The influence of Serum Zinc level and Serum Copper level on melasma duration and severity among group of Iraqi patients.

Abstract:

Background: Several factors on melasma etiopathogenesis have been suggested, yet the exact pathophysiology of melasma is uncertain. However, controversial results exist now on Zn and Cu in serum of melasma patients. **Objectives:** This study was done to measure the serum level of Zinc and Copper and to evaluate their influence on the duration and severity of melasma. **Patients and methods:** A Case- control study involved a total of 200 patients (melasma group 100 and equal number of healthy controls), The two groups were matched for age and sex. A sample of blood was taken from both cases and controls for assessment of serum zinc and serum copper, sent to the same lab and read by the same method for both groups, and then to compare the relation of these values with demographic data. **Results:** the serum zinc level was low in 51% of the patients with melasma, 47% had normal serum zinc level and only 2% had high serum zinc level. While only 5% had low serum copper level, 66% had normal serum copper level, and 29% had high serum copper levels. Analysis of means of serum zinc and serum copper levels revealed significant difference in means of serum zinc between cases and controls ($p<0.001$) , and low serum zinc was highly associated with melasma cases ($p<0.001$). Furthermore, there was a significant association between duration of melasma and serum zinc level; duration $\leq$ 2 years was associated significantly with normal serum zinc level ($P=0.03$). However, there was no significant difference in means of serum copper between cases and controls ($P=0.5$). **Conclusion**: The results of the present study showed that there is a significant relation between serum zinc levels and melasma while showed no significant relation between serum copper and melasma. Low serum zinc level may be one of the risk factors that effects the melasma duration.

Key word: Melasma , serum zinc, serum copper , melasma duration

Introduction:

Melasma is an acquired irregular brown or sometimes grey-brown hypermelanosis , which affects areas of sun exposure ,the condition is seen most commonly on the face of women

with skin types IV to VI. Family history was found in most of the cases. The incidence of melasma range from 1.5% and 33.3%. Its incidence in pregnancy is about 50-70%. It is triggered by several factors including sun exposure, genetic influences, and female sex hormones [1] .Despite the fact that melasma is clinically characterized by epidermal hyperpigmentation, the histopathological changes involve both the epidermis and dermis. In addition, the pathogenesis of melasma is complex and includes both exogenous and endogenous factors. [2] The pathology of melasma extends beyond melanocytes and recent literature points to interactions between keratinocytes, mast cells, gene regulation abnormalities, neovascularization, and disruption of basement membrane [3 ,4]. Moreover, it has been suggested that dermal inflammation induced by accumulation of UV irradiation may be associated with activation of fibroblasts, which result in the up-regulation of stem cell factor in melasma dermal skin leading to increased melanogenesis. This complex pathogenesis makes melasma difficult to target and likely to recur post treatment. [5,6]

Melasma often represents a mixture of three patterns: centrofacial, malar and mandibular patterns, according to clinical appearance and on the basis of Wood's light examination (320-400 nm), melasma can be classified to three types: Epidermal type, dermal type and mixed type [7].

Zinc is one of essential trace elements that are required for physiological functions in amount less than 100 mg daily. The serum zinc levels may vary based on the age group of individuals among women in Sulaymaniyha city –Iraq. It is present in high concentrations in pigmented tissues. The adult body stores of zinc are about half the iron content and some 10-20 times more than other trace elements such as copper, magnesium and nickel. It is important for the cell growth, development, and differentiation. zinc also is an intracellular regulator that regulates the expression and activation of biological molecules such as transcription factors, enzymes, adapters, channels, and growth factors, along with their receptors. [8-10].

Zinc cations modulate melanogenesis, but the net effect of zinc in vivo is unclear, as the reported effects of zinc on melanogenesis are ambiguous: zinc inhibits tyrosinase and glutathione reductase (tyrosinase-related protein-2) and has agonistic effects on melanocortin receptor signaling. [11]

Galvanic zinc-copper microparticles inhibited melanogenesis in a human melanoma cell line (MNT-1), human keratinocytes and melanoma cells co-cultures, and in pigmented epidermal equivalents. [12] According to preliminary studies, abnormal levels of zinc, copper, and iron are observed in many skin diseases and their determinations in serum or hair can be used as auxiliary and prognostic tests in the course of various dermatoses. However, since data for

some conditions are conflicting, clearly defining the potential of trace elements as auxiliary tests or elements requiring restriction/supplement requires further research. [13]

This study was done to measure the serum zinc and serum copper levels in clinically confirmed cases of melasma in comparison with equal number of gender and age matched controls and to evaluate the influence of serum zinc levels and serum copper levels with melasma duration and severity.

Patients and method:

A total of two hundred participants were included in this case -control study, we divide them into two groups: one hundred melasma patients (cases) and one hundred healthy controls.

Group -I: a convenient sample of 100 melasma patients was selected from melasma patients (study population) who fulfilled the criteria and agreed to participate.

Group- II: a convenient sample of 100 healthy controls was taken from the patients attending clinic center, who agreed to participate.

Inclusion Criteria include any adult patients with melasma. With exclusion of any case taking zinc one month before diagnosis of melasma, patients complain of persistent diarrhea, hepatic insufficiency, renal insufficiency, heart failure and history of hormonal treatment.

Clinical Examination and data Collection; A questionnaire was fulfilled for all cases, which included information about demographic class, familial background of melasma, medical and drug intake, presence of melasma, and. Full dermatological assessment for melasma and other skin problems. The clinical diagnosis of the cases was made through the inspection of the affected area (light to gray brown macules and patches in the sun-exposed areas of the skin), and wood's lamp examination. The assessment of the size and severity of melasma was made by modified melasma area and severity index (mMASI) [14].

Measurement of serum zinc level and serum copper level was done after obtaining consent. Normal values of serum zinc are 70-115 µg/dl., normal values of serum copper are 80-140 µg/dl for males and 80-155 µg/dl for females. Zinc considered to be deficient if a serum zinc level lower than 70 [15].

Statistical analysis: All patients' data entered using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 23 was used. In all statistical analysis, level of significance (p value) set at ≤ 0.05 and the results presented as tables and or graphs. Multiple contingency tables conducted and appropriate statistical tests

performed, Chi-square used for categorical variables. Independent t-test was used for continuous variables.

Results: A total of two hundred participants were included in this study, divided into two groups: one hundred melasma patients (cases) and one hundred healthy controls. **1.<u>Group-I (melasma cases):</u>** Mean age of patients was between 29.2±6.1 years; half of them were within 20-29 years. Male to female ratio was 1: 15, more than two thirds (74%) of melasma patients were housewives, most of the patients (89%) were living in urban areas (Table 1).

Table 1: Demographic characteristics of melasma cases.

Variable	**No.**	**%**
Age mean±SD (29.2±6.1 years)		
< 20 years	2	2.0
20-29 years	50	50.0
30-39 years	43	43.0
≥ 40 years	5	5.0
Total	100	100.0
Gender male: female (1: 15)		
Male	6	6.0
Female	94	94.0
Total	100	100.0
Occupation		
Housewife	74	74.0
Other occupation	26	26.0
Total	100	100.0
Residence		
Urban	89	89.0
Rural	11	11.0
Total	100	100.0

Mean duration of melasma for studied patients was 2.7±2 years with median (2 years), 61% of the patients had duration ≤ 2 years and 39% of them had duration > 2 years. More than two thirds (73%) of the patients had used previous topical treatment

for melasma and 27% of them had no history of any topical melasma treatment (Table 2).

Table 2: Clinical characteristics of melasma cases.

Variable	No.	%
Duration mean±SD (2.7±2 years) median (2 years)		
≤ 2 years	61	61.0
> 2 years	39	39.0
Total	100	100.0
Previous topical treatment		
No	27	27.0
Yes	73	73.0
Total	100	100.0

Seven (7%) patients had dermal melasma type, 66 (66%) patients had epidermal type and 27 (27%) patients had mixed type. The site of melasma was mandibular in 6% of the patients, malar in 52% of the patients and centrofacial in 42% of them (Table 3).

Table 3: Melasma types & sites among cases.

Variable	No.	%
Melasma types		
Dermal	7	7.0
Epidermal	66	66.0

Mixed	27	27.0
Total	100	100.0
Melasma sites		
Mandibular	6	6.0
Malar	52	52.0
Centrofacial	42	42.0
Total	100	100.0

Mean serum zinc for melasma patients was 74.9±17 µg/dl, 51% of the patients had low serum zinc, 47% had normal serum zinc and only 2% had high serum zinc. Mean serum copper for patients was 131.6±31.3µg/dl, 5% of the patients had low serum copper, 66% of the patients had normal serum copper level and 29% had high serum copper level (Table 4 and Figure 1).

Table 4: Zinc & Copper level for melasma cases.

Variable	**No.**	**%**
Serum Zinc mean±SD (74.9±17 µg/dl)		
Low	51	51.0
Normal	47	47.0
High	2	2.0
Total	100	100.0
Serum Copper mean±SD (131.6±31.3µg/dl)		
Low	5	5.0
Normal	66	66.0
High	29	29.0
Total	100	100.0

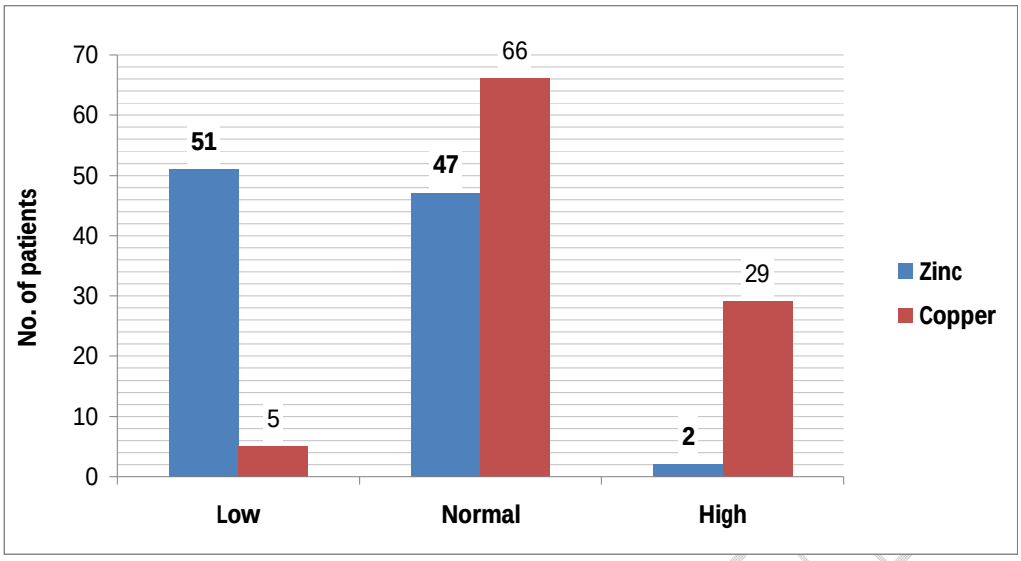

Figure 1: Distribution of zinc and copper among cases.

2. <u>Group II (controls):</u> mean age of controls was 28.2±6.1 years, 52% of them were between 20-29 years, male to female ratio was 1:15 (Table 5).

Table 5: Demographic characteristics of controls.

Variable	**No.**	**%**
Age mean±SD (28.2±6.1 years)		
< 20 years	7	7.0
20-29 years	52	52.0
30-39 years	35	35.0
≥ 40 years	6	6.0
Total	100	100.0
Gender male: female (1: 15)		
Male	6	6.0
Female	94	94.0
Total	100	100.0

Mean serum zinc of controls was 89.5±22.8 µg/dl, 12% of them had low serum zinc, 79% had normal serum zinc and 9% had high serum zinc level. Mean serum copper for controls was 135±39.1 µg/dl, 3% of them had low serum copper, 70% had normal serum copper and 27% had high serum copper (Figure 2).

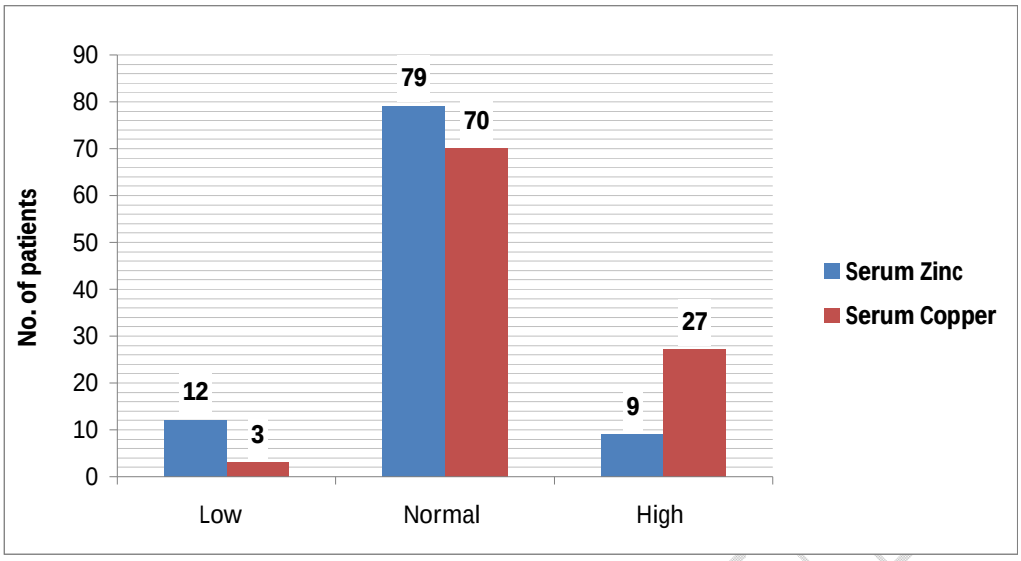

Figure 2: Serum zinc and copper for controls.

A significant association was observed between cases and controls regarding serum zinc ($p<0.001$). Low serum zinc was highly associated with melasma cases ($p<0.001$) (as it is shown in Table 6 and Figure 3).

Table 6: Distribution of demographic variables, Zinc and Copper levels among cases and controls.

Variable	Case		Control		χ^2	P
	No.	%	No.	%		
Serum Zinc					36.7	**<0.001**
Low	51	80.9	12	19.1		
Normal	47	37.3	79	62.7		
High	2	18.2	9	81.8		
Serum Copper					0.7*	0.7
Low	5	62.5	3	37.5		
Normal	66	48.5	70	51.5		
High	29	51.8	27	48.2		

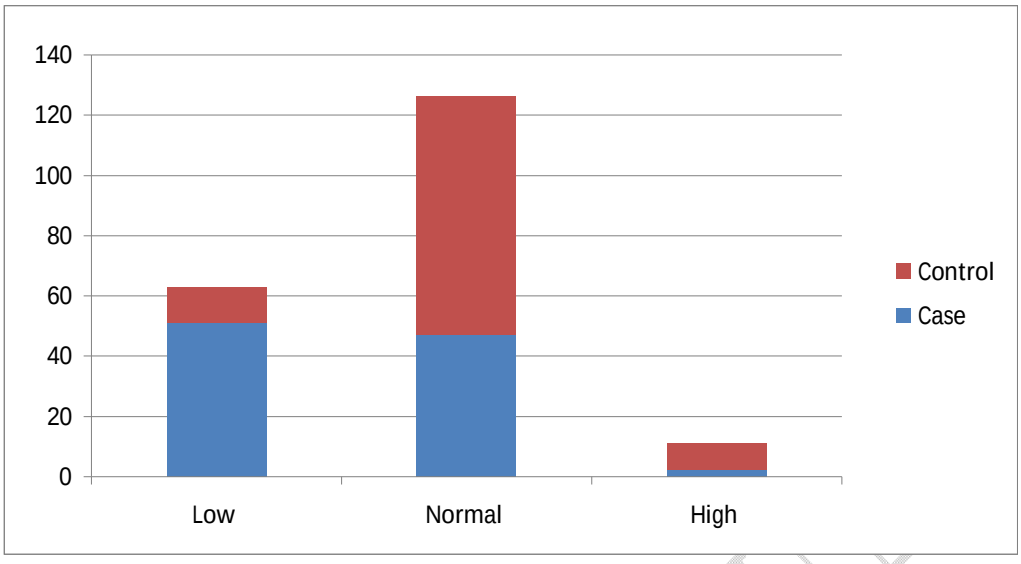

Figure 3: Distribution of Zinc levels among cases and controls.

Analysis of serum zinc and copper means by independent t-test revealed significant difference in means of serum zinc between cases and controls, low mean of serum zinc was observed among melasma cases ($p<0.001$). No significant difference was observed in means of serum copper between cases and controls (Table7 and Figure 4).

Table 7: Zinc & Copper mean levels among cases and controls.

Variable	**Case**	**Control**	**t-test**	**P**
	Mean± SD	**Mean± SD**		
Serum Zinc	74.9±17	89.5±22.7	5.1	**<0.001**
Serum Copper	131.6±31.3	135±39.1	0.68	0.5

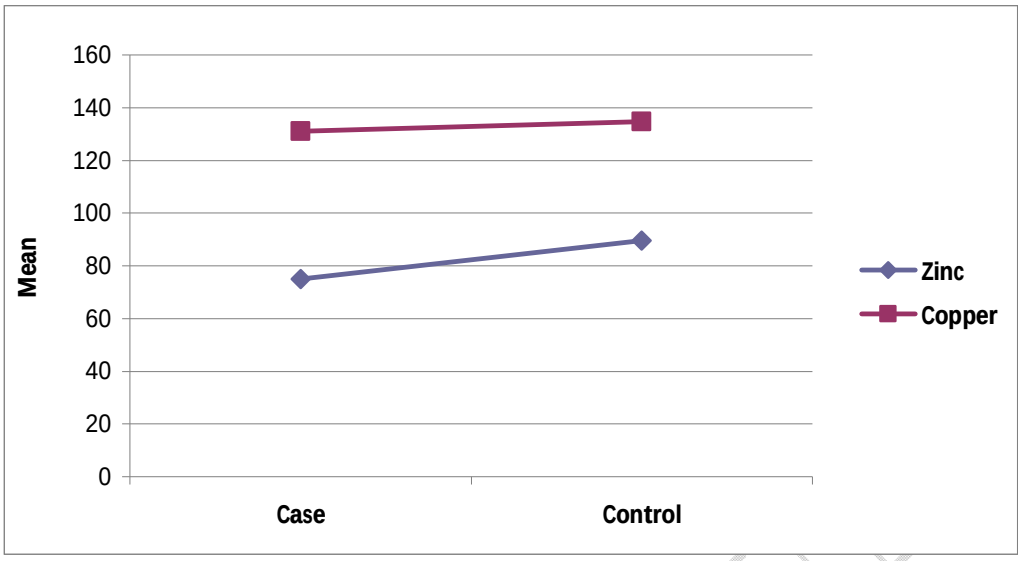

Figure 4: Difference in means of Zinc & Copper among cases and controls.

There was a significant association between duration of melasma and serum zinc level, duration ≤ 2 years was associated significantly with normal serum level (p=0.03) (Figure 5).

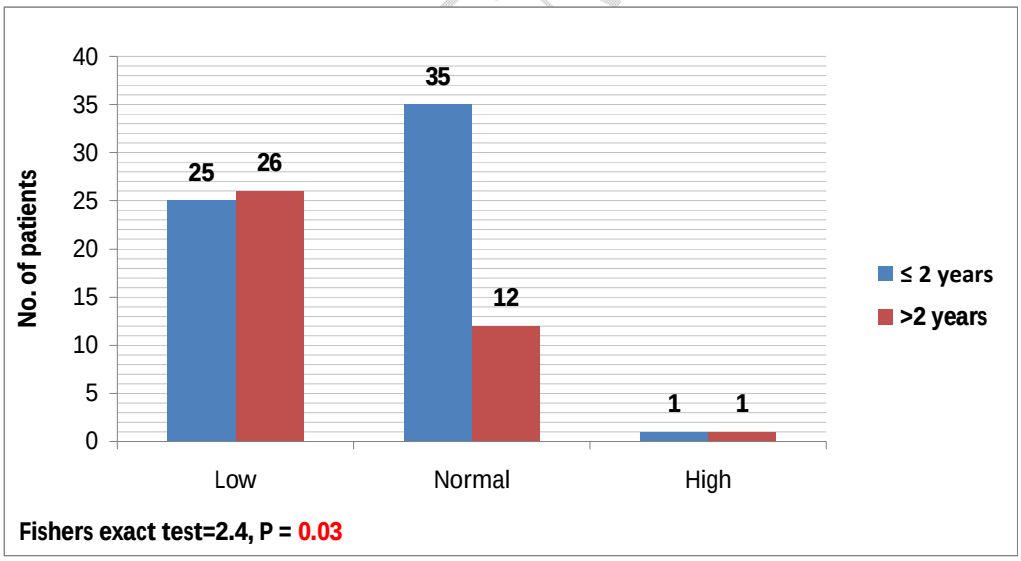

Figure 5: Serum zinc & duration of melasma.

Discussion:

Melasma is an acquired irregular brown or sometimes grey-brown hypermelanosis, which affects areas of sun exposure. The condition is seen most commonly on the face of women with skin types IV to VI. Family history was found in most of the cases [16].

This study was done to evaluate the serum Zinc levels and copper serum levels in cases with melasma compared to age and sex matched controls. The average age of our patients was (29.2±6.1) compared to those reported in Iraq (mean age 28.4 years) [16] while in India (38.5±7.8 years) and Brazil (38.43±6.75 years) [17].

Female gender was the prevalent with male: female ratio 1: 15. This finding is close to results of Ali R, et al study in Pakistan [18] and Al-Hamdi KI, et al study in Iraq [19] which reported the predominance of female gender on melasma cases. Men represented only 6% of cases and demonstrated the same clinicohistological characteristics as those for women. This could be attributed to the fact that hormonal factors probably do not hold a causative significance in men and they are considered one of the most important causative factors in melasma in women [20].

More than two thirds of melasma cases were housewives. This finding is close to results of Leeyaphan C, et al study Thailand [21] that recorded the predominance of housewives among melasma cases. The correlation between individual demographic, clinical, and socioeconomic characteristics and quality of life demonstrates that younger patients and patients with economically inactive occupations have the worst quality of life due to melasma [22]. Most of studied melasma cases in our study were living in urban areas. Asghar A & Rasheed T study in Pakistan reported that 79.7% of their melasma patients were originated from urban areas [22].

Most of melasma females in this study were having regular menstrual cycle. This finding is close to results of Mahmood K, et al study in Pakistan which reported that all studied melasma women were having regular menstrual cycle [23].

Most of melasma women in this study were not taking oral contraceptive pills (OCP). This finding is inconsistent with Shweta K, et al study in USA that recorded the effect of OCP on melasma among women [24]. This inconsistency might be attributed to difference in culture and health habits between countries especially family planning practice.

Mean duration of melasma in present study was 2.7 years that is close to results of Almosuly IM & Butros RO study in Erbil [25] with mean duration (2.8 years). More than two thirds of melasma patients were taking topical treatment.

The production of pigment in mammalian melanocytes requires the contribution of at least three melanogenic enzymes, tyrosinase and two other accessory enzymes called the tyrosinase-related proteins (Trp1 and Trp2), which regulate the type and amount of melanin. The last two proteins are paralogues to tyrosinase, and they appeared late in evolution by triplication of the tyrosinase gene. Tyrosinase is a copper-enzyme, and Trp2 is a zinc-enzyme. Trp1 has been more elusive, and the direct identification of its metal cofactor has never been achieved. However, due to its enzymatic activity and similarities with tyrosinase, it has been assumed as a copper-enzyme. There are several studies of serum zinc levels in cutaneous disorders. Modification of melanogenesis by zinc still uncertain. Also, an inhibits tyrosinase in vitro, but it also activates dopachrome tautomerase (Trp2). It is accepted that oral ingestion of zinc affects the amount of pigmentation and the melanosome configuration. High doses of zinc sulfate inhibit eumelanogenesis and cause severe murine hair hypopigmentation. Low zinc diets make irregular huge melanosomes in choroidal melanocytes of adult pigs resulting in abnormal and irregular melanin distribution [(26)]

The present study revealed a significant association between low serum zinc level and melasma cases ($p<0.001$). Bae YS, et al study in USA (2010) reported that zinc deficiency might be a predisposing factor of melasma especially among pregnant women as they are the risk group for zinc deficiency [(27)].

In this study the melasma patients with normal zinc level were associated significantly with shorter duration of melasma ($p=0.03$). This finding is similar to results of Yousefi A, et al study in Iran [(28)]. As a physical blocker, zinc can provide broader protection against the sun than chemical blockers.

Zinc deficiency may be involved in the pathogenesis of melasma. Also, treatment with oral zinc supplements can be tried in these patients to see the outcome. [(29)]

A large number of treatment modalities have been tried for the treatment of melasma ranging from depigmenting agents like hydroquinone to lasers. Topical zinc sulphate has also been tried in the management of melasma owing to its peeling and sunscreen properties. Sharquie et al. [(30)] reported a significant reduction in MASI (melasma area and severity index) scores in 14 melasma patients after three months of therapy with 10% topical zinc sulphate without any significant adverse effects. However, this mode of treatment did not find much favor as

results could not be reproduced in other studies and no statistically significant improvement was seen with topical zinc therapy [28,31]. Moreover, it is not cosmetically elegant and acceptability remains poor. Nevertheless, zinc oxide, in micronized forms, remains a common ingredient of most sunscreens used for treatment of melasma [32].

Significantly higher levels of serum copper and non-ceruloplasmin copper have been detected in hyperpigmentary disorders. Copper promotes melanin production in the skin and high levels can cause excess pigmentation [33]. Regarding the serum copper in this study; only 3% of them had low level, 70% had normal serum copper and 27% had high serum copper level, so there is no significant association between copper level and melasma (p=0.7). This finding is inconsistent with Kwon SH & Park KC study in South Korea that reported significant effect of copper in treatment of melasma [34]. This inconsistency might be attributed to difference in dietary patterns between communities. So we may need to do the serum of zinc and copper level for patients with melasma.

Conclusions: The results of this study showed that serum zinc level was low in patients with melasma compared to sex- and age-matched controls and normal serum zinc is more likely to be associated with shorter duration of melasma. Also, in comparison with recent cases with melasma and old cases, old cases have lower mean serum zinc levels.

Highly significant inverse correlations were found between serum zinc level and disease duration but not its severity. Therefore, serum zinc level may be a helpful marker of disease duration in melasma, and zinc supplements may have useful therapeutic outcome. Serum copper level may not be a risk factor for melasma , but still we may need to do the serum of zinc and copper level for patients with melasma .
Further national larger sample studies on effect of serum zinc level and serum copper level on melasma should be considered to assess the role of zinc supplements in patients with melasma, especially those with long standing, severe, or resistant lesions.

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