Original Research Article

OXIDATIVE STRESS AND ANTIOXIDANTS IN PATIENTS OF VITILIGO

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Abstract:

Background: Vitiligo is a chronic, common disease of still unknown etiology characterized by circumscribed ivory or chalky white macules of different size and shape. The pathogenesis of vitiligo is complex and still not well understood. According to autocytotoxic hypothesis, oxidative stress has been suggested to be the initial pathogenic event in melanocyte degeneration.

Objective: The present study was conducted with an objective to evaluate the role of oxidative stress and antioxidant status in the pathogenesis of vitiligo.

Materials and Methods: A total of sixty (60) subjects (age group 14-50 years) of both sexes were enrolled in this study. Of 60, 30 were clinically diagnosed cases of vitiligo of all types and remaining 30 were normal healthy controls. The indicator of oxidative stress was determined by measuring serum level of MDA. The role of antioxidant was determined by measuring serum levels of SOD and Vitamin C.

Results: The mean serum level of MDA was significantly increased in vitiligo patients compared to controls while the mean serum level of SOD was significantly decreased in patients of vitiligo than controls. Also, Vitamin C was decreased in vitiligo compared to controls but was insignificant.

Conclusion: The result of the present study demonstrates imbalance between oxidant-antioxidant system and support the associations between oxidative stress and free radical-mediated damage in the pathogenesis of vitiligo.

Key words: Vitiligo, Oxidative stress, Antioxidants.

Introduction:

Vitiligo is a well-recognized pigmentary disorder of the skin and/or mucous membrane, characterized by circumscribed ivory or chalky white macules of different size and shape that affects approximately 0.1-2% of world population[1]. It can develop at any age, but 50% of cases appear before the age of 20 years regardless of sex or racial background[1,2].

Clinical presentation of vitiligo include (a) segmental vitiligo, characterized by lesions that occur in a dermatomal, asymmetrical distribution (of limited clinical significance); (b) focal vitiligo, characterized by limited number of small lesions; (c) generalized vitiligo, the most common type of vitiligo where lesions occur with bilateral symmetrical distribution; and (d) universal vitiligo, almost complete depigmentation[3].

Vitiligo has major impact on quality of life of patients, many of whom experience distress and stigmatization[4]. The pathogenesis of vitiligo is complex and still not well understood. The question posed is about "vitiligo pathogenesis": is it due to autoimmune mechanism, genetic defect, excessive reactive oxygen species (ROS), calcium imbalance, or what else[5]. Autoimmune hypothesis is the most widely accepted since autoantibodies to melanocytes and tyrosinase have been demonstrated and also due to the association of vitiligo with other autoimmune diseases[6]. A defective antioxidant defense is also postulated to lead to the unhindered cytotoxic action of reactive oxygen species such as superoxide anion, hydroxyl radical, etc[7]. After formation, these highly reactive free radicals 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 malondialdehyde (MDA)[8]. These decomposition products can cause damage to cell membrane or DNA leading to cytotoxicity, mutagenicity and cell death. These reactive oxygen species are generated following ultraviolet rays induced damage to the epidermis. They are also cytotoxic to melanocytes and can inhibit tyrosinase[8]. The purpose of the present study was to evaluate the role of oxidative stress and antioxidant status in the pathogenesis of vitiligo. Hence, we investigated the serum levels of Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Vitamin C in vitiligo patients and compared them with normal healthy individuals.

Materials and Methods:

Subjects and study design:

This was a type of case control study and conducted during the period of one year. The present study was conducted in the Department of Biochemistry, SS Medical College, Rewa. Sixty (60) subjects (age group 14-50 years) of both sexes were enrolled in this study with an informed consent. They were divided into two groups. Group I: Consisted of 30 clinically diagnosed cases of vitiligo of all types and these patients were chosen from the Department of Skin and Venereal Disease, SS Medical College and Hospital, Rewa, India. Group II: Consisted of 30 normal healthy controls of same age group, selected from the healthy staff, their family and students of our college. Patients 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 with a history of smoking or alcoholism or taking any drugs for any other reason were also excluded.

Collection of blood sample:

5 ml venous blood sample was collected from the antecubital vein from each of the vitiligo patients and control subjects in a plain tube after an overnight fasting. The blood was then allowed to clot at room temperature for 30 minutes and centrifuged for 15 minutes at 5000 rpm to extract the serum. The serum was used for determination of MDA, SOD and Vitamin C.

Parameters measured:

Following parameters were assessed in the present study:

1. MDA was measured by the method described by Kei Satoh[9].
2. SOD was measured by the method described by Marklund and Marklund[10].
3. Vitamin C was measured by the method described by Roe and Kuther's[11].

Statistical analysis:

Results were statistically analyzed by 'SPSS version 20'. Student's t-test was used to assess the significance of difference between the groups. All results are presented as mean ± S.D. A *'p'* value of less than 0.05 was considered significant.

Observations and Results:

Table 1: Showing mean and standard deviation of MDA, SOD and Vitamin C in patients with vitiligo and normal healthy control group.

Parameters	**Patients (mean±SD)**	**Controls (mean±SD)**	***'p'*-value**
MDA (nmol/ml)	2.83±0.73	1.44±0.14	<0.0001 **S**
SOD (U/ml)	0.73±0.04	0.87±0.04	<0.0001 **S**
Vitamin C (mg/dl)	1.64±0.06	1.65±0.08	0.4422 **NS**

S= Statistically Significant, **NS**=Statistically Insignificant, MDA=Malondialdehyde, SOD= Superoxide Dismutase

The mean MDA level in patients with vitiligo was increased compared to control group and was statistically significant where as there was significant decrease in the mean serum level of SOD in vitiligo patients compared to controls. The mean serum level of Vitamin C was decreased in vitiligo patients but was statistically insignificant.

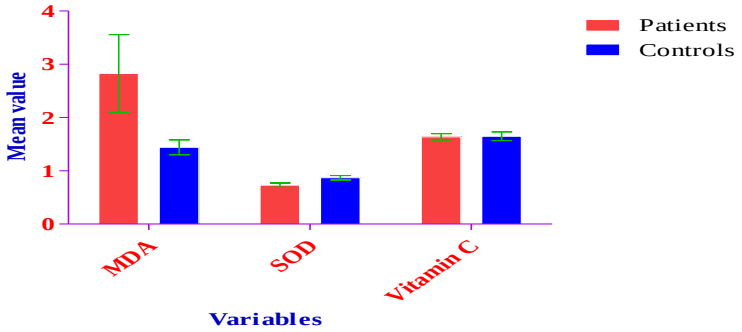

Figure 1: Showing comparison of MDA, SOD and Vitamin C between patients and controls.

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. ROS produces free radicals such as superoxide (O_2^-), H_2O_2, and nitric oxide. These molecules occur during several physiological and pathological processes. 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[12].

Measurement of MDA in the blood provides evidence of lipid peroxidation induced by reactive oxygen species and an indicator of oxidative stress[13]. In the present study, MDA level was significantly higher in vitiligo patients as compared to normal healthy population. This finding supports the study done by others[4,5,8,14]. Increased MDA represents increased lipid peroxidation i.e. enhanced free-radical-mediated processes. Free-radical reactions may be the chemical link between cell damage and the inflammatory response[8]. However, Kamel *et al* revealed no significant difference in the level of MDA when measured in plasma between vitiligo patients and control group. But, in skin specimen, they found that there was a significant increase in the level of MDA in vitiligo patients compared to control group. They suggested that this increase is due to exposure of ROS in vitiliginous lesion than that in the systemic circulation[15]. In the present study, the levels of oxidants and antioxidants have not been investigated in lesional skin of vitiligo patients. Yildirim *et al*[16] and Dammak *et al*[17] studied in tissue and had found the MDA levels in the skin of vitiligo patients to be higher than the controls and the difference was statistically significant, they explained this that it is a condition of oxidative stress. Moreover, Dammak *et al*[17] added that lipid peroxidation in the cellular membrane of melanocytes may play an important role in the rate of depigmentation observed in the skin of patients with active vitiligo.

In the present study, serum SOD level was significantly decreased in patients as compared to controls. This finding is in agreement with the study conducted by El-Refaei *et al*[14] who also showed significant decrease in serum SOD in vitiligo patients compared to controls. Similarly, Koca *et al*[18] found significant lower levels of serum SOD activity in vitiligo patients compared to healthy control. They explained this by the fact that superoxide and hydroxyl radicals are the most important radicals in peroxidation and decreased SOD activity could be responsible for the increase of superoxide radicals which may explain the increased level of MDA. Kamel *et al*[15] revealed a decrease in the level of SOD in vitiligo patients than control group both in plasma and skin specimens but this decrease was statistically insignificant. They explained that such decrease may be due to its utilization in detoxication of released ROS (superoxide anion). On the contrary, Chakraborty *et al*[19] and Jain *et al*[20] reported significantly higher serum levels of SOD in vitiligo patients than in controls. They suggested that there is an imbalance in the oxidant-antioxidant system (oxidative stress) due to disease activity resulting in high level of blood superoxide dismutase level.

We found lower level of Vitamin C in vitiligo patients than the control but statistically insignificant ($p>0.05$). Our result is in agreement with other studies[8,21]. Due to the lower level of vitamin C, free radicals may accumulate and contribute to the buildup of oxidative stress in the system. Vitamin C

is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized[22]. Dietary vitamin-C consumption can lead to some stabilization and in some cases repigmentation[23].

Conclusion:

The result of the present study demonstrates imbalance between oxidant-antioxidant system which is indicated by significantly increased serum level of MDA and decreased serum levels of SOD and Vitamin C in patients of vitiligo. These findings further support the associations between oxidative stress and free radical-mediated damage in the pathogenesis of vitiligo. However, further studies with adequate sample size are needed to explore the role of oxidative stress in vitiligo.

Author's contributions:
All the authors made substantial contributions to the design and conception of the study. All the authors have been involved in drafting and revising the manuscript, have read, and approved the final manuscript.

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