OXIDATIVE STRESS, ANTI-MELANOCYTE AND ANTI TYROSINASE ANTIBODY IN VITILIGO AND RESPONSE TO TREATMENT

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ABSTRACT

Vitiligo is an acquired, usually asymptomatic pigmented disorder that results in the loss of functional melanocytes and is often associated with other autoimmune diseases. At the onset of the disease white patches of different sizes appear on different parts of the body. Vitiligo usually affects the skin, but it can develop anywhere we have pigment. Patches of hair can turn white. Some people lose color inside their mouths. The affected skin can lighten or turn completely white. Vitiligo affects approximately 1% of the world population of all skin types, usually before the age of 20. In this case-control study (100) patients with vitiligo which divided into two subgroups (treated with topical, systemic steroid and treated with narrow band phototherapy), and (50) healthy control were examined. VASI score for each patient was calculated. Serum levels of GPx activity & TAC were measured by spectrophotometer, but MC-Ab & TYR-Ab were measured by ELIZA. After measurements and comparison between vitiligo patients and age-and-gender matched control group, results showed significant differences in glutathione peroxidase activity, total antioxidant capacity, anti-melanocyte antibody and anti tyrosinase antibody among these groups. There was significant increase in serum GPx activity, TAC, MC-Ab and TYR-Ab (P value = < 0.001, 0.003, < 0.001 and < 0.001 for glutathione peroxidase activity, total antioxidant capacity, anti-melanocyte antibody and anti tyrosinase antibody respectively) for vitiligo patients as compared with control group. After measurements and comparison between vitiligo patients subgroups results showed significant increase in serum GPx activity among NB-UVB group, but no significant differences in TAC, MC-Ab and TYR-Ab were showed when comparison between vitiligo patients subgroups. There was significant increase in serum GPx activity (P value = <0.001) for vitiligo patients treated with NB-UVB group as compared with vitiligo patients treated with steroid group.

Keywords: vitiligo, glutathione peroxidase activity, total antioxidant capacity, anti-melanocyte antibody and antityrosinase antibody, VASI


INTRODUCTION

Vitiligo is an acquired, usually asymptomatic pigmented disorder that results in the loss of functional melanocytes and is often associated with other autoimmune diseases. At the onset of the disease white
patches of different sizes appear on different parts of the body. Its psychological impact on the quality of life can be disastrous, as dissatisfaction with body image can smother self-esteem and develop a depressive state, especially among darker tan-skinned patients\(^1\). The disease may affect both genders and all skin types and may also be associated with systemic autoimmune diseases such as lupus erythematosus, scleroderma, autoimmune thyroiditis and alopecia areata\(^2,3\). The disorder has a profound negative psychological impact, especially in coloured races. Although exact mechanism is not known but due to genetic predisposition, immune-mediated injury, or other unidentified toxins, the melanocytes stop their function or they physically disappear in the affected epidermis while those in the hair follicle are usually spared. The treatment of vitiligo is based on the principles of stimulating the existing melanocytes in the affected area or repopulating it with functioning melanocytes\(^4\).

**MATERIALS AND METHODS**

A total of 100 patients cases of vitiligo, 50 case treated with steroid, (28 males and 22 females) were included in this study; the overall mean age of vitiligo patients treated with steroid was (33 ±10) years and 50 patients cases of vitiligo patients treated with phototherapy, (25 males and 25 females) were included in this study; the overall mean age of vitiligo patients treated with phototherapy was (32.22 ±12.46) years; while the control group was 50 subjects(24 males and 26 females) were included in the study; the overall mean age of control group was (33.86 ± 12.21) years. Subjects were collected from clinic of dermatology of Merjan teaching hospital and Al-Imam Al-Sadiq hospital in Hilla city, and all of patients and control gave their consent. Severity of vitiligo was determined by vitiligo area and severity index score (VASI). Body mass index was calculated by the equation (BMI= Kg/m\(^2\)). Blood samples were drawn from patients and control subjects, three to five milliliters of blood were obtained from vitiligopatients’ subgroups, and controls, then collected in tube without anticoagulants and were left for 15 minutes at room temperature to clot. After that, the blood samples were centrifuged at 1000-2000 xg for approximately 10 minutes. Then the sera were aspirated and stored at (-80\(^\circ\)C) until time of use. Samples were measured by spectrophotometer&ELIZA. Criteria excluded in this study were hypertensive patients, diabetic patients, pregnant women, patients with other skin diseases, BMI < 18 ->30, alcoholic patients, smoker patients and Patients taking treatment or supplements containing zinc, copper or selenium.

**RESULTS**

Regarding to serum glutathione peroxidase activity, total antioxidant capacity, anti-melanocyte antibody and anti tyrosinase antibody these showed significant increase in vitiligo patients as compared with control group (P value= < 0.001, 0.003, < 0.001 and < 0.001 ), with mean (38.42 ± 8.97 nmol/min/mL, 2128.88 ± 489.55 μM,1.37 ± 0.85 IU/mL and 32.65 ± 13.05 ng/ml respectively GPx activity, TAC, MC-Ab and TYR-Ab ) for patients and (43.95 ± 4.60nmol/min/mL, 2487.98± 822.42 μM,0.94 ± 0.28 IU/mL and 25.06 ± 11.64 ng/ml respectively GPx activity, TAC, MC-Ab and TYR-Ab) for controls, as shown in table (1)
Table 1: Study of serum glutathione peroxidase activity, total antioxidant capacity, anti-melanocyte antibody and anti tyrosinase antibody between psoriatic patients and control.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subject</th>
<th>No.</th>
<th>Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPX activity</td>
<td>Patients</td>
<td>100</td>
<td>38.42 ± 8.97</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>43.95 ± 4.60</td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td>Patients</td>
<td>100</td>
<td>2128.88 ± 489.55</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>2487.98 ± 822.42</td>
<td></td>
</tr>
<tr>
<td>MC-Ab</td>
<td>Patients</td>
<td>100</td>
<td>1.37 ± 0.85</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>0.94 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>TYR-Ab</td>
<td>Patients</td>
<td>100</td>
<td>32.65 ± 13.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>25.06 ± 11.64</td>
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</tr>
</tbody>
</table>

Comparison of serum glutathione peroxidase activity among vitiligo patient’s groups showed highly significant differences among these groups (P value < 0.001) with mean (41.75 ± 10.49, 35.09 ± 5.46 nmol/min/mL) for vitiligo patients treated with steroid and NB-UVB respectively, as shown in table (2). No significant differences in the concentration of serum total antioxidant capacity, anti-melanocyte antibody and anti tyrosinase antibody were observed between vitiligo patient’s groups.

Table 2: Comparison of serum glutathione peroxidase activity, total antioxidant capacity, anti-melanocyte antibody and anti tyrosinase antibody among vitiligo patient’s groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subject</th>
<th>No.</th>
<th>Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPX activity</td>
<td>Steroid</td>
<td>50</td>
<td>41.75 ± 10.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>NB-UVB</td>
<td>50</td>
<td>35.09 ± 5.46</td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td>Steroid</td>
<td>50</td>
<td>2142.54 ± 423.77</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>NB-UVB</td>
<td>50</td>
<td>2115.22 ± 551.58</td>
<td></td>
</tr>
<tr>
<td>MC-Ab</td>
<td>Steroid</td>
<td>50</td>
<td>1.38 ± 0.88</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>NB-UVB</td>
<td>50</td>
<td>1.37 ± 0.84</td>
<td></td>
</tr>
</tbody>
</table>
There was a significant positive correlation between serum anti-melanocyte antibody level, anti tyrosinase antibody level, and vitiligo area score index (VASI score) of the patient groups ($P = 0.01$, $r = 0.243$, $r = 0.383$, $p < 0.001$ respectively ). That indicates the positive association between level of serum MC-Ab, TYR-Ab and the severity of vitiligo, as shown in figure (1), (2).

![Figure 1: Correlation of serum MC-Ab and VASI score.](image1)

![Figure 2: Correlation of MC-Ab and extent in vitiligo patients groups](image2)
Correlation of glutathione peroxidase activity and age in vitiligo patient's NB–UVB group.

Correlation of glutathione peroxidase activity and age in vitiligo patient's steroid group.

Correlation of glutathione peroxidase activity with the age of vitiligo patient's groups viewed significant positive relationship ($r = 0.481$, $p < 0.001$, $0.763$, $< 0.001$ respectively), as shown in figure (3),(4). That indicates the positive association between level of serum glutathione peroxidase activity and age.

**DISCUSSION**

In our study there was significant decrease of serum glutathione peroxidase activity in patients compared with control group. This results is in agreement with (5,6), who recently found a significant decrease of serum glutathione peroxidase activity in patient with vitiligo. Our results are discrepant with (7,8), who recently found no significant differences of serum glutathione peroxidase activity in vitiligo patients when compared with control group. Is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydro peroxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. The main reaction that glutathione peroxidase catalyzes is (9):

$$2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GS}–\text{SG} + 2\text{H}_2\text{O}$$

Antioxidant: compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as
thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g. catalase, Glutathione peroxidase and superoxide dismutase), produced internally, or the dietary antioxidants vitamin C, and vitamin E \(^{(10)}\). In our study significant differences of serum TAC was found between vitiligo patients and control. \(^{(5,8)}\), also recently found no significant differences in serum TAC between vitiligo patients. Significant increase in serum MC-Ab and TYR-Ab of vitiligo patients compared with control group. This result is an agreement with \(^{(11-13)}\). This is suggested that vitiligo is accompanied by abnormal humoral and cellular immunity and high levels of serum circulating autoantibodies have been detected in 5 to 10% of patients, predominantly of the IgG class and particularly anti-tyrosinase one and two, however the role of anti-melanocyte antibodies in vitiligo pathogenesis remains uncertain and it has been suggested that their presence may be secondary to keratinocyte and melanocyte damages \(^{(14)}\). Melanocytes are capable of presenting antigens in the presence of MHC class II, which would allow HLA-DR+ cells present in the perilesional vitiligo area to present antigens in response to trauma or local inflammation, in the latter case leading to autoimmune destruction of melanocytes \(^{(15)}\). In our study no significant differences of serum TAC, MC-Ab and TYR-Ab between vitiligo patients groups. This may be explain that one mechanism by which corticosteroids can cause repigmentation in vitiligo is by decreasing the level of vitiligo antibodies, and support the notion that vitiligo antibodies are involved in the pathogenesis of this disease. Systemic corticosteroids are effective in the treatment of vitiligo the mechanism by which they do so is not known. The exact mechanism of action of NB-UVB in vitiligo is unknown \(^{(16,17)}\).

**CONCLUSION**

Since glutathione peroxidase activity may decrease due to oxidative damage to lipid, protein, and DNA, decrease serum glutathione peroxidase may play a major role in increasing the inflammatory response of vitiligo disease. Depletion of serum total anti-oxidant capacity may cause decreasing of antioxidant and anti-proliferative capacity, and increase keratinocyte proliferation as well as skin desquamation, so increase vitiligo progression. Anti-melanocyte antibody and anti tyrosinase antibody is a good marker for measuring the severity of vitiligo. Narrow band UV is a good treatment due to stimulates the activation, proliferation and migration of melanocytes from the outer root sheath to the adjacent epidermis.

**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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**REFERENCES**
1. Abreu, AC et al., Immunological parameters associated with vitiligo treatments: a literature review based on clinical studies on autoimmune diseases, 2015.


3. KARAGÜN, Ebru, et al. The role of serum vitamin D levels in vitiligo. Advances in Dermatology and Allergology/Postępy Dermatologii Alergologii, 2016, 33.4: 300.


