Research Article

Evaluation of oxidative stress and lipid profile in patients of generalized vitiligo

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Abstract

Background: Restricted depigmented macules that lack melanocytes signify vitiligo. Complicated genetic, immunological, neural, and self-destructive mechanisms are involved in its pathogenesis. It has been proposed that oxidative stress is the primary pathogenic event according to autocytotoxic hypothesis.

Objective: To study the antioxidant defenses in patients of generalized vitiligo.

Materials and Methods: A total of 120 subjects were enrolled for the study; of which sixty were patients with generalized vitiligo and sixty were age- and sex-matched healthy controls. All the subjects were evaluated for the lipid profile and erythrocyte antioxidant enzyme activities such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx).

Result: The low-density lipoprotein(LDL) and very low-density lipoprotein(VLDL) cholesterol levels were found to be significantly higher ($p < 0.001$) in vitiligo patients. The activities of CAT and GPx were significantly higher ($p < 0.001$), whereas there was no significant difference observed in SOD activity in vitiligo patients.

Conclusion: There are depleted activities of the antioxidant enzymes CAT and GPx in generalized vitiligo, suggesting a high oxidative stress. Thus, a proper antioxidant therapy should be incorporated as an adjuvant in the management of vitiligo.

KEY WORDS: Vitiligo, catalase, superoxide dismutase, glutathione peroxidase, lipid profile

Introduction

Vitiligo is an acquired condition, which features well-circumscribed milky white cutaneous macules deficient of distinguishable melanocytes.[1] Globally, about 0.1%–2% of the world population, regardless of race and gender, is affected by vitiligo, the most common pigmentary disorder.[1] Vitiligo generally presents in one of the three patterns: focal, segmental, and generalized.[2] The precise causative mechanism of vitiligo is not known. Autoimmune hypothesis is the most widely accepted, because autoantibodies to melanocytes and tyrosinase have been demonstrated and owing to the usual association of vitiligo with other autoimmune diseases.[3] A defective antioxidant defense is also postulated to lead to the unhindered cytotoxic action of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, etc.[4] After formation, these highly reactive free radicals can trigger a chain reaction, induce cytotoxic damage to the melanocytes, and can inhibit tyrosinase enzyme.[5] Imbalances in the oxidant/antioxidant system, such as the accumulation of hydrogen peroxide (H_2O_2), the increased level of lipid peroxidation, and the altered lipid profile, have recently been reported in vitiligo patients. It has been proposed that oxidative stress is the primary pathogenic event according to autocytotoxic hypothesis.[6] The role of free radicals and oxidative

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damage in the pathophysiology of vitiligo has been documented in recent studies.[7–9] However, the results have been inconsistent in the three different types of vitiligo. The purpose of this study was to evaluate the role of oxidative stress and lipid disturbances by studying the erythrocytic catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities along with the lipid profile in the pathogenesis of generalized vitiligo so as to provide an insight to the treating clinician for the incorporation of antioxidants as a therapeutic strategy.

Materials and Methods

The study comprised a total of 120 subjects aged 20–60 years, of which 60 were patients diagnosed with generalized vitiligo in the Outpatient Department of Dermatology, Kamineni Institute of Medical Sciences, Narketpally, Telangana, India, over a time period of 18 months. All the patients were clinically examined by the dermatologist, and the site and pattern (generalized or localized) of the lesions were noted. Patients with diabetes mellitus, thyroid disease, any autoimmune disorder, or concomitant dermatological diseases were excluded. Patients who had taken systemic or topical treatment within 3 months before this study, with the history of smoking or alcoholism, or taking drugs for any other reason were not included. Sixty age- and sex-matched healthy individuals, who were nonsmokers and not consuming alcohol, were included as controls. All the subjects were briefed about the purpose of the study, and written consents were obtained from the patients and the controls, before the collection of the blood sample. The study was approved by the Institutional Ethics Committee. About 10 mL of venous blood sample, after overnight fasting, was collected from the anterior cubital vein of each of the vitiligo patient and the control subjects for carrying out the following biochemical analysis.

Sample Preparation

Heparinized blood was centrifuged at 1,000 g for 10 min at 4°C; the buffy coat was discarded, and the isolated RBC pellet was hemolyzed in four times its volume of ice-cold high-performance liquid chromatography-grade water and again centrifuged at 4°C. The erythrocyte lysate was, then, used to evaluate the CAT, SOD, and GPx activities.

Catalase Assay

CAT activity was assayed based on the method of Johansson and Borg,[10] using the Cayman kits (item no. 707002; Ann Arbor, MI, US). The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced was measured colorimetrically with purpald as the chromogen at 340 nm.

Glutathione Peroxidase Activity Assay

The GPx activity was determined with Cayman kits (Item no. 703102; Ann Arbor, MI, US) at 25°C by colorimetry at 340 nm, based on the method of Paglia and Valentine,[11] which requires cumene hydroperoxide as a substrate.

Superoxide Dismutase Activity Assay

The SOD activity was determined with Cayman kits (item no. 706002; Ann Arbor,MI 48108, US) at 25°C by colorimetry at 340 nm, based on the method of Marklund.[12] This method employs xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(-4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride to form red formazan dye. The SOD activity is, then, measured by the degree of inhibition of this reaction.

Lipid Profile

Serum total cholesterol (TC), triglycerides (TGs), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were done by autoanalyzer (Hitachi 912). Very low-density lipoprotein (VLDL) was calculated by Friedewald equation.[13]

Anthropometric Data

The height and weight were recorded for all the subjects. The bodymass index (BMI) was calculated by the accepted formula weight [(kg)/height m^2].

Statistical Analysis

The statistical analysis was performed using the SPSS software, version 19 (SPSS, Chicago, IL). The variables were expressed as mean and standard deviation, and p value <0.05 was considered statistically significant. Unpaired *t*-test was used to compare the results between the patient and control groups.

Result

The baseline data with the lipid profile are shown in Table 1. The antioxidant activities are shown in Table 2. There was a significant difference observed in LDL- and VLDL-cholesterol ($p < 0.001$) in vitiligo patients in comparison with controls. The difference in the TC concentration between the vitiligo and control groups was close to the statistical significance ($p = 0.052$). The concentrations of other lipids, including TG and HDL-cholesterol, were not statistically different between the patient and control groups [Table 1]. While comparing the antioxidant activity, it was observed that the erythrocytic CAT and GPx levels were significantly lower ($p < 0.001$) in vitiligo patients, whereas no significant difference was observed in SOD activity.

Discussion

Vitiligo is essentially a cosmetic problem. It often causes social and emotional consequences including low self-esteem, social anxiety, depression, stigmatization, and, in extreme cases, rejection by those around them.[14] The pathogenesis of vitiligo is complex and still not well understood.[9] In this

Table 1: Baseline characteristics and lipid profile of vitiligo patients and controls

Parameters	Controls, *N* = 60	Vitiligo patients, *N* = 60	*p*
Age (years)	42.9 ± 1.02	43.1 ± 1.04	0.289 (NS)
BMI (kg/m^2)	19.72 ± 1.87	19.55 ± 1.39	0.573 (NS)
TC (up to 200 mg/dL)	162.65 ± 16	168.8 ± 18.1	0.052 (NS)
HDL (30–60 mg/dL)	46.07 ± 6.98	44.45 ± 7.33	0.217 (NS)
LDL (80–150 mg/dL)	97.1 ± 24.7	121.34 ± 14.77	0.001**
VLDL (10–30 mg/dL)	22.73 ± 5.72	26.88 ± 3.8	0.001**
TG (up to 150 mg/dL)	115.04 ± 26.57	120.44 ± 12.45	0.156 (NS)

TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; TG, triglycerides; NS, nonsignificant.
Data expressed as mean ± SD
*$p < 0.05$; **$p < 0.001$.

Table 2: Comparison of antioxidant enzyme activities among vitiligo patients and controls

Parameters	Control (*N* = 60)	Vitiligo patients (*N* = 60)	*p*
CAT (nmol/min/mL)	552.85 ± 77.25	492.25 ± 53.11	0.001**
SOD (IU/g of Hb)	1612 ± 252	1592.21 ± 129.78	0.589 (NS)
GPx (IU/g of Hb)	51 ± 9.6	43.33 ± 4.9	0.001**

SD, standard deviation; CAT, catalase; SOD, superoxide dismutase, GPx, glutathione peroxidase.
Data expressed as mean ± SD.
*$p < 0.05$; **$p < 0.001$.

study, while analyzing the lipid profile in vitiligo patients, a significantly lower LDL-cholesterol and VLDL-cholesterol ($p < 0.001$) levels were observed, whereas other parameters did not show much change, in comparison with controls. The significantly higher LDL and VLDL levels in vitiligo patients suggest lipid disturbances, which according to Karadag et al.[15] may be owing to the complex interaction of inflammatory, cytotoxic, and immunological factors in vitiligo patients inducing the systemic disturbances. Moreover, Pietrzak et al.[16] in their study on girls with vitiligo have reported similar findings of disturbances in the lipid profile. However, they had observed significantly lower levels of HDL-cholesterol in vitiligo patients along with significantly higher levels of LDL and VLDL. The differences in the studies may be owing to other associated confounding factors that are likely to interfere with lipid disturbances.

According to the self-destructive theory of melanocytes in the pathogenesis of vitiligo, oxidative stress is thought to play a significant role.[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.[17] These free radicals are scavenged continuously by antioxidant enzymes such as SOD, CAT, GPx, glutathione reductase, beta carotene, vitamin C, and vitamin E In oxidative stress, there is insufficient antioxidant activity leading to an excessive accumulation of free radicals, which damage cellular compounds such as protein, carbohydrate, DNA, and lipids.[17] Hydrogen peroxide, thus produced from superoxide anion (O_2^-), can readily cross cell membranes, causing much of the damage. Hence, measuring the levels of CAT, SOD, and GPx in the skin, melanocytes, erythrocytes, peripheral blood mononuclear cells, and blood indicates the status of oxidative stress in vitiligo patients.

In this study, the activity of CAT and SOD was found to be significantly lower ($p < 0.001$) [Table 2] among the vitiligo patients, whereas no significant change was observed in the SOD activity, while comparing with healthy controls. This decrease in CAT and GPx levels among vitiligo patients could be owing to the increased oxidative stress, which was in accordance to the findings of Arican and Kurutas, where erythrocyte CAT level was found to be significantly lower than controls.[18,19] Sravani et al.[7] also found the level of CAT to be significantly lower among the skin of vitiligo patients when compared with the healthy populations. However, Hazneci et al.[20] did not find any difference between the erythrocyte CAT and SOD levels among the vitiligo patients and healthy controls. Notably, CAT and SOD, acting in concert with GPx, constitute the major defense or primary antioxidant enzymes against the superoxide radicals. The protective effect of GPx activity on lipid peroxidation is reinforced by the fact that this enzyme not only detoxifies the H_2O_2 produced by SOD action but also converts lipid hydroperoxide to nontoxic alcohols, thus acting as a chain-breaking antioxidant.[21] Our findings are in concurrent with those reported by Passi et al.,[22] Picardo et al.,[23] who reported no significant difference in SOD activity, whereas Koca et al.[18] reported decreased levels of SOD and GPx in vitiligo patients in contrast to our study, when compared with control subjects. As oxidative stress is found

to be present in the course of vitiligo, it is held responsible for the depleted levels of erythrocytic antioxidant defenses. The high oxidative stress promotes lipid peroxidation, which may be a plausible explanation for the lipid abnormalities detected in our study.

Limitations

The strength of the study lies in the stringent selection criteria of the subjects, and the limitation is a relatively smaller sample size that is drawn from one limited geographical area. Therefore, future studies with larger sample size are warranted to further strengthen our results.

Conclusion

The findings from this study and evidence from the literature demonstrate that there is an impairment in the prooxidant/antioxidant balance in vitiligo leading to lipid disturbances. 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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