



Original Contribution

SEASONAL VARIATIONS IN THE ACTIVITY OF ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN PSORIATIC AND VITILIGO PATIENTS

Evgenia Hristakieva¹ and Veselina Gadjeva^{2*},

¹Department of Dermatology, ²Department of Chemistry and Biochemistry,
Medical Faculty, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

In the current study we explored the seasonal influence on the activity of the antioxidant defense enzymes superoxide dismutase (SOD) and catalase (CAT) in erythrocytes and the levels of lipid peroxidation (MDA products) in plasma of patients with vitiligo and psoriasis. Patients were separated in two groups: "summer" (investigated from May to October) and "winter" (from November to April). We found significantly increased MDA products in plasma (mean 2.49 μ M/l vs 1.87 μ M/l, $p < 0.001$ t-test) and SOD in erythrocytes (mean 5599.403 U/gHb vs 4493.110 U/gHb $p = 0.05$ t-test) for patients with vitiligo "summer" in comparison with those in "summer" controls. Increased CAT activity in the "summer" group of psoriasis compared to those of "winter" psoriasis group (mean 24771.10 U/gHb vs 12745.67 U/gHb, $p < 0.0001$ t-test) and to "summer" controls were also detected (mean 24771.10 U/gHb vs 15962.52 U/gHb, $p < 0.01$, t-test). In conclusion, our results indicate that exposure to environmental climatic factors due to seasonal changes induces increased systemic oxidative stress during the "summer" for patients with vitiligo and psoriasis. The improvement of antioxidant ability of erythrocytes in vitiligo and psoriasis patients during the warm seasons suggests a possible role of sunlight towards controlling or suppressing the diseases and might be one of the mechanisms explaining the positive effects of the helio- and thalassotherapy.

Key words: Psoriasis, Vitiligo, SOD, CAT, MDA, Seasons

INTRODUCTION

Bulgaria is a country with temperateness climate. The most comfortable temperatures are found from May to October. The country's climate is influenced by the Mediterranean and the Black Seas, making for generally mild conditions throughout the country and with two predominant seasons. Summer and springtime days rarely get too hot with intensive sun irradiation. The winter and late autumn can be bitterly cold, snowy and damp and with reduced sun irradiation. The increase of motor vehicles has caused a higher amount of nitrogen oxide and hydrocarbon emissions. Under particular weather conditions (high solar radiation, high temperatures, temperature inversions) these compounds may undergo complex chemical transformations resulting in formation of photochemical smog and a build-up of potentially toxic ozone concentrations.

The skin is the primary interface between

the body and the environment. The spectrum of insults to which skin is susceptible includes disorders caused by chemical and microbial agents, thermal and electromagnetic radiation (1). In addition, the changing climatic factors, especially the amount of precipitation, temperature and solar radiation (including ultraviolet-B), modify responses of humans to air pollution, and must be considered when these effects are evaluated. The regulation of UV-mediated melanogenesis and the importance of melanins in the photoprotection of human epidermis are not yet fully understood. Cellular interactions in antioxidant defences may pertain to some diseases with a loss of melanocytes, such as vitiligo (2). More and more evidence comes to prove accumulating that a proper balance between oxidants and antioxidants is involved in the maintenance of health and longevity and that altering this balance in favour of oxidants may result in pathological responses causing functional disorders and disease. Numerous recent studies have suggested that oxidative stress may be important in the pathogenesis and treatment of both, psoriasis

*Correspondence to: Vesselina Gadjeva, 11 Armeiska Str. 6000 Stara Zagora, Bulgaria, tel.: +359 42 79012; E-mail: vgadjeva@mf.uni-sz.bg

and vitiligo (3, 4).

Psoriasis anywhere involves from 1-5 % of the world's population depending on what racial group is studied. The disease as a considerable geographical variability in its prevalence (5). Although the incidence is a generally lowest at the equator and increases towards the poles, it is unlikely that climatic differences are responsible for this geographical variability, as there is prevalence of psoriasis in populations who live at similar latitudes. Although the above observations suggest that patients are genetically predisposed to psoriasis, it is also clear that environmental factors and psychological stress are involved in the expression of the disease (6). Most patients observe that their psoriasis improves during the summer with exposure to sunlight.

Vitiligo is an acquired skin disorder that affects between 0.5 and 4% of the world population, caused by the disappearance of pigment cells from the epidermis. Oxidative stress could be an important phenomenon leading to melanocyte death in vitiligo. Few abnormalities of antioxidants have been found in the blood of black patients with active generalized vitiligo (7). The cause is unknown, but might involve genetic factors, autoimmunity, toxic metabolites, and/or a higher vulnerability of melanocytes, (8). There are several possible approaches in the management of vitiligo and psoriasis. Helio- and thalassotherapy are both a kind of treatment proposed for these diseases. Warm weather summertime and rest and relaxation at beach-type vacation environments may provide significant periods of improvement without accompanying medical treatments in psoriasis patients (9). Most studies on photoradiation have come to a conclusion that more than 75% repigmentation can be achieved only when vitiligo patients are treated on a regular basis and for longer periods (10).

In the present study we report the seasonal influence on the level of systemic oxidative stress in patients with vitiligo and psoriasis through the level of plasma lipid peroxidation products and activities of the erythrocytes antioxidant enzymes SOD and CAT. Further, we have tried to explain a possible role of the oxidative stress and antioxidant ability during the seasons on the progress of these diseases.

MATERIALS AND METHODS

Patients

One-hundred and nineteen patients (40 with vitiligo and 79 with psoriasis) were selected

in this study. All of them were investigated prior any treatment: phototherapy, climatotherapy or other type of medication. The diagnosis was made by standard clinical and surface marker criteria. The mean age was 35 years (range 13-58). Patient's clinical characteristics (gender, age, disease duration,) are listed in Table 1. Patients were divided into two groups: "summer" (investigated from May to October) and "winter" (investigated from November to April). One hundred healthy volunteers, aged between 14 to 55 years of either sex formed the control group. They were nonsmokers and free of disease and pathological antecedents.

Blood Samples and Lysates

Blood was collected in tubes containing ethylenediamine-tetraacetic acid (EDTA), centrifuged at 3000 rpm for 15 min and plasma was carefully separated. The erythrocyte pellets were washed three times with saline, and 0.5 ml of the cell suspension was diluted with 2 ml cold water to lyse the erythrocytes. To 0.2 ml lysate 1.8 ml water and ethanol/ chloroform (3:5/v:v) were then added to precipitate hemoglobin. The tubes were shaken vigorously for 5 min and centrifuged at 2500 rpm for 20 min. The supernatant was used for determination of SOD and CAT enzyme activity and hemoglobin concentration in the corresponding sample.

Quantification of Lipid Peroxidation

Total amount of lipid peroxidation products in the plasma of healthy volunteers and patients was estimated using the thiobarbituric acid (TBA) method, which measures the malondialdehyde (MDA) reactive products (11). In brief, 1.0 ml of plasma, 1.0 ml of normal saline and 1.0 ml of 25% trichloroacetic acid (TCA) were mixed and centrifuged at 2000 for 20 min. One ml of protein free supernatant was taken, mixed with 0.25 ml of 1% TBA and boiled for 1 h at 95° C. After cooling, the intensity of the pink color of the product obtained was read at 532 nm. Results were expressed as $\mu\text{M/l}$.

Determination of Superoxide Dismutase

CuZn-SOD activity was determined as described by Sun et al.,(12) with minor modifications. The xanthine/xanthine oxidase system was used to generate the superoxide anion. This anion reduces nitroblue tetrazolium (NBT) to formazan, which is monitored at 560 nm. SOD of the sample removes the superoxide anion and inhibits the reduction. The level of this reduction was used as a measure of SOD activity. The final concentrations of xanthine, xanthine oxidase and nitroblue tetrazolium in the assay were 50

μM , 10 units/ml and 0.125 mM. One unit of enzymatic activity was defined as an amount of that causes 50% inhibition of NBT reduction to formazan. The results were expressed as U/gHb.

Monitoring of Catalase Activity

CAT activity in the erythrocyte lysats was assessed by the method described by Beers and Sizer (13). Briefly, hydrogen peroxide (30 mM) was used as a substrate and the decrease in its concentration at 22°C in phosphate buffer (50 mM, pH 7.0) was followed spectrophotometrically at 240 nm. One unit of CAT activity was defined as enzyme amount that degrades $1\mu\text{M}$ H_2O_2 per min. Results were presented as units per g hemoglobin (U/g Hb).

The hemoglobin concentration was determined by the cyanmethemoglobin method (14). The results were reported as means $\pm$ S.D. for the two groups of patients and the control group.

Statistical analysis was performed with Student's *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

The results of MDA levels in plasma of patients with vitiligo and psoriasis are compared in Figure 1. We found significantly increased MDA products in plasma (mean $2.49 \mu\text{M/l}$ vs $1.87 \mu\text{M/l}$, $p < 0.001$ *t*-test) for patients with vitiligo in the "summer" group compared to those in "summer" group of the controls. In the "summer" group was found a tendency to increase in comparison to those in the "winter" group of vitiligo. There was no statistically difference of MDA plasma levels between the "summer" group of psoriasis and "summer" controls ($2.103 \mu\text{M/l}$ vs $1.87 \mu\text{M/l}$, $p > 0.05$), also compared to the "winter" group of psoriasis ($2.103 \mu\text{M/l}$ vs $1.96 \mu\text{M/l}$, $p > 0.05$).

The results of the study of the red blood cell SOD activity are presented in Figure 2. We found a significantly increased SOD activity for the vitiligo "summer" group patients in comparison with that in "summer" group of the controls (mean 5599.403 U/gHb vs 4493.110 U/gHb , $p < 0.05$ *t*-test). SOD activity in the vitiligo "summer" group was significantly increased compared to "winter" vitiligo group (mean 5599.403 U/gHb vs 4263.844 U/gHb , $p < 0.05$ *t*-test) and with a tendency to increase in the "summer" psoriasis group compared to the "winter" psoriasis group (mean 4906.768 U/gHb vs 3669.302 U/gHb , $p > 0.05$), Figure 2.

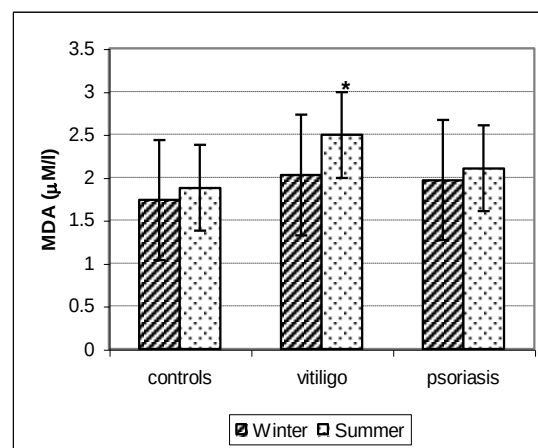


Figure 1. Comparison of MDA reactive products, presented in the plasma of vitiligo and psoriasis patients: "winter" group (from November to April) and "summer" group (from May to October). The results are expressed as mean $\pm$ S.D. $p < 0.001$ (vitiligo "summer" vs controls "summer"), *t*-test.

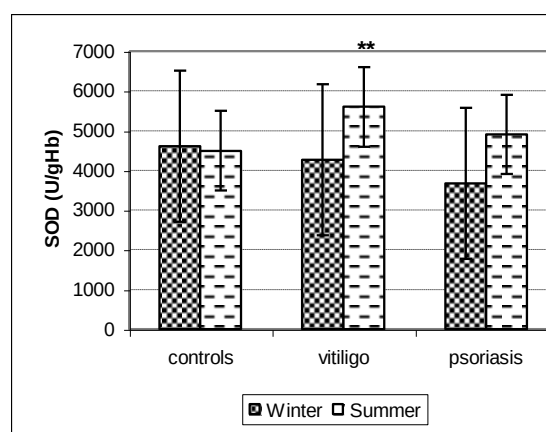


Figure 2. Comparison of SOD activity in erythrocytes of vitiligo and psoriasis patients. "winter" group (from November to April) and "summer" group (from May to October). The results are expressed as mean $\pm$ S.D. $**p < 0.05$ (vitiligo "summer" vs controls "summer" and vitiligo "winter"), *t*-test.

The other antioxidant enzyme CAT presents a clear tendency to increase the activity itself in the vitiligo "summer" group compared to controls in the "summer" months (mean 20365.47 U/gHb vs 15962.52 U/gHb , $p > 0.05$), Figure 3. There was not observed a seasonal dependency in CAT activity in vitiligo patients between the "summer" and the "winter" groups but we found a significantly increased activity in vitiligo "winter" compared to the control "winter" (19684.90 U/gHb vs 12754.62). In the "summer" group of psoriasis our results demonstrated an increased CAT compared to the "winter" group of psoriasis (mean 24771.10 U/gHb vs 12745.67 U/gHb , $p < 0.0001$ *t*-test) and to the "summer" group of the controls (mean 24771.10 U/gHb vs 15962.52 U/gHb , $p < 0.01$, *t*-test), Figure 3.

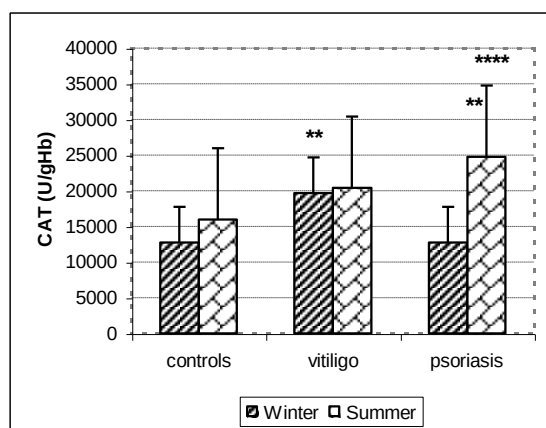


Figure 3. Comparison of CAT activity in erythrocytes of vitiligo and psoriasis patients. “winter” group (from November to April) and “summer” group (from May to October). The results are expressed as mean $\pm$ S.D. ** $p < 0.05$ (psoriasis “summer” vs controls “summer” and vitiligo “winter”; vitiligo “winter” vs controls “winter”); *** $p < 0.0001$ (psoriasis “summer” vs psoriasis “winter”), *t*-test.

There was no correlation between the oxidative stress parameters and the age in either patients or control group (data are not shown). To study the time-lapse effect of the disease on the investigated parameters, the patients were divided into three groups: those of less than 1 year of illness, those of 1 to 5 years, and those of more than 5 years of the disease since the date of diagnosis (Table 1). No correlations were observed between antioxidant status and the disease duration.

Table 1. Clinical characteristics of the patients with psoriasis and vitiligo.

Characteristics	Number of patients with psoriasis	Number of patients with vitiligo
Gender		
Male	41	17
Female	38	23
Age		
≤ 20	14	13
21-49	33	20
≥ 50	32	7
Time from diagnostic		
< 1 year	17	12
1-5 years	22	20
> 5 years	40	8

DISCUSSION

We established for the first time increased system oxidative stress during the seasons with warm weather for patients with vitiligo and psoriasis. Patients suffering from vitiligo, investigated from May to October had significantly increased MDA level in plasma and SOD activity in erythrocytes. During these months, the weather conditions in

Bulgaria are characterized with high solar radiation, high temperatures, temperature inversions. The peroxidation of the cell membranes may be the basis for conformational changes triggered by UV energy absorption, inducing signaling for melanogenesis and other photoprotective mechanisms (15). The SOD/CAT ratio has been evidenced to be a suitable marker of cell sensitivity of cultured human skin fibroblasts to UV exposure (16). We have previously reported an increased oxidative stress in patients with vitiligo and psoriasis before any kind of therapy demonstrated through the high plasma formation of lipid peroxidation products and it is accompanied with compensatory changes in the levels of the antioxidant defense enzymes: increased activity of CAT and SOD (17, 18).

In the present work, we demonstrate significantly higher erythrocyte levels of CAT, and not significant changes in MDA in summer for patients suffering from psoriasis. This suggests a compensatory rise in antioxidant enzyme activities in response to the increased free radical formation and the oxidative stress. Moreover, antioxidant systems interact in a complex fashion, so that changes in activity or concentration in one component can affect the whole system. CAT, however, is the enzyme most susceptible to the effect of UV irradiation in the epidermis (19). Its activity can be affected by high peroxide concentrations and by visible light (20). Enhancement of SOD activity in erythrocytes and MDA levels in plasma of vitiligo patients in seasons with high UV-irradiation indicates that the oxidative stress can play a role in the pathophysiology of the vitiligo. Our results reveal that the oxidative stress is a systemic problem in patients with vitiligo, so a systemic antioxidant therapy should be considered. We agree with a current hypotheses that lowering oxidative stress can have a clinical benefit in psoriasis and vitiligo.

The improvement of antioxidant ability of erythrocytes SOD and CAT in patients with psoriasis during the warm weather summertime suggests a possible role of sunlight towards controlling or suppressing the diseases and may be one of the mechanisms explaining the positive effects of the helio- and thalassotherapy. However, changes of an antioxidant levels are likely to play a role in the activity of the psoriasis disease. Since, the natural antioxidant defense system SOD limits the development of inflammatory and immune processes, we suppose that the tendency to its reducing

during the “winter” compared to the “summer” group of psoriasis patients is one of the reasons for the aggravated dermatological status typical for the cold seasons. We can speculate, that the reduction of the antioxidant defense systems, found during the “winter” months, could be directly responsible for an enhanced susceptibility of patients with vitiligo to oxidative stress, as it indicated by the high MDA concentration observed in next “summer” months.

In view of these results, our study represents a new approach to investigate susceptibility of patients to oxidative stress before treatments such as helio- and phototherapy where free radicals damage is involved. Studies are in progress to evaluate the additional supplementation of antioxidants in order to prevent oxidative free radical damage and to evaluate the effectiveness of the therapy.

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