

Determination of oxidative stress in vitiligo by measuring superoxide dismutase and catalase levels in vitiliginous and non-vitiliginous skin

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ABSTRACT

Background: Vitiligo is an acquired disorder characterized by circumscribed depigmented macules devoid of identifiable melanocytes. Complex genetic, immunological, neural and self destructive mechanisms interplay in its pathogenesis. According to autocytoxic hypothesis, oxidative stress has been suggested to be the initial pathogenic event in melanocyte degeneration. **Aims:** The aim of our investigation was to evaluate the role of oxidative stress by measuring levels of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in lesional and normal skin of patients with vitiligo and in the skin of normal controls. **Methods:** We determined the activity of SOD in lesional and non-lesional skin and CAT in lesional skin only of 25 vitiligo patients and 25 controls by using the spectrophotometric assay and Aebi's method, respectively. **Results:** There was statistically significant increase in the levels of SOD in vitiliginous and non vitiliginous skin of patient group compared to the control group ($P < 0.001$). No significant difference was found between the levels of SOD in lesional skin and non-lesional skin of vitiligo patients. The levels of CAT in the skin of patients were found to be significantly lower than those of controls ($P < 0.001$). **Conclusions:** There is increased oxidative stress in vitiligo as is indicated by high levels of SOD and low levels of CAT in the skin of vitiligo patients.

Key words: Catalase, Oxidative stress, Superoxide dismutase, Vitiligo

INTRODUCTION

Vitiligo is an acquired disorder characterized by well-circumscribed milky white cutaneous macules devoid of identifiable melanocytes.^[1] Across the globe, vitiligo is the most common pigmentary disorder affecting 0.1%-2% of the world's population irrespective of race and gender.^[2]

Although the precise etiology of vitiligo is not known, it has become quite clear in recent times that complex genetic, immunological, neural and self-destructive mechanisms interplay in its pathogenesis.^[2,3] According to autocytoxic hypothesis, oxidative stress has been suggested to be the initial pathogenic event in melanocyte degeneration with H₂O₂ accumulation in the epidermis of patients with active disease.^[4] In recent studies, the

involvement of oxidative stress in the pathophysiology of vitiligo has been investigated. An alteration in the antioxidant pattern, with significantly higher levels of superoxide dismutase (SOD) has been observed in the skin,^[5] erythrocytes,^[6-8] peripheral blood mononuclear cells^[6,9] and serum^[10,11] of vitiligo patients. Reduction in catalase (CAT) activity has been demonstrated in the epidermis,^[12,13] peripheral blood mononuclear cells^[6] and in melanocytes.^[4] These findings support the concept of possible systemic oxidative stress in vitiligo.

As there are very few studies showing the extent of oxidative stress at tissue level, the present study has been undertaken to determine the status of oxidative stress by measuring levels of antioxidant enzymes, i.e., SOD and CAT, in lesional and normal skin of patients with vitiligo and in the skin of normal controls.

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METHODS

The study was conducted in the Departments of Dermatology, Andhra Medical College, and Biochemistry, Andhra University, Visakhapatnam. The study group included 25 cases of generalized and localized vitiligo. Patients with diabetes mellitus, thyroid disease, any autoimmune disorder or concomitant dermatological diseases were excluded. Patients who had taken systemic or topical treatment within three months before the present study were also excluded. Patients with a history of smoking or alcoholism or taking drugs for any other reason were not included. Twenty-five age- and sex-matched healthy individuals, who were non-smokers and non-alcoholics, were included as controls.

After obtaining consent from the participants, split-thickness skin specimens were obtained from lesional and non-lesional skin of 18 patients, from only vitiliginous skin of 7 patients and from normal skin of 25 controls. Split-thickness skin specimens were obtained from the middle of the lesional skin and from the normal skin at least 10 cm away from the lesional skin in the patient group. The control skin specimens were taken from the inner aspect of the forearm in healthy volunteers. After the desired amount of specimen was obtained, it was washed in ice-cold saline and was immediately carried in an ice box to the Department of Biochemistry.

Tissue samples were blotted on paper, weighed and minced in ice-cold 0.25 M sucrose solution. The resulting pieces were homogenized and diluted with ice-cold 0.25 M sucrose solution to reach a final dilution of 10% (w/v) for tissues. The resulting homogenates were centrifuged at an average speed of 8500 *g* for 10 min (at 2°C) to spin down tissue fragments, nuclei and mitochondria. The resulting supernatant samples were obtained for enzyme assays^[14] and measured by UV-visible spectrophotometer.

Measurement of Cu/Zn superoxide dismutase activity

The Cu/Zn superoxide dismutase (E.C.1.15.1.1) enzyme activity was determined by the spectrophotometric assay using Oxford Biomedical Research kit.^[14-16] This method is based on the superoxide-mediated increase in the rate of autoxidation of 5,6,6a,11b-tetrahydroxybenzo (c) fluorine R1 in aqueous alkaline solution to yield a chromophore with maximum absorbance measured at 525 nm. Results were expressed as units (U) of SOD activity/mg protein.

Measurement of catalase activity

Catalase (E.C.1.11.1.6) activity was measured by Aebi's method.^[17] This method is based on the principle that the absorbance will decrease due to dismutation of H₂O₂ at 240 nm. (UV-visible spectrophotometry). The amount of H₂O₂ converted into H₂O and 0.5 O in 1 minute under standard conditions is accepted as the enzyme reaction velocity. Results were expressed as micromoles H₂O₂ metabolized/min/mg protein.

SOD was estimated in both lesional and non-lesional skin of patients and skin of controls, whereas CAT was measured only in lesional skin of patients and skin specimens of controls.

Statistical analysis

The statistical analysis was performed by using Student *t* test. The differences were considered significant when *P* < 0.05.

RESULTS

The study group included 25 cases of generalized and localized vitiligo (14 men and 11 women). The age of the patients varied from 18 to 53 years (mean age = 33.32 years). Duration of vitiligo ranged from 4 months to 40 years, with a mean duration of 11.3 years. Twenty patients had generalized vitiligo and 5 had focal vitiligo. Five patients had progressive vitiligo and 20 had stable vitiligo.

In this study, the levels of SOD in vitiliginous skin of vitiligo patients (2,474 + 966 U/mg of protein) were found to be higher than the levels of SOD in normal skin of controls (969.15 + 388.56 U/mg of protein). The difference was found to be statistically significant (*P* < 0.001). Similarly, it was also observed in our study that the levels of SOD in non-vitiliginous skin of vitiligo patients (2351.97 + 984.37 U/mg of protein) were significantly higher than the levels of SOD in normal skin of controls (969.15 + 388.56 U/mg of protein) (*P* < 0.001). No significant difference was found between the levels of SOD in vitiliginous skin and non-vitiliginous skin of patients (*P* > 0.05).

The levels of CAT in the lesional skin of patients (5.36 + 0.83 μM/min/mg protein) were found to be significantly lower than those of controls (13.18 + 0.53 μM/min/mg protein) (*P* < 0.001). There was no significant difference in the levels of SOD and CAT in the skin of vitiligo patients in relation to sex, age, duration or clinical type of vitiligo.

DISCUSSION

One of the hypotheses to explain vitiligo is the self-destructive theory of melanocytes, which suggests a role for oxidative stress. Oxidative stress is thought to be the initial pathogenic event in melanocyte destruction.^[3,4] Free radicals such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and nitric oxide are molecules that occur during several physiological and pathological processes.^[18] These free radicals are scavenged continuously by antioxidant enzymes such as SOD, CAT, glutathione peroxidase, glutathione reductase, beta carotene, vitamin C, vitamin E and other trace elements.^[18] In oxidative stress, there is insufficient antioxidant activity leading to excessive accumulation of free radicals, which damage cellular compounds such as protein, carbohydrate, DNA and lipid.^[19] In normal conditions, SOD, an antioxidant enzyme catalyzes the dismutation of superoxide anion (O_2^-) into O_2 and H_2O_2 ^[15,16] and CAT converts H_2O_2 to O_2 and H_2O . In oxidative stress, to counteract or scavenge increased levels of superoxide anions (O_2^-), SOD, an antioxidant enzyme, is increased, whereas CAT levels are decreased.^[20] Hydrogen peroxide, thus produced from superoxide anion (O_2^-), can readily cross cell membranes, causing much of the damage. Hence, measuring the levels of SOD and CAT in the skin, melanocytes, erythrocytes, peripheral blood mononuclear cells and blood indicates the status of oxidative stress in vitiligo patients.

In this study, the levels of SOD in vitiliginous and non-vitiliginous skin of vitiligo patients were found to be higher than the levels in normal skin of controls ($P < 0.001$). A lower level of CAT activity was found in vitiligo patients when compared with controls ($P < 0.001$). These high SOD levels and low levels of CAT in vitiligo patients support the concept of oxidative stress in the pathogenesis of vitiligo. In the present study, SOD levels were measured not only in lesional skin but also in non-lesional skin of patients to know whether the oxidative stress is localized or generalized. It was found that high levels of SOD were present both in lesional and non-lesional skin of patients. Schallreuter *et al.*,^[12] reported higher levels of glutathione reductase and low levels of CAT in both lesional and non-lesional skin of vitiligo patients. These findings, inclusive of ours, suggest that oxidative stress is not a localized phenomenon but a more generalized process. This may be one of the explanations for developing newer lesions in vitiligo patients in the course of the disease.

Very few studies have been done to measure antioxidant enzymes at tissue level. Yildirim *et al.*,^[5,11] found significantly increased levels of SOD not only in lesional skin but also in erythrocytes of patients with generalized vitiligo. They supported the concept of free radical-mediated damage to melanocytes in generalized vitiligo. Similar higher levels of SOD were reported not only at tissue level (epidermis) but also in erythrocytes,^[6-8] peripheral mononuclear cells,^[6,9] serum^[10,11] and whole blood.^[21] These findings are in agreement with the results of the present study. However, Passi *et al.*,^[13] Picardo *et al.*^[22] and Maresca *et al.*,^[4] reported no significant difference, whereas Koca *et al.*,^[21] reported decreased levels of superoxide dismutase in vitiligo patients compared with controls. Schallreuter *et al.*^[12] have demonstrated epidermal H_2O_2 accumulation in association with low CAT levels in both involved and uninvolved skin of vitiligo patients. Decreased CAT levels in vitiligo patients compared with healthy controls have also been reported in other studies.^[4,9,10,13] Recently, a study on the catalase gene (*CAT*) and its mutations leading to quantitative deficiency of CAT activity in the epidermis and accumulation of excess H_2O_2 and susceptibility to develop vitiligo has been reported.^[23]

Our findings and several studies demonstrate that there is impairment in the antioxidant system in vitiligo, leading to free radical-mediated damage to melanocytes. Our findings revealed that this oxidative stress is not a localized phenomenon but a generalized process and may be one of the reasons for the progressive nature of the disease. In view of these findings, antioxidants may play an adjuvant role in the management of vitiligo in addition to specific therapies.

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