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OXIDATIVE STRESS IN VITILIGO- A COMPARATIVE STUDY OF SERUM SUPEROXIDE DISMUTASE AND MALONDIALDEHYDE IN VITILIGO PATIENTS AND HEALTHY PEOPLE

YOGAPRIYA V[1*], JISHA R[2], JYOTHIRMAYI B[3]

1: Assistant Professor, Department of Biochemistry, Madha Medical College and Research Institute

2: Assistant Professor, Department of Biochemistry, Government TD Medical College, Alapuzha

3: Professor and Head, Department of Biochemistry, Madha Medical College and Research Institute

***Corresponding Author: Yogapriya V: E Mail: yogapriya154@gmail.com**

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ABSTRACT

Vitiligo is a pigmentary autoimmune disorder of the skin affects 1-2% of world population. Oxidative stress is one of the causes for vitiligo resulting in melanocyte degeneration, hydrogen peroxide (H_2O_2) accumulation in the epidermis and an insufficient antioxidant system with increased levels of reactive oxygen species (ROS). Superoxide dismutase (SOD) protects cells from toxic effects of superoxide radical. Malondialdehyde (MDA), an end-product of lipid peroxidation induced by ROS. The aim of this study is to compare the levels of serum SOD and serum MDA among newly diagnosed vitiligo patients and healthy people and to study the correlation between serum SOD and MDA levels in vitiligo. It is a cross sectional study, where 40 newly diagnosed vitiligo patients as study group and 40 healthy people as controls aged between 14-50years were included . Serum SOD and MDA were estimated and statistical analysis was performed. *p* value of < 0.05 is considered significant. The Mean ± SD of serum SOD (u/ml) (0.75± 0.24) in study group were significantly lower than controls (1.64 ± 0.72). The level of serum MDA (nmol/dl) in vitiligo patients (103.61 ±

17.84) were significantly higher than controls (83.36 ± 9.72; p<0.0001) and there was a statistically significant correlation between serum SOD (u/ml) and serum MDA (nmol/dl) among vitiligo patients (*P* value < 0.001). Our study demonstrate that there was an impairment in the oxidant/ antioxidant balance in vitiligo. So the markers of oxidative stress can be employed to assess disease activity and to plan and monitor antioxidant therapy**.**

Keywords: Vitiligo, Oxidative stress, Serum malondialdehyde (MDA), Serum superoxide dismutase (SOD)

INTRODUCTION

Vitiligo is a common pigmentary autoimmune disorder of the skin which affects 1-2% of world population, with selective destruction of melanocytes. Vitiligo was given a Diagnosis Code (l80) by the World Health Organization (WHO) in its 10th revision of International Statistical Classification of Diseases **[1]**.

Skin is a major target for toxic insults by various chemical and physical agents which can alter its structure and function. Augmentation of reactive oxygen species (ROS) by the environmental pollutants act as oxidants in the skin that promotes the development of many dermatological diseases **[2]**.

Several theories suggests that oxidative stress is the cause for the pathogenesis of vitiligo, which includes the autoimmune theory **[3]**, the autocytotoxic theory **[4]**, the neural theory **[5]**, the "impaired epidermal cytokine" theory **[6]**.

Oxidative stress causes melanocyte degeneration with hydrogen peroxide (H_2O_2) accumulation in the epidermis of skin and an insufficient antioxidant system activates vitiligo **[7]** .

Superoxide dismutase (SOD) is a group of metalloenzymes that protects cells from toxic effects of superoxide radical that catalyze the dismutation of superoxide into O_2 and H_2O_2 **[8]**. They form an important antioxidant defense in all cells exposed to O_2. There are 3 major families of SOD: Cu/Zn, Fe/Mn and the Ni type.

Malondialdehyde (MDA), an end-product of lipid peroxidation induced by ROS, is well correlated with degree of lipid peroxidation **[9]**.These findings support the systemic oxidative stress in vitiligo.

Alternatively, oxidatively stressed vitiligo melanocytes can produce self-antigens and/or cytokines to activate further autoimmune response **[10]** and disease progression **[11]**.

The aim of present study is to evaluate the role of oxidative stress in pathogenesis of vitiligo and therefore we evaluate the status of serum MDA and antioxidant enzyme serum SOD levels in vitiligo patients and compare it with

healthy people and study relation between SOD and MDA levels in vitiligo.

This can guide us in choosing the appropriate antioxidant enzymes to support antioxidant system which may be useful in the prevention of melanocyte degeneration that occurs due to oxidative damage in vitiligo **[12]**.

MATERIALS AND METHODS:

Study design: Hospital based cross sectional study done at Department of Biochemistry and Dermatology, Government Medical College Hospital, Thiruvananthapuram. Study period was one year after getting approval from ethical committee (IEC.No.05/61/2015/MCT).

Sample size: 40 cases of new vitiligo patients diagnosed by a dermatologist and 40 healthy people (without vitiligo) serve as controls aged between 14-50years are included for the study. Sample size was calculated by applying the following formula **[13]**:

$$n = 2 [Z_{(1-\alpha/2)} + Z_{(1-\beta)}]^2 \times \sigma^2 \over (\mu_d)^2$$

Inclusion criteria:

Study group: Newly diagnosed cases of all types of vitiligo who have active disease at the time of study (i) duration of the disease < 6 months (ii) appearance of new lesions (iii) increase in the size of the existing lesions in the last 6 months.

Control group: Age and Sex matched healthy people without vitiligo taken from general population served as controls.

Exclusion criteria:

Patients with any systemic illness, autoimmune disorder, concomitant dermatological disease, smoking or alcohol abuse, Treatment (systemic or topical) in prior three months and other supplements.

Collection of samples:

About 5 ml of fasting blood sample was drawn under strict aseptic precautions and centrifuged serum sample was stored at -20^0C.

- SOD activity is measured by colorimetric kit method **[14, 15, 16]**.
- Malondialdehyde is measured by method of Draper and Hadley **[17]**, chemical method (Thiobarbituric acid) **[18]**.

ANALYSIS AND RESULTS

- Statistical analysis was performed using SPSS for windows version 22
- The mean and standard deviation was calculated for study and control group
- Chi square test was used to compare the differences in the percentage of qualitative variables and student t' test was applied to find the differences in means of quantitative variables between the groups
- *p* value of less than 0.05 is considered significant.

Serum SOD and MDA among vitiligo patients and healthy controls were shown in Table 1: The mean distribution of biochemical parameters in vitiligo patients and controls are expressed in Mean ± SD and *P* value < 0.001 shows statistically significance.

Mean serum SOD among vitiligo patients and healthy controls were shown in Figure 1: The mean serum SOD values among vitiligo patients is 0.75 units/ml and among healthy controls is 1.64 units/ml. The difference was found to be statistically significant (*P* value < 0.001)

Mean serum MDA among vitiligo patients and healthy controls were shown in Figure 2: The mean serum MDA values among all 40 vitiligo patients is 103.61nmol/dl and among healthy controls is 83.36nmol/dl. The difference was found to be statistically significant (*P* value < 0.001).

Correlation between Serum SOD (u/ml) and Serum MDA (nmol/dl) among vitiligo patients were shown in Figure 3: Pearson Correlation r = -0.412, p<0.001. There was a statistically significant correlation between serum SOD (u/ml) and serum MDA (nmol/dl) among vitiligo patients (*P* value < 0.001).

Table 1: Serum SOD and MDA among vitiligo patients and healthy controls:

Parameter	Case (40) Mean ± SD	Control (40) Mean ± SD	t	P
SOD (U/ml)	0.75± 0.24	1.64 ± 0.72	-7.406	<0.001
MDA (nmol/dl)	103.61 ± 17.84	83.36 ± 9.72	6.302	<0.001

(*P* value < 0.001: statistically significant)

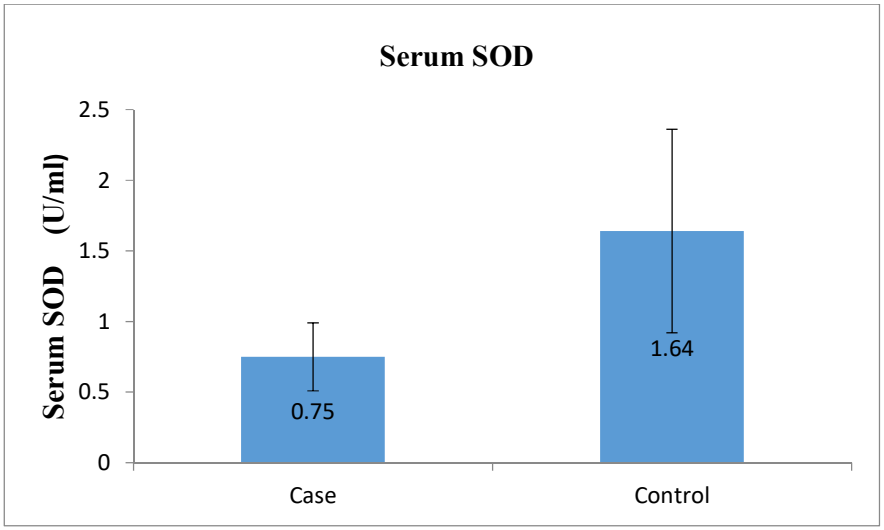

Figure 1: Mean serum SOD among vitiligo patients and healthy controls

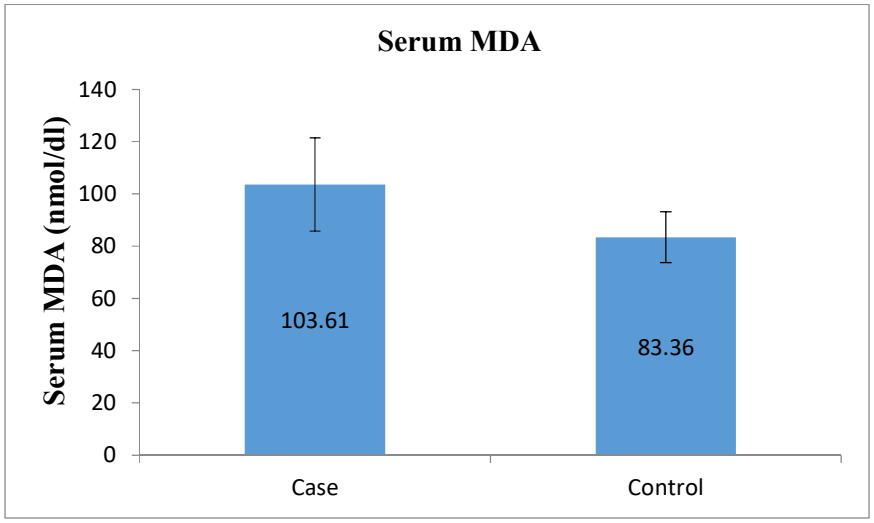

Figure 2: Mean serum MDA among vitiligo patients and healthy controls

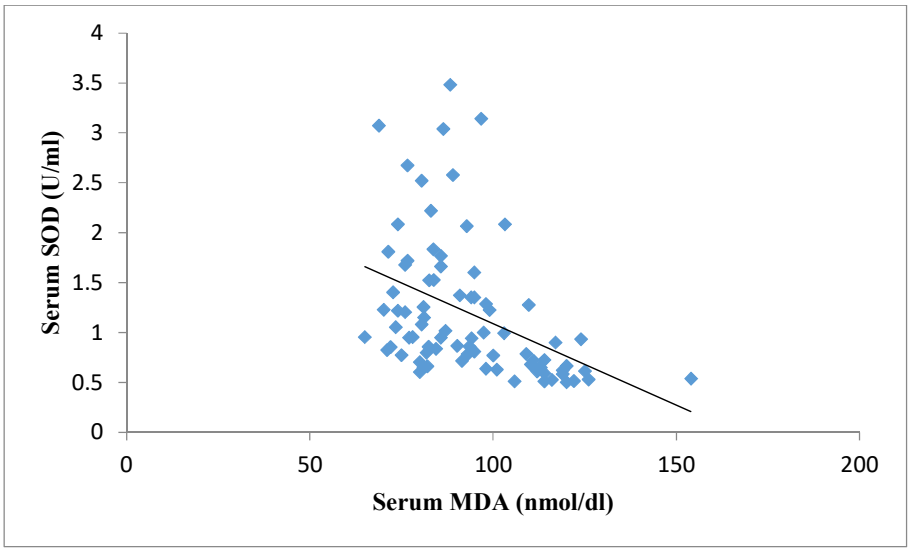

Figure 3: Correlation between Serum SOD (u/ml) and Serum MDA (nmol/dl) among vitiligo patients: Pearson Correlation r = -0.412 p<0.001

DISCUSSION

Vitiligo is characterized by progressive loss of melanocytes, due to the oxidative damage of polyunsaturated fatty acids (PUFA), known as lipid peroxidation, can be considered as hallmark of oxidative stress.

Malondialdehyde (MDA), an end-product of lipid peroxidation induced by ROS, is correlated with degree of lipid peroxidation. In the present study, significantly higher level of serum MDA in vitiligo patients compared with controls were documented.

Superoxide dismutase (SOD), an antioxidant enzyme, catalysis the conversion of superoxide radicals to H_2O_2 and O_2. In the present study significantly

lower levels of serum SOD activity in patients with vitiligo compared to healthy controls were documented. Decreased SOD activity is responsible for the increase of superoxide radicals, which in turn explain the increased level of MDA.

In the present study, highest prevalence of vitiligo was found to be in the age group 31-40 years which is 32.5%. Singh *et al*. **[19]** and Jain *et al*. **[20]** observed higher prevalence in younger age group 11–25years.

In this study, female cases and control shares equal prevalence 62.5% and male cases and control shares equal prevalence 37.5% and there was female preponderance (62.5%) and this was similar to studies by Jain *et al*. **[20]** and Shajil *et al*. **[21]**.

The oxidative stress biomarkers serum SOD and MDA has no statistically significant relation with different age and gender groups of vitiligo patients. This was in agreement with the study conducted by Arican and Kurutas **[22]**.

The present study showed, the SOD values among vitiligo patients (0.75± 0.24) is significantly decreased when compared to controls (1.64 ± 0.72). The difference was found to be statistically significant (*P* value < 0.001).This is in accordance with various other studies done previously[**13, 23, 24**] and a study done by Picardo *et al*. **[25]** found normal levels of SOD activity in erythrocytes of vitiligo patients. A study conducted by Passi *et al*. **[26]** in the epidermis and Maresca *et al*. **[7]** in the cultured melanocytes of vitiligo patients, found no significant differences between vitiligo patients and controls.

These results in the above studies suggest that the increased SOD activity in vitiligo patients might be due to increased oxidative stress, in the presence of high levels of O_2^- which leads to high levels of SOD, followed by high amounts of H_2O_2 leading to destruction of defective melanocytes in vitiligo patients.

In our study we observed that MDA values among vitiligo patients (103.61 ± 17.84) is significantly increased when compared to controls (83.36 ± 9.72). The difference was found to be statistically significant (*P* value < 0.001). This is been supported in various studies conducted by Yildirim *et al*. **[27]**, Dammak *et al*. **[28]** and Shabaka *et al*. **[29]**. Differing results about MDA activity has been documented in few studies such as Nabil Kamel **[23]** and Picardo *et al*. **[25]** observed not much statistically significant decrease in the levels of SOD and MDA. Variations in the duration and activity of the disease phase may be responsible for the varying results.

The lipid peroxidation products such as lipid peroxides, lipoxides and MDA could damage the cell membrane or DNA of melanocytes, inhibits tyrosinase enzyme

leading to cytotoxicity, mutagenicity and cell death.

The newer perspective of vitiligo is due to autoimmunity where melanocytes are destroyed by CD8+ T cells causing depigmentation **[30]**. Recent studies also suggests that H202 increases mitochondrial calcium influx, resulting in mitochondria-dependent apoptosis of melanocytes leading to vitiligo **[31]**.

We observed in our study that a statistically significant correlation between serum SOD (u/ml) and serum MDA (nmol/dl) among vitiligo patients (*P* value < 0.001). A study by Asmaa M. El-Refaei, *et al*. **[32]** and Sushil Pandae *et al*. **[33]** observed similar results and a study by Singh *et al*. **[19]** differ in their results with our present study.

LIMITATIONS OF THE STUDY

This present study does not measure the serum levels and effects of oxidative stress markers after effective treatment with antioxidant.

CONCLUSION

Our present study shows that there is an impairment in the oxidant/ antioxidant balance in vitiligo, resulting in lipid disturbances. Oxidative stress is a generalized process and might be one of the reasons for the progressive nature of the disease. Based on these findings, it can be concluded that antioxidants may play a role as adjuvants in management of vitiligo. Therefore, markers of oxidative stress (SOD & MDA) can be employed to assess disease activity and to plan and monitor antioxidant therapy.

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