigmented type of pityriasis versicolor patients (19.8%) ,4 pityriasis alba patients (4.2%) and 8 post inflammatory hypopigmentation patients (8.3%). While out of 93 hyperpigmented disorders patients there were 72 melasma patients (77.4%), 13 hyperpigmented type of pityriasis versicolor patients

(14%), and 8 post inflammatory hyperpigmentation patients (8.6%). **Table (2)**

Serum zinc level among the studied groups: the mean value of serum zinc levels in the hypopigmented disorders group was 71.87 ± 20.23, and the mean value of serum zinc levels the hyperpigmented disorders group was 72.36 ± 18.69, while mean value of serum zinc levels in the healthy control group. 87.48 ± 31.50 .The overall p-value was 0.013, indicating that statically significant differences were found between the three groups. This significant difference was found between the hypopigmented disorders group and the healthy control group. **Table (3).**

Serum zinc levels in patients with hypo pigmented disorders showed that only 12 vitiligo patients and 2 hypo pigmented type PV had serum zinc level less than 46 µg/dl. There was no significant correlation of serum Zn level between the hypo pigmented disorders of the studied cases. (p= 0.508). In patients with hyper pigmented disorders, there were 7 melasma patients who had serum zinc have serum zinc level less than 46 µg/dl, there was no significant correlation of serum Zn level between the hyperpigmented disorders of the studied cases. (p= 0. 462).

ROC curve using serum zinc level was conducted to discriminate hypopigmented disorders patients from controls. The curve shown that serum level below 87 µg/dl (cut off value) can discriminate between controls and hypopigmented patients with 82.86% sensitivity and 50.0 % specificity. Area under ROC curve was 0.660 with (p= 0.018 and CI was 0.534 – 0.785), PPV was 59.2 % and NPV was 76.9%. **(Table 4, Figure 1)**.

Roc curve using serum zinc level was conducted to discriminate hyperpigmented disorders patients from controls. The curve shown that serum level below 75.1 µg/dl (cut off value) can discriminate between controls and hyper pigmented patients with 68.0% sensitivity and 67.50% specificity. Area under ROC curve was 0.648 with (p= 0.047, CI was 0.514 – 0.781), PPV was 56.7% and NPV was 77.1%. **(Table 5**, **Figure 2).**

Discussion

Pigmentary disorders are considered one of the most common skin diseases which may occur due to abnormal melanocytes structures, distributions or functions. Pigmentary disorders can be expressed in two forms of skin pigmentation increase (hyperpigmentation) and decrease (hypopigmentation).[(12)]

Zinc, an essential trace element, insures health of different tissues - particularly epithelia and skin. Zn is involved in a lot of homeostatic mechanisms, including immunity, inflammation and oxidative stress. Zn in combination with other micronutrients has an essential function in melanogenesis process as Zn can catalyze the rearrangement of dopachrome to form 5,6-dihydroxy indole- -2′-carboxylic acid. Also, Zn has a vital role in elimination of free radicals. [13]

There are many studies that evaluate serum Zn levels in different skin disorders, and reduced serum Zn level was reported in many skin disorders, while other studies revealed normal or even high serum zinc level. [14,15]

In the present study serum zinc level in hypopigmented disorders which represented by vitiligo, hypopigmented type of PV, pityriasis alba and post inflammatory hypopigmentation showed normal ranges. Vitiligo which represented by 65 patients (67.7**%** of the total hypopigmented disorders study patients) showed normal ranges of serum zinc level but still lower than healthy control level ($p < 0.005$) and this finding is in agreement with that of ***Shameer et al***. [16], who showed significance correlation between low zinc levels in vitiligo cases compared to healthy control ($P < 0.0002$). Also, ***Mirnezami et al,*** [13] ***Mogaddam et al*** [17] and

Azzam OA et al [18], showed that serum zinc levels of their patient's group were lower than controls.

However, this finding was not in agreement with that of ***Arora et al.*** [19], who demonstrated no significant change in serum Zn level in vitiligo patients.

Also, our results were contradictory to that of ***Helmy et al,*** [20] who demonstrated that serum Zn and copper levels were significantly higher in active vitiligo cases in comparison to controls. The explanation was the presence of higher percentage of apoptotic peripheral blood mononuclear cells in active vitiligo with increased release of zinc and copper in serum.

There were contradictory results in serum zinc level in different hypopigmented disorders including vitiligo. Most of studies revealed low levels while others showed normal or even high level, this may be explained by some nutritional factors as excessive supplement therapy, differences in diet quality, zinc concentration in soil, consumption of large amount of phytate, in addition to an important factor which is the inequality in cut- off point of normal zinc levels.[21] Our study considered 46 ug/dl as the low limit of normal zinc level, while other studies revealed a higher reference level, for example ***Zaki et al.*** [21] considered the low limit of normal zinc level was 65 ug/dl. Also, ***Rostami***

mogaddam et al.[22] considered the low limit of normal zinc level 70 ug/dl and Zn deficiency was described as a serum Zn level <70ug/dL. This different in serum zinc reference level may subjected to variation of laboratory techniques statistical procedures and eco-environmental factors.

Hyperpigmented disorders which represented by melasma, hyperpigmented type of PV, post inflammatory hyperpigmentation showed normal ranges of serum zinc levels. Melasma represented by 72 patients (77.4 **%** of total hyperpigmented disorders study patients) showed normal ranges of serum zinc level and this finding was in agreement with ***Sastrini Sekarnesia et al***.[23], who found no significant differences between serum zinc levels in the melasma and non-melasma groups, on other hand our study was not similar to ***Rostami et al***.[22] who found a significant relationship between low zinc levels and melasma.

Of note, both plasma and serum Zn levels are the most frequently utilized to evaluate Zn deficiency, however the tight homeostatic control mechanisms making these levels don't essentially reflect cellular zinc condition. Also, clinical effects of Zn deficiency could be present in the absence of abnormal laboratory indices.[24]

Conclusion

In conclusion, although serum zinc level was within normal ranges in both patients and control groups, pigmentary disorders showed lower levels than that of control group with a statistically significant difference only between hypopigmented disorders and control group. This may highlight the role of zinc in the pathogenesis of such disorders. Large-scale and multicenter studies are needed to confirm these findings.

Ethical Approval and consent:

The study was submitted for approval by Ethical Committee of Human Rights in Research of Tanta University before study initiation. A written consent was obtained from every participant prior to specimen collection.

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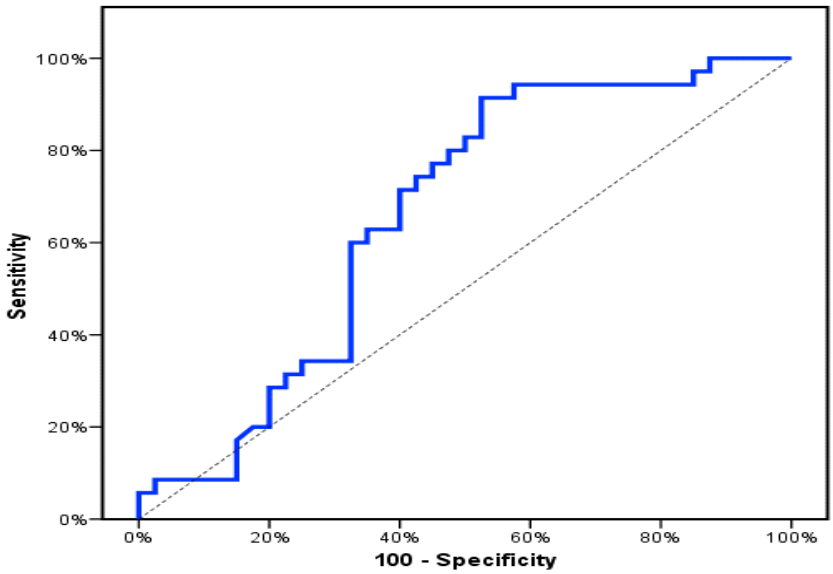

Figure (1): ROC curve for Serum level of zinc (ug/dl) to discriminate Hypo pigmented disorders patients (n = 96) from control (n = 50).

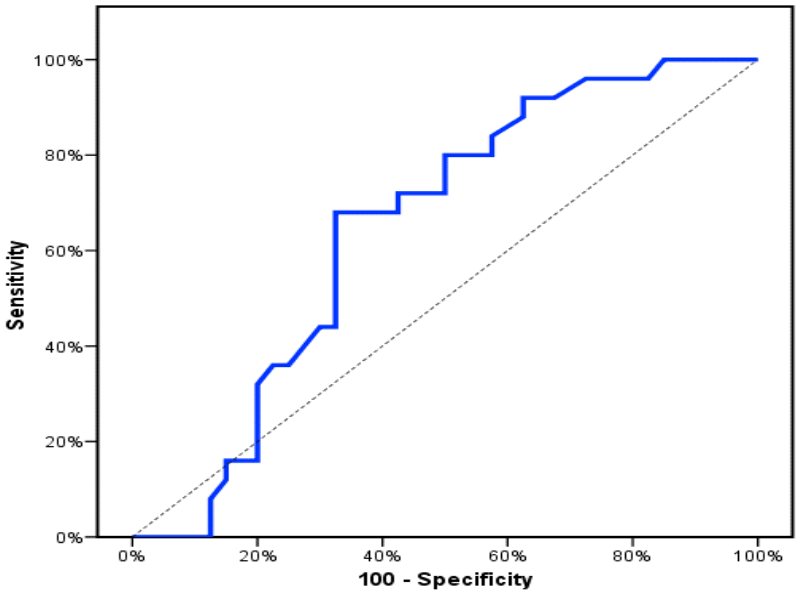

Figure (2): ROC curve for Serum level of zinc (ug/dl) to discriminate Hyper pigmented disorders patients (n = 93) from control (n = 50)

Table (1): Comparison among the studied groups according to demographic data.

	Hypo pigmented disorders (n = 96)		Hyper pigmented disorders (n = 93)		Control (n= 50)		Test of Sig.	p
	No.	%	No.	%	No.	%		
Gender								
Male	21	29.2	11	11.8	20	40	χ^2= 5.013	0.082
Female	68	70.8	82	88.2	30	60		
Age (years)								
Min. – Max.	15.0 – 59.0		17.0 – 50.0		17.0 – 61.0			
Mean ± SD.	30.46 ± 14.68		35.72 ± 10.73		35.02 ± 12.32		H=4.464	0.107
Median (IQR)	23.0(19.5 – 42.0)		39.0 (28.0 – 45.0)		33.50(24.50 – 44.0)			

- χ^2: **Chi square test** IQR: **Inter quartile range**
- H: H for **Kruskal Wallis test**
- **p**: p value for comparing between the studied groups.

Table (2): Comparison between the studied groups according to pigmentary disorder.

Pigmentary disorders	Hypo pigmented disorders (n = 100)		Hyper pigmented disorders (n = 100)		χ^2	^{MC}p
	No.	%	No.	%		
Vitiligo	65	67.7	0	0.0	35.912	0.647(NS)
Melasma	0	0.0	72	77.4		
Pityriasis alba	4	4.2	0	0.0		
Post inflammatory	8	8.3	8	8.6		
Pityriasis versicolor	19	19.8	13	14		

χ^2: **Chi square test** MC: **Monte Carlo**

p: p value for comparing between the studied groups
NS: non-significant

Table (3): Comparison among the studied groups according to serum level of zinc.

	Hypo pigmented disorders (n = 100)		Hyper pigmented disorders (n = 100)		Control (n= 50)		Test of Sig.	p
	No.	%	No.	%	No.	%		
Serum level of zinc (ug/dl)								
<46	14	14.6	7	7.5	3	6	χ^2= 0.763	^{MC}p= 0.685
>46	82	85.4	86	92.5	47	94		
Min. – Max.	27.50 – 128.0		42.80 – 114.0		35.0 – 151.0		F= 4.531*	0.013*
Mean ± SD.	71.87 ± 20.23		72.36 ± 18.69		87.48 ± 31.50			
Median (IQR)	71.80 (61.60 – 81.35)		68.70 (61.20 – 85.0)		86.0 (64.05 – 109.20)			
Sig.bet.Grps	p_1=0.997, p_2=0.023*, p_3=0.052							

χ^2: **Chi square test** FE**: Fisher Exact**
F: F test (ANOVA) with repeated measures, Sig. bet. periods were done using **Post Hoc Test** (**Tukey)**
p: p value for comparing between the studied groups
p_1: p value for comparing between **hypo pigmented disorders and hyper pigmented disorders**
p_2: p value for comparing between **hypo pigmented disorders and control**
p_3: p value for comparing between **hyper pigmented disorders and control**
*: Statistically significant at $p \leq 0.05$
IQR: Inter quartile range

Table (4): Validity (AUC, sensitivity, specificity) for Serum level of zinc (ug/dl) to discriminate Hypo pigmented disorders patients (n = 96) from control (n = 50).

	AUC	p	95% C. I	Cut off	Sensitivity	Specificity	PPV	NPV
Serum level of zinc (ug/dl)	0.660	0.018*	0.534 – 0.785	≤87	82.86	50.0	59.2	76.9

AUC: Area Under a Curve **P value**: Probability value
CI: Confidence Intervals **PPV:** Positive predictive value
NPV: Negative predictive value *: **Statistically** significant at $p \leq 0.05$

Table (5): Validity (AUC, sensitivity, specificity) for Serum level of zinc (ug/dl) to discriminate Hyper pigmented disorders patients (n = 93) from control (n = 50)

	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
Serum level of zinc (ug/dl)	0.648	0.047*	0.514 – 0.781	≤75.1	68.0	67.50	56.7	77.1

AUC: Area Under a Curve **P value**: Probability value
CI: Confidence Intervals **PPV:** Positive predictive value
NPV: Negative predictive value ***: Statistically significant** at $p \leq 0.05$