Evaluation of Melasma Severity through Serum 8-Hydroxy-2′-Deoxyguanosine Biomarker and MC1R Gene Polymorphisms (rs1805006, rs772551710): A Case–Control Study in Adult Women: A Case-Control Study of Women in Baghdad

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Article Info

ISSN (online): 2582-8940
Volume: 06
Issue: 03
July - September 2025
Received: 21-05-2025
Accepted: 22-06-2025
Published: 17-07-2025
Page No: 260-266

Abstract
Melasma is a chronic hyperpigmentation condition caused by ultraviolet (UV) exposure, light skin pigmentation, and variations in the melanocortin 1 receptor (MC1R) gene. Clinically, it manifests in mandibular, malar, and centrofacial patterns. The study aims to clarify whether elevated levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a biomarker correlate with increased melasma severity and if gene polymorphism (rs1805006 and rs772551710) variations in the MC1R gene influence this relationship. In a case-control study, 120 women from 20 - 50 years 60 took part, melasma and 60 of who had been age-matched healthy controls. Clinical diagnosis was established using Wood's light examination. Tetra-ARMS PCR was used to determine the MC1R genotypes, and ELISA had been utilized to measure levels of 8-OHdG of the serum. Of statistical analyses were one-way ANOVA, chi-square tests, independent t-tests, odds ratios (OR), and confidence intervals (CI). The Results rs772551710 II genotype (OR=3.28, p=0.002) and rs1805006 CA genotype (OR=4.67, p=0.0004) were significantly associated with melasma. Serum 8-OHdG conc. was markedly increased in patients (3.80 ± 0.22 ng/mL) compared with controls (0.603 ± 0.03 ng/mL, p<0.0001) and showed a strong positive correlation with Melasma Area and Severity Index (MASI) scores (r=0.929, p<0.0001). The optimal diagnostic cut-off for 8-OHdG was 1.59 ng/mL (96.7% sensitivity, 100% specificity; AUC=99.9%). Conclusions Both MC1R polymorphisms (rs1805006 and rs772551710) and elevated serum 8-OHdG are strongly associated with melasma severity. The 8-OHdG biomarker demonstrates high diagnostic accuracy and may be integrated into clinical assessment protocols.

DOI: https://doi.org/10.54660/IJMBHR.2025.6.3.260-266

Keywords: Melasma, MC1R Gene Polymorphisms rs1805006, rs772551710, 8-OHdG, Electrophoresis

Introduction

In the effort to understand and address the complex issues surrounding melasma, a recent study has shed light on intriguing investigations that have indicated that ultraviolet A (UVA) up-modulates the long-lasting synthesis of alpha-melanocyte stimulating hormone (α-MSH), leading to enhanced melanocyte pigmentation and senescence expression markers (Mpofana *et al*., 2023; Sevilla & Grichnik, 2024) [15, 24]. When it comes to fighting melasma, the potential to inhibit tyrosinase and the various functions it plays in controlling melanin formation. prompting us to leverage this information for more effective dermatology. The quest for understanding the intricate interaction between higher 8-OHDG levels (X. Liu *et al*., 2019; Suhim *et al*., 2023) [14, 26], mutations of SNPs rs1805006 and rs772551710 in the Melanocortin 1 Receptor gene (MC1R) genetic polymorphism and their role in melasma hold the potential key to therapy.

Oxidative stress and inflammation perform important functions in pigmentation regulation with the MC1R incomplete (W. Liu *et al*., 2023) [13]. Numerous illnesses have been linked to oxidatively produced DNA damage and 8-OHDG; more thorough investigations are required in order to treat this resistant illness. Furthermore, it then upregulates melanin-stimulating receptors (MSR) such as MC1R (Khanna *et al*., 2023) [11]. Previous scholars emphasize that melasma is becoming recognized as a common challenge, particularly among those with darker skin tones, like Asians. However, doctors still face considerable difficulties in treating and diagnosing it. In addition, they have suggested that the unresolved matter of uncertainty and ambiguity gene (Hussein *et al*., 2019) [9]. The current study focuses on the biomarker 8-OHDG levels and the genetic polymorphism of MC1R; notably, SNPs rs1805006 and rs772551710 were targeted to decipher the complicated pathways that lead to melasma (Alam *et al*., 2023) [1]. Understanding the significance of hormone fluctuations, especially during pregnancy or contraceptive (Smith, 2021; Wu *et al*., 2021) [25, 30]. Polymorphisms in the MC1R gene are a major factor in melanin production control. Molecular inquiry into MC1R target gene single nucleotide polymorphisms is vital for preventing melasma development (Petralia *et al*., 2021a) [19]. Challenges and problem statements that activate the reactive oxygen species of oxidative stress, which break down DNA structures and accelerate apoptosis (Hamashareef *et al*., 2020) [8]. In addition to its antioxidant properties, melanin shields the skin from solar ultraviolet radiation (UVR). Skin lightness was linked with indicators of oxidative-generated (8-OHDG) DNA damage and RNA damage (Omari Shekaftik & Nasirzadeh, 2021) [17].

The pathophysiology of this disorder is not yet fully understood despite the identification of various components such as family history, exposure, and molecular pathways (Mpofana *et al*., 2023) [15]. Melasma is difficult to diagnose because of its poorly understood pathophysiology and lack of uniform therapy. Researchers have been studying the connection between oxidative stress and melasma for decades (Albandar & Mbalaha, 2024) [2]. However, melasma is difficult to treat because of its complex etiology. Although topical therapies can help the disease momentarily (Neagu *et al*., 2022) [16]. Therefore, in this study, the leading causes of melasma disease were highlighted, and the real causes were identified, the most important of which are increased DNA damage and increased 8-OHDG levels. Explore the significant contributions of this research, which aims to tackle the urgent issues posed by melasma. The study advances treatment options by clarifying the underlying causes of melasma and offering a practical diagnostic methodology. This study presents a new, economically efficient diagnostic approach for dermatologists. To provide an assessment analysis of dermatologists' good biomarker 8-OHDG relation with the severity of melasma. Using a new method for diagnosis and treatment from dermatologists using the ELISA method, which is quick, accurate, and low-cost. To the best of our knowledge, this is the first study to utilize SNP rs772551710 by polymorphism. Investigates how psychological variables, long-term illnesses, and hormone imbalances contribute to elevated 8-OHDG levels, which exacerbate the symptoms of melasma. Increased mutation in MC1R exacerbates the symptoms of melasma gene SNPs rs1805006 and rs772551710 by polymorphism. Investigation: an evaluative analysis of 8-OHDG levels as assessed by an ELISA with mutation SNPs rs1805006 and rs772551710 measured by PCR polymorphism. To investigate a new fast methodology for melasma based on the biomarker, SNPs rs1805006 and rs772551710 were measured by PCR at different stages of melasma (mild, moderate, and severe). Assess the effectiveness of the new biomarker 8-OHDG in melasma for detection. To increase the accuracy of diagnosis and treatment with 8-OHDG using ELISA. Prior research has demonstrated their activity is impacted, DNA structure is damaged, and apoptosis is accelerated by oxidative stress (Hamashareef *et al*., 2020) [8]. Melasma is mostly caused and made worse by UVR. Taking into account oxidative stress, melanin metabolism, and gene expression (Yang *et al*., 2022) [32]. Another study conducted in Japanese populations discovered that the etiology of melasma is complex and might result from interactions between environmental factors and genetics. Studies have indicated that the Melanocortin-1 Receptor gene (MC1R) controls skin pigmentation. Due to their skin types II–III, people with MC1R gene polymorphism genotypes are more likely to develop freckles. Odds Ratio (OR) and Chi-squared test were used to examine the data. With an OR of 2.53 (95% CI: CI:1.21–5.29), the genotype was shown to be more prevalent in melasma participants than non-melasma subjects (p=0.005). With a chi-squared test, we were able to demonstrate that sun exposure and melasma in the family were risk factors (OR:1.99; 95% CI:1.04–3.78) and (OR:35.32; 95% CI:10.25–121.70) for melasma (Suryaningsih *et al*., 2019) [27]. Additionally, a genome-wide high-throughput SNP was evaluated in terms of its suitability for damaged DNA. By making changes to the pre-amplification process (Schmidt *et al*., 2020). However, in the human genome, one of the most prevalent kinds of genetic changes is SNPs (Deng *et al*., 2017) [5]. Furthermore, the regulation of melanin synthesis is significantly affected by the MC1R gene. Since it has been linked to skin disease risk, molecular research is crucial to halting the disease's progression; the genotyping assays demonstrated high rs1805005 V60 L (252 C→T) and the sensitivity with discrimination factor >4 for rs1805006 D84E (425 G→A) for all variants tested (Petralia *et al*., 2021a) [19]. Elevation of 8-OHDG levels is linked to elevated oxidative stress. The pathophysiology of disorders characterized by hyperpigmentation is linked to oxidative stress(Gabash *et al*., 2024) [7]. Current therapies have a low therapeutic impact and a high recurrence rate. This is largely because our knowledge of the disease's origin is still should still be seen as an ongoing difficulty. Furthermore, the previous investigation used SNPs rs1805006 in order to diagnose and detect the severity of melasma; however, there are rarely studies that utilized SNP rs772551710. Therefore, the current study will use this SNP, rs772551710, for diagnosis and detection.

Patients and methods

The provided methodology as includes two main phases.

Subjects and research methodologies

A case-control study was conducted from March to November 2023. The study included (60) patients with melasma and (60) controls from Baghdad women aged 20-50 years; all participants were examined using Wood's light examination and matched as a control. All samples were collected from Al-Emamain Al-Kadhemyain Teaching Medical City, Baghdad. The College of Medicine approved the study. A consent form was obtained from each

participant. Our methodology of the study takes A 6 mL blood sample was collected, separated into two parts, and kept in tubes for the purpose of separation:

1. **First phase:** Tubes containing Ethylene Di-amine Tetra Acetic acid (EDTA) tube anti-coagulated samples were transported immediately to be put in deep freeze under (-20) ℃ for DNA extraction for the genotyping of MC1R SNPs rs1805006 and rs772551710 of the polymorphisms ARMS-PCR method.
2. **Second phase:** A gel tube was used to separate sera through centrifugation at 3000 rpm for 10 minutes after coagulation. They were analyzed for biomarkers measure ROS human 8-OHDG levels in sera by the ELISA method.

The study included sixty healthy control subjects who were unrelated and had similar ages and sexes. The control group was drawn from the same geographic region as the patients; they had to have no family history of melasma or other chronic illness spanning the past three generations, and they had to have been off their usual drugs for at least two years at the time of sampling. The practical portion was carried out in the Chemistry and Biochemistry Department research laboratories at the College of Medicine, Al-Nahrain University. They were diagnosed at least 6 months ago by a dermatologist, depending on history, examination, and response. All patients included met the criteria that they didn't receive drugs, supplements, or contraceptive pills; were pregnant; smoked; had a chronic disease for the last two weeks prior to the study; and were subjected to a complete history taking, such as age, sex, menstrual cycle, and current antibiotic medication.

The exclusion criteria were represented as follows: Patients received supplements of vitamin B12, vitamin C, vitamin H (biotin), or iron or took glutathione or antioxidant drugs; pregnant women; women on hormonal contraceptives; and subjects with TSH, T3, T4, chronic disease, smoking, antibiotic intake, or any subject who used whitening cream over the final two weeks before the research. The disease's severity was determined using the Melasma Area and Severity Index (MASI). The MASI score 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: A+ right malar 0.3 (D+H) + left malar 0.3 (D+H) = forehead 0.3 (D+H) + chin 0.1 (D+H) A = A. These days, modified versions are used when homogeneity is not taken into account (Lakhani *et al*., 2016).

Phase One

Genomic DNA extraction: Genomic DNA was isolated in this study from samples collected from the genotyping of SNPs rs1805006 and rs772551710 of the MC1R polymorphisms of individuals using a Biosphere® Spin Kit. Genomic DNA extraction is done manually from blood samples in accordance with the manufacturer's instructions. Spectrophotometric techniques were used to assess the concentration and purity of DNA, which is:

1. Nano Drop™ system.

Using the Nano drop device, the absorbance technique was used to determine the concentration and purity of extracted DNA samples. The protein absorbs light most strongly at 280 nm, while DNA absorbs light most strongly at 260 nm. An indicator of DNA purity was the A260/A280 ratio. The well-recognized A260/A280 ratio of 1.8–2.0 denotes a high-quality DNA sample.

2. Agarose gel electrophoresis

Agarose gel electrophoresis was performed, as mentioned previously (Sambrook J., E.F. Fritsch, 1989) [21]. The submerged gel electrophoresis unit's casting tray was filled with the molten agarose. Following the gel's solidification, the casting tray was submerged in the submarine tank. The agarose gel was seen under ultraviolet (UV) light after the electrophoresis was terminated. Interpretation and documentation: - An UV trans illuminator (220 - 310 nm) was used to show the PCR amplification for documentation purposes. HISTO MATCH software was used to evaluate the outcomes.

Genotyping of case-control for two single nucleotide polymorphisms SNPs rs1805006 and rs772551710 of MC1R

The Tetra ARMs-PCR procedure was used to identify the MC1R genetic polymorphism SNPs rs1805006 and rs772551710. Traditional ARMS-PCR for genotyping single nucleotides.

Protocol: The recipes and primer design employed for ARMS-PCR for the aforementioned two SNPs of rs1805006 and rs772551710 were displayed below.

1. SNP rs1805006 (A/C) polymorphism: - Rs1805006 (A/C): Fw-Rs1805006 (A/C)- allele A reaction was 5'CTGGGGCTGGCTGCCAACCAGACAGGAG -3'-Rv-Rs18050065'-GGTCACGATGCTGTGGTAGCGCAGTGCG -3' PCR product(bp)in204, and the Rs1805006 (A/C). ARMS-PCR (C allele) …2nd PCR reaction was Fw-Rs1805006-allele C 5'-TGCTTCATCTGCTGCCTGGCCTTGTCGAAC-3'Rv- Rs1805006- (A allele): 5'-AGCACGTTGCTCCCGCTCACCAGCGGT -3' PCR product(bp) in 245
2. *SNP rs772551710 (INS/DELS) polymorphism:* - s772551710 (INS/DELS) Allele specific PCR (INS allele) …...1st PCR reaction was Fw (INSallele):5'GTGCCTCGTGGTCTTCT TCCTGGCTATGCTGGTGCTCATGGCCGTGCTGT-3'Rv-(DELSallele):5'ACGTCC ACATGCTGGCCCGGGCCTGCCAGCACGC CCAGGGCATCGCCCGG -3' PCR product(bP) in 345, and the rs772551710-(INS/DELS). Allele specific PCR (DELS allele) ...2nd PCR reaction Fw- (INS allele):5-'GTGCCTCGTGGTCTTCTTC CTGGCTATGCTGGTG CTCATGGCCGTGCTGT-3'Rv- (DELS allele):5'ACGTCCAC ATGCTGGCCCGGGCCTGCCAGCACGCCCAGGG CATCGCCCGG-3'PCRproduct (bP) in322

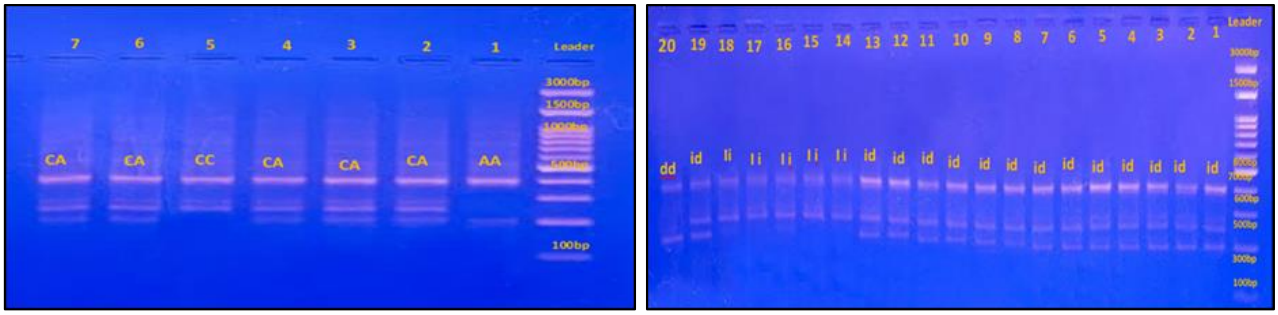
Phase Two

The first and second steps in this phase are the same as the steps in the determination of human 8-hydroxy-deoxyguanosine (8-OHdG) by ELISA. The kit assays 8-OHdG in human serum using a double-antibody sandwich enzyme-linked immunosorbent method. It involves adding the reference test sample. At 450 nm in wavelength, the color shift is determined spectrophotometric ally. The concentration of 8-OHDG in samples can be determined

through the comparison of their optical density (O.D.) with the standard curve.

Results & Discussion

Pheomelanin and eumelanin are the two forms of melanin stored and produced in the melanosome. Hair, skin, and eyes are all colored with two types of melanin. According to a study from Baghdad women aged 20-50 years. By using Wood's light inspection as a control, all samples. Two phases of study preparation. Moreover, any gene that directly or indirectly affects the melanosome proteins may be responsible for the different pigmentation (Carratto *et al*., 2020) [4]. The first phase genotyping of MC1R SNPs rs1805006 and rs772551710 by Tetra ARMs–PCR protocol was used for SNP detection of MC1R, and the second phase used the serum examined by ELISA. We found few studies that dealt with 8-OHDG markers and SNPs rs1805006 and rs772551710. There was no study that combined them together, so we used the methodology in our study of two-phase. In the current study, the clinical significance of the genotype of two single nucleotide polymorphisms, rs1805006 and rs772551710, of MC1R in patients with melasma was analyzed. An imbalance in the amount of reactive oxygen produced and antioxidant systems due to hyperpigmentation agree with study(Gabash *et al*., 2024) [7]. The Figure (1) explained agarose gel electrophoresis image exhibited the ARMS-PCR product analysis of rs1805006 (A/C) at 2% agarose. Where M: marker (3000-100 bp). Distribution of Genotype (CC), (AA) type homozygote at 250 bp, and 209 bp bands showed amplification. Meanwhile, the allele (CA) mutant heterozygote genotypes at the 459 bp band, SNP of rs772551710 (A INS / DELS). Where the distribution of genotype (II), the mutant type homozygote, at 349 bp bands showed genotype (DD) at the 327 bp band. Meanwhile, the genotype (ID) heterozygote at the 676 bp band showed positive genotype amplification at the 400 bp product size. 5-8 volts and 80 AM for 1 hour (Ozola *et al*., 2019) [18]. shown in Figure 1. A&B.



A:SNPrs1805006 **B:SNPrs772551710**

Fig 1: Electrophoresis ARMS-PCR of A: SNPrs1805006 & B: SNPrs772551710 (INS / DELS).

Every DNA sample underwent two PCR procedures tailored to its respective alleles. A primer pair consisting of a forward primer with the allele SNP base at the 3' prime end and a reverse primer is used to guide each ARMS-PCR reaction. Our study demonstrated that it inhibits melasma through the suppression of melanin pigmentation potentially mediated by the reduction of oxidative stress, such as 8-HDG markers, a mutation in the genotype by inhibiting HIF1-α via ROS. For the investigation of the genotype of two single nucleotide polymorphisms, rs1805006 and rs772551710. Compared with the control, genotype-phenotype association analysis produced findings consistent with previous research and confirmed the association of SNPs of MC1R.

Distribution of SNP rs1805006 & rs772551710 genotypes in control and study groups

In the present study, the results exhibited (Table 1), showed genotype CC with an OR of 0.20% and) of 0.0816 to 0.4962, and allele AA was less frequent, with an OR of 0.65%.0.65%. In (C) of in 0.1055 to 44.0687, that reduces the risk, but genotype CA was more frequent at OR 4.67%, of 2.0052 to 10.9106. high risk, the rates of allele C varied significantly (P=0.024) across the study groups. With a very significant difference in the rate of allele C between the study groups (P=0.024) and an OR.54% with a CI 0.3211 to 0.9244, it serves as a risk factor for ALL. The genotype AA had an OR of 0.65% and a CI of 0.1055 to 4.0687, With an OR of 1.83% and a CI 1.0818 to 3.1139, it is a risk factor for A. The genotype DD had an OR of 0.31% and a CI of 0.0600 to 1.6042, and the genotype ID was less frequent with an OR of 0.37% and a CI of 0.1657 to 0.8486, reducing the risk, but the genotype II was more frequent than in the control group (OR of 3.28% and CI of 1.5008 to 7.1955), indicating a high risk (Espósito *et al*., 2022) [6]. There was extremely substantial (P=0.002) variation in the allele I rate between study groups. It is a risk factor for me, with a very significant difference in the rate of allele INS between the study groups (P=0.002) and an OR of 2.78% with a CI of 1.4465 to 5.3648. DELS, with an OR of 0.35% and a CI of 0.1864 to 0.6913, serves as a risk factor for DELS.

Table 1: Comparison of SNP rs1805006 & rs772551710 genotypes case & control groups

Genotype	Control N=60		Patients N=60		X^2	P value	OR	CI
	No.	%	No.	%				
CC	26	43.33	8	13.33	13.29	0.0005*	0.20	0.0816 to 0.4962
CA	31	51.66	50	83.33	13.71	0.0004*	4.67	2.0052 to 10.9106
AA	3	5	2	3.33	0.209	0.64	0.65	0.1055 to 4.0687
Alleles								
C	83	69.16	66	55	5.11	0.024*	0.54	0.3211 to 0.9244
A	37	30.83	54	45	5.11	0.024*	1.83	1.0818 to 3.1139

Genotype	Control N=60		Patients N=60		X^2	P value	OR	CI
	No.	%	No.	%				
II	30	50	46	76.66	9.18	0.002*	3.28	1.5008 to 7.1955
ID	24	40	12	20	5.17	0.018*	0.37	0.1657 to 0.8486
DD	6	10	2	3.33	2.14	0.162	0.31	0.0600 to 1.6042
Alleles								
I	84	70	104	86.66	9.82	0.002*	2.78	1.4465 to 5.3648
D	36	30	16	13.33	9.82	0.002*	0.35	0.1864 to 0.6913

* n: number of alleles; OR: Odds ratio; CI: confidence interval; Chi-square test; HS: Highly Significant difference at P<0.05.

The OR (95% CI) was calculated to assess the strength of the link. (CI). -statistics. Differences that were statistically significant were indicated by P<0.05. Categories (rs1805006:C>A, rs772551710: I>D in MC1R) showed a high probability of relation with skin color hyperpigmentation(Rahimi *et al*., 2024). These SNPs may serve as genetic markers for melasma risk assessment. The result is a significant difference in the rate of the allele with MASI score according to the genotype C\A between study groups genotype CC, CA, AA as an acts risk factor

Nevertheless, variant allele C was more frequent in the control than the study group (Petralia *et al*., 2021a) [19]. The statistical package for social sciences software version 23 (SPSS Inc., Chicago, IL, U.S.A.), the variant allele INS in the patient was more frequent in the control than in the study group.

for all different stages of melasma mild, moderate, severe that heterozygous CA it is a clear mutation in CA. Homozygous AA, CC. This result is consistent with (Petralia *et al*., 2021b; Sarah *et al*., 2022) [19, 22]

Phase two: The result section includes three sub-sections. Determination of the conc. of 8-OHDG between the patient's melasma and the control group. Study revealed a significant correlation between serum 8-OHDG levels and the severity of melasma. The level was measured for each woman who participated in this study. An independent t-test showed a significantly increased 8-OHDG of each serum level in the patient group relative to the control group ($p < 0.0001$). The mean ± SD of serum 8-OHDG for sera according to groups was (3.80±0.22) and (0.603±0.03), respectively results exhibited (Table 2). The statistical package for social sciences software version 23 (SPSS Inc., Chicago, IL, U.S.A.), Serum 8-OHDG levels were elevated in melasma patients compared to controls, with higher levels correlating with disease severity(Yang *et al*., 2022) [32]. As shown in Table 2.

Table 2: Comparison of the conc 8-OHDG between patients and controls by independent t-test

Groups	8-OHDG ng/ ml
Control, N=60, Mean ±SD	0.603±0.03
Patients, N=60, Mean ±SD	3.80±0.22
P value	<0.0001

Correlation of serum 8-OHDG with MASI score in the melasma women group.

The level was 8-OHDG compared with MASI score levels in sera of women with melasma to investigate the possible relationships between them, as shown in Table 3.

Table 3: Correlation of serum 8-OHDG with MASI score in the melasma women group

Parameters	8-OHDGng/ml	MASI score
8-OHDG ng/ml (8-Hydroxydesoxyguanosine)	1	
MASI score	0.929*	1

The MASI scores and sera 8-OHDG levels showed a statistically significant positive connection, which suggests that the more severe the melasma, the higher the 8-OHDG sera levels. Distribution

of the levels 8-OHDG in the melasma and control as shown in Figure 2.

Determination of the normal range (cut-off value).

When the serum 8-OHDG concentration of 1.59 was used as the cut-off value for this marker in melasma women patients, the sensitivity was 96.7%, and the specificity was 100%, Significantly showed The Receiver Operator Characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of serum 8-OHDG concentration (ng/ml) in differentiating melasma patients from controls. The analysis revealed that the optimal cut-off value for 8-OHDG was 1.59 ng/ml. The area under the curve (AUC) was 99.9%, indicating an excellent discriminatory ability.as shown in Table 4.

Table 4: Sensitivity and specificity of 8-OHDG in sera of melasma compared with control subjects

Parameters	No.	AUC	Best cut-off value	Sensitivity%	Specificity%
8-OHDG (ng/ml)	60	99.9%	1.59	96.7	100

These results show that 8-OHDG is a highly sensitive and specific biomarker for melasma diagnosis(Katiyar & Yadav, 2022). The results indicated a significant correlation between the rs1805006 and rs7772551710 variants in the MC1R gene. The findings also validated an established correlation between SNPs in the MC1R gene and 8-OHDG, which was identified as a risk factor for melasma. Additionally, a one-way ANOVA test demonstrated a statistically significant correlation between serum 8-OHDG levels and MASI scores, supporting its potential role in assessing disease severity, as shown in Figure 3.

Determination levels of 8-OHDG according to variables MASI score by one-way ANOVA test. level of serum Conc. 8-OHDG in melasma women was measured for each woman who participated in this study. Additionally, it showed a significantly Figure 3 increased 8-OHDG of each serum. Level at (p<0.0001).

The mean (Mean ± SD) of serum 8-OHDG between the patient and control group by one-way ANOVA test according to severity of groups was mild (2.34±0.09), moderate (4.15±0.15), and severe (6.42±0.25), respectively, as shown in Table 5.

Table 5: Comparison of levels of 8-OHDG according to variables by one-way ANOVA test

Variables	Category	8-OHDG Mean ± SD	P value
MASI score	Mild	2.34±0.09	<0.0001*
	Moderate	4.15±0.15	
	Severe	6.42±0.25	

Through these tests, accuracy, and sensitivity were measured to know the severity of the disease and the possibility of diagnosing it accurate and comparing the results of the PCR SNPs rs1805006 rs772551710 examination with the 8-OHDG examination and them in terms of accuracy and sensitivity and the possibility of conducting the examination at the lowest cost and in the fastest time and to help dermatologists with ease of accurate diagnosis. Distribution of the SNPrs1805006 C/A gene polymorphism was detected by the ARMS-PCR primer technique from all study samples; at this locus, this result is consistent with oxidative stress, a condition when there is an imbalance between the production of ROS and the ability of antioxidants (Tariq & Hussein, 2016) [28]. There was a significant and strong link seen between the levels of serum 8-OHDG, which suggests that the higher the serum 8-OHDG, the more severe the melasma. An imbalance due to hyperpigmentation (Xing *et al*., 2022) [31]. The data showed increased serum 8-OHDG levels in patients with mild, moderate, and severe melasma compared to control women. In contrast, severe 8-OHDG was a study that agrees with the study. Level of serum 8-OHDG conc. An independent t-test showed a significantly increased 8-OHDG of each serum level in the patient group in comparison with the control group ($p < 0.0001$). The data showed increased serum 8-OHDG levels in patients with mild, moderate, and severe melasma severity in malar, central, and mandibular areas compared to control women. The mean (Mean ± SD) of serum 8-OHDG between the study groups was (3.80±0.22) and (0.603±0.03), respectively, according to the independent t-test. A study investigated the role of 8-OHDG in women with melasma. Agree with the study that agrees with the study (Uzunbajakava *et al*., 2023; Yang *et al*., 2022) [29, 32]. However, none of these versions has taken into account the mutation in SNPs rs1805006, rs772551710 number, and level biomarker 8-OHDG ELISA method despite its capability to handle two types of uncertainty and provide additional valuable information. Melasma is a complex and dangerous dermatological disorder that needs research on the complexities of association, diagnostic criteria, and treatment methods. These discoveries lead to a better understanding of the problem and make more effective diagnosis options possible. Here, we present an in-depth analysis of the relationship between MC1R variants and the melasma skin phenotype hyperpigmentation, as well as report on an important warning regarding the relative direction of genetic associations of the single variants in the incidence of structure, as MC1R gene. However, although all past studies agreed on the contribution of C alleles to hyperpigmentation skin color, there is an additional association between the rs772551710 (II) genotype and 8-OHDG melasma P value (0.977). These genes are all coupled within a single signaling pathway that controls pigmentation. In fact, these SNPs have great effectiveness in diagnosing this tenacious disease. However, none of these versions has taken into account the mutation in SNPs rs1805006 and rs772551710. All of these previous studies did not address SNP rs772551710 in diagnosing melasma; however, none of these versions have taken into account the mutation in SNPs rs1805006 and rs772551710 and the level of biomarker 8-OHDG ELISA method despite its capability to handle two types of uncertainty and provide additional valuable information.

Conclusion

The research presented here confirms the hypothesis that the production of the antioxidant 8-OHDG within the body increases the risk of melanogenesis and its association with SNP mutations. The objectives include identifying 8-OHDG which could potentially act as a biomarker that dermatologists may utilize for proactive diagnosis and intervention. It is a significant connection serum 8-OHDG levels and the MASI score with the MC1R SNP genotyping (rs1805006, rs772551710). This study showed a strong association between the MC1R gene polymorphisms (rs1805006 and rs772551711) and the severity of melasma at various stages. Elevated serum 8-OHDG levels were shown to strongly correlate with MASI scores, suggesting that 8-OHDG could serve as a reliable biomarker for diagnosis and severity evaluation. The higher prevalence of the CA and II genotypes among patients indicated an increased genetic predisposition. ROC curve analysis determined 1.59 ng/ml to be the optimal diagnostic 8-OHDG cut-off value with a sensitivity of 96.7% and 100% specificity, confirming its precision. These findings strengthen the hypothesis of oxidative stress and genetic risk factors contributing to the pathogenesis of melasma, as well as suggesting areas for further study. Similar to earlier works, this study also shares the same limitations, which present opportunities and possibilities for further research.

Recommendations

This study recommends considering serum 8-OHDG as a reliable, cost-effective biomarker for early diagnosis and severity assessment of melasma. Its strong relationship with MASI scores and MC1R SNPs (rs1805006, rs772551710) supports its clinical advantage. Combining 8-OHDG testing with genetic analysis may improve diagnostic accuracy and assist dermatologists in making faster, more precise evaluations.

Source of Funding:
Self-funding

Ethical clearance

The research was conducted after obtaining the approval of the standard Committee at Al-Nahrain College of Medicine, Dr. Seham Abdulsahib Muhsen, under Decision No: 2022 \037, Ref. No: M.M.M.\15 dated 07\02\2023.

Conflict of interest

The author declares no conflict of interest.

Acknowledgement

The authors highly appreciate the effort of all staff in center at Al Imamain Al-Kadhimain teaching medical city, Baghdad, Iraq. Diseases/Baghdad during samples collection.

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