The Role of Oxidative Stress in Vitiligo and Alopecia Areata

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Abstract

Background: The pathogenetic mechanism in vitiligo and alopecia has not been completely clarified. One of the major hypotheses in the pathogenesis of these diseases is oxidative stress hypothesis. Oxidative stress may be induced by increasing the generation of reactive oxygen species (ROS) and other free radicals.

Objectives: The objective of the present study is to evaluate the role of oxidative stress in pathogenesis of vitiligo, and Alopecia areata.

Materials & Methods: Twenty patients with generalized and localized vitiligo, 20 patients with alopecia areata, and 25 controls were included in this study. We examined serum levels of malondialdehyde (MDA), super oxide dismutase (SOD) and vitamins E and A in patients with vitiligo and alopecia areata and control subjects.

Result: The level of MDA in serum with patients with vitiligo and alopecia (2.07±0.43, 1.73±0.31, respectively) were significantly higher than those of control (0.85±0.12; p<0.0001). The SOD activity (8.94±0.38, and 8.809±0.44, correspondingly) in serum of patients with vitiligo and AA were significantly lower than that of controls (10.142±2.61). The level of vitamin A in serum of patients with vitiligo and AA (53.47±2.27, 52.85±2.34, respectively) were significantly lower than control (59.48±0.92, p<0.0001), while vitamin E levels in serum did not differ statistically.

Conclusion: increased lipid peroxidation may relate to decrease in SOD activity and vitamin A levels. These results demonstrate the presence of an imbalance in the oxidant-antioxidant system and provide further support for a free radical-mediated damage as an initial pathogenic event vitiligo and AA.

Key Words: Oxidative Stress, Vitiligo, Alopecia Areata

Introduction

The skin is constantly exposed to oxidative stress induced by reactive oxygen species (ROS), that are generated both from endogenous sources, such as enzyme activity or activated neutrophils and external pro-oxidant stimuli such as ultraviolet radiation (UV) [1-4].

Oxidative stress is now considered to be important in the pathogenesis of skin disease like vitiligo and Alopecia areata AA, an insufficient antioxidant system together with increased levels of reactive oxygen species (ROS) have been suggested to be important in the pathogenesis of these diseases.

Vitiligo is a common pigmentary disorder of the skin with selective destruction of melanocytes according to autolysis hypothesis. Oxidative stress has been suggested to be the initial pathogenic event in melanocyte degeneration with 
H2
O2 accumulation in the epidermis of patients with active disease [7].

An alteration in the antioxidant pattern with significantly higher levels of superoxide dismutase (SOD) has been observed in the skin [9] erythrocytes, peripheral blood mononuclear cells [9,12] and serum [13,14] of vitiligo patients. Reduction in catalase (CAT) activity has been demonstrated in the epidermis [15,16] peripheral blood mononuclear cells [9] and in melanocytes [7] These findings support the concept of possible systemic oxidative stress in vitiligo.

In the other hand Alopecia areata (AA) is a common, recurrent, chronic inflammatory disorder of the hair and nails. It occurs in either sex and any age can be affected [17]. The etiology and pathogenesis of AA is still uncertain but many factors have been assumed concerning its pathogenesis e.g. the patient’s genetic constitution, family history, the atopic state, nonspecific immune and organ-specific autoimmune reactions, possible emotional stress, infections agents, and neurological factors [17-23].

The aim of present study was to evaluate the role of oxidative stress in pathogenesis of some dermatology disease (vitiligo, and Alopecia areata), therefore we evaluate the status of serum level of malondialdehyde (MDA) and the status levels of antioxidant enzymes such as super oxide dismutase (SOD); and; non-enzymatic antioxidants like vitamins E and A.

Material & Methods:
The study was conducted in the Departments of Dermatology, Al-Khadymia Teaching Hospital, and Chemistry and Biochemistry, College of Medicine / Al-Nahrain University.
The study group included 40 cases (vitiligo and alopecia). Twenty-five age- and sex-matched healthy individuals, were included as controls. Patients and controls 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 and controls with a history of smoking or alcoholism or taking drugs for any other reason or taking antioxidant or vitamins were not included.

All blood samples, taken after 10-12 hours of fasting in the morning between 9-11 hours. The blood from forearm vein was collected in plain tube and allowed to clot at room temperature for 30 minutes and centrifuged for 15 minutes at 3000 rpm (755xg). The serum was divided into proper aliquots which were frozen at -20°C until used for measuring of MDA, SOD, vitamins A, and E.

MDA determination:
Serum MDA levels were determined by the method of Draper and Hadley [24] based on the reaction of MDA with thiobarbituric acid (TBA) at 95°C. In the TBA test reaction, MDA and TBA react to form a pink pigment with an absorption maximum at 532 nm.

SOD activity determination
We used (RANSOD kit, from Randox) the principle of the determination of SOD (EC 1.15.1.1) enzyme activity was based on the production of O₂⁻ from xanthenes by xanthenes oxidase, and reduction of iodophenyl-nitrophenol-phenyltetrazolium (I.N.T) by the H₂O₂ produced.

Vitamin A & E levels:
Serum levels of vitamin A and E were evaluated by high performance liquid chromatography (HPLC), in briefly for vitamin A the serum first deproteinized by 15% 5-sulphosalicylic acid, mixed and centrifuged, the sample was diluted and analyzed by HPLC system using (column) C-18 the mobile phase used acetonitrile (100%) at a flow rate of 1ml/min and wave length 290nm, while for vitamin E the mobile phase used ethanol-water (95:5 v/v) at a flow rate 1ml/min and the wave length 229nm.

Statistical analysis:
All data were given as mean ± standard deviation (SD). Statistica version-6 for windows was used for statistical analysis. Levels of MDA, SOD, vitamin A and vitamin E in serum of patients and control subjects were compared by paired student’s t-test. The differences were considered to be significant when the p value was less than 0.05.

Results:
The study included 20 patients with generalized and localized vitiligo (9 women and 11 men) age of those patients varied from 14 to 50 years (mean age=32.27 years), 20 patients with alopecia (9 women and 11 men) with age varied from 11 to 49 years (mean age=28.37 years), and 25 control age for control were between (11-49 years old) mean age (30.96).

Vitiligo and alopecia are universal diseases. The present study has been attempted to find out the antioxidant statues in the serum of patients with vitiligo and alopecia, levels of SOD and beta-carotene were significantly (p<0.05 and p<0.00001 respectively) lower in patients with vitiligo and alopecia than in controls, and the level of MDA were significantly (p<0.0001) higher in patients than control, while vitamin E levels in serum did not differ statistically. The mean results as mean ± SD and statistical values are presented in table 1.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>SOD(U/ml)</th>
<th>MDA(nmol/ml)</th>
<th>VE(µg/ml)</th>
<th>VA(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL (n=25)</td>
<td>10.14±2.61</td>
<td>0.83±0.12</td>
<td>9.20±1.8</td>
<td>59.48±0.92</td>
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<tr>
<td>VITILIGO Patients (n=20)</td>
<td>8.94±0.38  ** P&lt;0.04</td>
<td>2.07±0.43  *** P&lt;0.000</td>
<td>10.00±1.4</td>
<td>53.47±2.27  *** P&lt;0.000</td>
</tr>
<tr>
<td>ALOPECIA Patients(n=20)</td>
<td>8.80±0.44  *** P&lt;0.02</td>
<td>1.73±0.31  *** P&lt;0.000</td>
<td>10.27±1.77</td>
<td>52.85±2.34  *** P&lt;.000</td>
</tr>
</tbody>
</table>

* t-test: comparison of control group with vitiligo and AA patients

Table 1: The Mean ± Standard Deviation values SOD, MDA, VE, and VA in vitiligo and alopecia patients and normal control subjects.
Discussion:

Alopecia and vitiligo conserved as autoimmune disease. Many studies have reported the involvement of oxidative stress in alopecia and vitiligo disease and accumulation of hydrogen peroxide (H$_2$O$_2$) in the epidermal layer of affected skin [11]. Oxidative stress can be induced by increasing generation of reactive oxygen species (ROS) and other radicals. The generation of ROS can be associated with a decrease in antioxidant levels at the skin [6]. Recent studies have shown that FRs was increased and the antioxidant systems were insufficient in vitiligo and alopecia [7, 15, 28]. For this reason, we investigated the serum levels of MDA for the evaluation of oxidative stress and activity of SOD and vitamins E &A for the evaluation of antioxidant system in alopecia and vitiligo patients.

MDA, an end-product of lipid peroxidation induced by ROS, is well correlated with degree of lipid peroxidation [23]. We found significantly higher level of serum MDA in AA and vitiligo patients compared with controls. Naziroglu et al. [26] reported that the level of thiobarbituric acid-reactive substances (TBARS) in plasma and erythrocytes was significantly higher in patients with alopecia than in controls. Akar et al. [27] investigated TBARS levels in scalp tissue they found significantly higher levels of TBARS in the scalp tissue of patients with AA than in controls. They also observed that TBARS levels in scalp tissue were higher in patients in early phase than those in the late phase.

Our higher level of serum MDA in vitiligo supports the findings of Yildirim et al. [8] how found higher serum levels of MDA in patients with vitiligo and Koca et al. [28] showed higher serum MDA levels in generalized vitiligo patients. We also obtained a higher level of MDA in sera of vitiligo patients than the control group in a previous study [14] while Picardo et al. [29] found normal serum MDA levels in erythrocytes of combined types of vitiligo. Whereas Tastan et al. [30] found the MDA level in vitiliginous tissue to be normal.

In our study, the significantly higher levels of serum MDA support previous findings and indicate that lipid peroxidation may have a role in the pathogenesis of AA and vitiligo.

SOD is a group of metalloenzymes that protects cells from the toxic effects of superoxide radicals are produced as endogen. Superoxide converted to H$_2$O$_2$ reaction is accelerated by SOD [31] in this study we found low significant level of SOD in sera of vitiligo and AA patients as compared with control. Varying results have been found in different studies associated with SOD activity in vitiligo and AA patients.

This finding SOD in sera of AA patients is similar to Rafet et al. [32] who found a significant lower level of SOD sera of AA patients than control, Akar et al. [27] reported a significant increase in the activities of SOD and GSH-Px in the scalp tissue of patients with active AA. They suggest that antioxidant defense is not impaired in AA. But our findings do not support their results concerning SOD. We analyzed the parameters in the serum samples, instead of scalp tissue, which could explain the discrepancies.

There are different reports on SOD activity in patients with vitiligo compared to the healthy controls. SOD activity in erythrocytes was found to be normal [7, 29], in some studies and higher in others [1, 10, 14, 13] [34]. On the other hand, one study [24] reported lower levels in erythrocytes. Furthermore, Dell’Anna et al. [12] found higher SOD activity in leukocytes of vitiligo patients. Although SOD activities in the vitiliginous tissue were found to be normal in one study, [10] Maresca et al. [7] and Yildirim et al. [8] found it to be high.

These different results could be related to differences in serum and erythrocyte levels, duration and activity of the disease and differences in increased lipid peroxidation may relate to decrease in SOD activity and vitamin A levels.

These results demonstrate the presence of an imbalance in the oxidant-antioxidant system and provide further support for a free radical-mediated damage as an initial pathogenic event vitiligo and AA.

Laboratory techniques Superoxide and hydroxyl radicals are the most important radicals in lipid peroxidation. Decreased SOD activity could be responsible for the increase of superoxide radicals, which may explain the increased level of MDA [24].

Beta-carotene is found in plants and during dietary intake is converted to vitamin A. Beta-carotene is considered as the pro-vitamin A. It consists of two vitamin A molecules, split by hydrolysis to vitamin A. If the reverse occurs vitamin A could also be a precursor of beta carotene [19]. A plausible biological mechanism is provided by beta carotenoids ability to block oxidative damage produced by singlet oxygen or certain organic reactive oxygen radicals [35, 36].

Also, beta-carotene has positive effects with regard to the prevention and therapy of various dermatologic and neoplastic disorders [2, 3, 36].

Our study shows a significant lower in sera of AA and vitiligo patients than control. Mustafa et al. [37] found a low plasma beta-carotene levels in patients with AA than in healthy subjects.

Vitamin E is a lipid-soluble antioxidant; our results show significant high levels in sera of AA and vitiligo patients than control. Mustae et al. [37] wrote that plasma vitamin E levels were found to be lower in
patients with alopecia than control, while some study showed that vitamin E and vitamin A have been found to be normal in the blood and reduced or increased in the epidermis of vitiligo [33,36]. Hence AA and vitiligo can be an inflammatory disease and this hypothesis is confirmed by our vitamin a and vitamin E results.

Our conclusion is that increased lipid peroxidation may relate to decrease in SOD activity and vitamin A levels. These results demonstrate the presence of imbalance in the oxidant-antioxidant system and provide further support for a free radical-mediated damage as an initial pathogenic event vitiligo and AA.

References:
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