



## ASSESSMENT OF OXIDATIVE STRESS DURING NARROW BAND UVB PHOTOTHERAPY IN VITILIGO PATIENTS OF NORTH INDIA

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**Abstract:** Vitiligo is an acquired epidermal pigment loss which occurs anywhere in the body. The disease occurs worldwide with an estimated overall prevalence of less than 0.5%. The biological basis of vitiligo has been an intractable problem to confront. Among several therapies for the disease, narrow band UVB phototherapy is preferred modality and is effective. Recently, oxidative stress has been speculated to be the primary event in the pathogenesis of vitiligo. The ability of free radicals to destroy melanocytes is considered as missing link. This study is aimed to assess the oxidative stress and antioxidant status in vitiligo patients with and without NB-UVB phototherapy for 4 weeks. 30 Vitiligo patients were recruited for study from OPD of Department of Dermatology, Era's Lucknow Medical College & Hospital, Lucknow. Blood of all subjects was investigated for anti-oxidant parameters such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Lipid peroxides (LPO). Results indicated significant increase in the CAT and SOD activity in stable vitiligo patients after NB-UVB phototherapy as compared to its baseline activity. However, in active vitiligo patients, the increase in the CAT and SOD activity were found non-significant. GPx activity was found statistically non-significant in active vitiligo group after NB-UVB phototherapy compared to its baseline value, however in stable vitiligo patients the GPx activity was statistically significant. Following the NB-UVB phototherapy the LPO levels increased significantly in both active and stable vitiligo patients as compared to their baseline values.

**Key Words:** oxidative stress, vitiligo, narrow band UVB phototherapy, India

### INTRODUCTION

Vitiligo is commonly characterized by the depigmentation of skin anywhere in the body. It occurrence is approximately less than 0.5% in the world [1]. The disease usually begins in childhood or young adulthood; almost 50% of the patients present before the age of 20 years, and nearly 70–80% before the age of 30 years. Both sexes are equally affected by the severity of the disease. The disease is a slow and progressive and may have remissions and exacerbations correlating with triggering events [2]. The pathogenesis of vitiligo has been intriguing researchers for decades. The basic defect in vitiligo is disappearance of melanocytes [3], but even the concept of “disappearance” is a matter of debate [4]. Some evidence-based hypotheses have been proposed to explain the loss of melanocytes in epidermis. The most prevailing hypothesis include Genetic Hypothesis [3], Autoimmune Hypothesis [5], Neural Hypothesis [6], Viral Hypothesis [7], Self-Destruct Hypothesis [8], Convergence Hypothesis [9] etc.

There are several treatment for this disease. The primary goal of therapy is to restore melanocytes to the skin. Such repigmented skin regains its normal appearance, morphology, immune and inflammatory functions. Several treatment modalities are currently available including medical, surgical and adjunctive

therapies, each having certain indications as well as restrictions [10]. Now a days, Narrowband UVB (NB-UVB) phototherapy is preferred over the conventional therapies. NB-UVB phototherapy exerts its effects by stabilizing the local and systemic abnormal immune response through immuno-modulatory effects of UV radiation and by stimulating the dopa-negative, amelanotic melanocytes in the outer hair root sheaths to proliferate, produce melanin and migrate outward into depigmented skin, resulting in perifollicular repigmentation [11]. The advantages of this therapy over other therapies are represented by shorter time of treatment, less side effects, no oral drug administration, no contraindications [11,12] etc. We have taken up this study to observe effect of NB-UVB phototherapy in vitiligo patients, and to compare the oxidative stress and antioxidant status in vitiligo patients before and after NB-UVB phototherapy.

### MATERIALS AND METHODS

#### Study Place and design

The present study was carried out in the Department of Dermatology in collaboration with Department of Biochemistry of Era's Lucknow Medical College and Hospital, Lucknow (U.P) India. Study design was observational case control study.

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### Sample Size and Patient Enrollment

Sample size was statistically calculated [13]. Thirty patients were enrolled for the present study, with their ages ranging from 3 to 75, which were clinically diagnosed case of vitiligo supported by Wood's lamp examination. Patients were categorized into 2 groups viz. Active and stable vitiligo. Patients with increase in size of lesion or appearance of new lesions in past 6 months were kept in former category while patients with no increase in size of lesion or appearance of new lesions in past 6 months were kept in later category. Patients with pregnancy, cancer, diabetes, hypertension, cardiovascular diseases, thyroid disorders, epilepsy, infections, current habitual smokers, alcoholics, or those using antioxidant supplementation or estrogens/ progestins at the start of the study were not included in the study. Patients with family history of skin carcinoma, photosensitivity disorders e.g. systemic lupus erythematosus, xeroderma pigmentosa were also excluded from study. Written Informed consent was taken from all the patients. The study proposal was approved by the Institutional Ethics Committee. All the patients were examined clinically and information pertaining to age, gender, habits and health status was recorded in patient data sheet.

### Sample Collection

After obtaining the consent, 5ml blood was drawn from normal subjects as well as vitiligo patients by venipuncture, and collected in EDTA containing vials to collect plasma and RBC. Plasma and RBC were separated by centrifugation at 2500 rpm for 15 minutes at room temperature. Plasma was then transferred to sterile tubes for biochemical analysis. Packed cell volume was used to prepare the lysate for anti-oxidant enzyme assay. The samples were kept at -20°C till analysis.

### NB-UVB phototherapy

Whole body Phototherapy unit (V-care Medical System Pvt. Ltd., Bangalore, Karnataka) having 22 NB-UVB fluorescent TL-01 (Philips - 100W) tubes was used to administer phototherapy to the patients in the Department of Dermatology, S.T.D. & Leprosy. Standard starting dose of 280 mJ/cm<sup>2</sup> with stepwise increment of 20% of last dose on every next visit was done. In case of mild erythema, the irradiation dose was held constant for subsequent treatments or until resolution of symptoms. The goal of therapy was to achieve persistent asymptomatic erythema. In case of painful erythema with/without edema or blistering, further treatment was withheld with addition of topical/systemic steroids till symptoms subside. After resolution of symptoms, dose administered was 50% of the last dose and subsequent increments was done by 10% [14].

### Sample Analysis

RBC lysate was used to estimate antioxidant enzymes such as Catalase [15], Superoxide dismutase [16], Glutathione peroxidase [17] and plasma was used to estimate lipid peroxide [18] and total proteins [19]. Standard methodology was followed to estimate all the biochemical parameters as mentioned against each.

### Statistical Analysis

Data analysis was done using SPSS 17.0 software. The descriptive analysis was done using means and standard deviations. The differences in means from baseline to cases & controls were evaluated using the ANOVA test. Unpaired t-test was used to compare the means of the parameters between cases and controls. The confidence level of the study was kept at 95%, hence 'p' value less than 0.05 was considered as statistically significant for intergroup difference.

## RESULTS

### Characteristics of Study Participants

The age distribution of cases and controls is given in table 1. Most of cases and controls were in the age group of 21-40 years. The age of youngest patient was 11 years, whereas that of oldest patient was 40 years. Majority of cases in our study were females (76.67%). Of these 10 were present in the active group, whereas 13 were present in the stable group. 42.8% of the male patients were present in the stable group and 57.2% were in the active group. In our study, 63.33% patients had vulgaris type of vitiligo of which 11 had active disease whereas 8 had stable disease. Focal type of vitiligo was present in 2 patients with active disease and 5 patients with stable disease. Acrofacial type was seen in 4 patients with only 1 having active disease. 33.3% patients had disease for less than 5 years, whereas 9 and 8 patients had disease for 5-10 and 11-15 years respectively. Only 10% patients had disease for more than 15 years. 70% patients in our study had 0-10% body surface area involved as evaluated by Wallace rule of 9. Only 1 patient had more than 40% involvement whereas 3 patients having active type of vitiligo had 21-30% involvement. In our study, legs were the commonest site affected followed by face and arms. Genitalia were involved in 4 patients whereas oral mucosal involvement was seen in 3 patients with stable vitiligo.

**Table 1:** Characteristics of control and patients

Characteristic		Controls	Patients	
Age (in years)	11-20	3	5	
	21-30	13	14	
	31-40	10	11	
	>40	4	0	
Type of Vitiligo	Focal	-	2	5
	Acrofacial	-	1	3
	Vitiligo vulgaris	-	11	8
			Active	stable
Duration of Disease (in years)	< 5	-	7	3
	5-10	-	2	7
	11-15	-	3	5
	16-20	-	2	1
% Body surface area involved	0-10	-	8	13
	11-20	-	2	3
	21-30	-	3	0
	>40	-	1	0
Distribution of lesions	Face	-	9	12
	Scalp	-	5	2
	Chest	-	10	5
	Back	-	6	6
	Arms	-	9	10
	Legs	-	13	10
	Genitalia	-	2	2
	Oral	-	0	3
Mucosa	-	0	3	

**Anti-Oxidant Status before NB-UVB Phototherapy**

All the biochemical results are illustrated in the Table 2. Our results shows a significant ( $p < 0.05$ ) decrease in the catalase (CAT), superoxide dismutase (SOD) and Glutathione peroxidase (GPx) activity in RBC lysate of both the patient groups viz, active and stable vitiligo as compared to the healthy controls. CAT activity in healthy control was  $48.95 \pm 7.56$  units/mg protein, while in active and stable vitiligo patient it was  $37.65 \pm 7.77$  and  $41.25 \pm 9.47$  units/mg protein, respectively. Similarly, SOD activity in healthy control was  $1.15 \pm 0.18$  units/mg protein, while in active and stable vitiligo patient it was  $0.91 \pm 0.14$  and  $1.00 \pm 0.17$  units/mg protein, respectively. Further, GPX activity also showed the significant decrease in active and stable vitiligo patients as compared to normal healthy controls. Significant increase in plasma lipid peroxide level was observed in active and stable vitiligo patient ( $4.56 \pm 0.44$ ,  $3.84 \pm 0.25$  nmole of MDA/ml plasma respectively) as compared to the normal healthy controls ( $2.11 \pm 0.16$  nmole of MDA/ml plasma).

**Table 2:** Anti-oxidant status in Controls and vitiligo cases before NB-UVB phototherapy

Parameter	Control	Before NB-UVB therapy		After NB-UVB therapy	
		Active	Stable	Active	Stable
Catalase (U/mg protein)	$48.95 \pm 7.56$	$37.65 \pm 7.77^*$	$41.25 \pm 9.47^*$	$39.59 \pm 7.93$	$44.11 \pm 8.97^{**}$
Superoxide dismutase (U/mg protein)	$1.15 \pm 0.18$	$0.91 \pm 0.14^*$	$1.00 \pm 0.17^*$	$0.99 \pm 0.148^{**}$	$1.05 \pm 0.16^{**}$
Glutathione peroxidase ( $\mu\text{g}$ GSH utilized/ min/mg protein)	$38.51 \pm 9.62$	$24.99 \pm 4.03^*$	$28.28 \pm 5.42^*$	$25.57 \pm 4.21$	$29.73 \pm 5.70^{**}$
Lipid peroxides (MDA / ml plasma)	$2.11 \pm 0.16$	$4.56 \pm 0.44^*$	$3.84 \pm 0.25^*$	$4.98 \pm 0.46^{**}$	$4.37 \pm 0.34^{**}$

\*significant at p value  $< 0.05$  as compared to control

\*\*significant at p value  $< 0.05$  as compared to baseline values

**Anti-Oxidant Status after 4 Week NB-UVB Phototherapy**

After the NB-UVB phototherapy treatment, we observed significant improvement ( $p < 0.05$ ) in the CAT activity in stable vitiligo patients ( $44.11 \pm 8.97$  units/mg protein) as compared to its baseline activity ( $41.25 \pm 9.47$  units/mg protein). However, in active vitiligo patients the increase in the CAT activity was statistically non-significant. In active vitiligo patients, there was a significant improvement ( $p < 0.05$ ) in SOD activity after 4 weeks of NB-UVB phototherapy ( $0.99 \pm 0.148$  units/mg protein) as compared to its baseline activity ( $0.91 \pm 0.14$  units/mg protein), similar significant increase was observed in stable vitiligo patients. There was non-significant change in GPx activity in active vitiligo patients after 4 weeks of NB-UVB phototherapy ( $25.57 \pm 4.21$   $\mu\text{g}$  GSH utilized/min/mg protein) as compared to its baseline activity ( $24.99 \pm 4.03$   $\mu\text{g}$  GSH utilized/min/mg protein), however in stable vitiligo patients the improvement in activity was statistically significant ( $p < 0.05$ ). After 4 weeks of NB-UVB phototherapy, the lipid peroxide levels were found significantly elevated ( $p < 0.05$ ) in active ( $4.98 \pm 0.46$  nmole of MDA/ml plasma) and stable ( $4.37 \pm 0.34$  nmole

of MDA/ml plasma) vitiligo patients as compared to their baseline values ( $4.56 \pm 0.44$ ,  $3.84 \pm 0.25$  nmole of MDA/ml plasma, respectively).

**DISCUSSION**

There are several reports indicating the altered biochemical parameters in vitiligo patients [20,21]. Also the antioxidant imbalance has been demonstrated due to  $\text{H}_2\text{O}_2$  production inside the epidermis and systemically distributed by the blood indicating oxidative stress in vitiligo [22]. Disturbed antioxidant status has been reported in terms of CAT, SOD, GPx and LPO in vitiligo patients [21]. The imbalance of antioxidants was associated with hyper production of ROS due to a mitochondrial impairment. In another study, increased levels of erythrocyte SOD, serum LPO, and NO have been found associated with a marked reduction of erythrocyte GPx and GSH activities in generalized vitiligo patients. Based on the findings they suggested that the presence of an imbalance in the oxidant-antioxidant system might play a role in the pathogenesis of vitiligo [23]. One more study has revealed the oxidant/antioxidant imbalance in vitiligo

patients by measuring serum and tissue CAT and comparing them with the degree of lipid peroxidation. Their study included 15 vitiligo patients and 10 normal healthy volunteers acting as controls. Their results showed that the mean levels of serum and tissue CAT were significantly lower in vitiligo patients than in the control group [24]. In a study on age dependent antioxidant status in vitiligo patients of different age groups demonstrated that whole blood glutathione levels, erythrocyte GPx and G6PD activity were decreased significantly, whereas erythrocyte CAT activity and plasma vitamin E levels were not different in vitiligo patients as compared with age-matched healthy controls [25]. Our results also demonstrates the imbalance in the anti-oxidant enzymes, revealing that the reactive oxygen species are capable of deteriorating these anti-oxidant enzymes. Hence, implicating the role of reactive oxygen species in pathogenesis of vitiligo.

### CONCLUSION

The result of our study indicates that there is systemic oxidative stress in the vitiligo patients as evidenced by decreased activity of catalase, superoxide dismutase and glutathione peroxidase in erythrocytes of vitiligo patients compared to controls. Increased lipid peroxides in the plasma of vitiligo patients compared to control also reveals the role of reactive oxygen species in vitiligo. We also found that active vitiligo patients have increased derangement of antioxidant defense system as shown by significantly increased lipid peroxides in active vitiligo patients as compared to stable vitiligo patients. Vitiligo patients when treated with NB-UVB phototherapy, the result shows the improvement in activity of catalase, superoxide dismutase and glutathione peroxidase in vitiligo patients compared to their baseline activity, thus reaffirming the oxidative stress in vitiligo with improvement in the biochemical parameters. The results of the present study show that systemic oxidative stress is present in the blood of vitiligo patients. However, to prove and conclude the systemic oxidative stress in vitiligo patients and the supportive effect of antioxidants in its treatment, larger multicentric studies with large number of patients and longer duration of treatment are required.

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