Study of total antioxidants status in Indian vitiligo patients

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Abstract

Background: Vitiligo is an acquired, idiopathic disorder characterized by milky white patches devoid of identifiable melanocytes. Despite much research, the precise etiology of vitiligo is not clear, but it has become quite clear in recent years that complex genetic, neural, immunological and autocytoxic mechanisms play a role in its pathogenesis. In recent studies, a defective antioxidant defense is also postulated in the pathophysiology of vitiligo.

The purpose of this study was to evaluate the role of the oxidative stress in the pathogenesis of vitiligo and also to support the proposed theory which suggests that the destruction of melanocytes in vitiligo is induced by increased oxidative stress which in turn activates an autoimmune response.

Methods: A total of 80 patients of both sexes with vitiligo and 40 control subjects were enrolled in this study. Sera from patients and controls were assayed for total antioxidant status by antioxidant assay kit. The collected data were analyzed by SPSS version 16.

Results: The mean serum total antioxidant status (TAS) level in the patient group was significantly lower than in the control group. There was no significant relationship between TAS and sex of patients. The same findings were observed within the control group. Regarding the activity of the vitiligo, the mean TAS in patients with progressive disease (active vitiligo) was significantly lower than in the control group. In patients with stable disease also the mean TAS level was significantly lower than in the control group. There was
no significant difference in mean TAS level between active and stable vitiligo patients.

**Conclusion:** We conclude that there is impairment in the antioxidant system in vitiligo, leading to free radical mediated destruction of melanocytes or dysregulation of melanogenesis which may activates an autoimmune response. Thus antioxidants may be beneficial as therapeutic agents in vitiligo.

**Introduction**

Vitiligo is an acquired, idiopathic disorder characterized by milky white patches devoid of identifiable melanocytes. [1] Incidence of vitiligo is found to be 0.5-2.5% in India with a high prevalence of 8.8% in the states of Rajasthan and Gujarat. [2] Despite much research, the precise etiology of vitiligo is not clear, but it has become quite clear in recent years that complex genetic, neural, immunological and autocyctotoxic mechanisms play a role in its pathogenesis. [3,4] In recent studies, a defective antioxidant defense is also postulated in the pathophysiology of vitiligo. [5-9] Human skin which acts as an interface between the environment and the body is constantly exposed to a broad spectrum of physical, chemical and biological agents. These agents either inherent oxidants or catalyze the generation of reactive oxidants known as reactive oxygen species. [10] The reactive oxygen species (ROS) modulate the physiological state of cells and influence cell death. [11] Mammalian cells are equipped with both enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic (vitamin E, ceruloplasmin, uric acid etc) antioxidant activities to minimize the cellular oxidative damage. Imbalances in the oxidant-antioxidant system, such as the increased malondialdehyde (MDA) and hydrogen peroxide (H2O2) levels and low catalase levels have been reported in the epidermis and blood of vitiligo patients [12,13,14]. The purpose of this study was to evaluate the role of antioxidants in the pathogenesis of vitiligo and also to support the proposed etiology which suggests that the destruction of melanocytes in vitiligo is induced by increased oxidative stress which in turn activates an autoimmune response. We investigated the role of antioxidant system by measuring the total antioxidant status, instead of measurement of individual components, as it may provide more relevant biological information by considering the cumulative effect of all antioxidants present in plasma and body fluids.

**Material and methods**

The study was undertaken in the department of Dermatology and Venereology of Sir Sunderlal Hospital, Banaras Hindu University from September 2010 to February 2011. Ethical committee of institute has approved the study. Eighty vitiligo patients (41 Male and 39 female) and forty healthy volunteers (23 males and 17 females) participated in the present study with age group between 13-47 years and 13-44 years respectively. Vitiligo patients were subdivided into two groups: 44 (55%) with active vitiligo (new lesions within the 2 months prior to the study as observed by the patients) and 36(45%) with stable vitiligo (no change in the vitiligo lesions during the 2 months prior to the study as observed by the patients).The patients were not under therapeutic regimen for the previous two months and there was no history of smoking.

Determination of Total antioxidant status: Serum TAS was measured using the antioxidant assay kit of Cayman chemical company, Ann Arbor, MI, U.S.A. This kit assessed the combined antioxidant activities of all its constituents including vitamins, proteins, lipids,
glutathione, uric acid etc. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS (2,2'-Azino-di-[3-ethylbenthiazolinesulphonate]) to ABTS+ by metmyoglobin. The amount of ABTS+ produced can be monitored by reading the absorbance at 750nm or 450nm. The antioxidants in the sample cause suppression of the absorbance at 750nm or 405nm to a degree which is proportional to their concentration. [15]

**Statistical Analysis:** Data were expressed as mean ± SD. Differences between variables were calculated using student's t test on SPSS for windows (version 16.0) statistical package (SPSS Inc., Chicago, IL) computer statistics program. P values less than 0.05 was considered as significant.

**Results**

There was a positive family history of vitiligo in first degree relatives in 19 patients (23.75%). The duration of vitiligo ranged between years 1-15 years (6.60±6.30). The mean age of vitiligo patients and healthy controls are 28.72±9.81 and 29.43±7.32 respectively.

The mean serum TAS level in the patient group was significantly lower than in the control group (0.19±0.02 Vs 0.33±0.06) P value < 0.05 (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range (mM)</th>
<th>Mean ± SD (mM)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitiligo(80)</td>
<td>0.15-0.24</td>
<td>0.19±0.02</td>
<td>15.86</td>
<td>0.000*</td>
</tr>
<tr>
<td>Controls(40)</td>
<td>0.28-0.60</td>
<td>0.33±0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P< 0.05, Significant

**Table 1:** Showing total antioxidant status in vitiligo and controls.

There was no significant relationship between TAS and sex of patients (0.27±0.09 Vs 0.25±0.07) P value < 0.05. The same findings was observed within the control group (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD (mM)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active vitiligo(44)</td>
<td>0.19±0.03</td>
<td>12.9</td>
<td>0.000*</td>
</tr>
<tr>
<td>Controls(40)</td>
<td>0.33±0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P< 0.05, Significant

**Table 2:** Showing total antioxidant status in active vitiligo and controls.

Regarding the activity of the vitiligo, the mean TAS in patients with progressive disease (active vitiligo) was significantly lower than in the control group (0.19±0.03 Vs 0.33±0.06) P value < 0.05 (Table 3).
### Table 3: Showing total antioxidant status in stable vitiligo and controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD (mM)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable vitiligo</td>
<td>0.20±0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.33±0.06</td>
<td>11.1</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*P< 0.05, Significant

In patients with stable disease again the mean TAS level was significantly lower than in the control group (0.20±0.03 Vs 0.33±0.06) P value < 0.05 (Table 4). There was no significant difference in mean TAS level between active and stable vitiligo patients.

### Table 4: Showing total antioxidant status in male and female vitiligo patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD (mM)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (41)</td>
<td>0.27±0.09</td>
<td>0.907</td>
<td>0.367 (NS)</td>
</tr>
<tr>
<td>Female (39)</td>
<td>0.25±0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: Non-significant

**Discussion**

Vitiligo is a chronic, common disease of still unknown etiology. One of the recent hypotheses to explain the triggering event of melanocyte destruction in vitiligo is the oxidative stress induced by ROS. [16-20] ROS produces free radicals such as superoxide (O2⁻), H2O2, and nitric oxide. These molecules occur during several physiological and pathological processes. [21] Although a system of enzymatic and non-enzymatic antioxidants scavenges these free radicals and provides protection but an imbalance between oxidants and antioxidants leads to accumulation of free radicals which damages cellular components such as protein, carbohydrate, DNA and lipid. [22] In addition to this, a defective recycling of tetrahydrobiopterin has been reported in the vitiligo epidermis resulting in oxidative stress. [23,24] Our study has found that the serum composition of total antioxidant status in vitiligo patients significantly decreased from those of healthy controls. This is in accordance with the study of Jalel and Hamdaoui [25] and Khan et al [26] on 60 and 30 vitiligo patients respectively. Jalel et al [27] reported higher malondialdehyde (MDA) levels and lower CAT, SOD and GPx activities in experimental vitiligo mice. Similarly Jain et al [28] conducted a case-control study on 100 vitiligo patients and found high levels of SOD in blood of patients as compared to controls. In another study on 40 vitiligo patients Jain et al [29] revealed that MDA level was significantly raised while those of vitamin E, uric acid and ceruloplasmin were significantly lowered in blood of vitiligo patients as compared to 40 controls. Khan et al [26] also supported the role of oxidative stress in the pathophysiology of vitiligo. Sravani et al [30] determined significant increase in the SOD levels and significant decrease in catalase (CAT) levels in vitiliginous and non vitiliginous skin of 25 patients group as compared to
control group.

**Conclusion**

Thus our findings suggest that there is impairment in the antioxidant system in vitiligo, leading to excess free radical which causes destruction of melanocytes or dysregulation of melanogenesis and activates an autoimmune response. Thus antioxidants may be beneficial as therapeutic agents in vitiligo. It should be explored in depth in future by large multicentric studies.

**References**


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