

Serum Levels of Trace Elements (Zinc, Copper and Iron) in Pakistani Patients with Oculocutaneous Albinism (OCA)

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ABSTRACT

Aim: To determine trace elements levels (Zinc, copper and iron) and their correlation in patients with Oculocutaneous albinism (OCA).

Methods: Total eighty (80) subjects were selected for this study comprising of 40 healthy controls and 40 cases of OCA. The serum concentrations of trace elements zinc (Zn), copper (Cu) and iron (Fe) were carried out by atomic absorption spectrophotometer.

Results: Results showed that the serum Zn and Cu levels were significantly lower in patients ($p < 0.05$) than controls, while no significant difference was observed for serum iron (Fe) levels between patients and controls ($p > 0.05$). Although, there was the reduced concentration of trace elements in patients, but no correlation was established.

Conclusion: In conclusion, trace elements (Zn and Cu) were significantly lowered in OCA patients but no correlation was determined with different OCA phenotypes in patients.

Keywords Oculocutaneous albinism (OCA), Zn, Cu and Fe, Pakistan.

INTRODUCTION

Oculocutaneous albinism (OCA) is a rare genetic disorder considered by abnormal production of melanin which usually gives color to hair, skin and eyes. OCA is originated due to the mutation in specific genes, which are involved in its synthesis inside the melanocytes^{1,2}. Melanin synthesis is regulated by several factors such as bioactive peptides like alpha-Melanocyte-stimulating hormone (α -MSH) and its intermediary form, adrenocorticotrophic hormone (ACTH)³. Melanin deficiency in OCA also causes various ophthalmological features like photophobia, nystagmus, reduced visual acuity, and foveal hypoplasia due to developmental defects of the eyes^{2,4}.

These trace metals take part in several functions of the retina such as photo-transduction, neurotransmission and the visual cycle⁵⁻⁹. Variable levels of elements have toxic effects on retinal cells and abnormalities may develop due to mutations in genes taking part in the metal homeostasis inside retina⁶. Several mechanisms are interlinked to maintain the normal levels of trace elements by affecting their oxidation/reduction states, compartmentalization and concentrations¹⁰.

Abnormalities in the zinc and copper metabolism have been explained in certain pigmentary retinopathies. In these eye disorders, change in the RPE is a common finding. Albinism is an expression of a metabolic disorder with abnormalities of melanin pigment formation which also occurs in this RPE layer. From the previous literature, it has been documented and explained that degeneration or abnormality of retinal epithelium pigment can result in various eye diseases due to changes in homeostasis or metabolism of the trace elements^{9,10}.

Albinism is also categorized as a metabolic defect in which lacking of melanin can result in depigmentation due to changes in retinal epithelium. In a previous study, zinc (Zn), copper (Cu), and ceruloplasmin levels in the serum and the Cu levels in urine of albino patients were

measured, and it was documented that there were elevated Cu levels in serum and urine, but there was no effect on serum concentration of Zn¹¹. In another study of Caucasian albinos, the serum Zn concentration was elevated, but no correlation was observed with tyrosinase¹². Also, the low serum zinc was found in the ocular manifestation with poor dark adaptation, and the zinc level responses were normalized after the treatment¹³. The results of the previous studies proposed that there might be the involvement of primary dysfunction of copper metabolism in the albinism patients.

The aim/objective of this study was to determine the role of the serum Zn, Cu and Fe in patients with Oculocutaneous albinism (OCA) in Pakistani population.

METHODS

Ethical approval was taken from the Research and Ethical Board of University of Health Sciences (UHS), Lahore, Pakistan and written informed consent was taken. Helsinki guidelines (2008) were followed for the human subjects.

A total of 80 participants, both the males and the females were recruited in this study from the different areas of the Punjab, Pakistan. Out of the total individuals, forty individuals were the patients with OCA, and the forty individuals were the sex and age-related healthy controls (normal relatives of patients). The data of patients was obtained from the teaching hospitals of Lahore like Layton Rehmatullah Benevolent Trust (LRBT) Eye Hospital, Ittefaq Hospital and Services Hospital, Lahore, Pakistan. For the blood collection, the field visits were done to take the history and drew the blood after fulfilling the inclusion criteria (clinically diagnosed cases of OCA with hallmark features of OCA like nystagmus, photophobia, decreased visual acuity, foveal hypoplasia with light color skin and hair) and exclusion criteria (ocular albinism and syndromic albinism). Written informed consent was obtained from the adults and in case of the minors; the consent was obtained

from the head of the family. The complete demographic data like name, age, hair colour, skin colour, skin rashes and Iris colour and visual acuity of patients were documented on pre-designed pro-forma.

About 3-5 ml of the blood sample was collected/drawn aseptically from each subject for the determination of Zn, Cu and Fe. The serum was separated after 20 minutes of keeping at room temperature and then centrifugation at 5000 rpm for 5 minutes. The serum was transferred carefully into Eppendorf 1.5 ml tube. It was stored at -20°C until further analysis.

Serum levels of trace elements (Zn, Cu and Fe) in the subjects were carried out by the atomic absorption spectrophotometer (AAS, Hitachi Z2000) with the polarized Zeeman flame. The sample preparation was done with 10 % nitric acid to digest the protein and incubated overnight in microwave temperature. The procedure was performed according to the previously described protocol¹⁴.

Data was analysed by using the SPSS version 22.0. Shapiro-Wilk test was applied to document the distribution of the data. The data was considered normally distributed with p-value ≥0.05 and not normally distributed with the p-value ≤ 0.05. For the normally distributed data, Independent Student t-test and Pearson was applied to estimate the association and correlation, and p-value ≤0.05 was considered statistically significant. For non-normally distributed data, the Mann-Whitney U-test and Spearman test were applied to estimate the association and correlation.

RESULTS

A total of 80 study participants were recruited from different areas of Punjab. Out of total individuals, about 40 were oculocutaneous albinism (OCA) patients, and 40 individuals were their normal relatives without having albinism. In all recruited individuals, serum copper (Cu), zinc (Zn) and Iron (Fe) were determined.

The median age in patients and controls is represented in table I. The levels of serum Zn (83.9±33.0 µg/dL), and Cu (90.3±39.6 µg/dL) were reduced significantly in the OCA patients (p <0.05) as compared to the controls (Zn=119.5±84.0, Cu=26.3±80.82) with strong association between Cu and Zn (p=0.03 and 0.04), while there was no association (p=0.345) found with serum Fe (Table-I).

Chi-square analysis was done to find an association between qualitative variables like hair colour, skin colour, skin rashes and Iris colour in albinism patients. In male OCA patients, the white colour of hairs was more likely (35%) to be as compared to female patients (22%), but the association between males and females hairs colour was not statistically significant (p>0.05). The reddish-white skin colour (45 %) and skin rashes (50%) were more likely to be present in male OCA patients as compared to females OCA patients (20%) and (30%) respectively, which were statistical significant (p-value= 0.05 and 0.045) of association (Table- II).

For the non-normally distributed variables, non-parametric tests of correlation were applied. In patients group, a Spearman rho correlation was carried out but there was no correlation found among serum trace elements in OCA patients (Table- III).

Table I: The demographic and the laboratory data of normal distributed parameters in study groups.

Parameters	OCA cases (n = 40)		Healthy controls (n = 40)		p-value
	Mean±S.D	Median(Q1-Q3)	Mean±S.D	Median(Q1-Q3)	
Age (years)	17.8±14.98	14.0(9.0-24.75)	18.08±14.54	14(9.0-24.0)	0.800†
Zinc (Zn) µg/dL	83.9±33.0	81.0(59.2-108.7)	119.5±84.0	96.0(61.7-149.2)	0.040*
Copper (Cu) µg/dL	90.3±39.6	84(57.5-122)	126.3±80.82	99.52(75.7-152.7)	0.030*
Iron (Fe) µg/dL	99.45±59.0	85.5(57.0-123.7)	117.1±88.58	96(75.0-130.0)	0.345†
Corrected Visual Acuity	R	0.77±0.35	0.70(0.60-0.97)	-----	-----
	L	0.77±0.35	0.70(0.60-0.97)	-----	-----

p-value ≤0.05 is considered significant, † p-value >0.05 is considered non-significant IQR; interquartile range

Table II: The association of clinical phenotype in patients with OCA.

Characteristics	Categories	Male (25)	Female (15)	Statistics	
				x ²	p-value
Hair color	Golden	8 (20 %)	5 (12.5 %)	3.5	0.32
	Blonde	3 (7.5 %)	1(2.5 %)		
	White	14(35%)	9 (22.5 %)		
Skin colour	Red	2 (5 %)	0	3.9	0.05*
	Reddish White	18 (45 %)	8 (20 %)		
	White	5 (12.5 %)	7 (17.5 %)		
Skin rashes	No rashes	5 (12.5 %)	3 (7.5 %)	6.1	0.045*
	Mild rashes on sunlight exposure	16 (40 %)	7 (17.5 %)		
	Severe on sunlight exposure	4 (10%)	5 (12.5 %)		
Iris colour	Brown	3 (7.5 %)	2 (5 %)	0.015	0.99
	Grey	22 (55 %)	13 (32.5%)		

*p-value ≤0.05 is considered significant

Table III. Correlation of trace elements in OCulocutaneousu albinism (OCA) patients

Parameters		Age	Iron	Cu	Zn	
	Age	r	1			
		p				
Spearman's rho	Iron Levels	r	-0.213	1		
		p	0.186			
	Cu levels	r	-0.059	-0.054	1	
		p	0.719	0.742		
	Zn levels	r	-0.064	-0.011	-0.09	1
		p	0.695	0.947	0.564	

** Correlation is significant at the 0.001 levels (2 tailed), *Correlation is significant with p-value at 0.05 (2 tailed)

DISCUSSION

Oculocutaneous albinism (OCA) is a heterogeneous genetic disorder of abnormal melanin synthesis, resulting in absent or decreased pigmentation of eyes, skin and hair. In melanin synthesis pathways, the role of the essential trace elements has been described. The functions of trace elements have been established between the endogenous retinal trace element homeostasis and retinal disease, from inherited defects in their metabolic pathways to the cellular and molecular mechanisms specific to metal-induced retinal toxicity⁶.

In the present study, total 80 individuals comprising of 40 patients with OCA and a 40 healthy controls were studied for the serum Zn, Cu and Fe. The levels of serum Zn and Cu were found low in cases as compared to control with statistically significant difference (p 0.003 and p 0.004).

On the other hand, there was no statistical difference observed in serum Fe levels in patients and control groups (p >0.05). The association of the disease with skin color and skin rashes was significant (p=0.00), while other OCA clinical features were not associated in the OCA patients. No correlation of serum trace elements with the OCA disease was observed. The present study is the first study which was conducted on a large sample size with the rare disorder (1:20000), and correlation studies were performed between different disease phenotypes and trace elements¹⁵.

In a previous study, the trace element levels were determined in urine and blood of albino patients of black origin. In those patients, the levels of Cu were found higher both in urine and blood samples. Also, the concentration of Cu specific protein; ceruloplasmin was found significantly higher in albinos as compared to controls. According to this study, it was proposed that the defect in Cu metabolism might result in the albinism pathogenicity¹¹. In another study of Caucasian population, the concentrations of Zn, Cu and ceruloplasmin were determined in serum and urine samples. The levels of trace elements were statistically not associated between patients and controls. However, seven albinism patients showed higher levels of serum Zn and ceruloplasmin, although no definitive correlation regarding the positivity or negativity of tyrosinase was established. These findings might support their previous observations regarding albinism as a possible primary abnormality of zinc and copper metabolism¹².

The results of the present study are different to the previous studies. The serum concentrations of Zn and Cu found low in OCA patients than the normal controls (p 0.004). For serum Fe levels, no significant difference was

found between controls and patients. Overall, no correlation was found between the trace elements levels and OCA clinical characteristics (skin color, skin rashes and eye color).

In previous studies, the low levels of serum Zn have been reported in ocular features like poor dark adaptation^{16,17}. The evidence of Zn and Cu level studies has been demonstrated in some previous reports. Erie et al. reported the low levels of Zn and Cu in different eye structures like RPE, choroid complex and neuro-retina of age-related macular degradation¹⁸. The results of the previous study support our results as Cu and Zn levels are reduced in oculocutaneous albinism (OCA) which is also associated with abnormalities in retinal pigment epithelium (RPE).

Overall, the various studies have been conducted to establish the role of trace elements in various biological pathways and also in disease pathology. This study presents the addition of data in the scientific community, and this is the first ever report from Pakistan about the studies of trace elements in albinism patients.

CONCLUSION

In conclusion, levels of serum trace elements like Zn and Cu are significantly reduced in OCA patients as compared to healthy controls while the levels of serum Fe were not altered. No correlation was observed between the trace elements and phenotypic features of OCA patients.

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