



Original Article

EVALUATION OF OXIDATIVE STRESS LEVELS AS BIOMARKERS IN VITILIGO PATIENTS

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ABSTRACT

BACKGROUND: Vitiligo is an acquired depigmenting disorder caused by selective loss of melanocytes. Oxidative stress is believed to play a critical role in melanocyte destruction, yet its utility as a biomarker for disease activity remains underexplored. This study aimed to evaluate oxidative stress levels in vitiligo patients and assess their potential as biomarkers of disease severity.

MATERIALS AND METHODS: A hospital-based case-control study was conducted including 50 clinically diagnosed vitiligo patients and 50 age- and sex-matched healthy controls. Serum levels of malondialdehyde (MDA) and antioxidant enzymes-superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)-were measured using standard biochemical methods. Disease severity was assessed using the Vitiligo Area Scoring Index (VASI). Statistical analysis included Student's t-test, one-way ANOVA, and Pearson's correlation; $p < 0.05$ was considered significant.

RESULTS: Vitiligo patients exhibited significantly higher MDA levels compared to controls (4.85 ± 1.12 vs 2.92 ± 0.87 nmol/ml, $p < 0.001$) and lower activities of SOD (1.85 ± 0.56 vs 2.75 ± 0.62 U/ml, $p < 0.001$), CAT (32.4 ± 8.2 vs 46.1 ± 7.6 U/mg Hb, $p < 0.001$), and GPx (21.8 ± 5.4 vs 29.6 ± 6.1 U/ml, $p < 0.001$). Patients with generalized or active vitiligo had more pronounced oxidative stress imbalance. MDA positively correlated with disease duration ($r = 0.46$, $p = 0.002$) and VASI score ($r = 0.41$, $p = 0.004$), while SOD, CAT, and GPx negatively correlated with disease severity.

CONCLUSION: Elevated oxidative stress and reduced antioxidant enzyme activities are evident in vitiligo, particularly in generalized and active disease. Oxidative stress markers may serve as biochemical biomarkers for disease activity and severity, providing potential guidance for monitoring and antioxidant-based therapeutic interventions.

Keywords: Vitiligo, Oxidative Stress, Malondialdehyde, Superoxide Dismutase, Catalase, Glutathione Peroxidase, Biomarkers.

INTRODUCTION:

Vitiligo is a common, acquired, chronic depigmenting disorder of the skin and mucous membranes, characterized by selective loss of functional melanocytes, resulting in well-demarcated depigmented macules and patches. The global prevalence of vitiligo is estimated to be around 0.5–2%, with no predilection for sex or race, though higher prevalence is reported in certain geographic and ethnic populations [1,2]. The disease often begins before the age of 20 years in nearly half of the patients, and has profound psychosocial implications, especially in darker-skinned individuals where the contrast is more visible [3].

The pathogenesis of vitiligo is complex and multifactorial. Several hypotheses have been proposed, including autoimmune destruction of melanocytes, genetic predisposition, neural dysregulation, and oxidative stress [4]. Among these, **oxidative stress is considered a key initiating factor**, which may also act in concert with autoimmune mechanisms. Oxidative stress results from an imbalance between excessive production of reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂), superoxide radicals, hydroxyl radicals, and impaired antioxidant defense mechanisms [5,6]. Excessive ROS accumulation damages melanocyte DNA, proteins, and lipid membranes, ultimately triggering melanocyte apoptosis and autoimmune recognition [7].

Several studies have demonstrated elevated levels of lipid peroxidation products, particularly **malondialdehyde (MDA)**, in the blood and skin of vitiligo patients, suggesting enhanced oxidative damage [8,9]. At the same time, activities of key antioxidant enzymes, including **superoxide dismutase (SOD)**, **catalase (CAT)**, and **glutathione peroxidase (GPx)**, have been found to be decreased in many patients [10,11]. SOD normally catalyzes the conversion of superoxide radicals into hydrogen peroxide, while catalase decomposes hydrogen peroxide into water and oxygen, thus protecting cells from ROS-mediated injury. Deficiency of these enzymes leads to accumulation of toxic ROS within melanocytes [12].

This biochemical evidence supports the hypothesis that oxidative stress plays a pivotal role not only in melanocyte destruction but also in disease progression and activity. Furthermore, the extent of oxidative stress imbalance may serve as a **potential biomarker of disease severity and prognosis**. Identifying such biomarkers is important because they can aid in monitoring disease activity, predicting progression, and evaluating responses to therapeutic interventions, particularly antioxidant-based treatments [13,14].

Therefore, the present study was designed to evaluate oxidative stress markers—MDA, SOD, CAT, and GPx in patients with vitiligo and compare them with healthy controls.

MATERIALS AND METHODS:

Study Design and Setting

This was a hospital-based, case-control study carried out in the Department of Dermatology in collaboration with the Department of Biochemistry at a tertiary care teaching hospital over a period of one year after obtaining clearance from the Institutional Ethics Committee. Written informed consent was obtained from all participants before enrolment.

Study Population

- **Cases:** A total of 50 clinically diagnosed vitiligo patients were recruited from the outpatient dermatology clinic. Diagnosis was established based on typical clinical features including depigmented macules with well-defined margins, confirmed by Wood's lamp examination. Both stable and active cases of vitiligo were included.
- **Controls:** 50 age- and sex-matched healthy individuals without vitiligo or other dermatological/systemic disorders were recruited from hospital staff and patient attendants.

Inclusion Criteria

- Age $\geq$ 18 years.
- Newly diagnosed or previously diagnosed vitiligo patients who were not on any systemic therapy in the past 3 months.
- Both localized and generalized vitiligo cases.
- Controls free from dermatological, systemic, or autoimmune disorders.

Exclusion Criteria

- Patients with systemic illnesses known to influence oxidative stress (diabetes mellitus, chronic liver or renal disease, cardiovascular disease, malignancy).
- Patients on antioxidant supplements, corticosteroids, or immunosuppressive drugs within the last 3 months.
- Pregnant and lactating women.
- Smokers and chronic alcoholics.

Clinical Data Collection

Detailed demographic and clinical data were recorded using a structured proforma, including:

- Age, sex, duration of disease, family history of vitiligo, type of vitiligo (localized, generalized, segmental).
- **Disease severity** was assessed using the **Vitiligo Area Scoring Index (VASI)**, which measures depigmentation as a percentage of body surface area.
- **Disease activity** was graded based on patient history of new lesions, enlargement of existing lesions, or stability over the past 6 months.

Blood Sample Collection

- **Sample volume:** 5 ml of venous blood was collected from each participant after overnight fasting.

- **Processing:** Samples were collected in plain vacutainers. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until biochemical analysis.
- **Precautions:** All procedures were performed under aseptic conditions, and hemolyzed samples were excluded.

Biochemical Assays

All assays were performed in the Department of Biochemistry using standardized methods:

1. **Malondialdehyde (MDA):**
 - Measured as thiobarbituric acid reactive substances (TBARS) following the method of Ohkawa et al [15].
 - Results expressed in **nmol/ml of serum**.
2. **Superoxide Dismutase (SOD):**
 - Estimated using the Marklund and Marklund method [16], based on inhibition of pyrogallol auto-oxidation.
 - Results expressed in **U/ml of serum**.
3. **Catalase (CAT):**
 - Measured by the Aebi method, [17] using the rate of decomposition of hydrogen peroxide (H_2O_2) at 240 nm.
 - Results expressed in **U/mg of hemoglobin (Hb)**.
4. **Glutathione Peroxidase (GPx):**
 - Determined by the Paglia and Valentine method [18], based on oxidation of NADPH in the presence of glutathione reductase.
 - Results expressed in **U/ml of serum**.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 20. Continuous variables were expressed as mean $\pm$ standard deviation (SD) and categorical variables as **frequencies and percentages**.

- **Independent Student's t-test** was used for comparison between cases and controls.
- **One-way ANOVA** was applied to compare biomarkers across subgroups (localized vs generalized vitiligo; active vs stable disease).
- **Pearson's correlation coefficient (r)** was used to analyze correlations between oxidative stress markers and clinical parameters (disease duration, VASI score).
- A p-value < 0.05 was considered statistically significant.

RESULTS:

A total of 100 participants were enrolled, comprising 50 clinically diagnosed vitiligo patients (cases) and 50 age- and sex-matched healthy controls.

Demographic Profile

The mean age of patients (34.8 ± 10.2 years) was comparable to that of controls (33.6 ± 9.8 years), with no statistically significant difference (**Independent Student's t-test, $p = 0.58$**). Similarly, the male-to-female distribution did not differ significantly between the groups (**Chi-square test, $p = 0.84$**). The mean disease duration in cases was 5.6 ± 3.2 years, and 18% of patients reported a family history of vitiligo (Table 1).

Table 1. Demographic Profile of Study Participants

Variable	Cases (n=50)	Controls (n=50)	p-value
Age (years, mean $\pm$ SD)	34.8 ± 10.2	33.6 ± 9.8	0.58
Sex (M/F)	27 / 23	26 / 24	0.84
Duration of disease (years)	5.6 ± 3.2	—	—
Family history (%)	18%	—	—

Oxidative Stress Markers in Cases and Controls

Comparison of oxidative stress parameters revealed:

- **MDA levels** were significantly higher in cases (4.85 ± 1.12 nmol/ml) than in controls (2.92 ± 0.87 nmol/ml) (**Independent Student's t-test, $p < 0.001$**).
- **SOD activity** was significantly reduced in cases (1.85 ± 0.56 U/ml) compared to controls (2.75 ± 0.62 U/ml) (**Independent Student's t-test, $p < 0.001$**).
- **Catalase activity** was also lower in cases (32.4 ± 8.2 U/mg Hb) compared to controls (46.1 ± 7.6 U/mg Hb) (**Independent Student's t-test, $p < 0.001$**).
- **GPx activity** was reduced in cases (21.8 ± 5.4 U/ml) compared to controls (29.6 ± 6.1 U/ml) (**Independent Student's t-test, $p < 0.001$**).

These findings confirm a significant oxidative stress imbalance in vitiligo patients (Table 2).

Table 2. Oxidative Stress Markers in Cases and Controls

Parameter	Cases (Mean $\pm$ SD)	Controls (Mean $\pm$ SD)	p-value
MDA (nmol/ml)	4.85 $\pm$ 1.12	2.92 $\pm$ 0.87	<0.001
SOD (U/ml)	1.85 $\pm$ 0.56	2.75 $\pm$ 0.62	<0.001
Catalase (U/mg Hb)	32.4 $\pm$ 8.2	46.1 $\pm$ 7.6	<0.001
GPx (U/ml)	21.8 $\pm$ 5.4	29.6 $\pm$ 6.1	<0.001

Subgroup Analysis

When vitiligo patients were subgrouped:

- Those with **generalized vitiligo** had significantly higher MDA levels and lower antioxidant enzyme activities compared to localized vitiligo (**One-way ANOVA with post-hoc Tukey test, $p < 0.05$**).
- Patients with **active disease** had significantly higher MDA and lower SOD, CAT, and GPx levels compared to stable vitiligo (**Independent Student's t-test, $p < 0.05$**).

This demonstrates that oxidative stress correlates with both **disease type and activity** (Table 3).

Table 3: Subgroup Analysis of Oxidative Stress Markers in Vitiligo Patients

Parameter	Localized Vitiligo (n=22)	Generalized Vitiligo (n=28)	p-value (t-test/ANOVA)	Stable Disease (n=24)	Active Disease (n=26)	p-value (t-test)
MDA (nmol/ml)	4.32 $\pm$ 0.98	5.28 $\pm$ 1.06	0.002*	4.36 $\pm$ 0.91	5.31 $\pm$ 1.12	0.001*
SOD (U/ml)	2.05 $\pm$ 0.42	1.69 $\pm$ 0.48	0.004*	2.01 $\pm$ 0.44	1.71 $\pm$ 0.46	0.006*
Catalase (U/mg Hb)	34.9 $\pm$ 7.2	30.4 $\pm$ 8.5	0.03*	35.1 $\pm$ 6.9	30.6 $\pm$ 7.8	0.02*
GPx (U/ml)	23.7 $\pm$ 5.1	20.2 $\pm$ 5.6	0.01*	23.3 $\pm$ 5.3	20.1 $\pm$ 5.2	0.01*

Correlation of Biomarkers with Clinical Parameters

Correlation analysis was done using **Pearson's correlation coefficient (r)**:

- MDA showed a positive correlation with disease duration (**$r = 0.46$, $p = 0.002$**) and VASI score (**$r = 0.41$, $p = 0.004$**).
- SOD correlated negatively with VASI score (**$r = -0.44$, $p = 0.003$**).
- Catalase (**$r = -0.39$, $p = 0.01$**) and GPx (**$r = -0.33$, $p = 0.02$**) also showed significant negative correlations with disease severity.

These findings indicate that oxidative stress markers may serve as useful **biomarkers of disease progression and activity** (Table 4)

Table 4. Correlation of Biomarkers with Disease Duration and VASI Score

Biomarker	Disease Duration (r, p-value)	VASI Score (r, p-value)
MDA	+0.46, $p = 0.002$	+0.41, $p = 0.004$
SOD	-0.38, $p = 0.01$	-0.44, $p = 0.003$
Catalase	-0.35, $p = 0.02$	-0.39, $p = 0.01$
GPx	-0.29, $p = 0.04$	-0.33, $p = 0.02$

DISCUSSION:

The present study evaluated oxidative stress parameters in vitiligo patients and healthy controls, with the aim of assessing their potential as biomarkers of disease activity and severity. Our findings demonstrate that vitiligo patients exhibit significantly elevated malondialdehyde (MDA) levels and reduced activities of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) compared to controls. Furthermore, subgroup analysis revealed that patients with generalized and active vitiligo had more pronounced oxidative stress alterations, and correlation analysis showed strong associations between biomarker levels, disease duration, and severity.

These results are consistent with a growing body of literature highlighting the role of oxidative stress in the pathogenesis of vitiligo. Elevated MDA levels, indicative of lipid peroxidation, have been consistently reported in vitiligo patients [19–21]. Our findings align with those of Schallreuter et al. [22], who demonstrated excessive hydrogen peroxide (H_2O_2)

accumulation in lesional skin, leading to oxidative damage. Similarly, Arican et al. [23] and Yildirim et al. [24] reported significantly lower activities of SOD, CAT, and GPx in vitiligo patients, suggesting impaired antioxidant defense mechanisms.

The observation that oxidative stress markers were more deranged in generalized and active vitiligo corroborates earlier studies, which have suggested that disease progression is accompanied by a greater imbalance in redox homeostasis [25,26]. Our correlation analysis between MDA and disease duration/VASI score is in agreement with earlier reports by Khan et al. [27] and Dell'Anna et al. [28], further supporting the hypothesis that oxidative stress contributes not only to disease initiation but also to its chronicity and severity.

Oxidative stress has been implicated as a critical factor in melanocyte destruction in vitiligo. Reactive oxygen species (ROS) may trigger melanocyte apoptosis through mitochondrial dysfunction, DNA damage, and protein oxidation [29]. Furthermore, accumulation of ROS such as H₂O₂ leads to inactivation of key melanogenic enzymes, such as tyrosinase, thereby impairing melanin synthesis [30]. Deficient antioxidant defenses, as evidenced by reduced SOD, CAT, and GPx activities in our study, may further exacerbate melanocyte vulnerability to oxidative damage. This redox imbalance is thought to interact with autoimmune pathways, amplifying melanocyte loss [31].

The significant correlations observed between oxidative stress markers and disease duration and severity indicate that these parameters could serve as biochemical biomarkers for disease monitoring. Such biomarkers may be particularly useful in predicting disease activity, assessing treatment response, and guiding therapeutic strategies aimed at restoring redox balance. Indeed, therapeutic interventions such as antioxidant supplementation (vitamins C and E, zinc, alpha-lipoic acid) and topical or systemic pseudocatalase have been investigated as potential adjuvant therapies in vitiligo [32,33].

CONCLUSION:

In conclusion, our findings reinforce the hypothesis that oxidative stress plays a pivotal role in the pathogenesis and progression of vitiligo. Elevated MDA levels and reduced antioxidant enzyme activities may serve as reliable biomarkers of disease activity and severity. Future research with larger cohorts and interventional studies targeting oxidative stress pathways will be essential to validate these biomarkers and to develop targeted therapies for vitiligo.

Declaration:

Conflicts of interests: The authors declare no conflicts of interest.

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