EVALUATION OF PLASMA OXIDATIVE STRESS IN THE CASE OF PATIENTS WITH VITILIGO AND NICOTINE-DEPENDANCE

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Abstract: Vitiligo represents a skin pathology characterized by white areas. The mechanism of occurrence of this disease has many unknown. There are the authors who consider that oxidative stress might be involved in the pathogenesis of this disease. Smoking as risk factor would accelerate the emergence and evolution of this disease. The purpose of this paper was to assess markers of oxidative stress in this context. In a dermatology outpatient between February 2008 and May 2009 we evaluated 25 patients with Vitiligo and nicotine-dependence by means of Fagerstrom test and 24 healthy controls subjects of a similar age and sex distribution. We measured their indicators of oxidative stress such as catalase (CAT), superoxidedismutase (SOD), glucose 6-phosphate dehydrogenase (G6PD) in erythrocytes, and plasma malondialdehyde (MDA) by spectrophotometry.

INTRODUCTION

Vitiligo is an acquired depigmentary disorder affecting around 1% of the world's population. Approximately 50% of the cases have the onset of their disease prior to the age of 20 years and 25% prior to the age of 14 years. (Hann et al., 2000; Kovacs SO., 1998) Vitiligo is characterized by selective destruction of melanocytes of the basal layer of the epidermis and/or occasionally the hair follicle resulting in white patches on the skin, the mucous membranes and/or white hair.

Various theories have been proposed for the etiology of vitiligo, including genetic, neural, autocytotoxic/ metabolic and autoimmune theories, all of which have been encompassed in the convergence theory. It seems that vitiligo has a multifactorial etiology, where genetic factors, various kinds of stress (emotional stress, oxidative stress with the accumulation of free radicals), accumulation of toxic melanin precursors in melanocytes, disturbance of melanocytes homeostasis (e.g., impaired intracellular and extracellular calcium), and autoimmunity can all contribute to the development of the disorder. (Njoo et al., 2001; Passeron et al., 2005; Dell'anna et al., 2006)

Some findings show that oxidative stress may be an important phenomenon in the pathophysiology of vitiligo (Dell'anna et al ., 2003; Yildirim et al, 2003 ; Koca et al., 2004; Agrawal et al., 2004; Yildirim et al., 2004; Hazneci et al., 2005)

Imbalances in the oxidant/antioxidant system, such as the accumulation of hydrogen peroxide (H_2O_2) and low catalase (CAT) levels have been demonstrated in the epidermis and blood of vitiligo patients (Schallreuter et al., 2001; Schallreuter et al., 1999; Hasse et al., 2004; Rokos et al., 2002). The antioxidant status has been studied in the case of segmental and non-segmental vitiligo (Shajil et al., 2006).

It should be noted that smoking is an important risk factor with implication in the etiology of many diseases. The mechanisms of induction/inhibition enzymatic simultaneously present with disruption melanocytic balance may amplify the pathological effects.

The purpose of this study was to assess markers of oxidative stress in the case of patients diagnosed with vitiligo and nicotine dependence compared with a control group consisting of healthy subjects.

MATERIALS AND METHODS

The study comprised 25 patients with localized vitiligo that had visited the Department of Dermatology of Municipal Hospital between February 2008 and May 2009 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.

24 healthy volunteers with no systemic disease were included as a control group.

To assess nicotine dependence, Fagerstrom test was applied to all subjects included in this study. According to the score, the level of dependence on nicotine was: a) the score 0-2 - very low dependence; b) the score 3-4 - low dependence; c) the score 5 - medium dependence; d) the score 6-7 - high dependence; e) the score 8-10 - very high dependence (Heatherton et al., 1991)

Blood from the forearm vein was collected into 5 ml tubes containing potassium EDTA (ethylenediaminetetraacetic acid). The blood samples were centrifuged at 1,000 × g for 10 minutes at 4 °C to remove plasma.

CAT activity was assayed by measuring the degradation rate of H_2O_2 using Beutler's method (Beutler E., 1975). The rate of disappearance of H2O2 was monitored spectrophotometrically at 230 nm. 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 (Fridovich I., 1974). 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 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 (Beutler E., 1975). One unit of enzyme activity is the amount catalyzed the reduction of 1 mM of nicotinamide adenine dinucleotide phosphate (NADP) per minute. Results were expressed as U/g hemoglobin.

The lipid peroxidation level in the plasma samples was expressed in malondialdehyde (MDA). Results were expressed as nmol/ml.

Statistical assessment was carried out with the SPSS 10.0 for Windows statistical software. All data were given as mean ± standard deviation (SD). The statistical significance was accepted as $p < 0.05$.

RESULTS AND DISCUSSIONS

A total of 25 patients (13 males, 12 females), with a mean age of 33.5 ± 15.4 years were enrolled in the study. The control group ($n = 24$) included 12 males and 12 females, with a mean age of 33.9 ± 14.1 years. The mean duration of illness for the patients' group was 5.1 ± 2.3 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.

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

	CAT (U/gHb)	**SOD (U/gHb)**	**G6PD (U/gHb)**	**MDA (nmol/ml)**
Patients ($n = 16$)	13.7 ± 2.0 (12–18)	4,546 ± 920 (2,750–6,000)	6.4 ± 0.7 (4.9–7.8)	3.7 ± 0.5 (2.8–5.0)
Controls ($n = 16$)	16.76 ± 1.4 (15–20)	2,128 ± 403 (1,550–3,100)	9.4 ± 1.2 (7.4–12)	2.1 ± 0.2 (1.9–2.7)
***p* value**	< 0.05	< 0.001	< 0.001	< 0.001

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.

Comparative evaluation of specific enzymatic activity in oxidative stress and nicotine dependence for two study groups was featured in the figure 1.

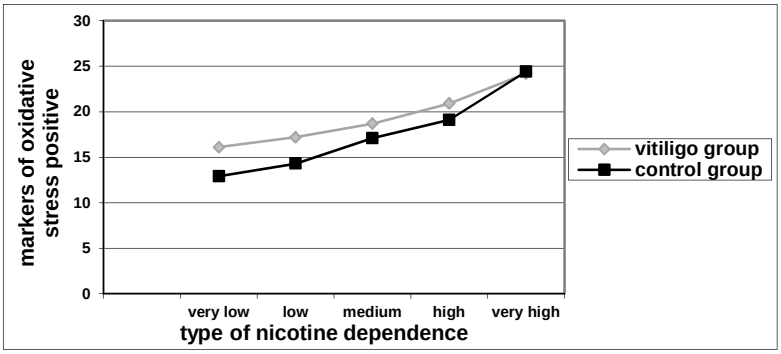

Figure 1 Comparative assessment of specific enzymatic activity according to the type of nicotine dependence

It was observed as specific enzyme activity for oxidative stress was more intense in vitiligo group compared with the control group.

Cigarette smoke is a complex mixture of more than 4700 chemical compounds including free radicals and oxidants. Toxicity exhibited by cigarette smoke may be due to combined action of these compounds inducing many cellular processes mediated through reactive oxygen species (ROS). Major player probably nicotine as it is present in tobacco, in higher concentrations. The compounds that induce intracellular oxidative stress recognized as the important agents involved in the damage of biological molecules, also melanocytes. (Johnny et al., 2007)

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. According to autocytotoxic hypothesis, oxidative stress has been suggested to be the initial pathogenic event in melanocyte degeneration (Yildirim et al., 2004).Some studies have also showed that melanogenesis produces significant levels of reactive oxygen species (ROS) (Riley et al., 1988). ROS and other radicals can induce oxidative stress (Procter et al., 1984). In oxidative stress, there is insufficient antioxidant activity leading to excessive accumulation of free radicals, which damage cellular compounds.

SOD catalyzes the conversion of superoxide anions to oxygen and hydrogen peroxide. It protects cells from the toxic effect of superoxide radicals (Beyer et al., 1991). 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 (Prasad et al., 1995; Passi et al., 1998).

Related to other enzymatic activity our study was comparable with literature data. (Yildirim et al., 2003 ; Koca et al., 2004; Yildirim et al., 2004; Boisseau-Garsaud et al., 2003; Schallreuter et al., 1991)

CONCLUSIONS

The results of this study showed that oxidative stress may play a role in the pathogenesis of vitiligo and cause the melanocyte damage in vitiligo.

The data of specialized literature suggests that the oxidant/antioxidant system may be affected in all types of vitiligo. The changed antioxidant enzyme activities of erythrocytes in the patients might be peripheral responses of the organism to an increased oxidative stress. However, further larger studies are necessary to confirm our results and to verify whether antioxidant treatments may be beneficial. 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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