Oxidative stress in the blood of patients with active localized vitiligo

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A BSTRACT

Objectives: Vitiligo is an acquired skin disease characterized by white areas on the skin. The pathogenesis of the disease is still unclear. Some findings show that oxidative stress could be an important phenomenon in the pathophysiology of vitiligo.

Methods: We evaluated 16 consecutive localized vitiligo patients and 16 healthy controls of a similar age and sex distribution. We measured their indicators of oxidative stress such as catalase (CAT), superoxide dismutase (SOD), glucose 6-phosphate dehydrogenase (G6PD) in erythrocytes, and plasma malondialdehyde (MDA) by spectrophotometry.

Results: SOD activities and MDA levels of patients were significantly higher than controls ($p < 0.001$). CAT and G6PD activities of patients were significantly lower than controls ($p < 0.05$ and $p < 0.001$, respectively).

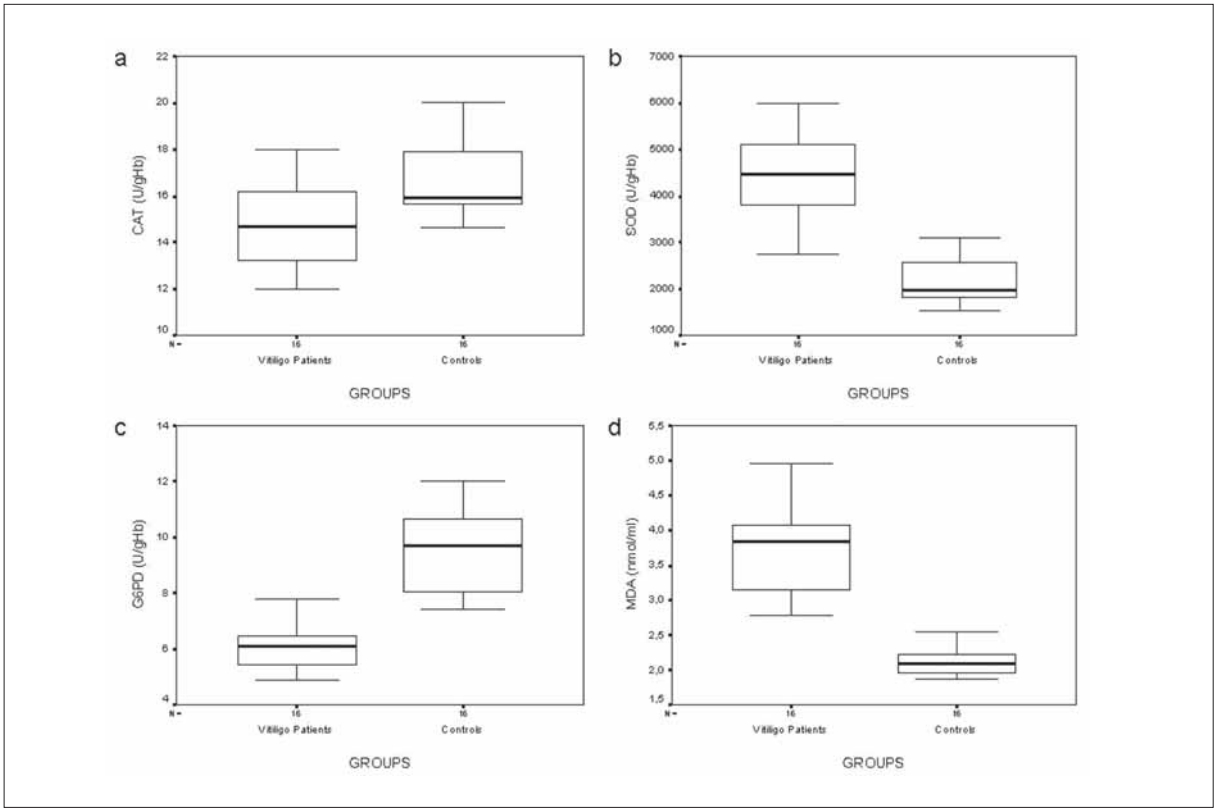
Conclusion: Our results confirmed that oxidative stress may play an important role in the pathogenesis of vitiligo. Melanocyte damage in vitiligo might be linked to generalized oxidative stress. This study is the first report on some antioxidant parameters of localized-type vitiligo patients.

K E Y W O R D S

vitiligo, oxidative stress, antioxidant status, catalase, superoxide dismutase, malondialdehyde, glucose 6-phosphate dehydrogenase

Introduction

Vitiligo is an acquired skin disease characterized by white areas of the skin that can be observed in 0.1 to 8.8% of the population. The disease may affect individuals of both sexes and is mostly characterized by loss of melanocytes (1). Despite much research, the etiology of vitiligo and the causes of melanocyte death are not clear. At least three pathogenic mechanisms – immunological, neural, and biochemical – have been suggested, but none can completely explain the disease (2, 3). Some findings show that oxidative stress may be an important phenomenon in the pathophysiology of vitiligo (3–14). Imbalances in the oxidant/antioxidant system, such as the accumulation of hydrogen peroxide (H_2O_2) and low catalase (CAT) levels, have recently been demonstrated in the epidermis and blood of vitiligo patients (15–18). Recent studies have shown antioxidant systems to play a role in the pathogenesis of generalized vitiligo (5, 6). Antioxidant status has also been studied in segmental and non-segmental vitiligo (19); however, the literature contains no information about the status of antioxidant systems in the blood of localized vitiligo patients.



Figures 1a–1d. Box plot graphic representations of antioxidant enzyme activities and MDA levels in both groups.

The purpose of this study was to evaluate the role of oxidative stress in the pathogenesis of active localized vitiligo. We investigated the role of antioxidant systems by measuring the levels of CAT, superoxide dismutase (SOD), glucose 6-phosphate dehydrogenase (G6PD) in erythrocytes, and the plasma levels of malondialdehyde (MDA) in vitiligo patients with active localized disease, and in healthy controls.

Material and methods

Patients and controls

The study comprised 16 consecutive patients with active localized vitiligo that had visited the KSU Medical Faculty Department of Dermatology between 2004 and 2005 and were diagnosed by clinical examination and Wood's lamp. None of the patients had segmental or generalized vitiligo, an autoimmune disease, a concomitant dermatological disease, or thyroid dysfunction. Patients that had used systemic or topical treatment within at least 1 month prior to study entry were excluded. Sixteen healthy volunteers with no systemic disease were included as a control group. The patients and controls had no history of smoking, alcohol intake, vitamin intake, or use of anti-inflammatory or other drugs. Skin phototypes of patients and controls were Fitzpatrick's II–IV. A signed informed consent was obtained from each subject.

Methods

Blood from the forearm vein was collected into 5 ml Vacutainer® tubes containing potassium EDTA (ethylenediaminetetraacetic acid). The blood samples were centrifuged at 1,000 × g for 10 minutes at 4 °C to remove plasma. The buffy coat on the erythrocyte sediment was separated carefully after plasma was removed. The erythrocytes were washed three times with 0.9% NaCl solution to remove the plasma remnant. After each procedure, the erythrocyte-saline mixture was centrifuged at 1,000 × g for 10 minutes at 4 °C. The hemolysates were prepared from the washed cells to measure the biochemical workup parameters.

CAT activity was assayed by measuring the degradation rate of H_2O_2 using Beutler's method (20). The rate of disappearance of H_2O_2 was monitored spectrophotometrically at 230 nm. The assay medium consisted of 50 µL 1M Tris HCI buffer (pH 8), 930 µL 10mM H_2O_2, 930 µL deionized water, and 20µL hemolysate sample. One unit of CAT activity is defined as the amount of

enzyme causing about 90% destruction of the substrate in 1 min in a volume of 1 ml. CAT activity in the erythrocyte was expressed as U/g hemoglobin.

SOD activity was measured according to the method described by Fridovich (21). To determine SOD activity in hemolysate preparations, the degree of inhibition of a reaction that catalyses the generation of superoxide radical by xanthine and xanthine oxidase was monitored spectrophotometrically at 505 nm for 3 min. The assay medium consisted of the 50 µL 0.01 M phosphate buffer, 1.7 mL substrate solution (0.05 mM xanthine and 0.025 mM INT in 3-cyclohexilamino-1-propanesulfonicacid (CAPS) buffer pH 10.2), 250 µL 80 U/L xanthine oxidase, and 50µL hemolysate sample. A 1-unit SOD sample inhibits the reaction by approximately 50% of the initially measured xanthine-oxidase reaction. The activity is given in SOD units (1 SOD unit = 50% inhibition of the xanthine oxidase reaction). SOD activity in the erythrocyte was expressed as U/g hemoglobin.

G6PD activity was determined at 37 °C using Beutler's method (20). The reaction mixture contained 1 M Tris-HCl pH 8, 6 mM glucose 6-phosphate sodium, 2 mM nicotinamide adenine dinucleotide phosphate (NADP), 0.1 M $MgCl_2$, and hemolysate in a total volume of 3 ml. One unit of enzyme activity is the amount catalyzed the reduction of 1 mM of NADP per minute. Results were expressed as U/g hemoglobin.

The lipid peroxidation level in the plasma samples was expressed in MDA. It was measured according to the procedure developed by Ohkawa et al. (22). The reaction mixture contained 0.1 ml sample, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid, and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid. The mixture's pH was adjusted to 3.5 and the volume was then made up to 4 mL with distilled water, whereupon 5 mL of a mixture of n-butanol and pyridine (15:1, v/v) was added. The mixture was shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm. The rate of lipid peroxidation was expressed as nmol of thiobarbituric acid reactive substances (TBARS) formed of ml plasma using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$. Results were expressed as nmol/ml.

The hemoglobin level was measured by the cyanomethemoglobin method with a Spectronic-UV 120 spectrophotometer (20). Bovine serum albumin was used as a standard.

Statistical analysis

Statistical assessment was carried out with the SPSS 10.0 for Windows statistical software. All data were given as mean ± standard deviation (SD). The chi-square test was used to compare differences between the frequencies. The Mann-Whitney U test was used to compare mean values between groups. The Spearman correlation test was used for the assessment of correlation. The statistical significance was accepted as $p < 0.05$.

Results

A total of 16 patients (9 males, 7 females), with a mean age of 24.9 ± 18.6 years (range 3–63 years) were enrolled in the study. The control group (n = 16) included 9 males and 7 females, with a mean age of 23.5 ± 15.1 years (range, 5–50 years). The mean duration of illness for the patients' group was 4.1 ± 7.3 years (range, 3 months–50 years). There were no significant differences in age, male/female ratio, or skin phototypes between the patients and controls ($p > 0.05$).

The mean, minimum, and maximum values of the blood activities of antioxidants and MDA levels of both groups are shown in Table 1. SOD activities and MDA levels of patients were significantly higher than in controls. CAT and G6PD activities of patients were significantly lower than in controls. The results are graphically presented in Figs 1a–1d. No correlation was observed between enzyme levels and age (r = 0.12, p = 0.98) or sex (r = 0.28, p = 0.67).

Discussion

Vitiligo is a common disease but, unfortunately, the pathogenesis of vitiligo is still unclear. Oxidative stress has been proposed as the triggering event in the melanocyte degeneration of vitiligo (3–14). Some studies have also showed that melanogenesis produces significant levels of reactive oxygen species (ROS) (23). ROS and other radicals can induce oxidative stress (24). Oxidative stress may be a good model for vitiligo pathogenesis.

G6PD is the first rate-limiting enzyme in the hexose monophosphate shunt pathway, playing an important role in the regeneration of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). It has the role of protecting the oxidant/antioxidant balance in the cell and reducing the oxidative stress. In addition, NADPH is necessary for the formation of reduced glutathione in erythrocytes, the reduction of methemoglobin to oxyhemoglobin, and CAT activity (25–27). Some authors have reported a significant decrease in G6PD activity similar to our results (7, 19, 27).

CAT converts hydrogen peroxide to water and oxygen (29). Some authors reported normal CAT activities in erythrocytes of vitiligo patients (7, 8, 11, 14). However, Dell'Anna et al. (3, 4) found lower CAT activity in leukocytes of vitiligo patients. In addition, Shajil and Begum showed lower CAT activity in segmental vitiligo patients, whereas in non-segmental vitiligo patients CAT activity was normal (19). We also found significantly lower CAT activity in erythrocytes of localized vitiligo patients. Previous studies of vitiliginous melanocytes showed lower CAT activity (11, 30). We believe that lower CAT activity may be associated with H_2O_2 accumulation, which may further inhibit CAT activity resulting in the destruction of melanocytes (16).

SOD catalyzes the conversion of superoxide anions to oxygen and hydrogen peroxide. It protects cells from

Table 1. Antioxidant enzyme activities and malondialdehyde (MDA) levels in vitiligo patients and controls (mean ± SD, minimal and maximal values).

	CAT (U/gHb)	SOD (U/gHb)	G6PD (U/gHb)	MDA (nmol/ml)
Patients (n = 16)	14.8 ± 2.0 (12–18)	4,457 ± 930 (2,750–6,000)	6.1 ± 0.8 (4.9–7.8)	3.8 ± 0.6 (2.8–5.0)
Controls (n = 16)	16.67 ± 1.5 (15–20)	2,219 ± 505 (1,550–3,100)	9.5 ± 1.4 (7.4–12)	2.2 ± 0.3 (1.9–2.7)
p* value	< 0.05	< 0.001	< 0.001	< 0.001

the toxic effect of superoxide radicals (28). This study found significantly higher levels of SOD activity of erythrocytes in patients with active localized vitiligo. Increased levels of erythrocyte SOD in patients with vitiligo may enhance the systemic production of H_2O_2. In addition, high SOD activities were correlated with high immune competence (31). Previous studies were performed in patients with generalized or combined types of vitiligo. There are different reports on SOD activity in patients with vitiligo compared to the healthy controls. SOD activity of erythrocytes was found to be normal (4, 8, 11, 12) in some studies and higher in others (5, 7, 14, 19, 32). On the other hand, one study (6) reported lower levels in erythrocytes. Furthermore, Dell'Anna et al. (4) found higher SOD activity in leukocytes of vitiligo patients. Although SOD activities in the vitiliginous tissue were found to be normal in one study (12), Maresca et al. (11) and Yildirim et al. (13) found it to be high. We hypothesized that these varying results could be related to differences in serum, leukocyte, erythrocyte, and epidermis levels, duration and activity of disease, and differences in laboratory techniques.

MDA is an end-product of a lipid peroxidation reaction and is accepted as a specific indicator of oxidative stress (33). Picardo et al. (8) and Tastan et al. (12) found normal serum MDA levels in erythrocytes of combined types of vitiligo. Yildirim et al. (5) and Koca et al. (6) showed higher serum MDA levels in generalized vitiligo patients. Whereas Tastan et al. (12) found the MDA level in vitiliginous tissue to be normal, Yildirim et al. (13) found it to be high. In this study we found statistically higher plasma MDA levels in localized vitiligo patients. Lipoperoxidation, the primary reaction sites of which involve membrane-associated polyunsaturated fatty acids of phospholipids, can be considered a major manifestation of oxidative stress (9). High SOD activity also has been found to correlate with high MDA levels.

In conclusion, our results showed that oxidative stress may play a role in the pathogenesis of vitiligo and cause the melanocyte damage in vitiligo. Published data suggests that the oxidant/antioxidant system may be affected in all types of vitiligo. The changes in oxidative stress parameters are not related to the types of the disease. Once the living organism is exposed to a disease, the oxidative state may be influenced in a different ways. The changed antioxidant enzyme activities of erythrocytes in the patients might be peripheral responses of the organism to an increased oxidative stress. No study has ever investigated how the imbalance of the oxidant/antioxidant system in vitiligo affects the process of the disease. This study is the first report on some antioxidant parameters of localized-type vitiligo patients. However, further larger studies are necessary to confirm our results and to verify whether antioxidant treatments may be beneficial for patients with vitiligo.

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