Assessment of Serum Homocysteine and Oxidative Stress in Vitiligo Patients

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Abstract

Introduction: Vitiligo is a pigmentation disorder affecting about 1–2% of the population with a multifactorial etiology. This condition may be acquired or often an inheritable disorder with polygenic inheritance pattern and complex pathogenic behavior. Increased Serum homocysteine levels and oxidative stress resulting from oxidant–antioxidant imbalance may play a vital role in the pathogenesis of vitiligo.

Objectives: The present study aims to evaluate the serum levels of homocysteine and serum malondialdehyde (MDA) and total antioxidant capacity (TAC) in vitiligo patients and also to study the role of the same in the pathogenesis of the disease.

Materials and methods: The study group comprised of 32 vitiligo patients of 16–40 years age group, attending outpatient department (OPD) in the Dermatology department of Mamata General Hospital, Khammam, Telangana, India. Thirty-two healthy individuals of the corresponding age group were selected as controls (control group) from the patient’s attendants and hospital staff. Approval was obtained from the Institutional Human Ethical Committee. Prior informed consent was obtained from the selected participants. Serum level of homocysteine was measured using Axis homocysteine enzyme immunoassay (EIA). The MDA was determined as thiobarbituric acid reactive substances (TBARS). The total antioxidant capacity was estimated using ferric reducing ability of plasma (FRAP) assay.

Results: Mean serum levels of homocysteine and MDA were significantly increased, and TAC was decreased considerably in vitiligo cases compared to controls.

Conclusion: In cases of vitiligo patients, along with routine screening for homocysteine and oxidative stress status, the usage of antioxidants and homocysteine lowering agents such as vitamin B⁶, B¹², and folic acid may prove beneficial as therapeutic agents.

Keywords: Homocysteine, MDA, Oxidative stress, Total antioxidant capacity, Vitiligo.

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Introduction

Vitiligo is a pigmentation disorder affecting about 1–2% of the population with a multifactorial etiology. This condition may be acquired or often an inheritable disorder with polygenic inheritance pattern and complex pathogenic behavior.¹ As the exact etiology of vitiligo is yet to be established, the possible pathology is attributed to melanocytes damage due to excess free radicals and compromised immunity.²

In a pilot study by Shaker et al., which studied the relationship between vitiligo and homocysteine levels, it was observed that the serum levels of homocysteine were considerably elevated in vitiligo patients.³ The increase in serum levels of homocysteine produces harmful reactive oxygen species molecules, which in turn can cause oxidative stress on melanocytes.⁴ The probable mechanism of melanocyte damage is through increased oxidative damage, production of Interleukin–6, and nuclear factor activation due to elevated levels of serum homocysteine.⁵ The other possible mechanism may be due to the inhibitory action of homocysteine on histidase and tyrosinase of the skin, which are essential for normal melanocyte production, thereby dampening the melanogenesis.⁶ Tyrosinase contains 75 kD copper, which is essential for the biosynthesis of melanin. Homocysteine’s binding capacity with the copper present in tyrosinase may also lead to inhibition of its active site, which in turn can cause decreased pigment production.⁷

In two different studies by Tang et al. and Souto et al., the genetic predisposition of vitiligo and also of hyperhomocysteinemia was observed to be linked to a common locus on chromosome 11q23.⁸⁹ Many studies indicate that the oxidant stress theory, which suggests the melanocyte damage due to toxic free radicals, is a possible pathological mechanism for vitiligo.¹⁰ The antioxidant status of serum is represented by the combined activity of all the antioxidants together, which denotes its antioxidant capacity.¹¹ However, some studies show that the role of TAS is conflicting in vitiligo cases.¹²⁻¹⁴ The reactive oxygen species molecules are toxic to normal body cells. Any defect in the antioxidant defense mechanism can cause unhindered cytotoxic activity of these cells. MDA, the end product of lipid peroxidation is potential enough to damage the cell membrane or the DNA, which in turn can cause mutagenicity or even cell death.¹⁵ The ultraviolet radiation also can cause damage to the epidermis by generating reactive oxygen species, which inhibit tyrosine activity and also damage melanocytes.¹⁶ Schallreuter et al. in a study on accumulation of H₂O₂ in the epidermal cells and the blood of vitiligo patients, observed that the oxidant–antioxidant system imbalance caused the accumulation of hydrogen peroxide, elevated serum levels of MDA and decreased levels of enzyme catalase.¹⁷¹⁸
AIM OF STUDY
The present study aims to evaluate the serum levels of homocysteine and serum MDA and TAC in vitiligo patients and also to study the role of the same in the pathogenesis of the disease.

MATERIALS AND METHODS
The study group comprised 32 vitiligo patients of 16–40 years age group, attending OPD in the Dermatology department of Mamata General Hospital, Khammam, Telangana, India. Thirty-two healthy individuals of the corresponding age group were selected as controls (control group) from the patient’s attendants and hospital staff. Approval was obtained from the Institutional Human Ethical Committee. Prior informed consent was obtained from the selected participants.

Fasting blood sample of 5 mL was collected and serum was separated after clot retraction. Until further analysis, serum was aliquoted and stored at –80°C. Diagnosis of the disease was done on the basis of clinical examination.

The participants with known diagnosis of with autoimmune disorders such as Hashimoto thyroiditis, pernicious anemia, type 1 diabetes, alopecia areata, psoriasis and other diseases like hypertension and conditions like pregnancy, lactation, intake of vitamin B6, B12, or folic acid, smokers, chronic alcoholics and also subjects taking antioxidant supplements were excluded from the study. Serum level of homocysteine was measured using Axis homocysteine EIA. The MDA was determined as TBARS. The total antioxidant capacity was estimated using FRAP assay.

Statistical Analysis
Statistical analysis of all the biochemical parameters of study and control groups was done using mean and standard deviation values. The mean difference between the two study groups was compared using student t-test.

RESULTS
The mean and standard deviation of serum homocysteine levels in study and control groups were 16.48 ± 6.24 µmol/L and 10.74 ± 5.72 µmol/L, respectively (Graph 1). When statistically compared, the difference in the increased serum levels of homocysteine in study and control groups, there was a significant increase (p <0.0003). The mean and standard deviation of MDA levels in study and control groups were 3.17 ± 1.14 nmol/mL and 1.68 ± 0.63 nmol/mL, respectively (Graph 2). The statistical difference between the two groups was significant (<0.0001). In case of total antioxidant capacity, the results of the control group showed 1.18 ± 0.38 µmol/mL, but in the study group, the mean TAC levels were significantly (p <0.0001) decreased to 0.58 ± 0.26 µmol/mL (Table 1).

DISCUSSION
In the present study of vitiligo patients and healthy control groups, the levels of serum homocysteine and oxidative stress were evaluated. The results showed a significant increase in the serum levels of homocysteine in vitiligo cases than in the control group, inconsistency with previous studies by various authors like Shaker et al., Karadag et al., Singh et al. Whereas, Balci et al., Kim et al. observed that there was no significant difference in the serum levels of homocysteine between the two groups.

The mean levels of MDA, when compared with the control group, showed a significant increase, while the TAC levels showed a significant decrease. These findings are in corroboration with previous studies by Mehaney et al., Khan, et al. and Jain, et al. in his study on vitiligo patients showed that there was an imbalance in oxidant and antioxidant system.

Vitiligo is a multifactorial disorder with a polygenic inheritance pattern. Vitiligo affects about 2% of the worldwide population. The disease manifests itself as white patches over skin and nails due to loss of melanocytes in the epidermis. The autoimmune conditions, neural mechanisms, viral infections, biochemical and oxidant–antioxidant imbalances, and genetic susceptibility are considered to be the possible etiological factors for the disease.

The increased levels of homocysteine are considered to inhibit the key melanogenic enzymes and increase the harmful reactive

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group Mean ± SD</th>
<th>Study group Mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>16.48 ± 6.24</td>
<td>10.74 ± 5.72</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>3.17 ± 1.14</td>
<td>1.68 ± 0.63</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TAC (µmol/mL)</td>
<td>0.58 ± 0.26</td>
<td>1.18 ± 0.38</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
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Oxygen radicals, which in turn can damage the existing melanocytes of the epidermis or hamper the new melanocyte genesis. The harmful reactive oxygen species is potential to induce lipid peroxidation, damage the DNA, or increase the proinflammatory and anti-melanogenic cytokines. It is also supposed that the increase in homocysteine levels can induce autoimmunity by altering the protein structure and may also induce melanocyte damage by increasing oxidative stress or production of interleukin-6 or by activation of kappa B. The destruction of epidermal melanocytes of vitiligo skin may be due to oxidation of homocysteine by peroxides, anion or hydrogen peroxide or by hydroxyl free radicals along with another abnormal biochemical biopterin metabolism, which can cause abnormal accumulation of melanocytotoxic compounds or inhibition of natural detoxifying processes.

Homocysteine also interacts with the copper at the active site of tyrosinase enzyme, which can inhibit the melanin synthesis by catalyzing the rate-limiting step of melanin biosynthesis. The free homocysteine in the serum is found to react with sulfhydryl residues of body protein by forming adducts with disulfide linkage, called thiolation, which can affect the normal function of body proteins and enzymes. Mutations in catalase gene (CAT) can lead to genetic heterogenicity in the metabolism of homocysteine thus reducing the catalase activity which is observed in the vitiligo patients. All the above-mentioned factors might add up to destroy the normal melanocytes, or decrease the melanin production in vitiligo patients.

Conclusion
The present study has demonstrated that the vitiligo patients showed increased mean serum homocysteine concentration and oxidative stress levels when compared to healthy controls, which suggest impairment in the antioxidant system of study group, leading to excess free radical production, which in turn caused destruction of available melanocytes, dysregulation of melanocyte production and activation of an autoimmune response. In cases of vitiligo patients, along with routine screening for homocysteine and oxidative stress status, the usage of antioxidants and homocysteine lowering agents such as vitamin B6, B12, and folic acid may prove beneficial as therapeutic agents. Large multicentric studies are also recommended to support the role of homocysteine and oxidant-antioxidant imbalance in vitiligo.

References
28. Jain D, Misra R, Kumar A, Jaiswal G (2008) Levels of malondialdehyde in vitiligo skin may be due to oxidation of homocysteine by superoxide anion or hydrogen peroxide or by hydroxyl free radicals along with another abnormal biochemical biopterin metabolism, which can cause abnormal accumulation of melanocytotoxic compounds or inhibition of natural detoxifying processes. The present study has demonstrated that the vitiligo patients showed increased mean serum homocysteine concentration and oxidative stress levels when compared to healthy controls, which suggest impairment in the antioxidant system of study group, leading to excess free radical production, which in turn caused destruction of available melanocytes, dysregulation of melanocyte production and activation of an autoimmune response. In cases of vitiligo patients, along with routine screening for homocysteine and oxidative stress status, the usage of antioxidants and homocysteine lowering agents such as vitamin B6, B12, and folic acid may prove beneficial as therapeutic agents. Large multicentric studies are also recommended to support the role of homocysteine and oxidant-antioxidant imbalance in vitiligo.