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Exploring the Correlation Between Tyrosinase Enzyme Levels and the Severity of Melasma in Women: A Case-Control Study in Baghdad

Seham A. Muhsen[1] *PhD*, **Estabraq A. Alwasiti**[1] *PhD,* **Iqbal G. Farhood**[2] *FICMS (D&V)*

[1]National Centre for Drug Control and Research, Ministry of Health, Baghdad-Iraq, [2]Dept. of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad-Iraq, [3]Dept. Medicine, College of Medicine, Al-Nahrain University, Baghdad-Iraq

Abstract

Background Melasma is a skin condition that disrupts the skin on the face. Tyrosinase enzyme (TYR) increase and decrease the antioxidants naturally produced in the body, leading to melasma. TYR is a key enzyme in melanogenesis. Tyrosinase's various mechanisms are enhanced by genetic, environmental, and endocrine factors.

Objective To investigate the relationship between serum TYR enzyme levels and the severity of melasma as a biomarker for diagnosis and treatment.

Methods A case-control study was executed during the term of March to October 2023. The study included 60 patients with melasma and 60 control participants, all female, ranging in age from 20 to 50 years. All participants were examined using Wood's light. Blood samples were collected from each individual, and sera were used after separation to measure TYR by enzyme-linked immunosorbent assay (ELISA) method levels in different stages of melasma severity.

Results Increased concentration TYR with different stages of severity (7.36±0.19 U/ml) compared to the control (3.89±0.08 U/ml) utilizing an independent t-test. Additionally, the serum TYR levels significantly increased as the severity of melasma increased. Furthermore, the one-way ANOVA test indicated substantial serum TYR level and melasma area and severity index (MASI) scores for mild, moderate, and severe were 6.19±0.1 U/mL, 7.69±0.07 U/mL, and 9.38±0.31 U/mL, respectively. These results strongly suggest that TYR plays a crucial role in the formation and progression of melasma.

Conclusion The result of this study refers to a significant correlation between the level of TYR and the degree of melasma.

Keywords Melasma, Tyrosinase enzyme, melanogenesis, MASI score, ELISA

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List of abbreviations: α-MSH = Alpha-melanocyte-stimulating hormone, BL = Blue light, DHICA = 5,6-Dihydroxyindole-2-carboxylic acid, ELISA = Enzyme-linked immunosorbent assay, L-DOPA = L-3,4-dihydroxy-phenylalanine, MASI = Melasma area and severity index, MITF = Microphthalmia-associated transcription factor, mMASI = Modified melasma area severity index, ROS = Reactive oxygen species, TRF = Tocotrienol-rich fraction, T4 = Thyroxine, T3 = Triiodothyronine, TSH = Thyroid-stimulating hormone, TYR = Tyrosinase enzyme, TRP-1= Tyrosine-related protein-1, TRP-2 = Tyrosine-related protein-2, UV = Ultra violate

Introduction

Tyrosinase (TYR) an oxidoreductase enzyme (E.C. 1.14.18.1), plays a central role in melanogenesis by catalyzing the

oxidation of L-DOPA and hydroxylation of L-tyrosine. Although melasma's precise cause remains unknown, multiple risk factors—including inflammation and oxidative stress—are known triggers [1,2]. The involvement of TYR in melanin formation has prompted research into its link with dermal melanocyte function [2]. Melasma can significantly affect mental well-being, with some patients experiencing suicidal ideation [3,4]. Thus, affordable diagnostics and therapies are vital for better disease understanding and management [5].

Effective treatment of melasma requires long-term topical therapies aimed at disrupting melanosome production, inhibiting melanocyte formation, and impeding their spread [6]. Despite the availability of TYR as a biomarker, its correlation with melanin hyperpigmentation remains poorly understood [7]. Topical treatments often yield temporary results, with recurrence common [8]. Studies have identified hormone imbalances and chronic diseases as contributors to elevated TYR activity, exacerbating melasma [9]. Tocotrienol-rich fraction (TRF) has shown promise by inhibiting TYR and slowing melanogenesis induced by blue light [10]. Determining sources of TYR inhibitors in pharmaceutical and cosmetic formulations continues to be a focus [11].

The TYR enzyme's relationship with hyperpigmentation extends beyond melasma to conditions such as dermatoses, age spots, and malignant melanoma. TYR inhibitors offer partial pigment suppression. Related proteins like Tyrosine-related protein-1 (TRP-1) and Tyrosine-related protein-2 (TRP-2) show modified activity depending on metal ion composition, and play roles in melanogenesis along with 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and microphthalmia-associated transcription factor (MITF) [12]. Melasma commonly affects individuals with Fitzpatrick skin types III–V, particularly Asian and African-origin females. Evidence on efficacy of chemical peels, lasers, and light therapies remains limited [13,14]. In Southeast Asia, prevalence rates may reach up to 40%, with patients often reporting low self-esteem and reduced social engagement [15]. TYR inhibitors remain the backbone of treatment [16], but more potent variants are needed for broader medical and cosmetic use [17].

Ultra violate A (UVA) radiation has been shown to elevate alpha-melanocyte-stimulating hormone (α-MSH) levels, aggravating melasma and pointing to the need for accurate diagnosis [18,19]. A TYR-dependent pathway is key to reducing melanin production [11]. Melanin safeguards skin from UV damage and oxidative stress, with TYR, TRP-1, TRP-2, and MITF essential to the melanogenesis cascade [20]. Hormonal triggers, such as pregnancy, contraceptive use, and menopause, elevate progesterone, estrogen, and MSH, boosting TYR production and pigmentation [21,22]. Unchecked melanin synthesis can lead to cosmetic concerns like freckles and dermatitis [23] and adversely impact life quality [24]. Metabolomics can deepen understanding of melasma's pathophysiology [25], which is influenced by genetics, hormones, and UV exposure [17].

This study targets TYR's association with melasma severity, aiming to validate a fast, cost-effective ELISA-based diagnostic method. It promises to improve both clinical accuracy and patient quality of life.

Methods

This case-control study was conducted from March to November 2023. The study included 60 patients with melasma and 60 healthy control subjects who were unrelated and had similar ages and sexes. The study included with 120 females aged between 20 and 50 years. They are all examined using Wood's light examination, which matches the control. Blood samples were collected from each individual. The current study's methodology involved collecting 5 ml of peripheral blood samples from venous blood from all participants, storing them in clean, sterile tubes, and using a gel tube to separate sera through centrifugation. Blood samples were left for 20

min at room temperature. Centrifugation at 3000 rpm separated the serums for 10 minutes after coagulation. Following aspiration, the sera were split into tiny aliquots and kept at -20°C until they were analyzed using ELISA technique. The control individuals with no history of any melasma or other chronic disease and who had not been on regular medications for at least 2 years from the date of sampling were recruited from the same geographic area as those patients. The practical part was conducted at research laboratories at the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University. A dermatologist determined their diagnosis at least 6 months ago based on their history, examination, and response. All patients included during the melasma attack didn't receive drugs, supplements, or contraceptive pills; were pregnant; smoked; had chronic disease for the last two weeks prior to the study; and were subjected to a complete history, such as age, sex, menstrual cycle, and current antibiotic medication. The study excluded patients who received supplements of vitamin B12, vitamin C, vitamin H (biotin), or iron; those who took glutathione; pregnant women; those taking hormonal contraceptives; those with abnormal thyroid-stimulating hormone (TSH), triiodothyronine (T3), or thyroxine (T4) levels; those with a chronic disease; those smoking; and those taking antibiotics. The severity of melasma was measured using melasma area and severity index (MASI) score which is computed by multiplying the numerical value of the affected regions (A) by the total of the severity grades for homogeneity (H) and darkness (D), as well as by the percentages of the four face areas (10-30%). Overall MASI rating: Head 0.3 (D+H) A + left malar 0.3 (D+H) A + chin 0.1 (D+H) A + right malar 0.3 (D+H) A. Nowadays, we employ a modified version where inhomogeneity is not considered [(26)].

Statistical analysis

The statistical package for social sciences software version 23 (SPSS Inc., Chicago, IL, U.S.A.) has been utilized to analyze the data. A P <0.05 was accepted as the level of significance. Each result was calculated as the mean±SD. An independent T-test was used to determine differences in means between patients and controls, while analysis of variance (one-way ANOVA) used in measurement the melasma severity.

Results

When comparing the patient group to the control group, an independent t-test revealed that the patient group had a significantly higher mean±SD TYR level than control group (7.36±0.19 vs 3.89±0.08) respectively (P <0.001) as shown in table (1).

Table 1. Comparison of the level of serum Tyrosinase enzyme

Groups	TYROSINASE U/ ml
Control	3.89±0.08
Patients	7.36±0.19
P value	<0.001

The level of serum TYR in women with melasma was measured according to the severity of disease; the mean±SD of the TYR level in serum was (6.19±0.1) in mild, in moderate (7.69±0.07), and in severe (9.38± 0.31). The difference among these levels was significant (P <0.001) (Table 2).

Table 2. Comparison of TYR levels according to variables in melasma by one-way ANOVA test

variables	Category	TYROSINASE U/ml (Mean ± SD)
MASI score	Mild	6.19±0.1
	Moderate	7.69±0.07
	Severe	9.38±0.31
	P value	<0.001

A comparison was made between the levels of TYR and the levels of MASI score in the sera of women who had melasma in order to study the possible links that exist between the two. The patients with melasma and hyperpigmentation had the highest serum levels of TYR; the results showed a significant positive correlation between TYR and MASI score (P <0.01) as shown in table 3.

Table 3. Correlation of serum Tyrosinase U/ ml with MASI score

Parameters	TYROSINASE U/ ml	MASI score
Tyrosinase U/ ml	1	
MASI score	0.878*	1

*Significant correlation at P <0.01

The receiver operator characteristic (ROC) curve shows a significant discriminated ability of increased serum TYR concertation (Figure 3). Table 4 shows that as the cut-off value of serum TYR concentration of 4.99 U/ml with sensitivity and specificity were 100% is the border line between melasma patients and control.

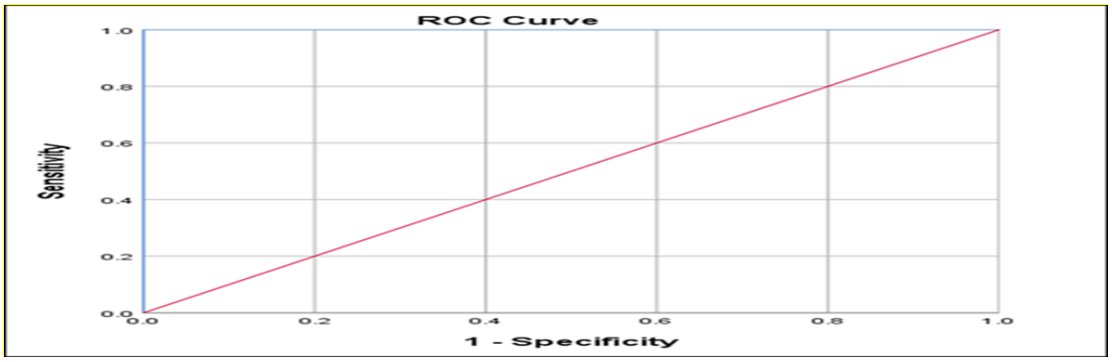

Figure 3. Patients' ROC curve of tyrosinase concentration in sera melasma compared with the control

Table 4. Sensitivity and specificity of Tyrosinase concentration

Parameters	No.	A.U.C.	Best cut-off value	Sensitivity %	Specificity %
Tyrosinase	60	100%	4.99	100	100

Discussion

Amongst the general enzyme classification, the TYR enzyme is an oxidoreductase that participates in the oxidation and reduction process in the epidermis. The melanogenesis process primarily relies on the chemical reactions that the TYR enzyme facilitates. This process of melanogenesis is associated with the synthesis of melanin, an indolic heteropolymer that gives skin its various characteristics and aids in UV protection. On the other hand, TYR-induced hyperpigmentation can result in several skin conditions associated with excessive synthesis of pigment [26]. UV radiation commonly induces melanogenesis in photoreceptor cells, while keratinocytes reside in the outer layer of the epidermis. The binding of α-MSH to its receptor, melanocortin 1 receptor (MC1-R), can increase melanogenesis by encouraging the production of more eumelanin than pheomelanin and raising TYR activity. Additionally, α-MSH promotes melanocyte growth [24]. Melanocytes are specialized dendritic cells that transport melanin to keratinocytes through organelles called melanosomes, which resemble subcellular lysosomes. Genetic, environmental, and endocrine factors are among the many processes and variables that regulate melanogenesis [27].

Increases in MSH, estrogen, and progesterone may contribute to the development of hyperpigmentation during pregnancy. This, in turn, may lead to an increase in TYR and dopachrome tautomerase transcription [15]. Hyperpigmentation (spotted) in face is called melasma. 90% of cases are female, and dark people are more likely to have it [9]. UV light appears to be significant contributors to its pathogenesis. There was a significant, strong correlation between sera TYR levels and the result control. This can be interpreted as increased TYR, which causes more severe melasma. These results confirmed that oxidative stress may be involved in the pathogenesis of hyperpigmentation melasma [28]. In the treatment of melasma, antioxidants inhibit free radicals, increase enzyme concentrations, and increase severity at different stages: mild, moderate, and severe. This could be interpreted as an increase in TYR leading to a more severe stage of the disease. These results confirmed that oxidative stress may be involved in the pathogenesis of melasma. The significance of difference of TYR according to severity of disease found in this study agrees with the study [24]. Researchers have used antioxidants to treat melasma because they inhibit free radicals, boost enzyme concentration, and intensify the condition at various stages, including mild and moderate, according to the study [29]. Investigate the possible relationships between them. Serum levels of TYR were found to be highest in patients with melasma and hyperpigmentation, which have environmental and genetic causes. These levels also increased in patients with chronic diseases, and there was a deficiency in supplements such as vitamin C, B12, vitamin H (biotin), and glutathione, which inhibit the enzyme TYR The level of TYR was compared to the MASI score in sera of women with melasma. These results confirmed that oxidative stress may be involved in the pathogenesis. We have applied antioxidants in the treatment of melasma to inhibit free radicals [30], increased concertation of the enzyme, and increased severity in different stages, mild, moderate, and severe, according to the study [31]. There is an increase in melanin, melanocytes, and melanosomes, along with a higher production of TYR Most of its pathophysiology is still unclear [32].

Practical Implications

It is critical to have a brief diagnostic window in order to determine the severity of the MASI score (mild, moderate, or severe). The laboratory uses the ELISA approach, which is low-cost, quick, straightforward, and accurate for detection and diagnosis. The study's findings should aid in diagnosing diseases under treatment, selecting appropriate therapies, detecting severity, and publicizing diagnosis states. The study's recommendations are put into practice to help patients who are struggling financially by promoting affordable choices for diagnosis and treatment. Early diagnosis and accurate assessment of illness severity reduce patients' financial burden, resulting in faster and less expensive remedies. Secondly, by using this information, dermatologists may allay public worries about treating elevated TYR. levels, therefore boosting patient trust and encouraging the use of effective TYR. marker control strategies. Improving patient compliance and trust is essential to successful therapy and better health outcomes. Armed with this knowledge, dermatologists can confidently determine the most effective methods of controlling the enzyme TYR for treating melasma. It enhances the public's understanding of the benefits of sustainable healthcare practices, emphasizing stress mitigation and overall health.

In conclusion, in women with melasma, there is a strong positive association between plasma TYR concentration and the MASI score. This work has successfully produced a thorough prediction that the body enzymatically synthesizes TYR and participates in the melanogenesis process. The study demonstrates a significant correlation between the patient's TYR levels and MASI score. Additionally, TYR in serum is used in this study, which is significant since it can help dermatologists identify patients more quickly by giving a visual depiction of their health.

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Author contribution

Dr. Muhsen and Dr. Alwasiti: Study design, data collection, measurement of TYR by ELISA, and writing the article. Dr. Farhood: Dermatologists; selected the patients who had melasma.

Conflict of interest

The author declares no conflict of interest.

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Correspondence to Dr. Seham A. Muhsen
E-mail: sehammuhsen12@gmail.com
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