**Journal of Neonatal Surgery**
***ISSN(Online): 2226-0439***
***Vol. 14, Issue 32s (2025)***
*https://www.jneonatalsurg.com*

OPEN ACCESS

# Effect of phototherapy on peroxidase, myeloperoxidase activities, oxidized protein and calcium levels in Iraqi vitiligo patients

**Noor Abdulhussain Naama[1], Jwan Abdulmohsin Zainulabdeen[2]**

[1]Department of Chemistry, College of Sciences, University of Baghdad, Baghdad, Iraq):
Email ID: noorabdulhussin42@gmail.com
[2]Department of Chemistry, College of Sciences, University of Baghdad, Baghdad, Iraq):
Email ID: Jwan.Zainulabdeen@sc.uobaghdad.edu.iq

Cite this paper as: Noor Abdulhussain Naama, Jwan Abdulmohsin Zainulabdeen, (2025) Effect of phototherapy on peroxidase, myeloperoxidase activities, oxidized protein and calcium levels in Iraqi vitiligo patients. *Journal of Neonatal Surgery*, 14 (32s), 7180-7189.

## ABSTRACT

**Background:** Vitiligo is a skin condition in which the skin loses its color due to the destruction of pigment cells , meantime oxidative stress plays a significant role in triggering this damage. Peroxidases are members of the oxidoreductases enzymes that reduce hydroperoxides via the decomposition of a great variety of substrates into harmlessly components. The oxidative cycle of peroxidases involves the reduction of an electron-donor substrate, in the presence of hydrogen peroxide . The heme enzyme; Myeloperoxidase (MPO) is released in the europhilic granules of neutrophils, and to a lesser degree in the lysosomes of monocytes to forming reactive oxygen intermediates (hypochlorous acid ; a powerful oxidant) to kill internalized bacteria and other pathogens.

**Objective:** The study aims to assess oxidative stress in vitiligo patients by measuring peroxidase and myeloperoxidase activities to explore their role in the diseases and the effect of phototherapy on the oxidative stress status represented by the oxidized proteins , enzymes activities and the level of calcium.

**Materials and methods**: One hundred fifty participants were included in the present study divided equally to three groups as follow: patients treated with NB-UVB(PUV), newly diagnosis groups(PV) , and apparently healthy as control(C). All studied parameters were determined by using appropriate spectrophotometric method.

**Results:** Results showed high significant increases in the serum oxidized proteins level , as well peroxidases and MPO activities in both patients groups (PUV and PN) in comparison to that of C group While, no significant was observed in PUV compared with PN group for oxidized proteins level and significant increase in PN over PUV in activities of the two enzymes. In contrast the results appeared a significant decrease in calcium level of PUV when compared with C. whilst, no significant changes were seen between the PN and C group as well as PUV with PN.

**Conclusion:** The study showed that phototherapy reduces oxidative stress in vitiligo patients, reflected in decreased activity of antioxidant enzymes, resulting from reduced defense needs. Changes in oxidized protein and calcium were also observed, indicating a clear effect of the treatment on internal balance.

***Keywords:*** *Peroxidases, Myeloperoxidase, Oxidized protein, Oxidative stress, phototherapy, Vitiligo*

## 1. INTRODUCTION

Vitiligo is a chronic pigmentary disorder causes the loss of skin color in blotches because of melanocytes destruction [1], it affects the melanocytes, which are synthesizing melanin color within the skin and providing the skin with its natural pigmentation. Melanocytes, which are located in the basal layer of the epidermis and the hair follicles, have the principal function of producing melanin. Under the influence of an ultraviolet light stimulus, melanocytes are able to synthesize more melanin[2]. vitiligo typically gets broader over time until there is a significant loss of melanocytes in the epidermis and occasionally in the hair follicles.; there were several classifications of the disease depending on whether it is segmental or non-segmental [1]. This disease affects between 0.38 - 2.9% of the world's population, in some populations, such as in India, the incidence is higher, affecting around 8% of the population [3]. Risk factors include family history, long exposure to stress, skin injuries, mental or physical illnesses, and in rare cases, smoking . Nonetheless, vitiligo is not infectious.The precise etiology of vitiligo remains unknown despite the existence of several theories regarding its pathogenesis[4]. Rapid advances in genetics, molecular biology, immunology, and intracellular signaling, in addition to oxidative stress (''oxidant–

antioxidant'' balance is tilted in favor of the ROS so that oxidative damage levels increase) have provided new information concerning the mechanisms implicated in vitiligo pathogenesis [5-7]. Oxidative stress, a key pigment-deficient parcel of the epidermal defense system against various environmental factors including drastic temperature changes, biological, physical, and chemical stress, can produce damage not only on isolated pigments but also in metabolic networks such as enzymes and hormones. The balance of oxidative and antioxidative system guarantees normal skin cells [8].

Peroxidases (EC 1.11.1.X) are a wide category of enzymes, and the prosthetic group of peroxidases is frequently made up of heme with a proximal ligand that is a histidine residue. Depending on the reaction type, there are 14 different peroxidases, which remove reactive oxygen species (ROS) like hydrogen peroxide[9]. It can be classified into heme and nonheme peroxidases, the majority (80% ) are heme peroxidases [10]. Myeloperoxidase (MPO) (E.C 1.11.1.7) is commonly expressed in immune cells, including neutrophilic polymorphonuclear leukocytes, Neu, Lym, Mono, and macrophages. It belongs to the heme peroxidases subfamily[11]. The storage of MPO in cytoplasm membrane-bound azurophilic granules which are during activation, these granules are secreted out by degranulation or exocytosis to the extracellular space. OS plays an essential role in the release of the MPO enzyme from these cells . The MPO constitutes 5% dry weight of Neu and 25% of the azurophilic granular proteins[12]. Released MPO from activated Neu, Mono, and macrophages at the site of inflammation, using $H_2O_2$ to oxidize many substrates, such as halides (Chloride and bromide), and pseudohalides such as thiocyanate ($SCN^-$). This reaction forms hypochlorous acid (HOCl), hypobromous acid (HOBr), and hypothiocyanous acid (HOSCN)[12].

This study aims to evaluate the effect of phototherapy on the levels of some biomarkers associated with oxidative stress, including peroxidase and myeloperoxidase activity, oxidized protein levels, and serum calcium level in Iraqi vitiligo patients. This study aims to highlight the potential role of phototherapy in reducing oxidative stress and improving biochemical balance.

## 2. MATERIALS AND METHODS

This study was conducted between October 2024 and February 2025, and it involved 150 participants (27-40 years old) who were attending Dermatology and Venereology Department of the Baghdad Teaching Hospital, located in the Medical City Complex, and Al-Imamain Al-Kadhimiya Teaching Hospital in Iraq. The participants were divided into three groups,50 patients receiving NB-UVB (50 to 100 sessions) treatment (PUV) were in the first group. There were 50 newly diagnosed patients(PN) in the second group and 50 people who appeared to be in healthy individuals (C) in the third. The PUV group was divided between active and stable vitiligo by a ratio of one to two, while the PN group all had active vitiligo. all participants underwent a personal interview utilizing a specifically created questionnaire format that included their complete medical history and all pertinent details. Protocol was approved by the Ethics Committee of the College of Science/ University of Baghdad (Ref:CSEC/1224/0123). The exclusion criteria of samples include autoimmune diseases ; eye cataract or skin cancer; psoriasis and history of cutaneous photosensitivity; pregnancy; lactation; diabetic; anemia . Ten milliliters of room-temperature venous blood were collected as samples, and the tube was centrifuged for five minutes at 3,000 cycles per minute. Before being examined, the serum was placed in an Eppendorff tube and kept in a freezer at -20°C. Peroxidases activity was assayed by colorimetric method[13]. Serum MPO activity was determined by the method of Klebanoff and Clark[14]. The carbonyls in oxidized proteins were determined using a spectrophotometric method according to the DNPH alkaline method[15]. Calcium level was measurement by using colorimetric analysis. SPSS version 26 was used to properly characterize the data utilizing licensed materials. Using one-way ANOVA, the study's data was presented as mean± standard deviation (mean± SD), and correlation between variables was determined by person correlation coefficients (r). The ROC(Receiver operating -characteristic curve) is an analysis of statistics that determines the optimal specificity and sensitivity of a diagnostic test by plotting the association between sensitivity and 1-specificity.

## 3. RESULTS

The study included 150 participants, and the samples were of both gender and distributed equal in all groups .The general anthropometric of the participants are represented in Table 1. age were of the (PUV,PN and C ) groups ranging from (27 to 40) years. Also the individual's BMI (an anthropometric assessment based on their weight and height that aids in determining their obesity status) was showed in Table (1). A non-significant variance ($p > 0.05$) in (mean ± S.D) of age, height, weight and BMI between the three groups were indicated; the results showed that individuals were overweight (BMI>26) for the three groups.

**Table (1): The demographic characteristics of the three groups.**

| **Parameter** (Mean ± S.D ) | PUV group **(25men+25women)** | PN group **(25men+25women)** | C group **(25men+25women)** | P-value | | |
|---|---|---|---|---|---|---|
| | | | | Pa | Pb | Pc |
| **Age (years)** | 32.80 ± 3.90 | 32.23 ± 4.17 | 32.35 ± 4.16 | 0.827 | 0.996 | 0.778 |
| **Height (cm)** | 166.25 ±10.93 | 168.03 ±10.65 | 166.4 ± 8.89 | 0.998 | 0.757 | 0.717 |
| **Weight (Kg)** | 69.5 ± 8.08 | 73.15 ± 11.61 | 70.25 ± 9.55 | 0.938 | 0.389 | 0.227 |
| **BMI (Kg/m²)** | 26.13 ± 1.66 | 26.75 ± 1.65 | 26.35 ± 2.73 | 0.890 | 0.665 | 0.386 |

**(BMI): Body mass index, (Pa): indicated a significant difference between PUV and C, (Pb): indicated a significant difference between PN and C, and (Pc): indicated a significant difference between PUV and PN, P-value less than 0.05 (significant).**

Serum total peroxidases activity was determined in all the studied groups ,and the results are presented in Table (2), which revealed the presence of a high significant increase in the serum peroxidases activity in both patients groups (PUV and PN) in comparison to that of C group and significant increase of total peroxidases in PN over PUV . The results of MPO showed that there was a significant increase in MPO activity in patients groups (PUV and PN) compared with C group , as well as, significant increase in PN compared with PUV group .The oxidized proteins level was increased significantly in PUV and PN group, compared to C group. While, no significant was observed in PUV compared with PN group. The results of $Ca^{+2}$ was appeared a significant decrease for PUV group when compared with C, whilst, no significant changes were seen between the PN and C groups as well as PUV with PN.

**Table (2): Comparison of serum total peroxidases activity , Myeloperoxidase activity, oxidized protein and calcium in sera of the three studied groups.**

| **Parameter** (Mean ± S.D ) | PUV group | PN group | C group | P-value | | |
|---|---|---|---|---|---|---|
| | | | | Pa | Pb | Pc |
| **Total peroxidases activity U/L** | 22.86 ± 7.48 | 26.40 ±6.61 | 12.09 ± 5.29 | <0.001 | <0.001 | 0.044 |
| **MPO activity U/L** | 37.05 ±5.70 | 40.30 ± 6.16 | 27.66 ±3.34 | <0.001 | <0.001 | 0.017 |
| **Oxidized protein (µM)** | 20.38 ± 3.83 | 21.78 ± 3.64 | 7.44 ± 1.59 | <0.001 | <0.001 | 0.127 |
| **$Ca^{+2}$ (mg/dL)** | 6.03 ± 0.45 | 6.32 ± 0.77 | 6.39 ± 0.46 | 0.019 | 0.849 | 0.074 |

**(Pa): indicated a significant difference between PUV and C, (Pb): indicated a significant difference between PN and C, and (Pc): indicated a significant difference between PUV and PN, P-value less than 0.05 (significant).**

The correlation between peroxidases activity , MPO activity and other studied for both patients groups (PUV, and PN) were examined as summarized in Table 3.

**Table (3): Correlation between peroxidases activity and MPO activity with other studied parameters for the PUVand PN groups.**

| | Parameter | PUV Group | | PN Group | |
|---|---|---|---|---|---|
| | | peroxidases activity | MPO activity | peroxidases activity | MPO activity |
| **Age** | **r** | 0.177 | 0.178 | -0.157 | 0.001 |
| | **P-value** | 0.472 | 0.272 | 0.333 | 0.998 |
| **BMI** | **r** | 0.008 | -0.087 | 0.064 | 0.142 |
| | **P-value** | 0.962 | 0.593 | 0.693 | 0.384 |
| **peroxidases activity** | **r** | 1 | 0.023 | 1 | 0.254 |
| | **P-value** | | 0.886 | | 0.114 |
| **MPO activity** | **r** | 0.023 | 1 | 0.254 | 1 |
| | **P-value** | 0.886 | | 0.114 | |
| **Oxidized protein** | **r** | 0.143 | -0.06 | -0.197 | 0.07 |
| | **P-value** | 0.378 | 0.715 | 0.224 | 0.666 |
| **$Ca^{+2}$** | **r** | 0.357 | -0.108 | -0.068 | -0.077 |
| | **P-value** | 0.024 | 0.508 | 0.676 | 0.636 |

**P-value less than 0.05 (significant).**

Peroxidases activity showed a medium positive correlation with $Ca^{+2}$in the PUV group with P-value (0.024) .

A diagnostic test's optimal specificity and sensitivity are determined via statistical analysis Receiver Operating Characteristic Chart (ROC), which plots the association between sensitivity and 1-specificity. The following Figure (1and 2) , Table( 3 and 4) represents ROC curve of total peroxidases activity & MPO activity in PUV and PN.

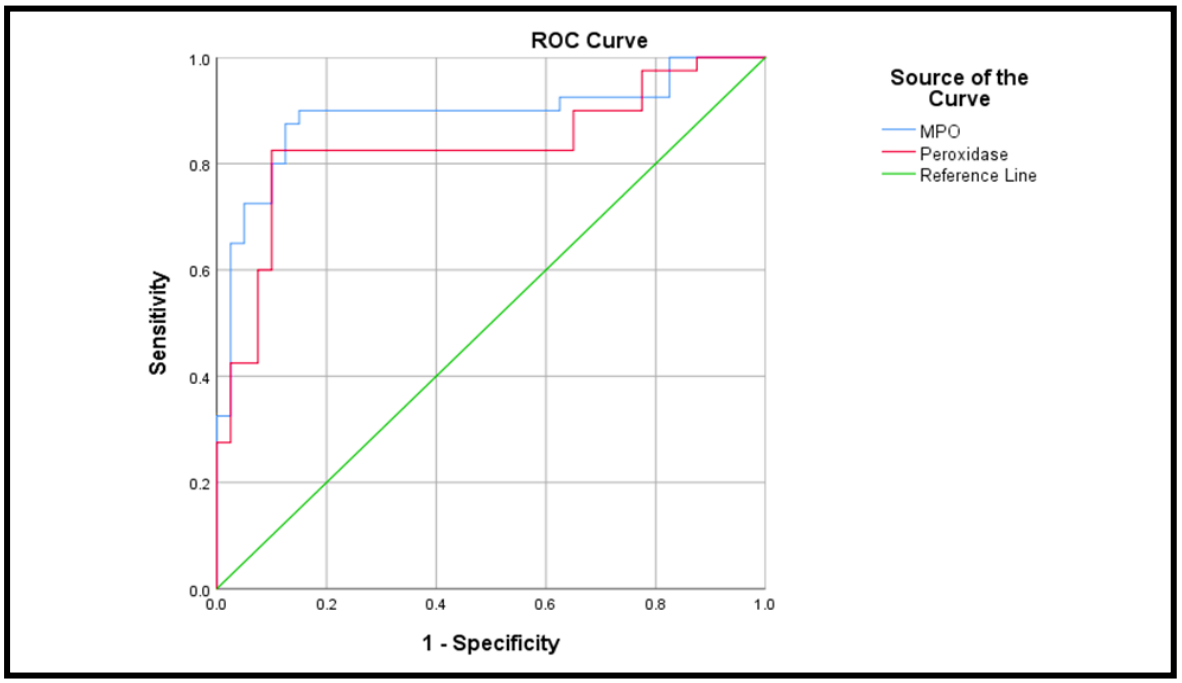


**Figure 1: ROC curve of total peroxidases activity & Myeloperoxidase activity in PUV and control groups .**

**Table( 3) : Measurements of AUC, P-value, Lower Bound , Upper Bound , Cut-Off Value, Sensitivity and Specificity for total peroxidases activity & Myeloperoxidase activity in PUV and control groups .**

| parameter | AUC | SE | P-value | Asymptotic 95% Cl | | Cut-Off Value | Sensitivity | Specificity |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | | |
| **peroxidase activity** | 0.832 | 0.049 | <0.001 | 0.736 | 0.928 | 14.81 | 82.5% | 90.0% |
| **MPO activity** | 0.890 | 0.041 | <0.001 | 0.811 | 0.969 | 30.34 | 80.0% | 87.5% |

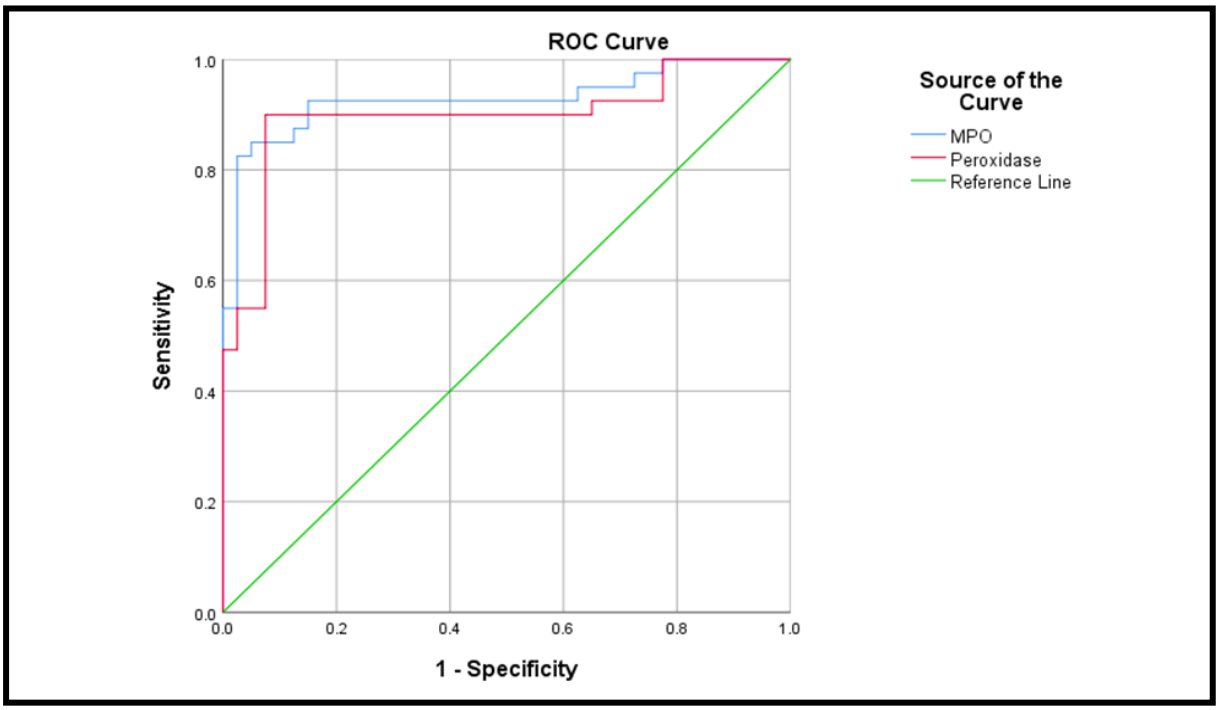


**Figure 2: ROC curve of total peroxidases activity & Myeloperoxidase activity in PN and control groups .**

**Table ( 4) : Measurements of AUC, P-value, Lower Bound , Upper Bound , Cut-Off Value, Sensitivity and Specificity for total peroxidases activity & Myeloperoxidase activity in PN and control groups .**

| parameter | AUC | SE | P-value | Asymptotic 95% Cl | | Cut-Off Value | Sensitivity | Specificity |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | | |
| **peroxidase activity** | 0.898 | 0.039 | <0.001 | 0.821 | 0.974 | 17.94 | 90.0% | 90.0% |
| **MPO activity** | 0.928 | 0.032 | <0.001 | 0.865 | 0.991 | 30.86 | 85.0% | 90.0% |

## 4. DISCUSSION

Vitiligo is defined as an autoimmune disease distinguish by loses color in additional to patches of several shapes driven by the caused by the skin's melanocytes being destroyed or ceasing to function, therefore, appearance of white spots with variable colors on areas of the body, including hair [1]. Melanocytes of vitiligo patients are greater susceptible to oxidative

damage. The presence of a genetic background that is susceptible combined with additional environmental stress results in extensive changes to the antioxidant system [2].Meanwhile , oxidative stress (OS) is considered to be one of the possible pathogenic events in melanocyte loss [16]. Vitiligo vulgaris has been reported to be more prevalent in women, however, greater response to the treatment in men has been observed, in spite of this women exhibited better quality of life and a lesser degree of stress than men vitiligo individuals [2] . When conducting statistical analysis between women and men, we did not observe any statistically significant differences. The results of Khudhair and coworkers appeared that no difference in the age of patients compared with control, confirming our findings [17]. This is because a particular age group was the focus of the study, and it was fixed for all groups. The age (mean ± S.D) of PUV, PN and control groups were (32.80 ± 3.90), (32.23 ± 4.17), and (32.35 ± 4.16) years, respectively. According to Table 1 of the current study, there was not a significant difference in the BMI of the patients and controls as well all participants in this wok were overweight. A study by Dragoni et al. found no significant association between obesity and vitiligo. However, the study noted that obese patients were more likely to develop itchy skin patches[18].The results that presented in Table (2) revealed the presence of high significant increases in the serum peroxidases activity in both patients groups (PUV and PN) in comparison to that of C group and a significant increase of total peroxidases in PUV over PN . During the OS, an imbalance between the production and degradation of reactive oxygen species (ROS) occurs. Peroxidases are widespread enzymes perform an important role as antioxidants or starters for oxidative reactions by generating ROS. The capacity of human peroxidases to generate cytotoxic hypohalous (HOX, X= $Cl^-$, $Br^-$, and $I^-$) and hypothiocyanous (HOSCN) acids account for their antimicrobial actions[9].However, the overproduction of these highly reactive oxidants due to unfavorable excessive peroxidases activity causes direct oxidation of the lipids, proteins and other macromolecules leading to OS [19]. On the other hand, this increasing in the peroxidases activity might be due to the (MPO), or other heme peroxidases, which may in turn increases the oxidant that leads to OS. All heme peroxidases have the ability to react with $H_2O_2$ to form an oxidized enzyme intermediate that subsequently oxidizes a substrate [20]. One of the major sources of ROS is NADPH oxidase family. Superoxide radicals ($O_2^{\bullet-}$) are produced by enzymes and are the precursor to many ROS. Thus, ($O_2^{\bullet-}$) can be spontaneously or catalytically (by Superoxide dismutase SOD) converted into hydrogen peroxide, the main substrate which peroxidases act on , and is one of the major oxidants, *in vivo*. Under cellular disorders, relatively high levels of $H_2O_2$ can be generated, fueling peroxidases activity [21].Melanocytes responsible for the pigment production are especially fragile when exposed to the high concentration of ROS. Hydrogen peroxide ($H_2O_2$) is especially damaging to melanocytes [22].the melanocytes do not undergo the mitophagy process and continue melanin production even if the mitochondria are damaged by high $H_2O_2$ concentration. It is found that melanocytes' mitophagy is also inhibited by the high activity of the P53 factor stimulated by the high production of $H_2O_2$. Damaged mitochondria produce excessive ROS, causing OS, until the well-known apoptosis process is induced [23]. It has been found that overproduction of ROS in melanocytes precedes an immune response in the form of increased levels of $H_2O_2$. The immune response following overproduction of ROS in melanocytes is an increased infiltration of T cells into the epidermis and a sharp rise in $H_2O_2$ concentrations [24]. Ultimately, vitiligo is a lesion resulting from the accumulation of $H_2O_2$, which is the substrate for peroxidase [25]. The results of MPO activity showed that there was a significant increase in patients groups (PUV and PN) compared with C group. Myloperoxidase (MPO) is an essential enzyme of the innate immune defense system. In neutrophils, MPO promotes the generation of anti-microbicidal reactive intermediates. Because of the strong oxidant potential of the side products, MPO is potentially harmful for host cells [26]; high MPO activity in aggressive neutrophils is associated with damage to healthy host cells. The cytotoxicity of MPO is not limited to bacterial cells. The enzyme can also promote the destruction of foreign and healthy host eukaryotic non-phagocytic cells by inducing the excessive generation of reactive halogenating species [27]. Advanced activation of neutrophils, however, causes spillover of MPO and other cellular components from broken cells to the environment. This, in turn, can decimate melanocytes and in this way trigger depigmentation (He et al., 2022). Vitiligo is associated with an increased autoimmune response, where immune cells (such as CD8+ T cells) attack melanocytes [28]. This immune stimulation leads to the activation of neutrophils and macrophages, which increases the secretion of MPO and other oxidants that contribute to melanocyte damage [29]. As mentioned previously in vitiligo patients, there is an imbalance between antioxidants and free radicals, leading to the accumulation of ROS, such as $H_2O_2$ [30]. $H_2O_2$ and other oxidants cause damage to DNA, proteins, and lipids in melanocytes, leading to their death or impaired function. Production of $H_2O_2$, which is a good substrate for the MPO to form (HOCl) [12]. Myeloperoxidase (MPO), one of the heme enzymes secreted by activated neutrophils during an inflammatory response, is responsible for converting $H_2O_2$ to hypochlorous acid (HOCl), a potent oxidizing agent. HOCl reacts with the amino acid tyrosine, oxidizing it to tyrosyl radicals, which are highly reactive and covalently bond with other cellular proteins, leading to the formation of abnormal bonds such as dityrosine. These structural modifications contribute to functional damage to proteins within cells[31] .Tyrosine oxidation and the subsequent formation of harmful compounds impair the effectiveness of the biological pathways responsible for melanin synthesis, leading to melanosome dysfunction and skin pigmentation disorders[32]. Studies have shown that HOCl has a high capacity to oxidize proteins, lipids, and DNA, causing direct damage to sensitive cell organelles, especially melanosomes, the organelles responsible for the synthesis, storage, and transport of melanin within melanocytes. Melanosome damage resulting from oxidation of their components, such as lipid membranes or the enzyme tyrosinase within them, leads to a disruption of the pigmentation process and impairs the melanocyte's ability to produce melanin normally. Furthermore, the accumulation of HOCl within the melanocyte environment may trigger a series of oxidative and inflammatory responses, promoting melanocyte death by

oxidative stress in vitiligo[28, 33]. OS has been implicated in the pathogenesis of vitiligo, and therapeutic interventions that address free radical reduction and antioxidant support could potentially bring about a greater improvement in repigmentation rates in this chronic condition. Via these modalities, phototherapy possibly plays a modulatory role in ROS production and improves the antioxidant defense system. Phototherapy works through light activation of tryptophan within the cell and a cascade of cellular pathways involving the formation of oxygen-derived species (ODS). As a result, the cellular metabolism is increased, and the DNA repair mechanisms are activated, which reduces ROS production, a possible explanation of how phototherapy can decrease the oxidative damage in patients with active vitiligo [34, 35]. Meanwhile the photomodulation obtained with the use of the main types of phototherapy, such as narrowband ultraviolet B radiation (NB-UVB), influences these mechanisms in such a way that ROS levels are significantly reduced after sessions of these treatments [36]. After phototherapy, the levels of $H_2O_2$ in lesions decrease significantly in the seventeen weeks of follow-up [37]. This explains the decreased activity of total peroxidase and MPO in PUV compared with PN group. The oxidized protein is one of the most common indicators of protein oxidation and results from the attack of free radicals on protein chains, leading to direct oxidation of amino acids such as cysteine, methionine, tyrosine and tryptophan, which changes their biological function and thus damages the tertiary structure of proteins, which disrupts their biological function [38]. Likewise it's level was increased significantly in PUV and PN group, compared to control group. When exposed to high OS, the level of oxidized proteins increases significantly due to the increase in ROS that lead to the modification of amino acids in proteins through the formation of protein carbonyls [39]. No significant was observed in PUV compared with PN group. Phototherapy may reduce the production of free radicals, but it does not have a direct mechanism to repair previously damaged proteins. Calcium ($Ca^{+2}$) is a mineral essential for skin health. It helps regulate skin cell growth and repair, and imbalances in its levels are linked to the development of certain skin diseases[40].The results appeared to show a significant decrease in $Ca^{+2}$ of PUV when compared with control, whilst, no significant changes were seen between the PN and Control groups as well as PUV with PN. Oxidative stress leads to the excessive production of ROS in melanocytes.This excessive production can disrupt mitochondrial function, affecting intracellular calcium balance [41].This leading to increased $Ca^{+2}$ entry into the cells and attempts to repair oxidative damage, thus decreasing blood levels. Vitiligo patients suffer from a deficiency of vitamin D, which is essential for regulating $Ca^{+2}$ levels [42]. Phototherapy stimulates the production of vitamin D, which leads to the absorption of $Ca^{+2}$ from the intestine, stimulates its storage in the bones, and reduces its levels in the blood [43]. A study by Takci et al. found that $Ca^{+2}$ levels were lower in vitiligo patients compared to the control group [42]. Also, a study in Egypt by Hussein et al, found that $Ca^{+2}$ levels were significantly decrease in vitiligo patients compared to the control group [44]. While a study in Iran by Mogaddam et al. found that $Ca^{+2}$ levels were no significant difference in vitiligo patients compared to the control group[45]. The correlation results in Table 3 indicate medium positive correlation peroxidases activity with $Ca^{+2}$ level in the PUV group. When exposed to OS resulting from the accumulation of $H_2O_2$, cells stimulate calcium-dependent signaling pathways, leading to $Ca^{+2}$ entering the cell to activate damage-repair mechanisms or stimulate the production of endogenous antioxidants. Conversely, an increase in the activity of the peroxidase enzyme, which decomposes $H_2O_2$, is observed as a protective response to reduce oxidative damage to pigment cells[21, 42]. It is believed, according to the data, that this simultaneous interaction between low calcium and high peroxidase with phototherapy reflects the cell's efforts to repair oxidative damage. The Roc curve's findings demonstrated the diagnostic susceptibility of peroxidase activity and MPO activity to PUV and PN. In PUV Patients Roc cut off point for peroxidase activity and MPO activity was calculated . Peroxidase activity shown cut off point was (14.81) and the AUC was (0.832). The best cut-off point shows a sensitivity of 82.5% and specificity of 90.0%. MPO activity shown cut off point was (30.34) and the AUC was (0.890) ,The best cut-off point shows a sensitivity of 80.0% and specificity of 87.5%.In PN Patients peroxidase activity shown the cut off point was (17.94) and the AUC was (0.898). The best cut-off point shows a sensitivity of 90.0% and specificity of 90.0%. MPO activity shown the cut off point was (30.86) and the AUC was (0.928).The best cut-off point shows a sensitivity of 85.0% and specificity of 90.0%. ROC curve results, peroxidase activity and MPO activity may help diagnose PUV and PN. Note that if the area under the curve is less than 0.5, this test is not used in diagnosing the disease.

## 5. CONCLUSION

Development of the chronic skin disorder (vitiligo ) is linked with complex mechanisms including oxidative stress. Meanwhile , phototherapy impact on some biomarkers associated with oxidative stress was evaluated by measuring peroxidases and myeloperoxidase activities, as well as the levels of oxidized protein and calcium for newly diagnosed (PN) and receiving phototherapy(PUV) Iraqi vitiligo patients and also for apperently control (C) person . The results showed significant increasing in antioxidant enzymes activities and oxidized protein for PN and PUV compared to the C . Like wise a decrease in calcium level was observed in PUV compared with C.The comparison between PUV and PN indicated clear decreases in two enzymes activities with no significant difference in oxidized protein and calcium levels. These results indicated changes in peroxidaaes and myloperoxidases activities in vitiligo improving the oxidative stress mechanism of this disease . On the other hand the positive effect of phototherapy was proved by decreasing the oxidative status in these patients and thus reducing the need for peroxidase and myeloperoxidase activity.

**Ethical Clearance**

The Iraqi ministries of the environment, health, higher education, and scientific research have approved the Research Ethical Committee for scientific research(Ref :CSEC/1224/0123).

**Acknowledgments**

The authors would like to extend their sincere gratitude to the entire team of Al-Imamain Al-Kadhimiya Teaching Hospital in Iraq and the Dermatology and Venereology Department of the Baghdad Teaching Hospital in the Medical City Complex. Additionally, the authors would like to express their gratitude to every participant who gave blood samples and information for this study.

## REFERENCES

[1] Mokhtari M, Bardhi R, Hamzavi I. A Canvas of Contemporary Insights into Clinical Vitiligo. Dermatologic clinics. 2025;43(1):67-76.

[2] Singh M, Mansuri MS, Kadam A, Palit SP, Dwivedi M, Laddha NC, et al. Tumor Necrosis Factor-alpha affects melanocyte survival and melanin synthesis via multiple pathways in vitiligo. Cytokine. 2021;140:155432.

[3] Al-Issa YA-H, Al-Hamamy HR, Hasan HR. Evaluation the effect of NB–UVB phototherapy on thyroid function in sera of vitiligo patients. Iraqi National Journal of Chemistry. 2015;15(2):97-102.

[4] Abdalla MA. The vitiligo is the human skin's pigmentary challenge still: an up-to-date review. Проблемы социальной гигиены, здравоохранения и истории медицины. 2024;32(5):985.

[5] Zainulabdeen JA, Al-kinani AA. New Modified Method for Determination of Nitric oxide Synthase Activity in Plasma of Vitiligo Patients. Oriental Journal of Chemistry. 2018;34(5):2502.

[6] Al-Tuama JA. Lack of association between PTPN22 1858 C> T gene polymorphism and susceptibility to generalized vitiligo in a Iraqi population. Iraqi journal of biotechnology. 2022;21(1.(

[7] Tariq SF, Hussein TA. Study of the Immunological Status of Iraqi Vitiligo Patients. Baghdad Science Journal. 2016;13(3):0454.-

[8] Chang W-L, Ko C-H. The role of oxidative stress in vitiligo: an update on its pathogenesis and therapeutic implications. Cells. 2023;12(6):936.

[9] Bhandari Y, Sajwan H, Pandita P, Koteswara Rao V. Chloroperoxidase applications in chemical synthesis of industrial relevance. Biocatalysis and Biotransformation. 2023;41(6):403-20.

[10] Krewall JR. Impacts of the oxidizable scaffold of catalase-peroxidase (KatG): Modulation of a heme peroxidase for catalytic versatility: Auburn University; 2021.

[11] Khan AA, Alsahli MA, Rahmani AH. Myeloperoxidase as an active disease biomarker: recent biochemical and pathological perspectives. Medical sciences. 2018;6(2):33.

[12] Lin W, Chen H, Chen X, Guo C. The roles of neutrophil-derived myeloperoxidase (MPO) in diseases: the new progress. Antioxidants. 2024;13(1):132.

[13] Song H-Y, Yao J-H, Liu J-Z, Zhou S-J, Xiong Y-H, Ji L-N. Effects of phthalic anhydride modification on horseradish peroxidase stability and structure. Enzyme and microbial technology. 2005;36(4):605-11.

[14] Klebanoff SJ, Clark RA. The neutrophil: function and clinical disorders. (No Title). 1978.

[15] Levine RL, Wehr N, Williams JA, Stadtman ER, Shacter E. Determination of carbonyl groups in oxidized proteins. Stress response: methods and protocols. 2000:15-24.

[16] Xuan Y, Yang Y, Xiang L, Zhang C. The role of oxidative stress in the pathogenesis of vitiligo: a culprit for melanocyte death. Oxidative medicine and cellular longevity. 2022;2022(1):8498472.

[17] Khudhair OS, Gorial FI, Hameed AF. Fibromyalgia syndrome and vitiligo: A novel association. Archives of Rheumatology. 2017;33(2):174.

[18] Dragoni F, Conti R, Cazzaniga S, Colucci R, Pisaneschi L, Naldi L, et al. No association between vitiligo and obesity: a case-control study. Medical Principles and Practice. 2017;26(5):421-6.

[19] Hajam YA, Rani R, Ganie SY, Sheikh TA, Javaid D, Qadri SS, et al. Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. Cells. 2022;11(3):552.

[20] Shen C, Wang Y. Recent progress on peroxidase modification and application. Applied Biochemistry and Biotechnology. 2024:1-25.

[21] Ahmad R, Surguchov A. Reactive oxygen species: advances and developments: BoD–Books on Demand; 2024.

[22] Chen J, Liu Y, Zhao Z, Qiu J. Oxidative stress in the skin: Impact and related protection. International Journal of Cosmetic Science. 2021;43(5):495-509.

[23] Chauhan K, Goel F, Singh S. Apigenin protects melanocytes and improve tyrosinase activity in a hydroquinone induced vitiligo mouse model targeting P38 MAP kinase signaling: histopathology and immunohistochemistry analysis. Naunyn-Schmiedeberg's Archives of Pharmacology. 2024;397(7):4859-69.

[24] Emanuelli M, Sartini D, Molinelli E, Campagna R, Pozzi V, Salvolini E, et al. The double-edged sword of oxidative stress in skin damage and melanoma: From physiopathology to therapeutical approaches. Antioxidants. 2022;11(4):612.

[25] Schallreuter KU, Elwary SM, Gibbons NC, Rokos H, Wood JM. Activation/deactivation of acetylcholinesterase by H2O2: more evidence for oxidative stress in vitiligo. Biochemical and Biophysical Research Communications. 2004;315(2):502-8.

[26] Rawat K, Syeda S, Shrivastava A. Neutrophil-derived granule cargoes: paving the way for tumor growth and progression. Cancer and Metastasis Reviews. 2021;40(1):221-44.

[27] Aroca-Crevillén A, Vicanolo T, Ovadia S, Hidalgo A. Neutrophils in physiology and pathology. Annual Review of Pathology: Mechanisms of Disease. 2024;19(1):227-59.

[28] Chen J, Li S, Li C. Mechanisms of melanocyte death in vitiligo. Medicinal Research Reviews. 2021;41(2):1138-66.

[29] Ali H, Haribabu B, Richardson RM, Snyderman R. Mechanisms of inflammation and leukocyte activation. Medical Clinics. 1997;81(1):1-28.

[30] Jaffri JM. Reactive oxygen species and antioxidant system in selected skin disorders. The Malaysian journal of medical sciences: MJMS. 2023;30(1):7.

[31] Heinecke JW, Li W, Francis GA, Goldstein JA. Tyrosyl radical generated by myeloperoxidase catalyzes the oxidative cross-linking of proteins. The Journal of clinical investigation. 1993;91(6):2866-72.

[32] Sasaki M, Kondo M, Sato K, Umeda M, Kawabata K, Takahashi Y, et al. Rhododendrol, a depigmentation-inducing phenolic compound, exerts melanocyte cytotoxicity via a tyrosinase-dependent mechanism. Pigment Cell & Melanoma Research. 2014;27(5):754-63.

[33] Denat L, Kadekaro AL, Marrot L, Leachman SA, Abdel-Malek ZA. Melanocytes as instigators and victims of oxidative stress. Journal of Investigative Dermatology. 2014;134(6):1512-8.

[34] Białczyk A, Wełniak A, Kamińska B, Czajkowski R. Oxidative stress and potential antioxidant therapies in vitiligo: a narrative review. Molecular diagnosis & therapy. 2023;27(6):723-39.

[35] Bouceiro Mendes R, Alpalhão M, Filipe P. UVB phototherapy in the treatment of vitiligo: State of the art and clinical perspectives. Photodermatology, photoimmunology & photomedicine. 2022;38(3):215-23.

[36] Guarneri F, Bertino L, Pioggia G, Casciaro M, Gangemi S. Therapies with antioxidant potential in psoriasis, vitiligo, and lichen planus. Antioxidants. 2021;10(7):1087.

[37] Dell'Anna M, Mastrofrancesco A, Sala R ,Venturini M, Ottaviani M, Vidolin A, et al. Antioxidants and narrow band-UVB in the treatment of vitiligo: a double-blind placebo controlled trial. Clinical and experimental dermatology. 2007;32(6):631-6.

[38] Chakravarti B, Chakravarti DN. Oxidative modification of proteins: age-related changes. Gerontology. 2007;53(3):128-39.

[39] Stadtman ER, Levine RL. Protein oxidation. Annals of the New York Academy of Sciences. 2000;899(1):191-208.

[40] Haftek M, Abdayem R, Guyonnet-Debersac P. Skin minerals: key roles of inorganic elements in skin physiological functions. International Journal of Molecular Sciences. 2022;23(11):6267.

[41] de França E, Dos Santos RV, Baptista LC, Da Silva MA, Fukushima AR, Hirota VB, et al. Potential role of chronic physical exercise as a treatment in the development of vitiligo. Frontiers in Physiology. 2022;13:843784.

[42] Takci Z, Tekin Ö, ERTUĞRUL DT, Karadağ AS, Akin KO. A case-control study: evaluation of vitamin D metabolism in patients with vitiligo. Turkish Journal of Medical Sciences. 2015;45(4):837-41.

[43] Juzeniene A, Grigalavicius M, Juraleviciute M, Grant WB. Phototherapy and vitamin D. Clinics in dermatology. 2016;34(5):548-55.

[44] Hussein S, Aly HF, Mohamed N, Youness ER. Estimation Of Serum Levels Of Vitamin D, Calcium , Phosphorous, Matrix Metalloproteinases 1, 2 And Tissue Inhibitor Matrix Metalloproteinase-1 In Patients With Acrofacial Vitiligo During COVID-19. Egyptian Journal of Chemistry. 2022;65(2):369-75.

[45] Mogaddam MR, Ardabili NS, Maleki N, Chinifroush MM, Fard EM. Evaluation of the serum zinc level in patients with vitiligo. Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii. 2017;34(2):116-9