Oxidative Stress in the Blood of Patients with Active Localized Vitiligo

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Abstract

**Background:** Several hypotheses have been made about the pathogenesis of vitiligo, and some of them were presenting evidence on the role of oxidative stress as an initial pathogenic event in melanocyte degradation.

**Objective:** In this study we evaluate the role of oxidative stress in the pathogenesis of active localized vitiligo by measuring the serum levels of superoxide dismutase (SOD) enzyme, glucose-6-phosphate dehydrogenase (G-6-PD) enzyme, and malondialdehyde (MDA) and plasma levels of catalase (CAT) by spectrophotometric assay.

**Patients and Methods:** This case control study was conducted on 20 patients with active localized vitiligo and 10 healthy participants as controls. The indicator of oxidative stress is determined by measuring serum levels of MDA. The role of antioxidant system is determined by measuring serum levels of G-6-PD, SOD and plasma levels of CAT.

**Results:** The mean serum levels of G-6-PD, SOD and plasma levels of CAT were significantly decreased in vitiligo patients than in controls (p<0.05 for all) while the mean serum levels of MDA was significantly increased in vitiligo patients than in controls (p<0.001). Significant negative correlation was found between SOD and MDA serum levels (p<0.05).

**Conclusion:** Vitiligo is probably mediated through a process of oxidative stress and imbalance of oxidant-antioxidant system represented by SOD, CAT, G-6-PD and MDA. The net results of this imbalance may lead to toxic melanocyte destruction.

**Key Words:** Oxidative stress – Superoxide dismutase (SOD) – Glucose-6-phosphate dehydrogenase (G-6-PD) – Catalase (CAT) – Malondialdehyde (MDA).

Introduction

VITILIGO is an acquired skin disease characterized by milky 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 [2]. Jimbow et al., [3] suggested that the early cell death of vitiligo melanocytes is related to their increased sensitivity to oxidative stress, which may arise from complex processes of tyrosine related protein-1 (TRP-1) abnormal synthesis which has been shown to be involved not only in melanin biosynthesis but also in the prevention of premature melanocyte death in animals.

Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species and a biological system’s ability to detoxify the reactive intermediates or to repair the resulting damage. Disturbance of this balance in tissues can cause toxic effects through the production of free radicals that damage all components of the cell including proteins, lipids and DNA [4]. Superoxide dismutase enzyme (SOD) protects cells from the toxic effect of superoxide radicals [5]. Catalase enzyme (CAT) works primarily to catalyze the decomposition of hydrogen peroxide to oxygen and water [6]. Glucose-6-phosphate dehydrogenase enzyme (G-6-PD) is a cytosolic enzyme in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells such as erythrocytes by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH) [7]. The NADPH in turn maintains the level of glutathione in these cells that protect the red blood cells against oxidative damage [8]. Malondialdehyde (MDA) is the end product of lipid peroxidation and one of the indicators of oxidative stress [9]. Imbalances in the oxidant/antioxidant system, such as the accumulation of hydrogen peroxide (H₂O₂) and low CAT levels...
have been demonstrated in the epidermis and in blood of vitiligo patients [10]. Significantly decreased blood levels of CAT, SOD and increased levels of MDA were observed in experimental active localized vitiligo mice measured by spectrophotometry [11].

**Aim of the work:**

The aim of this study was to evaluate the role of oxidative stress in the pathogenesis of active localized vitiligo by measuring the serum levels of SOD, G-6-PD, MDA and plasma level of CAT by spectrophotometric assay.

**Patients and Methods**

This case control study was conducted on 20 patients with localized (one or more patches in one area) active vitiligo and 10 healthy participants as controls. The disease activity was determined by history of the appearance of new lesions or enlargement of preexisting lesions during the previous 3 months [12]. Patients and controls were selected from the outpatient Clinic of Dermatology and Andrology of Benha University Hospital from October, 2010 to January, 2011. Informed consent was taken from each patient and control after approval from Research Ethics Committee of the faculty of medicine of Benha University before the study. Inclusion criteria were patients with active localized vitiligo including focal and segmental types with minimum disease duration of 1 year. The exclusion criteria were patients with generalized vitiligo, autoimmune disease, concomitant dermatological disease, thyroid dysfunction and systemic or topical treatment for vitiligo within at least one month prior to the study. Patients and controls with history of smoking, alcohol intake, vitamin intake, or use of anti-inflammatory or other drugs within at least one month prior the study were excluded from the study. Patients and control subjects were subjected to 1- History taking via purposely-designed sheet with emphasis on name, age, sex, family history of vitiligo, special habits, onset and duration of vitiligo, 2- Clinical examination to determine the type of vitiligo, 3- Laboratory investigations: The indicator of oxidative stress is determined by measuring serum levels of SOD, G-6-PD, MDA and plasma levels of CAT.

**Blood sampling:**

Five ml blood was withdrawn from every patient and control and divided into 2.5ml into a plain tube, left to clot, centrifuged and the sera obtained were kept frozen at –20°C until determination of MDA and SOD. The remaining 2.5ml put in EDTA tube and 0.5ml was withdrawn into epindorph and examined for G-6-PD and 2ml was centrifuged and plasma obtained were kept frozen at –20°C until determination of CAT.

- **Determination of superoxide dismutase:**
  This assay relies on the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Measure the increase in absorbance of dye at 560nm spectrophotometrically [13].

- **Determination of catalase assay:**
  **Principle:**
  Catalase reacts with a known quantity of H2O2. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase, the remaining H2O2 reacts with 3, 5-Dichloro-2-Hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample [14].

- **Determination of lipid peroxide (malondialdehyde):**
  **Principle:**
  Thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30min. to form thiobarbituric acid reactive product, the absorbance of the resultant pink product can be measured at 534nm spectrophotometrically [15].

- **Determination of glucose-6-phosphate dehydrogenase (G-6-PDH):**
  **Principle:**
  Glucose-6-phosphate dehydrogenase catalyses the oxidation of glucose-6-phosphate to 6-phosphogluconate with a concurrent conversion of NADPH. The enzyme activity is determined by measurement of the rate of increase in NADPH concentration. The rate of increase in absorbance at 340nm is the measure of enzyme activity [16].

**Statistical analysis:**

The collected data were tabulated and analyzed using Statistical Package for Social Science version 16 (SPSS Inc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean and standard deviation. Fisher’s exact test, student “t”, Mann Whitney U test and Person’s correlation coefficient (r) were used as tests of significance. The accepted level of significance in this work was stated at 0.05 (p<0.05 was considered significant).
**Results**

This study was conducted on 20 patients with active localized vitiligo and 10 healthy participants as control group. The age of the patients ranged from 14 to 41 years with mean±SD 24.80±7.281 years, and the age of the controls ranged from 11 to 30 years with the mean±SD 20.20±6.321 years with no statistically significant difference ($p>0.05$). As regards sex, patients included 11 females (55.0%) and 9 males (45.0%) while controls were 6 females (60.0%) and 4 males (40.0%) with no statistically significant difference ($p>0.05$). Patients were 16 (80%) focal vitiligo and 4 (20%) segmental vitiligo. Mean duration of vitiligo was 4.9±2.2 years (range 1-8 years). Five (25%) patients had a positive family history of vitiligo.

The mean serum G-6-PD level was significantly lower in patients than in controls (The mean±SD 7.67±2.91µ/ml and 13.37±3.93µ/ml respectively $p<0.001$). The mean plasma CAT level was significantly lower in patients than in controls (The mean±SD 84.15±9.34µ/ml and 103.80±10.74µ/ml respectively $p<0.001$). The mean serum SOD level was significantly lower in patients than in controls (the mean±SD 0.75±0.078µ/ml and 0.89±0.069µ/ml respectively $p<0.001$). The mean serum MDA level was significantly higher in patients than in controls (The mean±SD 2.75±0.42nmol/ml and 2.08±0.21nmol/ml respectively $p<0.001$) (Table 1).

Table (1): Comparing the studied groups regarding oxidative stress markers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (N=20)</th>
<th>Controls (N=10)</th>
<th>St. “r”</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum G-6-PD (µ/ml)</td>
<td>7.67±2.91</td>
<td>13.37±3.93</td>
<td>4.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma CAT (U/ML)</td>
<td>84.15±9.34</td>
<td>103.80±10.73</td>
<td>5.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum SOD (µ/ml)</td>
<td>0.75±0.078</td>
<td>0.89±0.069</td>
<td>4.86</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum MDA (nmol/ML)</td>
<td>2.75±0.42</td>
<td>2.08±0.21</td>
<td>4.66</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table (2): Correlation between different oxidative markers among vitiligo patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Serum G-6-PD</th>
<th>Plasma CAT</th>
<th>Serum SOD</th>
<th>Serum MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>1</td>
<td>0.056</td>
<td>-0.048</td>
<td>-0.101</td>
</tr>
<tr>
<td>$p$</td>
<td>0.815</td>
<td>0.841</td>
<td>0.942</td>
<td>0.797</td>
</tr>
</tbody>
</table>

Plasma CAT:

<table>
<thead>
<tr>
<th>$r$</th>
<th>-0.017</th>
<th>1</th>
<th>-0.048</th>
<th>-0.547</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$</td>
<td>0.942</td>
<td>0.672</td>
<td>0.797</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Serum SOD:

<table>
<thead>
<tr>
<th>$r$</th>
<th>0.048</th>
<th>-0.017</th>
<th>1</th>
<th>-0.547</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$</td>
<td>0.841</td>
<td>0.942</td>
<td>-</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Serum MDA:

<table>
<thead>
<tr>
<th>$r$</th>
<th>-0.101</th>
<th>0.061</th>
<th>-0.547</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$</td>
<td>0.672</td>
<td>0.797</td>
<td>0.013</td>
<td>-</td>
</tr>
</tbody>
</table>

G-6-PD: (Glucose-6-phosphate dehydrogenase).
CAT: (Catalase).
SOD: (Superoxide dismutase).
MDA: (Malondialdehyde).

$p<0.05$ was considered significant.

No significant correlation was found between oxidative stress markers and family history of vitiligo, disease duration and type of vitiligo ($p>0.05$ for all). Significant negative correlation was found between SOD and MDA serum levels ($r = -0.547, p=0.013$) (Table 2) (Fig. 1).

**Discussion**

During melanin synthesis some intermediates are generated such as 3,4-dihydroxyphenolamine (dopa), dopachrome, and 5,6-dihydroxyindole (DHI) that are known toxic substances to melanocytes [17]. The body protects itself by enzymatic and non-enzymatic antioxidants including SOD, CAT, glutathione peroxidase (GPX), ascorbic acid, b-carotene and vitamin A [18].

In this study, serum SOD level was significantly lower in patients than in controls. Kamel et al., [19] found a decrease in plasma SOD levels in vitiligo patients compared with controls but this decrease was statistically insignificant. On the contrary, Chakraborty et al., [20] and Jain et al., [21] reported significantly higher serum levels of SOD in vitiligo patients than in controls. They
suggested that the increased SOD activity in vitiligo patients might be an adaptation to the increased oxidative stress evident in these individuals and their result suggested that prominent oxidative stress particularly in the presence of high levels of O$_2^-$ leads to high levels of SOD followed by high amounts of H$_2$O$_2$ which might result in destruction of defective melanocytes in vitiligo patients.

The present study reported significantly lower serum levels of G-6-PD in vitiligo patients than in controls. This was supported by Saha et al., [22]. Their study explained that G-6-PD is the first rate-limiting enzyme in the hexose mono-phosphate shunt pathway, playing an important role in the regeneration of the reduced form of NADPH that maintain glutathione in its reduced form which essential for detoxification of reactive free radicals, lipid peroxides and toxic compounds.

Measurement of MDA in the blood provides evidence of lipid peroxidation induced by reactive oxygen species and an indicator of oxidative stress [23]. The present work found significantly higher serum levels of MDA in vitiligo patients than in controls. This was in agreement with Shabaka et al., [24]; Jain et al., [25] and Khan et al., [26]. They suggested that a defective antioxidant defense leads to unhindered cytotoxic action of reactive oxygen species (ROS) which can start a chain reaction and bring about lipid peroxidation producing lipid peroxides and lipoxides, whose further decomposition yields a variety of end products, including MDA. These end products can cause damage to cell membrane or DNA leading to cytotoxicity, mutagenecity and cell death. They are also cytotoxic to melanocytes and can inhibit tyrosinase enzyme.

In contrast to the present study, Picardo et al., [27] reported that blood levels of SOD and MDA were not significantly different in different forms (acrofacial, segmental, generalized) of vitiligo patients from those of healthy controls. They suggested that abnormal release of catecholamines from autonomic nerve endings might play an etiological role in the onset and development of vitiligo through an overproduction of toxic radicals in the microenvironment of melanocytes in the affected areas.

Catalase is one of the major primary antioxidant defense mechanisms. It works primarily to catalyze the decomposition of hydrogen peroxide to oxygen and water [6]. The current work found significant lower plasma levels of CAT in vitiligo patients than in controls. Other studies measure CAT in erythrocyte [28-30] and melanocytes [30] of vitiligo patients with variable results. However, there is no published data on its plasma level in active localized vitiligo patients.

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