Original Article

# Investigation of Biomarkers for Melasma: Baghdad's Women, a Case Study Baghdad

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## Abstract

**Background:** Melasma is a dermatological condition characterized by the appearance of skin discoloration, particularly on the face. Glutathione peroxidase (GSHPx) levels are a vital nonenzymatic antioxidant naturally produced in the body and intricately involved with the process of inhibition in melanogenesis. **Objective:** Investigate serum GSHPx levels with the severity of melasma disease in different stages as a biomarker for diagnosis and treatment. **Materials and Methods:** A case-control study was conducted from March to October 2023, and research was performed on (120 women) with an age between (20 and 50) years women, 60 patients with melasma, and 60 controls after consent from each participant. Blood samples were collected from each individual, and sera were separated through centrifugation. An Enzyme-linked Immunosorbent Assay (ELISA) was used to measure GSHPx levels in serum with severity. **Results:** There was a significant decrease in sera GSHPx levels in women ($18.15 \pm 0.81$ ng/mL) compared to control ($43.28 \pm 1.04$ ng/mL) utilizing an independent *t* test. A one-way ANOVA test also indicated a substantial drop in sera GSHPx MASI score levels for mild, moderate, and severe were $23.07 \pm 0.77$, $15.12 \pm 0.64$, and $11.62 \pm 1.28$ ng/mL, respectively. **Conclusions:** There was a strong correlation between GSHPx and the progression of stages. This research indicates GSHPx's efficacy as a key biomarker for dermatologists, enhancing the evaluation and treatment of mild-to-moderate facial conditions by treating the severity caused by GSHPx insufficiency. This investigation carries significant implications for dermatology and the development of therapies.

**Keywords:** ELISA, GSHPx, mMASI score

## Introduction

Skin discoloration disorder is associated with significant psychological distress and can occur on the face, arms, and neck. This problem becomes more prominent with age, which further highlights the need for appropriate treatments.[1] Glutathione peroxidase (GSHPx) is a powerful antioxidant that not only combats inflammation but also defends against harmful ultraviolet (UV) rays while reducing reactive oxygen species (ROS). Within the global community of selenoproteins, the enzyme called GSHPx stands up as a severe defender and essential antioxidant against the onslaught of ROS.[2,3] Consequently, we have concentrated on the biomarker GSHPx for the detection and treatment of.[4,5] GSHPx has been generally utilized for brightening skin treatments because of its capacity to repress tyrosinase. Its underlying causes remain predominantly unidentified. Despite the identification of various components such as family history, exposure to ultraviolet radiation, hormonal influences, neurological and vascular factors, visible light, and molecular pathways,[6] the pathophysiology of this disorder is not yet fully understood.

The management is characterized by uneven treatment outcomes and a high likelihood of recurrence, despite the growing demand for therapeutics.[7] Current research endeavors have been primarily directed toward examining

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the significance of oxidative stress in the development and progression of this condition. Nevertheless, the multifaceted and dynamic elements that contribute to its complexity pose an ongoing obstacle to its successful management.[8] This investigation provides an evaluative analysis of GSHPx levels as assessed by an Enzyme-linked Immunosorbent Assay (ELISA) sandwich technique. This package includes a microtiter plate that has already been pre-coated with an antibody. Fill wells with the standard, samples, and HRP-conjugated antibody. Add Chromogen Solutions A and B after incubation and washing to eliminate the uncombined enzyme. The blue color changed the liquid's color. Acid ultimately causes the color to become yellow. At 450 nm in wavelength, the color shift is determined spectrophotometrically. By contrasting the OD of the samples with the standard curve, the quantity of GSHPx in the samples was evaluated. Using Sandwich ELISA Human Glutathione Peroxidase (GSHPx) ELISA Kit (No. EKHU-0773, Melsin Medical China). This study is to provide an assessment and analysis of dermatologists' good biomarker relation with severity. In addition, assess the effectiveness of the new biomarker in detection. Moreover, it investigates a new fast methodology based on the biomarker with different stages (mild, moderate, and severe). Finally, increase the accuracy of diagnosis and treatment. The study presents a new and economically efficient diagnostic approach for dermatologists. By elucidating root causes, this study contributes to the development of treatment strategies, facilitating the development of more accurate and effective treatments. This study examines the role of psychological factors, chronic diseases, and hormonal irregularities in the depletion of GSHPx levels and the increase of tyrosinase enzyme activity.

Prior research has demonstrated that GSHPx is a prominent antioxidant that has a crucial function in safeguarding cells from oxidative harm and the harmful effects of foreign chemical substances and heavy metals that are nucleophilic in nature react with soft electrophilic but low with weak and strong electrophilic, while also maintaining a stable balance of redox reactions inside the cell. A tripeptide refers to a compound called glutathione, which is in its reduced form and consists of the amino acids glutamyl, cysteinyl, and glycine. A compound known as GSHPx hinders melanin production by binding to copper. Copper is crucial in converting tyrosine into DOPA (3, 4-dihydroxyphenylalanine) and DOPA into DOPA-Quinone. GSHPx can also form a complex with the intermediate substance involved in the conversion of tyrosine to melanin.[9] Research has examined the involvement of specific oxidative stressors in the etiopathogenesis of patients, despite the fact that the relevance of oxidative stress in the etiopathogenesis of several cutaneous illnesses has been determined and demonstrated.[10]

The elderly may produce up to 50% less 25-dihydroxy vitamin D due to age-related decreases in renal function. Reduced serum levels of vitamin D may be the cause of secondary hyperparathyroidism.[11] Additionally, female participants with the epidermal subtype were validated using Wood's light examination. Treatment efficacy was evaluated using the modified area and severity index (mMASI). There was a substantial decrease in the average MASI score across the treatment sessions on both sides of the face when using GSHPx, indicating an earlier response to therapy. The incidence varies from 8.8% to 40%. Primarily impacts females during their reproductive cycle. The prevalence is higher among individuals of Asian descent, and those with Fitzpatrick skin Type III, V. is a chronic skin condition characterized by darkness. Various pathogenic mechanisms include heightened activity of melanocytes and deposition of melanin in the epidermis and dermis.[6] There is knowledge of several dermatologists. For many years, there has been research focused on understanding the impact of oxidative stress.[12] Therapeutics for this condition are perplexing since it is characterized by high rates of recurrence that have a major impact on the individual's quality of life. There is no single therapy that is uniformly effective.[8,13] Mesotherapy is a widely used innovative treatment method that involves injecting small amounts of medication directly into the skin or underlying fat layer. While this unique treatment procedure is frequently employed in cosmetic dermatology, knowledge is scarce on the specifics of the injections.[14] The following have been identified as risk factors: sex, age, UV radiation exposure, Fitzpatrick skin types I and II, certain hereditary illnesses, and prolonged exposure to arsenic.[15]

GSHPx serves as an enzymatic antioxidant within cells, and its levels are utilized as a significant indicator of oxidative damage. Nevertheless, we examined instances and matched them with healthy volunteers in a case-control design. A significant difference was found between cases and normal skin in terms of the distribution ($P = 0.001$) and intensity ($P = 0.005$) of GSHPx in the epidermis. Furthermore, a statistically significant correlation was seen among the intensity, distribution of GSHPx in the epidermis, and severity, as evaluated by the MASI score.[16] GSHPx decreases the activity of enzyme tyrosinase, alters the process of melanogenesis by favoring the synthesis of phaeomelanin over eumelanin, and functions as a scavenger of ROS generated by UV radiation.[17]

Enzyme tyrosinase is generated through a series of oxidative processes involving amino acids. The common risk factors include genetic factors, female sex hormones, oral contraceptives, cosmetics, and medicines. UV radiation and visible light have the potential to induce lipid peroxidation in cellular membranes and trigger the production of free radicals, which can, in turn, increase melanogenesis.[1] Oral administration of GSHPx has demonstrated substantial effectiveness when used with a topical modified Kligman

regimen.[18] GSHPx is an innovative medicine for treating. The reaction was evaluated by utilizing a visual analog scale score relative to the baseline. However, the statistical analysis revealed an in-severity improvement.[19] The investigation determined that GSHPx is sufficiently effective as a skin-whitening agent. While the safety profile of topical and oral GSHPx appeared to be favorable, further research investigations with improved study designs are necessary to assess the effectiveness of antioxidants.[20] Additionally, reduced GSHPx is vital in the defense against UVB-induced damage to DNA.[21] Biomarkers of oxidative tension and a rise in GSH peroxidase lead to antioxidant defense markers.[22] Oxidative stress with low GSHPx is an effect of hyperpigmentation formation. It also changes DNA structure and speeds up apoptosis.[23] Most studies focused on treatment using GSHPx to compensate for the deficiency. Unfortunately, previous studies did not discuss the issue in terms of diagnosis. Therefore, our study focused on the examination method and its accurate results according to varying degrees of injury severity-worthy biomarkers.

## Materials and Methods

The provided methodology, a case-control study, was conducted for 120 women recruited in Baghdad, Iraq, from the Diseases Center in Al Imamain Al-Kadhimain Teaching Medical City. The study sampling was executed from March to October 2023. The demographic and clinical data, including age, duration, current medication, number of acute attacks/last year, and family history of atopy, were obtained from each patient. Other age-matched healthy women were enrolled as controls.

The College of Medicine, University of Babylon, approved its Institutional Review Board (I.R.B.) Resolution number 2022/037 on February 7, 2023. A verbal consent was obtained from each participant.

This study included two groups; the first group consisted of 60 outpatient visitors or participation patients (20–50) years old (female). The second group, control, contained 60 subjects age-matched with the group. Patients were divided into subgroups (mild, moderate, and severe) according to their severity. The practical portion was completed in research labs at the Department of Chemistry and Biochemistry College of Medicine, Al-Nahrain University, Iraq.

Our methodology included six steps. The first step was to prepare the patient, the second was to collect the sample, and the third was the separation from the whole blood tube (without anticoagulant) gel tube; the fourth saved the serum and examined by ELISA; and finally, the calculation of the result produced the severity of (mild, moderate, and severe). In addition, study females with the epidermal type (examined by Wood's light). Samples A venous blood sample (5 mL) was collected from the antecubital vein of each subject. The blood sample was kept undisturbed for at least 20 min and then centrifuged at 3000 rpm for 10 min. Serum samples were stored at −20°C for analysis of the GSHPx concentration measured by ELISA. This ELISA is used to measure human enzyme GSHPx concentration in sera with severity. The study evaluated a group of 60 unrelated healthy control people who were matched in terms of age and sex. The control group consisted of persons who had no prior history of or any other chronic illness in their family for the last three generations. Additionally, they had not been using any regular drugs for at least 2 years prior to the sample date. These individuals were recruited from the same geographic region as the patients. They were diagnosed at least 6 months ago by a dermatologist, depending on history, examination, and response. Included criteria: all patients didn't receive drugs, supplements, or contraceptive pills for the last 2 weeks prior to the study and had a complete history of taking them, such as age, sex, minstrel cycle, and current antibiotic medication. All subjects are interviewed with standard questionnaires, including their family history, by a dermatologist, depending on the examination and response. They are all either dermal, epidermal, or mixed, according to Wood's light examination, matched as controls. All patients' samples were collected after approval of the study. The severity was measured using the modified Area and Severity Index mMASI score[24]; the amount of the severity score for darkness (D) and homogeneity (H) is multiplied by the numerical value of the areas (A) involved and by the percentages of the four facial areas (10–30%). Total MASI score: Forehead 0.3 (D+H) A + right malar 0.3 (D+H) A + left malar 0.3 (D+H) A (+ chin 0.1 (D+H) A. The MASI assessment measured three factors, including the area of involvement (A), darkness or hyperpigmentation (D), and homogeneity (H) based on a facial skin examination.[25] Table 1 shows information on the grading of the area and its severity.

The clinical shape was rated by the dermatologists at five for the variables of MASI/mMASI: darkness (0–4), area (1–6), homogeneity (0–4) on the forehead, right cheek, left cheek, and chin, the MSS (global assessment 0–3), (pigmentation (0–4) and area (1–4), for the left and right face and nose, respectively.[26]

Calculated mMASI by the percentage of involved area and darkness in patches.

mMASI = forehead (0.15×D×A) + malar (0.30×D×A) + chin (0.05×D×A)

A =Area = 0–6, 0 = 0%, 1 = 1%–9%, 2 = 10%–29%, 3 = 30%–49%, 4 = 50%–69%, 5 = 70%–89%, 6 = 90%–100%, D = Darkness of patches = 0–4, 0 = absent or normal skin color without evidence of hyperpigmentation, 1 = slight visible hyperpigmentation, 2 = mild visible, 3 = marked, and 4 = severe.[27]

Nowadays, a modified version is employed where inhomogeneity is not considered.[28] The exclusion criteria were patients who received a supplement of vitamin B12,

**Table 1: Melasma area severity index distribution among patients with melasma**

| Variables | Category | Control | Patients | $X^2$/*P* value |
|---|---|---|---|---|
| Site | Malar | 0 (0) | 41 (68.33) | 120/<0.0001* |
| | Malar and central | 0 (0) | 8 (13.33) | |
| | Central | 0 (0) | 2 (3.33) | |
| | Central + mandibular | 0 (0) | 1 (1.66) | |
| | Mandibular | 0 (0) | 2 (3.33) | |
| | Malar &central & mandibular | 0 (0) | 6 (6.66) | |
| | No | 60 (100) | 0 (0) | |
| Family history | Yes | 9 (15) | 32 (43.33) | 14.1/<0.0001* |
| | No | 51 (85) | 28 (46.66) | |
| MASI score | Mild | 0 | 29 | 120/0.0001* |
| | Moderate | 0 | 19 | |
| | Severe | 0 | 12 | |

vitamin C, vitamin H (biotin), or IRON or take glutathione, antioxidant drugs, hormonal contraceptives, antibiotic intake, pregnancy, TSH, T3, T4, chronic disease, smoking, and any subject who used whitening cream for the last 2 weeks prior to the study. All the subjects identified the skin condition validated using Wood's light examination, and the area was examined with an mMASI score.[24]

## Results

The study revealed a significant inverse connection between serum GSHPx levels and the severity of. This split-face investigation revealed a statistically significant decrease in GSHPx levels throughout several stages of severity, including mild, moderate, and severe. GSHPx is a crucial molecule for preserving the integrity of cell membranes; the result section includes four subsections. The first section presents a comparison of the levels of serum GSHPx. The second section investigates the possible relationships of GSHPx with MASI score levels. The last section determines the normal range (cutoff value).

### Comparison of the level of serum GSHPx between patients and controls by an independent *t* test

The level of serum GSHPx. A was measured for each woman who participated in this study (whether women or control women). An independent *t* test showed a significantly decreased GSHPx for each serum level in the group compared to the control group ($P < 0.0001$). T valued (18.9). The mean and standard deviation (Mean±SD) of serum GSHPx between and the control group by independent *t* test for sera according to the severity of groups were (18.15 ± 0.81) and control (43.28 ± 1.04), respectively, by independent *t* test as shown in Table 2. The present study investigates the role of GSHPx in women. The data showed lower serum GSHPx levels in patients with mild, moderate, and severe. Severity in malar, central, and mandibular than in healthy women.

A pronounced inverse association was seen between serum GSHPx levels, indicating that as GSHPx serum levels decrease, the severity increases. The levels of GSHPx in both women and the control group

**Table 2: Comparison of the level of serum GSHPx**

| Groups | Glutathione (ng/mL( |
|---|---|
| Control, *N* = 60, Mean ± SD | 43.28 ± 1.04 |
| Patients, *N* = 60, Mean ± SD | 18.15 ± 0.81 |
| *P* value | <0.0001 |

The correlation was a significantly strong negative between serum GSH levels; this can be interpreted as the lower the GSHPx serum, the more severe the severity. The nonenzymic GSHPx plays an important role in the inhibition of tyrosinase enzyme activity, and melamine darkens. The relationship between GSHPx concentration and activity tyrosinase and oxidative status in patients and different correlations between the disease and activity levels were also reported for the skin. The distribution of the levels of glutathione GSHPx in women and the control group is shown in Figure 1.

### Investigate the possible relationships between GSHPx and MASI score levels in sera of women with melasma

The level has been compared to glutathione with MASI score levels in sera of women to investigate the possible relationships between them, as shown in Table 3.

Table 3 illustrates a significant relationship between GSHPx and the MASI score. A pronounced inverse association was seen between serum GSHPx levels and MASI scores, indicating that as serum GSHPx levels decrease, the severity of the disease increases. A comparison analysis was performed to evaluate the correlation between the levels of GSHPx and the degree of MASI score in the blood samples of women suffering from melasma. The findings demonstrated a statistically significant correlation between GSHPx and MASI Score (0.151) with a *P* value of less than 0.05. The findings of such a relationship claimed that the MASI score (the severity) in sufferers was demonstrated by the decreased plasma glutathione levels.

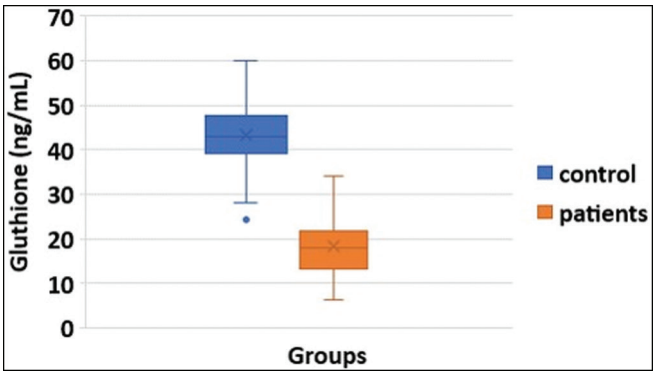


**Figure 1:** GSHPx distribution

**Table 3: The correlation between serum GSHPx and MASI score**

| Parameters | Glutathione (GSHPx) | MASI score |
|---|---|---|
| Glutathione | 1 | |
| MASI score | 0.151 | 1 |

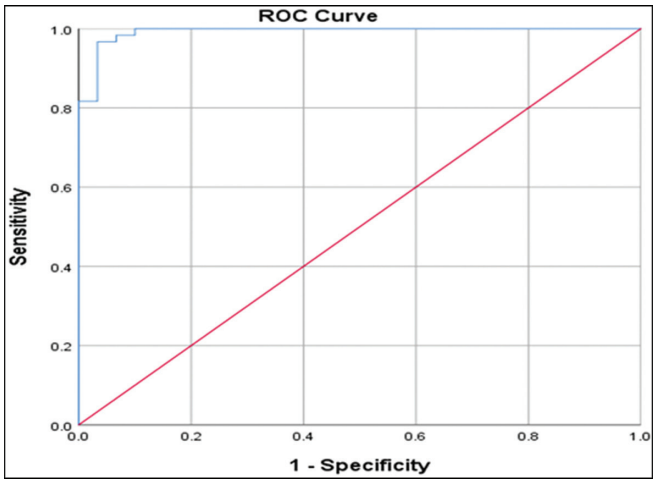


**Figure 2:** ROC curve of GSHPx (ng/mL)

### Purpose of the normal range (cutoff value)

This section produces a complete explanation of the cutoff value and includes two subsections.

### Determination of the normal range (Cutoff Value) of GSHPx in Sera of Women

The Receiver Operator Characteristic (ROC) curves show a significant discriminating ability of decreased serum GSHPx, as shown in Figure 2. When the serum GSHPx concentration of 24.14 was chosen as the threshold value for female patients, the sensitivity was found to be 81.7% and the specificity was 100%, as shown in Table 4.

### Determination of the normal range (cutoff value) of GSHPx in sera of women with melasma

The current study concluded that the cutoff value of the ROC curve shows a significant discriminated ability of decreased serum GSHPx in women when the serum GSHPx concentration of 24.14 was used as the cutoff value for this marker in patients; the sensitivity is 81.7% and the specificity is 100%, as shown in Table 4. The ROC curve is primarily employed to visualize the performance of a classification algorithm while the decision threshold is adjusted. Each point on the curve represents a possible operational point for the classifier and may be evaluated in the same manner as precision. The element we have measured and assessed is the region under the ROC curve (AUC); as a result, it was possible to depend on the test GSHPx for sensitivity and specificity as a good biomarker.

The results show that there is a difference observed between serum GSHPx levels and MASI scores because GSHPx is lower in severity. In terms of family history, this study adopted two main categories (i.e., yes and no), and our outcome captured a notable detrimental impact of family history (i.e., yes) Mean ± SD (19.35 ± 1.17). Meanwhile, the value of family history (i.e., no) was Mean ± SD (17.1 ± 1.11). The MASI score was assessed at different stages of severity using a one-way ANOVA test, indicating a significant relationship between the MASI score and serum GSHPx levels in patients. m\MASI scores for the severity of diseases regarding mild, moderate, and severe were 23.07 ± 0.77, 15.12 ± 0.64, and 11.62 ± 1.28, respectively. Table 5 shows the comparison of the GSHPx mMASI score.

## Discussion

Different levels and types of melanin pigment determine variations in skin color between individuals. Melanocytes synthesize melanin. Melanin production, known as melanogenesis, involves a series of biochemical and enzymatic reactions facilitated by tyrosinase and tyrosinase-related proteins. These reactions result in the creation of two distinct types of melanin: pheomelanin and eumelanin. UV light is the primary external element that controls the process of melanogenesis. Antioxidant systems, which have the capability to remove ROS,[29] may reduce the production of melanin.[30] This paper specifically examines the primary cellular antioxidant systems, including the GSHPx, and explores their functions in melanogenesis.[31] The GSHPx is linked with regulating pheomelanin formation.[32,33] Insufficiency of GSHPx was common in the study sample. Calculated mMASI by the percentage of involved area and darkness in patches of the equation of measurement is mMASI = forehead (0.15×D×A) + malar (0.30×D×A) + chin (0.05×D×A) depending on the result in Table 1. Whereas severe GSHPx deficiency was significantly higher in women with and diseases than in healthy women, different correlations between the disease and activity levels were also reported for skin, agreeing with the study done by Giustarini *et al.*[4] This study showed a strong relationship between GSHPx deficiency and the severity of the disease. GSHPx measured for each woman who participated in this study by an independent *t* test showed

a significantly decreased GSHPx of each serum level in the group compared to the control group ($P < 0.001$). Show the mean and standard deviation (Mean ± SD) of serum GSHPx between the control group by independent *t* test for sera according to the severity of groups (18.15 ± 0.81) and control (43.28 ± 1.04), respectively. It is a clear deficiency the level GSHPx in the serum in women with infection as shown in Table 2, agree with the study.[34,35] The correlation was a significantly strong negative between serum GSH levels; this can be interpreted as the lower the GSHPx serum, the more severe the severity [Figure 1]. GSHPx was found to be distributed at higher levels between two groups of patients and the control; this can be interpreted as the lower the GSHPx serum, the more severe the severity. The nonenzymic GSHPx plays an important role in the inhibition of tyrosinase enzyme activity, and melamine darkens.[36] A comparison analysis was performed to evaluate the correlation between the levels of GSHPx and the degree of MASI score in the blood samples of women suffering from melasma. The findings demonstrated a statistically significant correlation between GSHPx and MASI Score (0.151) with a *P* value of less than 0.05. The findings of such a relationship claimed that the MASI score (the severity) in sufferers was demonstrated by the decreased plasma glutathione levels. As shown in Table 3 illustrates a significant relationship between GSHPx and MASI score. The main purpose of the ROC curve is to show how well a classification algorithm performs when the decision threshold is changed. Every point on the curve denotes a potential classifier operational point, and it may be assessed similarly to precision. The region under the ROC curve is the component that we have measured and evaluated; hence, the test GSHPx was a reliable biomarker for both sensitivity and specificity, showing a significant discriminated ability of decreased serum GSHPx women when the serum GSHPx concentration of 24.14 was used as the cutoff value for this marker in patients; the sensitivity is 81.7% and the specificity is 100%, as shown in Table 4. There was a significant difference between cases and the control group ($P = 0.001$). The mean and standard deviation (Mean ± SD) of serum GSHPx between the control group and sera, according to the severity of the groups, showed a notable correlation between serum GSHPx levels and MASI scores. In different stages of severity, score mild (23.07 ± 0.77), moderate (15.12 ± 0.64), and severe (11.62 ± 1.28) at a *P* value < 0.001*.[37] The ROC curves show a significant discriminated ability of decreased serum GSHPx, as shown in Figure 2. When the serum GSHPx concentration of 24.14 was chosen as the threshold value for female patients, the sensitivity was found to be 81.7% and the specificity was 100%. The MASI score was assessed at different stages of severity, indicating a significant negative relationship between the MASI score and serum GSHPx levels in patients, as well as the degree of dermal inflammation that results in different severity stages. At *P* value < 0.0001*: mild (23.07 ± 0.77), moderate (15.12 ± 0.64), and severe (11.62 ± 1.28). There is a clear correlation between the MASI scores of patients and their serum GSH levels.[38]

It is frequently employed as a product for lightening the skin. This molecule consists of three amino acids: L-cysteine, glycine, and glutamate, all of which contain sulfhydryl groups. The GSHPx treatments are available for skin whitening, encompassing topical, oral, and parenteral formulations.[39] Despite its initial promising findings, more controlled trials with a larger sample size are required to demonstrate the efficacy of GSHPx in mesotherapy for treatment.[40] The distribution of the levels of GSHPx in the control group is shown in Figure 1. Oxidative stress reduces their activity, alters the structure of DNA, and accelerates apoptosis, ultimately decreasing their numbers, motility, and proper structural development while compromising their function.[41,42] The dietary supplement industry predominantly features oral glutathione as the most prevalent product. GSHPx catalyzes the decrease of hydrogen peroxides or natural hydroperoxides, utilizing GSHPx as a significant ROS forager in serum, agreeing with the study.[43,44] More precisely, women showed significantly reduced levels of GSHPx in their blood serum. The decrease in GSHPx levels is believed to be a contributing reason to

**Table 4: Sensitivity and specificity of Glutathione (ng/mL)**

| Parameters | No. | AUC | Best cutoff value | Sensitivity % | Specificity % |
|---|---|---|---|---|---|
| Glutathione (GSHPx) | 60 | 92.9% | 24.14 | 81.7 | 100 |

**Comparison of** GSHPx and MASI score according to variables in group by one-way ANOVA test

**Table 5: Comparison of Glutathione peroxidase and mMASI score**

| Variables | Category | Glutathione peroxidase, Mean ± SD |
|---|---|---|
| Family history | Yes | 19.35 ± 1.17 |
| | No | 17.10 ± 1.11 |
| mMASI score | Mild | 23.07 ± 0.77 |
| | Moderate | 15.12 ± 0.64 |
| | Severe | 11.62 ± 1.28 |

the heightened severity, as indicated by the significant negative connection in the context of this study.[19] Moreover, this outcome emphasizes the need to evaluate GSHPx deficiency, which has been linked to recurrent and severe infections, as explained in the study conducted by Nirgude and Choudhary.[43] Indicating a significant negative relationship between the MASI score and serum GSHPx levels in patients, as well as the degree of dermal inflammation, with results at a $P$ value of <0.0001, our study consents to the study of Khaled *et al.*[16] and Ashrafi *et al.*[45] One important enzyme for antioxidants is GSHPx[46]. This study has similarities with prior studies in terms of its limitations, which present opportunities and possibilities for further research. Studies suggest the potential effects of GSHPx on different skin types since it is commonly used as a skin-lightening agent to aid in the repair of cells damaged by stress and other detrimental factors. It is important to have a short time to detect and diagnose the disease and calculate the severity (mild, moderate, and severe) of the MASI score.[17] The ELISA method used for detection and diagnosis by the laboratory is accurate, simple, easy, short, and low. The results of this study would likely benefit the diagnosis of diseases involved in the treatment, including determining the type of treatment and fallow treatment, severity detection, and public diagnosis. Lower plasma glutathione levels indicate a stronger negative connection between the MASI score (the degree) and lower plasma glutathione levels in sufferers. Female patients had higher levels of enzyme tyrosinase and lower blood glutathione levels than controls. These suggest that elevated tyrosinase and low glutathione levels in the serum of women with patients may be utilized as useful prognostic markers and diagnostic tools for asthma patients.[1] Implementing the study's results alleviates patients' financial hardship by encouraging cost-effective diagnostic and treatment options. Early illness diagnosis and accurate severity evaluation lead to faster and less expensive therapies, thereby lessening the financial strain on patients. Secondly, dermatologists may use this data to address public concerns over the treatment of decreased GSHPx, thereby increasing patient confidence and promoting the adoption of efficient GSHPx marker management techniques. Enhancing trust and patient compliance is crucial for achieving effective therapy and improved health outcomes. Furthermore, equipped with this understanding, dermatologists may make well-informed choices on the most appropriate approaches for regulating GSHPx in treatment. Finally, it enhances public awareness of the benefits of sustainable healthcare practices, emphasizing stress relief and overall well-being.

## Conclusion

This study aimed to investigate the correlation between blood GSHPx levels and severity. The purpose was to identify GSHPx as a potential biomarker for dermatologist doctors to assess the degree and stage of the illness and for early detection in patients. This prospective case-control study was case-matched with healthy participants. Severity was measured using the mMASI. A considerable negative correlation exists between serum GSHPx levels and the mMASI score in individuals with. This work has effectively carried out a comprehensive prediction that GSHPx, a nonenzymatic antioxidant synthesized by the body, contributes to the process of melanogenesis. There was a significant difference between cases and normal skin ($P$ = 0.001). The mean and standard deviation (Mean ± SD) of serum GSHPx between the control group by independent $t$ test for sera according to the severity of groups were (18.15 ± 0.81), (7.36 ± 0.19), and (3.80 ± 0.22) respectively by independent $t$ test, there was a notable correlation seen between serum GSHPx levels and MASI scores. The study suggests that lower serum GSHPx levels are associated with greater severity. In different stages of severity, MASI score by one-way ANOVA test is mild (23.07 ± 0.77), moderate (15.12 ± 0.64), and severe (11.62 ± 1.28) at a $P$ value < 0.001*. There is a notable adverse correlation between the mMASI score of patients and their serum GSHPx levels. The significance of this study is the use of GSHPx in serum to provide a visual representation of a healthy patient's condition, which can aid dermatologists in making early diagnoses. Several methodologies have been suggested for the determination of GSHPx.

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## Conflicts of interest

There are no conflicts of interest.

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