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Original Article

Impact of Vitamin D and Trace Elements on Melanin Synthesis Pathway in Vitiligo Patients

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ABSTRACT

Melanin is a polymeric pigment found in many organisms, synthesized by specialized pigment-producing cells called melanocyte. melanogenesis is the pathway of melanin production which includes tyrosine oxidation and many polymerization reactions. Melanosme; is a special organelle in which tyrosine is oxidized by tyrosinase enzyme to eumelanin. Vitiligo; is an autoimmune disease characterized by melanin deficiency in certain skin areas with unknown cause. The study aims to explore the relationship between vitamin D and some trace elements with melanin deficiency, which may aid in predicting the cause of melanin deficiency in vitiligo disease. The study was performed in Al-Ramadi Teaching Hospital on 42 vitiligo patients and 42 healthy individuals (control group). Serum vitamin D was estimated for both patients and healthy individuals group by using enzyme-linked immunosorbent assay (ELISA) technique, the trace elements levels (iron, copper, zinc) were determined by atomic absorption device. The results of the current study showed a significant decrease in serum vitamin D, iron, copper and zinc for patients compared with healthy individuals group in statistical threshold of ($p<0.0001$). The studied parameters showed the decreasing of the area under the receiver operating characteristic (AUROC) curve: vitamin D (0.9974), iron (0.9060), copper (0.8118), and zinc (0.8250), new cut-off values were determined of vitamin D (< 21.96), iron (< 11.21), copper (< 11.96) and zinc (< 12.55). We; concluded from the results of the current study that melanin pigment synthesis pathway can be affected by the decreased levels of vitamin D, iron, copper, and zinc. These parameters, especially vitamin D, are related to melanogenesis since vitamin D decreases various cytokines expression responsible for vitiligo.

Keywords: Vitamin D, Iron, Copper, Vitiligo, Melanin.

1 Introduction

Vitiligo is an autoimmune disease characterized by the loss of skin melanin due to an unknown cause [1]. Many factors, including immune system disturbances, stress, and genetic predisposition, are believed to contribute to the pathogenesis of vitiligo, but its exact cause remains unknown [2]. The depigmented patches of skin may appear in all skin types and any place of skin in different sizes [3]. Melanin; is a biopolymer responsible for pigmentation in various organisms, including humans [4]. Melanocytes; are a group of cells responsible for melanin production via many biochemical reactions starting with oxidation of tyrosine and followed by polymerization process collectively called melanogenesis [5]. Eumelanin; is formed from the oxidation of tyrosine by tyrosinase enzyme in special organelles called melanosomes [6]. The first step in melanin synthesis is catalyzed by tyrosinase, which converts tyrosine into L-DOPA and subsequently into dopaquinone. Dopaquinone; can either be combine with cysteine to form pheomelanin or undergo further oxidation and polymerization to produce eumelanin, therefore, the synthesis of melanin depends on the availability of tyrosine, which is derived from phenylalanine, and cysteine in the presence of tyrosinase enzyme [7]. The determinant of skin color in human is the melanin pigment which is not only found in skin, but is also found in hair, inner ear and iris of the eyes [8]. Despite; both eumelanin and pheomelanin are existent in human hair and skin, the most abundant type is eumelanin [9].

Although of many Iraqi studies that have studied the relationship between various diseases and inflammatory variables [10,11], this study is the first to examine the relationship between serum levels of vitamin D and trace elements that are mainly involved in the synthesis of melanin pigment.

Vitamin D is a fat soluble vitamin that controls the absorption of calcium [12]. The most effective form of vitamin D in human is

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D3[13]. The vitamin of diet source and even the synthesized is inactive unless be activated by two hydroxylation reactions in both liver and kidneys [14].

Iron, copper and zinc are essential trace elements needed in specific quantities for human body to perform the metabolic activities [15]. Iron is an essential trace element for various physiological processes, including oxygen transport and enzymatic functions. Many enzymes, such as catalase, require iron as a cofactor [16]. Iron is absorbed from the duodenum, and its storage is precisely regulated by transferring [17]. Copper; is essential for aerobic respiration in all eukaryotes and is found in mitochondrial cytochrome oxidase responsible for oxidative phosphorylation[18]. Copper; facilitates iron absorption; therefore, the lack of copper may cause anemia [19]. Zinc; is important for activity of many enzymes and is stored and transferred in metallothioneins, zinc is the only trace element that appears in each enzyme [20].

This study aims to find the effect of vitamin D and trace elements concentrations on the melanin biosynthesis pathway. Vitamin D; decreases various cytokines expression responsible for vitiligo occurrence, in addition to; the measured trace elements have a diverse biological role.

2 Materials and methods

2.1 Study participants

This study was conducted at Al-Ramadi Teaching Hospital, where vitiligo patients were diagnosed by a dermatologist. A total of 84 participants were included, comprising 42 vitiligo patients (mean age: 29.24 years) and 42 healthy individuals (mean age: 29.49 years). Venous blood samples were collected from both vitiligo patients and healthy controls. The serum was separated and stored at -80°C until biochemical analysis. Patient consent was obtained to participate in the study. The ethical approval for the study was obtained from the ethical approval committee at the University of Anbar (Ref189).

2.2 Blood Collection

Venous blood samples (5 mL) were collected from healthy individuals and vitiligo patients using sterile disposable syringes. Blood samples were collected in gel tubes and left undisturbed for 15 minutes to allow clot formation. The samples were centrifuged at 1500 × g for 10 minutes to separate the serum. The obtained serum was aliquoted into three tubes and stored at -80°C for biochemical analysis. Vitamin D levels were measured using the enzyme-linked immunosorbent assay (ELISA) technique (competitive), while trace element levels (iron, copper, and zinc) were determined using atomic absorption spectrophotometry.

2.3 Statistical Analysis

The statistical analysis of current study data has been done by (GraphPad Prism version 8.02 and SPSS version 26). The results were illustrated as mean and standard deviation. Unpaired t-test was used to analyze the study data statistically, receiver operating characteristic (ROC) curve analysis was used to estimate AUC for each variable, cut off values, sensitivity and specificity were calculated. Differences in a set of data less than 0.05 were considered statistically significant.

3 Results

The general parameters including the age (29.49 vs 29.14 year), accommodation and family history did not show any significant differences between patients and control group. The results showed a significant decrease in each of vitamin D (14.78 vs 28.61 ng/ml), iron (8.945 vs 13.83 µg/mL), copper (10.39 vs 15.24 µg/mL), and zinc (10.83 vs 14.68 µg/mL), (P<0.0001) for vitiligous patients compared with control group as shown in Table 1.

Table 1: Data of measured parameters for melanin deficiency patients and healthy control group.

Biomarkers	Group	No	Mean	SD	p-value
Age (years)	Vitiligo patients	42	29.14	8.875	0.8503
	Controls	42	29.49	6.987	
Vitamin D (ng/ml)	Vitiligo patients	42	14.78	3.701	<0.0001
	Controls	42	28.61	3.519	
Iron (µg/mL)	Vitiligo patients	42	8.945	2.555	<0.0001
	Controls	42	13.83	2.618	
Zinc (µg/mL)	Vitiligo patients	42	10.83	2.982	<0.0001
	Controls	42	14.68	3.122	
Copper (µg/mL)	Vitiligo patients	42	10.39	0.559	0.0871
	Controls	42	15.24	4.765	

Table 2 refers to a comparison of the areas under the curve (AUC) for the measured parameters, the preferred biomarkers which were related to melanin deficiency compared to a healthy individuals were vitamin D [AUC=0.9974; P< 0.0001; Sensitivity=97.62%; Specificity=97.3%] Figure 1 D, iron [AUC=0.9060; P< 0.0001; Sensitivity=80.95%; Specificity=81.08%] Figure 1 B, zinc [AUC=0.8250; P< 0.0001; Sensitivity=78.57%; Specificity=78.38%] Figure 1 C and copper [AUC=0.8118; P< 0.0001; Sensitivity=74.42%; Specificity=75.68%] Figure 1 A respectively.

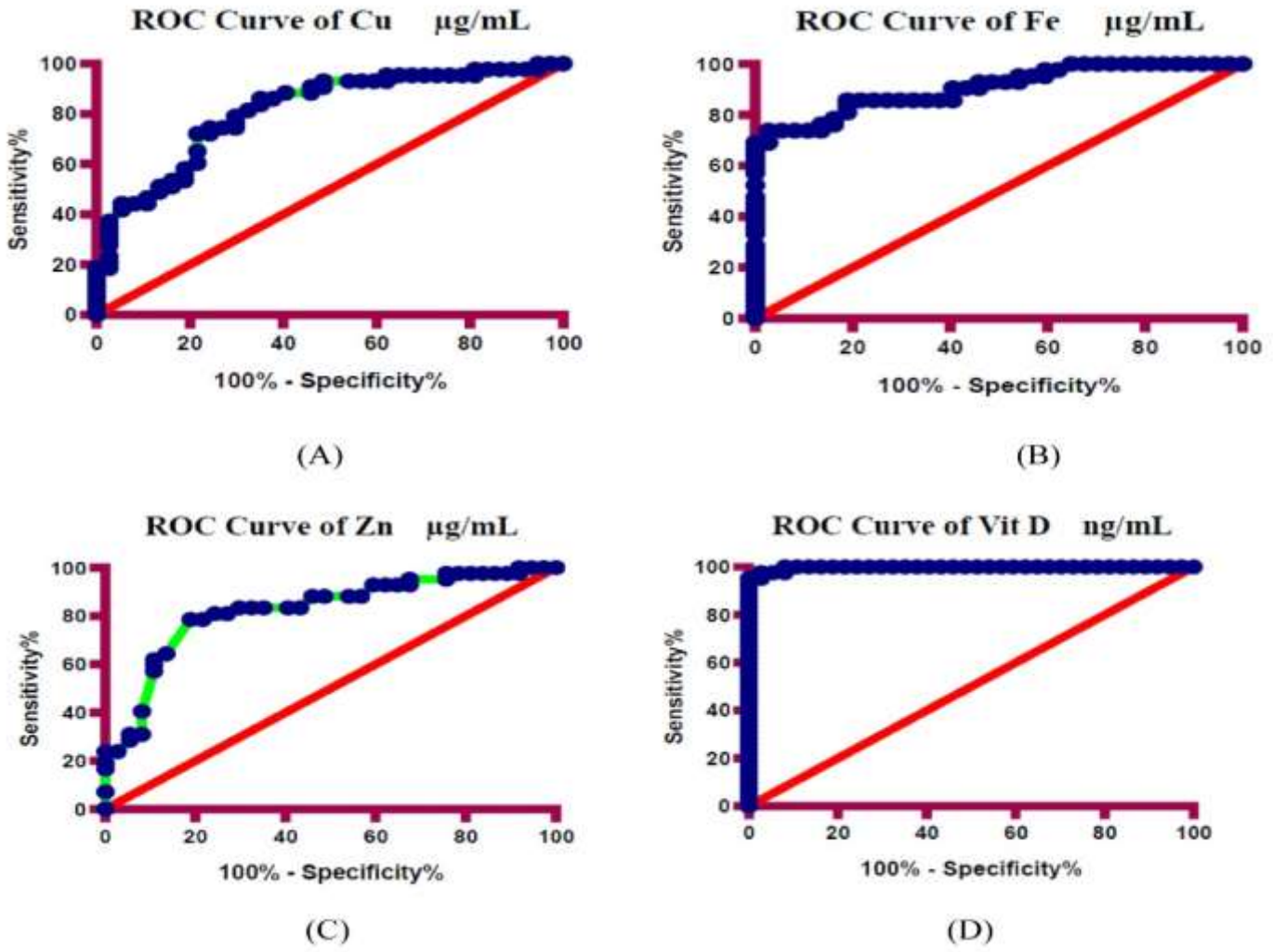

Figure 1: Area under curves for ROC for measured parameters.

Biomarkers	AUC	Positive if COV	Sensitivity %	Specificity %
Vitamin D (ng/ml)	0.9974	< 21.96	97.62	97.3
Iron (µg/mL)	0.9060	< 11.21	80.95	81.08
Zinc (µg/mL)	0.8250	< 12.55	78.57	78.38
Copper (µg/mL)	0.8118	< 11.96	74.42	75.68

Table 2: standard diagnostic for measured parameters in melanin deficiency of ROC analysis.

Table 2 and Figure 1 show a significant decrease in vitamin D, iron, copper and zinc in vitiligous patients compared to healthy individuals. Although; all four measured parameters were decreased significantly, the decrease in the concentration of vitamin D was more than the others with bigger AUC and more sensitivity and specificity making this vitamin the most relative biomarker to melanin deficiency compared to other measured parameters.

4 Discussion

Vitamin D is an essential hormone synthesized in the skin through photochemical reactions triggered by ultraviolet B (UVB) radiation from sunlight. Several factors can limit vitamin

D synthesis, including aging, increased skin pigmentation, and sunscreen use. Skin pigmentation is a risk factor for vitamin D deficiency because melanin filters ultraviolet radiation [21]. 1,25-dihydroxyvitamin D3 is the active form which controls calcium absorption, metabolism of bone, cell proliferation and immune regulatory activities [22].

Vitamin D deficiency is related to many diseases including Pemphigus Vulgaris, cardiovascular diseases especially myocardial infarction and bone diseases [23]. Vitamin D insufficiency is also associated with autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, diabetes mellitus, and multiple sclerosis [24]. In vitro studies, B16 melanoma cells showed an increase in the activity of tyrosinase enzyme and melanogenesis after treatment with vitamin D3 [25]. Other study states that vitamin D and ultraviolet radiation B promote melanocytes proliferation leading to that vitiligo can be treated with vitamin D and UVB. On the contrary of stimulatory activity, vitamin D has an inhibition effect to the growth of melanocytes [26]. Another study showed that vitamin D inhibited melanocyte proliferation and didn't affect the process of melanization [27].

Several studies reported that using of vitamin D without or with ultraviolet light enhances pigmentation in vitiligo [28]. Recent study showed that 68.9% of vitiligous patients were vitamin D deficient from which 55.6% were vitamin D insufficient and 13.3% were with very low vitamin D concentration [29]. Recent case report also found a low level (12 ng/mL) in vitiligous patients [30].

It is reported that calcipotriol activates melanocytes to increase melanin production [31]. The role of vitamin D activity in melanocytes is not fully understood, but vitamin D is involved melanocytes physiology by controlling cytokines of melanogenesis, in addition to the proposed mechanism of vitamin D as an antioxidant protects the skin from the oxygen reactive species that are formed in excess amounts in the epidermis of vitilgous skin [32]. Vitamin D; also plays a role in the formation of sphingosine-1-phosphate, a molecule that inhibits apoptosis. As a result, vitamin D may help protect melanocytes from apoptosis, thereby preventing skin pigment loss [33].

In addition to the physical role of iron on melanin, iron may alter the signaling pathway contributed to the biosynthesis and degradation of melanosome [34]. Other studies also suggest that iron level is related to melanogenesis, in hemochromatosis disease, when many organs are iron overload, skin darkening is a result of increased iron levels [35]. Because; superoxide dismutase antioxidant enzyme is zinc dependant, zinc is an antioxidant playing a vital function against the attack of free radicals, zinc is also responsible for the rearrangement of dopachrome to dihydroxy indol carboxylic acid involved in melanogenesis [36]. Zinc data of our study agrees with another study which showed interesting correlations between decreased zinc levels and vitiligo [37]. Oxidation of DOPA in melanin synthesis pathway is strongly stimulated by copper ions compared by other metals, this fact reflects the importance of copper in melanogenesis and explain the effect of copper deficiency in vitiligous patients.

Many limitations opposed our study and determined the number of samples, including the consent of patients and lack of dermatological centers, so the sampling was limited in Al Anbar teaching hospital, in addition to not all patients were agreed to participate in this study.

Conclusions

The statistical analysis of the measured parameters in this study indicates that decreased levels of vitamin D, iron, zinc, and copper are associated with melanin deficiency in vitiligo patients. Among these, vitamin D showed the most significant reduction, as it plays a key role in downregulating cytokines involved in vitiligo pathogenesis.

Conflict of interests

None

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Author Contributions

Conceptualization and project administration: MMA &SFT. Sample collection and analysis; BTR. Writing: BTR. Validation: all authors.

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