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Original Article

Exploring the Biochemical Markers for Vitiligous Patients: Tyrosinase and Amino Acids

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ABSTRACT

Melanin is an organic biomolecule arranged in polymers that form the pigment of various organisms, melanocytes are group of cells responsible for the production of melanin through several biochemical reactions including the oxidation of tyrosine followed by polymerization processes which are collectively called melanogenesis. Eumelanin is formed from the oxidation of tyrosine by tyrosinase enzyme in unique organelles called melanosomes. Vitiligo is an autoimmune disease characterized by melanin skin loss with unknown cause. This study aims to discover the relationship between biochemical parameters (tyrosinase enzyme, phenylalanine, cysteine and tyrosine) with melanin deficiency, which may be valuable in predicting the cause of pigment loss in vitiligous patients. The study was performed in Al-Ramadi Teaching Hospital on 42 vitiligous patients and 42 healthy individuals. Serum tyrosinase enzyme was estimated for both patients and healthy individual groups by enzyme-linked immunosorbent assay (ELISA) technique, amino acids levels were determined by amino acid auto analyzer device. Results of our study showed a significant decrease in serum tyrosinase enzyme, phenylalanine and tyrosine for patients compared with healthy group in a statistical threshold of ($p<0.0001$), in contrast, no significant difference was seen in cysteine levels showed between patients and healthy individuals. The studied parameters showed the decreasing of the area under the receiver operating characteristic (AUROC) curve: tyrosinase ($p<0.9516$), phenylalanine ($p <0.9125$) and tyrosine (0.8127), new cut-off values were determined of tyrosinase ($p <184.1$), phenylalanine ($p <367.5$) and tyrosine ($p <538.9$). We conclude from the results of the current study that melanin pigment synthesis pathway may be affected by the decreased levels of tyrosinase, phenylalanine, and tyrosine. These parameters are valuable in the diagnosis and/or treatment of vitiligo. Additionally; they may considered in further biochemical studies.

Keywords: Tyrosinase, Tyrosine, Cysteine, Vitiligo, Melanin.

1 Introduction

Vitiligo is an autoimmune disease characterized by melanin skin loss with unknown cause [1]. Many factors are believed to contribute to the pathogenesis of vitiligo including immune system disturbances, stress and genetic factors, but the real cause is still unknown [2]. The depigmented patches of skin may appear in all skin types and any place of skin in different sizes [3]. Melanin is an organic biomolecule arranged in polymers that form the pigment of various organisms [4], melanocytes are group of cells responsible for the production of melanin through several biochemical reactions including the oxidation of tyrosine followed by polymerization processes which are collectively called melanogenesis [5]. Eumelanin; is formed from the oxidation of tyrosine by tyrosinase enzyme in unique organelles called melanosomes [6]. The first step in melanin synthetic pathway is motivated by tyrosinase enzyme which converts tyrosine to DOPA then to dopaquinone; the latter combines with cysteine into manners forming pheomelanin and 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]. Melanin pigment is the determinant of human skin color which is not only found in human skin, but also the hair, inner ear and iris of eyes [8]. Despite; both eumelanin and pheomelanin existent in human hair and skin, the most abundant type is eumelanin [9]. Although there are a massive number of studies that have studied the relationship between various diseases and inflammation-related variables [10, 11], this study is the first to examine the relationship between serum levels of tyrosinase with amino acids that are mainly involved in the synthesis of melanin pigment.

Tyrosine is a conditional essential amino acid and one of the standard twenty amino acids, it contains polar side chain, and two codons are responsible for tyrosine in messenger RNA which is

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UAC and UAU [12]. Tyrosine in mammals is synthesized from phenylalanine by phenylalanine hydroxylase enzyme [13]. Phenylalanine is non-polar crucial amino acid obtained naturally from food, an accumulation in phenylalanine causes phenylketonuria due to a decrease in phenylalanine hydroxylase enzyme [14].

This study aims to find the effect of amino acids level, which are precursors in melanin formation, and the role of tyrosinase enzyme on the melanin biosynthesis pathway. Tyrosine, phenylalanine and cysteine are all included in pathway of melanin pigment biosynthesis, many steps in this pathway are controlled and catalyzed by tyrosinase enzyme.

2 Materials and methods

2.1 Study participants

Vitiligo patients were diagnosed by dermatologist in Al-Ramadi Teaching Hospital, the study included (84) individuals, vitiligo patients (n=42) in the mean age of (29.2) years and healthy people (n=42) in the mean age of (29.4) years. Patients consent was obtained to participate in the study. The ethical approval for the study was obtained from the ethical approval committee from the University of Anbar (Ref189).

2.2 Blood Collection

Venipuncture was used for blood samples collection by sterile disposable syringe of 5 mL capacity, after collection, blood was poured in two gel tube for fifteen minutes, after which it was separated by centrifuge at a speed of 1500 xg for ten minutes. The obtained serum was kept in two tubes; both tubes were stored at -80°C until it was used.

2.3 Statistical Analysis

The current data has been analysed 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 compare the study data, receiver operating characteristic (ROC) curve analysis was used to estimate AUC for each variable, cut off values, sensitivity and specificity were also calculated. Differences in set of data less than 0.05 were considered statistically significant.

3 Results

The population parameters including the age (29.49 vs 29.14 year), BMI (25.02 vs 25.89 kg/m^2), accommodation and family history showed no significant differences between patients and healthy individuals (control group). The results also showed no significant decrease in cysteine amino acid (1245 vs 1175 mg/L). The results showed significant decrease in each of tyrosinase enzyme (221.1 vs 123.2 pg/ml), phenylalanine (405.5 vs 330.3 mg/L) and tyrosine (570.9 vs 501.8 mg/L) amino acids (P<0.0001) for vitiligous patients compared with control group as shown in Table 1.

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

Biomarkers	Group	No	Mean	SD	p-value
Age (years)	Controls	42	29.49	6.987	0.8503
	vitiligous patients	42	29.14	8.875	
BMI (kg/m^2)	Controls	42	25.02	4.306	0.3911
	vitiligous patients	42	25.89	4.642	
Tyrosinase (pg/ml)	Controls	42	221.1	38.44	<0.0001
	vitiligous patients	42	123.2	40.33	
Phenylalanine (mg/L)	Controls	42	405.5	30.10	<0.0001
	vitiligous patients	42	330.3	44.59	
Cysteine (mg/L)	Controls	42	1245	55.71	0.0871
	vitiligous patients	42	1175	239.2	
Tyrosine (mg/L)	Controls	42	570.8	60.10	<0.0001
	vitiligous patients	42	501.8	59.15	

The areas under the curve (AUC) for the measured biomarkers are shown in Table 2. The preferred biomarkers which were related to melanin deficiency compared to healthy individuals were tyrosine [AUC=0.8127; P< 0.0001; Sensitivity=73.81%; Specificity=72.97%] (Figure 1 A), phenylalanine [AUC=0.9125; P< 0.0001; Sensitivity=83.33%; Specificity=83.78%] (Figure 1 B) and Tyrosinase enzyme [AUC=0.9516; P< 0.0001; Sensitivity=88.1%; Specificity=86.67%] (Figure 1 C).

Table 2: Standard diagnostic for measured biomarkers in melanin deficiency of ROC analysis.

Biomarkers	AUC	Positive if COV	Sensitivity %	Specificity %
Tyrosinase (Pg/ml)	0.9516	<184.1	88.10	86.67
Phenylalanine (mg/L)	0.9125	<367.5	83.33	83.78
Tyrosine (mg/L)	0.8127	<538.9	73.81	72.97

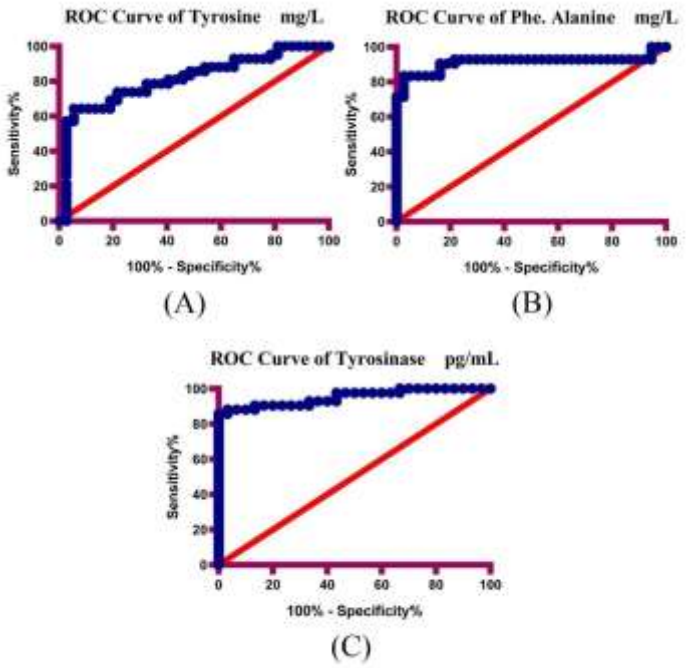

Figure 1: Receiver operating characteristic (ROC) curve analysis to estimate AUC for each variable.

The results showed significant decrease in each of tyrosinase enzyme, phenylalanine and tyrosine for vitiligous patients compared to healthy individuals, but no significant differences were observed in the level of cysteine between patients and healthy individuals. Despite three parameters were decreased significantly, but the decrease in the concentration of tyrosinase enzyme was more than the others with bigger AUC, sensitivity and specificity making this enzyme the most relative biomarker to melanin deficiency.

4 Discussion

The biosynthesis of both eumelanin and pheomelanin is catalyzed by tyrosinase enzyme in the first step of oxidizing tyrosine into DOPA and then to dopaquinone, dopaquinone will combine with cysteine by two different pathways forming pheomelanin, dopaquinone can be converted to leucodopachrome and then to eumelanin by two pathways [7]. Therefore, melanin synthesis requires tyrosine, which is yielded from phenylalanine, cysteine and the catalytic factor tyrosinase enzyme, any deficiency in the precursors or catalytic activity of tyrosinase can leads to melanin deficiency [15].

The availability of amino acid tyrosine and DOPA, which is the tyrosine hydroxylated form, is the starting point of melanin synthesis [16]. Tyrosinase is a membrane enzyme that is located only in melanosomes and is an indispensable factor in melanogenesis, this enzyme is created in the endoplasmic reticulum and packed in Golgi apparatus then transported to melanosome. The activity of tyrosinase enzyme is related to the degree of melanin synthesis [17]. The availability of tyrosine and the resulting DOPA controls the melanogenesis system balance [18]. In the treatment of hyperpigmentation of skin, azelic acid is used, which inhibits the creation of mitochondrial enzymes, which in turn will affect tyrosinase [19].

The dominant factors in melanogenesis is the activity of tyrosinase enzyme, which is involved in several biochemical reactions in melanin synthesis pathway, and the abundance of phenylalanine, tyrosine and cysteine. Any reduction in the concentration of enzyme, its catalytic activity or the concentration of one of the precursors results in similar decline in melanin pigment. Our study indicates a significant decrease in tyrosinase enzyme, phenylalanine and tyrosine, and this decease may be the direct cause of melanin deficiency and the appearance of depigmented spots in vitiligo patients.

Tyrosine deficiency may be due to phenylalanine deficiency, as the latter is converted to tyrosine by phenylalanine hydroxylase enzyme, therefore, any decrease in phenylalanine can cause a similar decrease in tyrosine. In addition to the significant decreases of amino acids, a significant decrease in tyrosinase enzyme is also detected in vitiligous patients and this deficiency may due to the decrease in amino acids.

Limited sample size is due to the low prevalence of the disease in the city. Inability to conduct sub-analyses based on sex, age, and/or economic status.

Conclusions

The data of the statistical analysis of the measured biomarkers in our study showed that the decrease in tyrosinase enzyme, phenylalanine and tyrosine amino acids is related in melanin deficiency in vitiligous patients. The most sensitive decrease was for tyrosinase and this can be illustrated by the major role of tyrosinase in the melanogenesis.

We recommend considering phenylalanine, cysteine and tyrosine as additional parameters in diagnosis of melanin deficiency.

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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