Original Article

Evaluation of Oxidative Stress Levels as Biomarkers in Vitiligo Patients

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Abstract

Objective: Vitiligo is a skin disease that causes the loss of melanocyte cells, which produce pigment in the skin. There is evidence that oxidative stress plays an important role in vitiligo disease of unknown pathogenesis. Evidence suggests that there is increased oxidative stress in melanocytes in patients with vitiligo. The aim of this study is to investigate oxidative stress markers in patients with vitiligo. **Materials and Methods:** Blood samples were taken from 23 vitiligo patients and 23 healthy control groups without any disease in Bezmialem Vakıf University Hospital Dermatology and Venereal Diseases Outpatient Clinic. Total antioxidant levels (TAS) and total oxidant levels (TOS) were measured photometrically method with blood samples. The oxidative stress index (OSI) was calculated as the TOS to TAS level ratio. **Results:** When the results of the vitiligo patient group were compared with the control group, TOS and OSI were found to be significantly higher than the control group ($P < 0.05$). No statistically significant result was found in TAS. **Conclusions:** Our results show that oxidative stress plays a role in the pathogenesis of vitiligo.

Keywords: Antioxidant, oxidant, oxidative stress, vitiligo

Introduction

Vitiligo is a dermatological disease characterized by limited depigmented patches resulting from the functional loss of melanocyte cells in the epidermis.[1] This condition typically manifests as non-scaly, chalk-white spots and is caused by selective melanocyte loss.[2] Studies on the epidemiology of vitiligo have found a wide range of incidence rates (0.14%–8.8%). However, the generally accepted view is that the incidence of vitiligo falls within the range of 1%–2%.[3] This disease is not associated with factors such as gender, age, or climate and can occur in individuals of all races. Nevertheless, it is more prevalent in individuals with a family history of vitiligo and those influenced by genetic factors.[4] The exact pathogenesis of vitiligo is not fully understood. Still, it is believed that genetic predisposition, autoimmune melanocyte damage, significant stress, infection, physical trauma, sunburn, and other factors may play significant roles in the disease's pathogenesis. Positive family history has been detected in 25%–50% of patients diagnosed with vitiligo.[5]

Vitiligo is characterized by acquired hypopigmentation and depigmentation, which can be attributed to several potential causes. These causes may include stress, accumulation of toxic compounds, infections, autoimmunity, mutations, melatonin receptor dysfunction, cellular microenvironmental changes, and disturbances in melanocyte migration and/or proliferation.[6]

Oxidative stress occurs due to imbalances in the production and degradation of reactive oxygen species

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(ROS). This condition is characterized by the oxidation and damage of biomolecules such as DNA, membrane lipids, enzymes, and structural proteins that cells cannot adequately repair.[7] Oxidative stress may play a significant pathogenic role in vitiligo. Some studies have shown that the accumulation of free radicals, which are toxic to melanocytes, leads to their destruction. Additionally, increased levels of nitric oxide have been observed in cultured melanocytes and the serum of vitiligo patients, suggesting that nitric oxide may contribute to the self-destruction of melanocytes. It has also been found that the red cells of vitiligo patients have lower levels of glutathione than control subjects, which helps prevent damage caused by free radicals. Therefore, vitiligo patients may experience higher levels of oxidative stress.[8] Our study aims to investigate the effects of vitiligo on oxidative stress.

Materials and Methods

This study was conducted in collaboration with the Dermatology and Venereology Clinic of Bezmialem Vakif University Hospital and the Department of Medical Biochemistry at Hamidiye Medical Faculty of Health Sciences University, following the approval of institutional ethical clearance with registration number 22/594.

Forty-six individuals were included in the study after obtaining informed consent. The study consisted of two groups: 23 vitiligo patients diagnosed clinically based on inclusion criteria and 23 voluntary control subjects. Inclusion criteria considered individuals under the age of 65 with no metabolic or skin diseases that share the same demographic characteristics as vitiligo and who do not consume alcohol and/or smoke. After the clinical examinations of all participants in the study groups, blood samples were collected for further analysis.

Blood samples

Blood samples were collected in gel clot activator sterile biochemical tubes. After centrifugation at 3000 × *g* for 10 min, the separated serum was stored at −80°C until further analysis.

Oxidative Stress Analysis

Determination of total antioxidant status

Measurement of Serum Total Antioxidant Status (TAS) is an automated method developed by Erel, which measures the total antioxidant capacity of the sample against strong free radicals.[9] In this method, 1 mM Ascorbic acid was used as a standard. Serum samples were measured in two stages using the Agilent Synergy HTX Multi-Mode Microplate Reader device at 660 nm. TAS results were expressed in mmol Ascorbic Acid Equivalent units per liter (mmol AA Eq/L).

Determination of total oxidant status

Measurement of Serum Total Oxidant Status (TOS) was measured using an automated measurement method developed by Erel. The color intensity, which can be measured spectrophotometrically, is associated with the total amount of oxidant molecules in the sample.[10] Hydrogen peroxide (H_2O_2) was used as the standard in this method. Serum samples were measured in two stages using the Agilent Synergy HTX Multi-Mode Microplate Reader device at 571/658 nm. TOS results were expressed in units of μmol H_2O_2 Equivalent per liter (μmol H_2O_2 Eq/L).

The oxidative stress index (OSI) in the samples was calculated as the ratio of TOS level to TAS level.

Statistical analysis

IBM SPSS version 28.0 software was used for statistical analysis. Mean ± standard deviation values were used to represent continuous variables. Mann–Whitney *U* test was performed to compare variables between the two groups. The relationships between variables were examined using Spearman's correlation coefficient. Confidence intervals (95%) were used to demonstrate differences between groups. Statistical significance was defined as $P < 0.05$.

Results

The TAS, TOS, and OSI parameters were compared between the Vitiligo and control groups [Figures 1–3]. As seen in Figure 1, TAS was lower in Vitiligo patients compared to the healthy control group, but this relationship was not statistically significant. However, TOS and OSI were significantly higher in Vitiligo patients compared to the control group ($P < 0.05$) [Figures 2 and 3].

The duration of vitiligo diagnosis for patients was recorded in months. The correlation between BMI, TAS, TOS, and OSI values with the duration of vitiligo diagnosis was

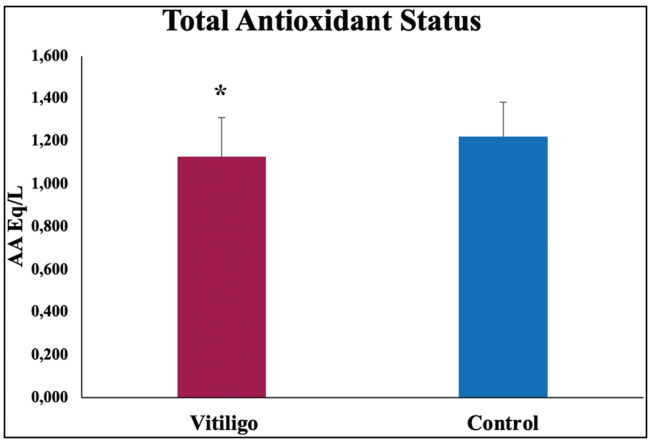

Figure 1: Total antioxidant status of vitiligo patients and healthy control group. Data are expressed as mean ± standard deviation ($n = 23$)

evaluated using the Spearman correlation test [Table 1]. No statistically significant correlations were found.

Discussion

Vitiligo is a disease characterized by the destruction of skin pigmentation and presents with depigmentation. The prevalence of vitiligo in Turkey is reported to be between 0.15% and 0.32%.[11] Familial cases of vitiligo are common, indicating a genetic predisposition, as 6% to 38% of vitiligo patients have affected family members.[12] The patches are initially small but expand over time. Skin lesions are predominantly observed on the face, hands, and wrists. Patients suffering from this disease often experience concomitant depression.[13]

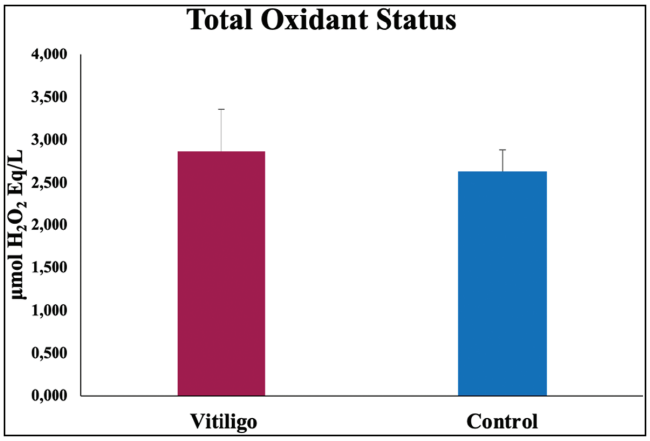

Figure 2: Total oxidant status of vitiligo patients and healthy control group. Data are presented as mean ± standard deviation (n = 23)

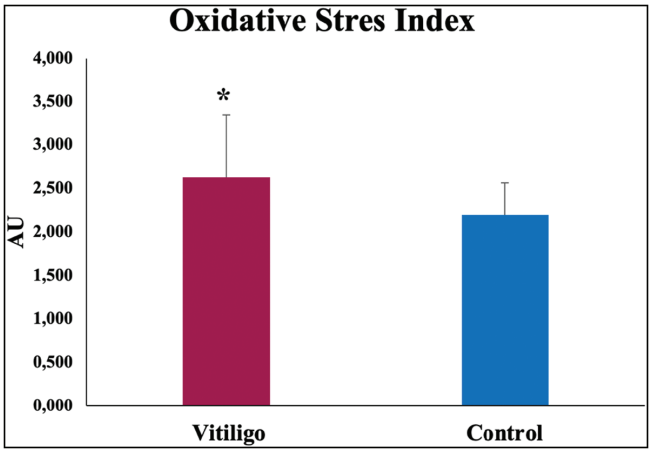

Figure 3: Oxidative stress index levels of vitiligo patients and healthy control group. Data are presented as mean ± standard deviation (n = 23). Differences in the vitiligo patient group were considered statistically significant at *P < 0.05, **P < 0.01, ***P < 0.001

Increased stress, life changes such as job loss, loss of a loved one, accidents, or illnesses, and physical traumas like exposure to ultraviolet (UV) radiation and pressure, contribute to the development or progression of vitiligo lesions. A study including five thousand vitiligo patients identified triggering factors in 51.4% of cases related to dietary factors, 34.26% to recurrent infections, 23.26% to medications, and 20.46% to emotional stress.[14]

Oxidative stress is characterized by forming and accumulating ROS, known as free radicals, during normal cellular processes. It leads to oxidative damage to cell membranes, proteins, lipids, and nucleic acids.[15] It is considered one of the possible pathogenic events in melanocyte loss. Defective recycling of tetrahydrobiopterin in the entire epidermis of vitiligo patients is associated with intracellular production of H_2O_2. Additionally, increased intracellular ROS production due to mitochondrial dysfunction and compromised antioxidant status supports the concept of systemic oxidative stress in vitiligo. This accumulated oxidative stress leads to DNA damage, lipid and protein peroxidation. Many proteins are modified through H_2O_2-mediated oxidation and exhibit partial or complete loss of functionality.[16]

Information obtained from different studies indicates that melanocyte loss in vitiligo results from increased epidermal oxidative stress through cytotoxic metabolites of melanogenesis. Several studies have demonstrated abnormalities in the antioxidant system and increased susceptibility of melanocytes to oxidative stress in the blood and skin of vitiligo patients.[17] Previous studies have shown the involvement of oxidative stress in the pathogenesis of vitiligo and higher levels of oxidative stress in vitiligo patients compared to the control group.[16,18] Our study yielded similar experimental results, although statistically significant findings were not obtained.

Furthermore, when considering the duration of vitiligo diagnosis in patients, no correlation was found between BMI, TAS, TOS, OSI parameters, and the duration of diagnosis.

Table 1: Correlation between BMI, TAS, TOS, and OSI in patients diagnosed with vitiligo

Correlation Parameters	BMI		TAS		TOS		OSI	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Vitiligo diagnosis (Months)	0,317	0,140	0,195	0,374	-0,071	0,748	-0,167	0,447

BMI: Body Mass Index, TAS: Total Antioxidant Status, TOS: Total Oxidant Level, OSI: Oxidative Stress Index, r: Spearman correlation coefficient

Conclusion

The results indicate oxidative stress's involvement in the pathogenesis of vitiligo.

Limitations

One limitation of this study is the small sample size in both groups, which may affect the generalizability of the findings.

Ethics committee approval

This study was conducted in collaboration with the Dermatology and Venereology Clinic of Bezmialem Vakif University Hospital and the Department of Medical Biochemistry at Hamidiye Medical Faculty of Health Sciences University, following the approval of institutional ethical clearance with registration number 22/594.

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Conflicts of interest

There are no conflicts of interest.

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