

Assessment of Serum Homocysteine and Oxidative Stress in Vitiligo Patients

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Abstract:

Background: Vitiligo is a chronic depigmenting disorder characterized by selective destruction of melanocytes, resulting in well-defined white patches on the skin. Increasing evidence suggests that oxidative stress and abnormal homocysteine metabolism may play a role in melanocyte damage.

Objective: To assess serum homocysteine levels and oxidative stress markers in patients with vitiligo and compare them with healthy controls.

Methods: A cross-sectional case-control study was carried out at Patna Medical College between April 2025 and December 2025. The study included 80 participants, comprising 40 patients diagnosed with vitiligo and 40 age- and sex-matched healthy individuals. Serum homocysteine levels and oxidative stress markers, namely malondialdehyde (MDA) and superoxide dismutase (SOD), were analyzed. Data were processed using SPSS version 25, with Student's t-test and chi-square test applied as appropriate. A p-value < 0.05 was considered statistically significant.

Results: Patients with vitiligo exhibited significantly elevated serum homocysteine and MDA levels compared to controls, whereas SOD levels were markedly reduced. These differences were statistically highly significant (p < 0.001).

Conclusion: Vitiligo patients demonstrate elevated serum homocysteine and oxidative stress markers, suggesting a possible role of oxidative imbalance in disease pathogenesis.

Keywords: Vitiligo, Homocysteine, Oxidative Stress, Malondialdehyde, Antioxidants.

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Introduction

Vitiligo is an acquired depigmentary disorder characterized by the loss of melanocytes from the epidermis, resulting in well-demarcated depigmented macules and patches [1]. The condition affects approximately 0.5–2% of the global population and can occur at any age, although onset frequently occurs before the age of 30 years [2].

Although the exact pathogenesis of vitiligo remains incompletely understood, several hypotheses have been proposed, including autoimmune destruction, neural mechanisms, genetic predisposition, and oxidative stress-mediated melanocyte damage [3,4]. Among these, oxidative stress has gained increasing attention as a major contributing factor in the development and progression of vitiligo [5].

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms of the body [6]. Excessive ROS can lead to lipid peroxidation, DNA

damage, and protein oxidation, ultimately resulting in cellular dysfunction and apoptosis [7].

In vitiligo, melanocytes appear particularly vulnerable to oxidative damage due to their high metabolic activity and melanin synthesis processes [8]. Studies have demonstrated increased levels of lipid peroxidation products such as malondialdehyde (MDA) in patients with vitiligo, indicating enhanced oxidative stress [9].

Homocysteine is a sulfur-containing amino acid formed during methionine metabolism. Elevated serum homocysteine levels have been associated with endothelial dysfunction, oxidative stress, and several dermatological conditions [10]. Increased homocysteine may contribute to melanocyte toxicity by inducing oxidative damage and interfering with melanin synthesis pathways [11].

Furthermore, homocysteine may inhibit tyrosinase activity, an enzyme essential for melanin production, thereby affecting pigmentation processes [12]. Elevated levels of homocysteine have been reported in vitiligo patients and may serve as a potential biomarker of oxidative stress in this condition [13].

Antioxidant defense systems play an essential role in neutralizing reactive oxygen species. Superoxide dismutase (SOD) is an important enzymatic antioxidant that catalyzes the conversion of superoxide radicals into hydrogen peroxide and oxygen [14]. Reduced antioxidant activity has been observed in vitiligo patients, suggesting impaired oxidative defense mechanisms [15].

Several studies have attempted to establish a relationship between oxidative stress and vitiligo, but findings remain variable across different populations [16]. Evaluating biochemical markers such as homocysteine and oxidative stress indicators may provide further insight into disease pathogenesis.

Therefore, the present study was conducted to assess serum homocysteine levels and oxidative stress markers in vitiligo patients attending Patna Medical College and to compare these parameters with healthy individuals.

Materials and Methods

Study Design: This study was conducted as a hospital-based observational case-control study to evaluate the association between serum homocysteine levels and oxidative stress parameters in patients with vitiligo. The study compared biochemical markers between clinically diagnosed vitiligo patients and age- and sex-matched healthy individuals.

Study Setting: The study was carried out in the Department of Dermatology in collaboration with the Department of Biochemistry at Patna Medical College and Hospital, Patna, Bihar, India.

Study Duration: The study was conducted over a period of eight months from April 2025 to December 2025.

Study Population and Sample Size

A total of 80 participants were included in the study. These were divided into two groups:

- **Group A (Cases):** 40 patients clinically diagnosed with vitiligo attending the dermatology outpatient department.
- **Group B (Controls):** 40 healthy individuals without vitiligo or other autoimmune or dermatological disorders.

The control group was selected to be age- and sex-matched with the vitiligo patients in order to reduce potential confounding effects.

Inclusion Criteria

Vitiligo group

- Patients aged 18 years or older.
- Patients with clinically diagnosed vitiligo confirmed by a dermatologist.
- Patients willing to participate in the study and provide blood samples.

Control group

- Healthy individuals without vitiligo.
- Individuals without any known autoimmune disease or chronic systemic illness.
- Age- and sex-matched with the vitiligo group.

Exclusion Criteria

Participants were excluded if they had:

- Any systemic inflammatory or autoimmune disease.
- Chronic renal, hepatic, or cardiovascular disorders.
- Diabetes mellitus or thyroid disorders.
- Current use of antioxidant supplements, corticosteroids, or immunosuppressive therapy.
- Pregnancy or lactation.

These exclusion criteria were applied to minimize factors that could influence oxidative stress markers or homocysteine levels.

Ethical Considerations: The study protocol was reviewed and approved by the Institutional Ethics Committee of Patna Medical College and Hospital. Written informed consent was obtained from all participants prior to enrollment. Confidentiality of patient information was strictly maintained throughout the study.

Clinical Evaluation

All participants underwent a detailed clinical assessment including:

- Demographic data (age and sex)
- Duration of vitiligo
- Distribution of depigmented lesions
- Relevant medical history

Diagnosis of vitiligo was made based on clinical examination by a qualified dermatologist, supported by Wood's lamp examination when necessary.

Sample Collection: Venous blood samples were collected from all participants under aseptic conditions after overnight fasting. Approximately 5 mL of venous blood was drawn from the antecubital vein.

The blood sample was divided into two portions:

- One portion was used for serum homocysteine estimation.
- The other portion was used for assessment of oxidative stress markers.

Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until biochemical analysis.

Biochemical Analysis

Estimation of Serum Homocysteine: Serum homocysteine levels were measured using an enzyme-linked immunosorbent assay (ELISA) method in the biochemistry laboratory. Results were expressed in micromoles per liter ($\mu\text{mol/L}$).

Measurement of Oxidative Stress Markers:

Two oxidative stress indicators were evaluated:

1. Malondialdehyde (MDA)

MDA was measured as an indicator of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) method. The results were expressed in nmol/ml .

2. Superoxide Dismutase (SOD)

SOD activity was measured as an indicator of antioxidant defense using a spectrophotometric enzymatic assay. Results were expressed in units per milliliter (U/ml).

Outcome Measures

The primary parameters evaluated in the study included:

- Serum homocysteine levels
- Serum malondialdehyde (MDA) levels
- Serum superoxide dismutase (SOD) activity

Demographic characteristics such as age and gender distribution were also recorded to compare baseline characteristics between the two groups.

Statistical Analysis

All collected data were compiled and analyzed using SPSS version 25.

- Continuous variables were expressed as mean \pm standard deviation (SD).
- Categorical variables were expressed as frequencies and percentages.

The Student's t-test was used to compare the mean values of biochemical parameters between vitiligo patients and healthy controls. The Chi-square test was applied for comparison of categorical variables such as gender distribution.

Statistical significance was determined at a p-value less than 0.05.

Results

A total of 80 participants were included in the study, comprising 40 clinically diagnosed vitiligo patients and 40 healthy controls. The demographic and biochemical parameters of both groups were analyzed and compared.

Demographic Characteristics: The demographic characteristics of the study participants are summarized in Table 1. The mean age of vitiligo patients was 34.6 ± 9.1 years, whereas the mean age of controls was 33.8 ± 8.7 years. The difference between the two groups was not statistically significant ($p = 0.72$).

In terms of gender distribution, 22 (55%) of vitiligo patients were male and 18 (45%) were female, while the control group included 21 males (52.5%) and 19 females (47.5%). There was no statistically significant difference in gender distribution between the groups ($p = 0.81$).

These findings indicate that both groups were comparable in terms of demographic characteristics.

Table 1: Demographic Characteristics of Study Participants

Parameter	Vitiligo Patients (n=40)	Controls (n=40)	p-value
Mean age (years)	34.6 ± 9.1	33.8 ± 8.7	0.72
Male (%)	55	52.5	0.81
Female (%)	45	47.5	0.81

Comparison of Serum Homocysteine Levels:

Serum homocysteine levels were significantly higher in patients with vitiligo compared to the control group. The mean serum homocysteine level in vitiligo patients was $18.2 \pm 4.6 \mu\text{mol/L}$, while in healthy controls it was $11.3 \pm 3.2 \mu\text{mol/L}$.

Statistical analysis using the Student's t-test demonstrated a highly significant difference between the two groups ($t = 7.92$, $p < 0.001$).

This finding suggests that elevated homocysteine levels may be associated with the pathogenesis of vitiligo.

The comparison of serum homocysteine levels between the two groups is illustrated in Figure 1.

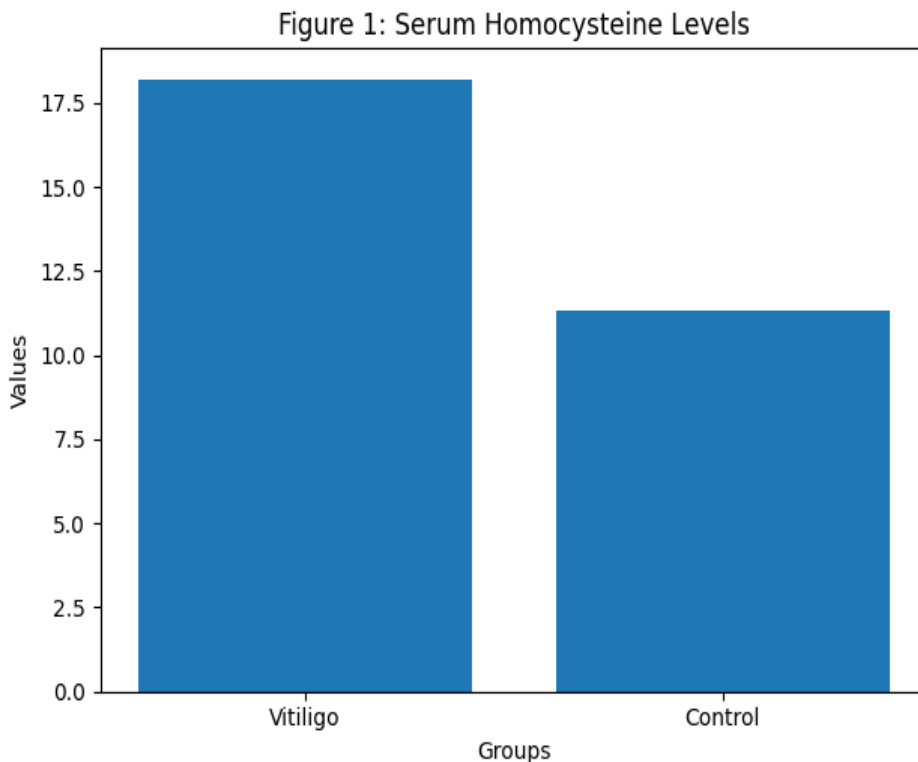


Figure 1: Comparison of Serum Homocysteine Levels Between Study Groups

Comparison of Oxidative Stress Markers:

Oxidative stress parameters were assessed by measuring malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity in both groups.

The mean MDA level in vitiligo patients was 4.9 ± 1.1 nmol/ml, whereas the control group showed a mean value of 2.7 ± 0.9 nmol/ml. The difference was statistically significant ($t = 9.18, p < 0.001$), indicating increased lipid peroxidation in vitiligo patients.

Conversely, the antioxidant enzyme superoxide dismutase (SOD) was significantly reduced in vitiligo patients (2.6 ± 0.7 U/ml) compared to controls (3.8 ± 0.8 U/ml). This difference was also statistically significant ($t = -7.12, p < 0.001$).

These results indicate a significant imbalance between oxidant and antioxidant systems in patients with vitiligo.

The biochemical comparison between the two groups is summarized in Table 2.

Table 2: Comparison of Biochemical Parameters Between Vitiligo Patients and Controls

Parameter	Vitiligo Patients (n=40)	Controls (n=40)	t-value	p-value
Homocysteine (µmol/L)	18.2 ± 4.6	11.3 ± 3.2	7.92	<0.001
MDA (nmol/ml)	4.9 ± 1.1	2.7 ± 0.9	9.18	<0.001
SOD (U/ml)	2.6 ± 0.7	3.8 ± 0.8	-7.12	<0.001

Distribution of Oxidative Stress Markers: The distribution of oxidative stress markers in both groups was further illustrated graphically.

Figure 2 demonstrates the comparison of MDA levels between vitiligo patients and healthy controls. Vitiligo patients showed significantly higher levels of lipid peroxidation compared with controls.

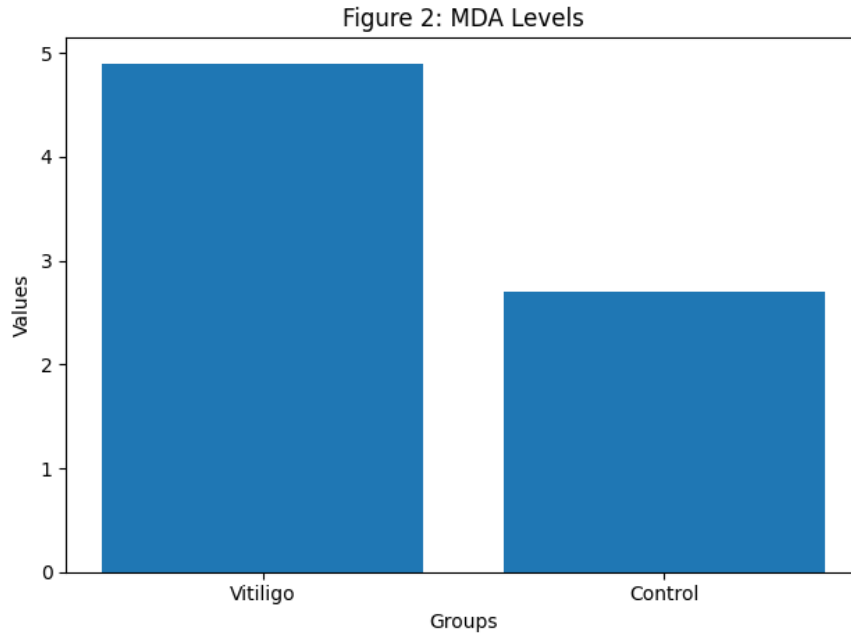


Figure 2: Comparison of Malondialdehyde (MDA) Levels Between Study Groups

Figure 3 illustrates the comparison of superoxide dismutase (SOD) levels between the two groups. A significantly lower antioxidant enzyme activity was

observed in vitiligo patients compared with healthy individuals.

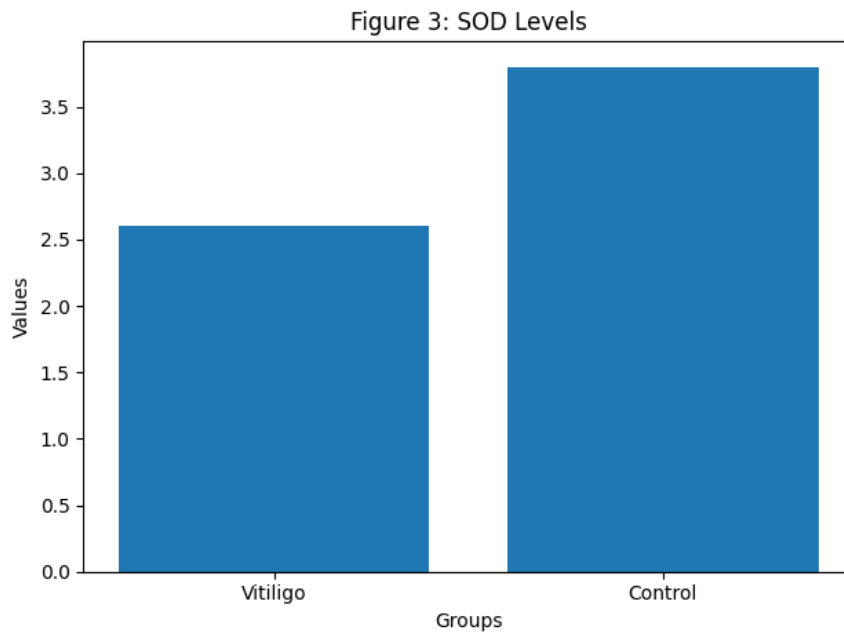


Figure 3: Comparison of Superoxide Dismutase (SOD) Levels Between Study Groups

Summary of Key Findings

The present study demonstrated that:

- Serum homocysteine levels were significantly elevated in vitiligo patients compared with controls.
- Malondialdehyde levels were significantly increased, indicating enhanced oxidative stress.

- Superoxide dismutase levels were significantly reduced, suggesting impaired antioxidant defense.

These findings collectively indicate that oxidative stress and homocysteine metabolism may play an important role in the pathogenesis of vitiligo.

Discussion

The present study evaluated serum homocysteine levels and oxidative stress markers in vitiligo patients and demonstrated significant biochemical differences compared with healthy controls.

Our findings showed significantly elevated serum homocysteine levels among vitiligo patients. These results are consistent with earlier reports suggesting that hyperhomocysteinemia may contribute to melanocyte damage through oxidative mechanisms [17].

Homocysteine has been shown to induce oxidative stress by generating reactive oxygen species and impairing antioxidant defenses [18]. This mechanism may contribute to melanocyte destruction and depigmentation observed in vitiligo.

The present study also demonstrated significantly increased malondialdehyde levels in vitiligo patients. MDA is a widely recognized marker of lipid peroxidation and oxidative damage [19]. Increased MDA levels suggest enhanced oxidative stress in vitiligo.

These findings support previous studies reporting increased lipid peroxidation in vitiligo patients [20]. Oxidative stress may damage melanocyte membranes and impair cellular function, ultimately leading to cell death.

In contrast, the antioxidant enzyme superoxide dismutase was significantly reduced in vitiligo patients in the present study. Reduced antioxidant defense mechanisms may render melanocytes more susceptible to oxidative injury [21].

Several researchers have proposed that oxidative stress may act as an initiating factor in vitiligo pathogenesis by triggering autoimmune responses against melanocytes [22].

Furthermore, elevated homocysteine may interfere with melanin synthesis by inhibiting tyrosinase activity and disrupting melanocyte metabolism [23].

Our findings are consistent with studies conducted in different populations that have reported similar biochemical alterations in vitiligo patients [24].

The combination of increased homocysteine levels and oxidative stress markers observed in this study supports the hypothesis that oxidative imbalance plays a significant role in vitiligo pathogenesis.

However, larger multicenter studies are required to further clarify the relationship between oxidative stress and vitiligo and to evaluate the potential role of antioxidant therapy in disease management [25].

Conclusion

Vitiligo patients demonstrate significantly elevated serum homocysteine and malondialdehyde levels

along with decreased antioxidant enzyme activity. These findings suggest that oxidative stress may play an important role in the pathogenesis of vitiligo. Monitoring biochemical markers such as homocysteine may provide additional insight into disease mechanisms and potential therapeutic strategies.

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