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EFFECT OF ANTI-OXIDANT SUPPLEMENTATION IN VITILIGO PATIENTS DURING NARROW BAND UVB PHOTOTHERAPY

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ABSTRACT: Vitiligo is an acquired idiopathic epidermal pigment loss occurring on the body. The prevalence of the disease is less than 0.5%. Pathogenesis of vitiligo has been intriguing researchers for decades. Basic defect in vitiligo is the disappearance of melanocytes. Several therapies are prescribed for the disease; the most effective one is narrowband UVB phototherapy. Several evidence-based hypotheses have been proposed to explain the loss of melanocytes in the epidermis. Oxidative stress has been speculated to play a pivotal role in disease pathogenesis. The present study was planned to determine the effect of oral antioxidant supplementation for 4 weeks in patients undergoing Narrow Band UVB phototherapy and comparison with patients receiving Narrow Band UVB phototherapy only. 30 Vitiligo patients were recruited for the study from OPD of Department of Dermatology, Era's Lucknow Medical College & Hospital, Lucknow. Blood was investigated for anti-oxidant parameters such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Lipid peroxides (LPO). After 4 weeks oral anti-oxidant supplementation, we observed significant ($p<0.05$) increase in CAT, SOD and GPx activity in vitiligo patients receiving anti-oxidant and NB-UVB phototherapy as well as in NB-UVB phototherapy alone receiving group, as compared to baseline values. We also observed a statistically significant ($p<0.05$) elevation in LPO levels of vitiligo patients with NB-UVB phototherapy alone as well as in patients receiving anti-oxidant and NB-UVB phototherapy. Our results show that on the one hand antioxidant supplementation decreases oxidative stress in vitiligo patients while on the other hand, NB-UVB phototherapy exerts the overproduction of lipid peroxides.

INTRODUCTION: Vitiligo is characterized by the depigmentation of the skin. The occurrence of the disease is estimated to be less than 0.5% of the world's total population. The disease onset begins in early childhood or young age; about 50% of the sufferers are under the age of 20 years, and nearly 70-80% under the age of 30 years [1].

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Male and female are equally affected by the disease. The disease is slow and progressive and may pose remissions and exacerbations correlating with triggering events [2].

Several researchers are trying to understand the pathogenesis of the disease for decades. The basic cause is the disappearance of melanocytes [3, 4]. Several hypotheses have been proposed to explain the loss of melanocytes in the 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*. Several treatment modalities are currently available including

medical, surgical and adjunctive therapies, each having certain indications as well as restrictions [10]. The primary goal of the therapy is to restore melanocytes to the skin. Such pigmented skin regains its normal appearance, morphology, immune and inflammatory functions. Now a day, Narrowband UVB (NB-UVB) phototherapy is the preferred mode of treatment. 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 the shorter time of treatment, fewer side effects, no oral drug administration, no contraindications [12,] *etc*.

Oral antioxidant supplementation has been proven to be good therapy in maintaining the antioxidant enzymes and reducing the oxidative stress during diseases such as diabetes [13], cardiovascular [14], asthma [15], rheumatoid arthritis [16] *etc*. Antioxidant supplementation (oral) is capable of fighting the reactive oxygen species as well as to boost the immune system [17]. Therefore we have taken up this study to observe the effect of oral antioxidant supplementation in vitiligo patients with and without NB-UVB phototherapy.

MATERIALS AND METHODS:

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

Study Participants and Enrollment: The sample size was statistically calculated [18]. 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 an increase in the size of lesion or appearance of new lesions in past 6 months were kept in former category while patients with no increase in the size of lesion or appearance of new lesions in past 6 months were kept in the 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 a family history of skin carcinoma, photosensitivity disorders, *e.g.* systemic lupus erythematosus, xeroderma pigmentosa were also excluded from the study. Written Informed consent was taken from all the patients. The Institutional Ethics Committee approved the study proposal. All the patients were examined clinically and information about age, gender, habits and health status was recorded in the patient data sheet.

NB-UVB Phototherapy: The whole body Phototherapy unit (V-care Medical System Pvt. Ltd., Bangalore, Karnataka) having 22 NB-UVB fluorescent TL-01 (Philips - 100W) tubes were used to administer phototherapy to the patients in the Department of Dermatology, S.T.D. & Leprosy. The standard starting dose of 280 mg/cm2 with a stepwise increment of 20% of the 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 the addition of topical/systemic steroids till symptoms subside. After resolution of symptoms, the dose administered was 50% of the last dose, and subsequent increments were done by 10% [19].

Antioxidant Supplementation: Antioxidant supplementation was done by administering one capsule twice a day for 4 weeks. Each capsule comprises of an antioxidant formulation containing vitamin A 5000 IU, Vitamin C 150 mg, Vitamin E 25 mg, Manganese 1.5 mg, Zinc 7.5 mg, Copper 1 mg, and Selenium 150 mcg.

Treatment Schedule: All the vitiligo patients were first subjected to NB-UVB phototherapy (as mentioned earlier) for 4 weeks, followed by blood sample collection. Further, all the same, patients were subjected to the oral antioxidant

supplementation (as mentioned earlier) along with the NB-UVB phototherapy for another 4 weeks, followed by blood sample collection.

Sample Collection: After obtaining the consent, 5 ml. blood was drawn from healthy 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 min 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 ºC till analysis.

Sample Analysis: RBC lysate was used to estimate antioxidant enzymes such as Catalase [20], Superoxide dismutase [21]; Glutathione peroxidizes [22] and plasma was used to estimate lipid peroxide [23] and total proteins [24]. 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 the intergroup difference.

RESULTS:

Characteristics of Controls: Recruitment of healthy subjects (control) and vitiligo patients (case) was done at the hospital of Era's Lucknow Medical College, Lucknow. Characteristics of controls and cases were thoroughly recorded. The age of controls and cases was in the range of 21-40 years. The youngest enrolled patient was 11 years, while the age 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. After proper diagnosis, we recorded 63.33% patients had vitiligo vulgaris, of which 11 had active disease whereas 8 had stable disease. Focal vitiligo was present in 2 patients with active disease and 5 patients with stable disease. Acrofacial type was observed in 4 patients with only 1 having active disease. When we analyzed for the duration of disease, we found that 33.3% of patients had the disease for less than 5 years, whereas 9 and 8 patients had the disease for 5-10 and 11-15 years respectively. Only 10% of patients had the disease for more than 15 years. We further evaluated the affected body surface area following Wallace rule of 9 in all patients and revealed that 70% of patients reported 0-10% affected body surface area. Only 1 patient had more than 40 % involvement whereas 3 patients of active vitiligo had 21-30% involvement. In our study, legs were the common affected site followed by face and arms. Genitalia were involved in 4 patients whereas oral mucosal involvement was seen in 3 patients with stable vitiligo.

Anti-Oxidant Status in Vitiligo Patients after 4 Week NB-UVB Phototherapy Alone: Following the NB-UVB phototherapy for 4 weeks, we observed significant ($p<0.05$) elevation in the CAT activity in stable vitiligo patients (44.11 ± 8.97 units/mg protein) as compared to its baseline value (41.25 ± 9.47 units/mg protein). All the results are explained in table no.1. However, in active vitiligo patients, the increase in the CAT activity was found to be statistically non-significant. Inactive vitiligo patients, there was a significant ($p<0.05$) increase in SOD activity (0.99 ± 0.148 units/mg protein) as compared to its baseline value (0.91 ± 0.14 units/mg protein), a similar significant increase was observed in stable vitiligo patients.

There was a non-significant change in GPx activity (25.57 ± 4.21 µg GSH utilized/min/mg protein) in active vitiligo patients as compared to its baseline value (24.99 ± 4.03 µg GSH utilized/min/mg protein), however, in stable vitiligo patients, the activity improved statistically significant ($p<0.05$). After 4 weeks of NB-UVB phototherapy, the lipid peroxide levels were found significantly ($p<0.05$) elevated in active and stable vitiligo patients (4.98 ± 0.46, 4.37 ± 0.34 nmole of MDA/ml plasma, respectively) as compared to their baseline values (4.56 ± 0.44, 3.84 ± 0.25 nmole of MDA/ml plasma, respectively).

Anti-Oxidant Status in Vitiligo Patients after 4 Week NB-UVB Phototherapy Along With Antioxidant Supplementation: After 4 weeks of both therapies, we observed a significant decrease in oxidative stress as evidenced by elevated antioxidant enzymes. All the results are explained in **Table 1**. Oral antioxidant supplementation has resulted in significant improvement in CAT, SOD and GPx activity as compared to the patients receiving phototherapy alone as well as baseline values. The improvement in CAT activity was recorded inactive and stable vitiligo group. Lipid peroxide was also measured and highly significant ($p<0.05$) increase was recorded in the patients receiving phototherapy alone. Lipid peroxidation was recorded decreased in the p[atient group receiving both therapies which indicates that the antioxidant supplementation has a positive effect in vitiligo patients. Antioxidant supplementation has tried to bring down the rate of lipid peroxidation as compared to patients receiving phototherapy alone.

TABLE 1: COMPARISON OF ANTI-OXIDANT STATUS IN VITILIGO CASES FOLLOWING NB-UVB PHOTOTHERAPY ALONE AND WITH ANTIOXIDANT SUPPLEMENTATION

Parameter	Control	Vitiligo patients		After 4 weeks NB-UVB therapy without antioxidant supplementation		After 4 weeks NB-UVB therapy with antioxidant supplementation	
		Active	Stable	Active	Stable	Active	Stable
Catalase (U/mg protein)	48.95 ±7.56	37.65 ±7.77*	41.25 ±9.47*	39.59 ±7.93	44.11 ±8.97**	44.05 ±7.22**	46.75 ±8.30 **
Superoxide dismutase (U/mg protein)	1.15 ±0.18	0.91 ±0.14*	1.00 ±0.17*	0.99 ±0.148**	1.05 ±0.16**	1.06 ±0.13**	1.13 ±0.17**
Glutathione peroxidase (µg GSH utilized/ min/mg protein)	38.51 ±9.62	24.99 ±4.03*	28.28 ±5.42*	25.57 ±4.21	29.73 ±5.70**	28.54 ±4.84**	31.95 ±5.75**
Lipid peroxides (MDA/ml plasma)	2.11 ±0.16	4.56 ±0.44*	3.84 ±0.25*	4.98 ±0.46**	4.37 ±0.34**	4.81 ±0.50**	4.22 ±0.40**

* significant at p value <0.05 as compared to control.
** significant at p value <0.05 as compared to baseline values.

DISCUSSION: Different researchers have shown the imbalance in an antioxidant enzyme in vitiligo patients [25, 26]. Similar results have been observed and explained the fact that due to H_2O_2 production inside the epidermis and systemically distributed in the blood of vitiligo patients [27]. Another study reveals the disturbance in CAT, SOD, GPx activity and LPO levels in vitiligo patients [26]. This imbalance in closely associated with overproduction of reactive oxygen species. One more study demonstrates the oxidant/antioxidant imbalance in vitiligo patients [28]. An age-dependent antioxidant status has been observed in vitiligo patients of different age groups, where the blood glutathione levels, GPx, and G6PD activity were decreased significantly [29]. Antioxidant

Supplementation therapy has been reported to improve the antioxidant imbalance in various diseases and experimental conditions. Selenium decreased the production of reactive oxygen species and inhibited the activation of NFκB-mediated transcription of pro-inflammatory mediators in diabetic cardiac hypertrophy [30].

Eight weeks of green tea supplementation showed a neuroprotective effect; it also reduced the cognitive deficits and was able to maintain the functional levels of antioxidant enzymes and glutathione [31]. In a double-blind, randomized placebo-controlled study in schizophrenia patients shows that supplementation with vitamin E may improve the antioxidative defense, especially glutathione system, while no major effect on symptoms severity was observed [32]. It has been claimed that vitamin A retards atherogenesis and Vitamin C decreases free radical-induced endothelial injury in cardiovascular disease patients [33]. Effect of antioxidant supplementation in an experimental model for aging and longevity has been extensively studied and have been found effective [34]. Our study also demonstrates the profound improvement in an antioxidant enzyme in vitiligo patients regarding CAT, SOD and GPx activity, while non-significant results in LPO levels, after the oral supplementation of antioxidants.

CONCLUSION: The results of the present study indicates 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. 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. Further, we also observed significant improvement in CAT, SOD and GPx activity following the oral antioxidant supplementation and NB-UVB phototherapy. However, to prove the supportive effect of antioxidants in vitiligo treatment, larger multicentric studies with large patient population and long duration treatment are needed.

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CONFLICT OF INTEREST: Nil

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