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Original Article

Co-supplementation of oral antioxidant to PUVASol therapy has no effect on oxidative stress index in patients of unstable vitiligo: A hospital based pilot study

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Abstract

Background In recent years, interest has grown in studying the role of oxidative stress in vitiligo, so measurement of the combined activities of all antioxidants or the Total antioxidant status (TAS) is often used to estimate the overall antioxidant status. Likewise, Total oxidant status (TOS) is measured to determine a patient's overall oxidation state. Furthermore, the oxidative stress index (OSI), which is calculated as the ratio of TOS to TAS, may be a more accurate index of oxidative stress in the body. This study was done to measure the effect of oral antioxidant supplementation therapy for 1 month on the oxidative stress index in patients of vitiligo.

Methods In this pilot study 40 consecutive patients presenting in departmental vitiligo clinic were enrolled and randomly allocated into 2 groups of 20 patients each were prescribed oral prednisolone at a dosage of 1mg/kg for 2 consecutive days/week along with oral 8-methoxypsoralen on 3 alternate days in a week at a dose of 0.6mg/kg body weight. Patients in group B received the following antioxidants orally along with treatment of group A. End point of the study was 1 month of continuous therapy in the respective groups. OSI was calculated before initiating treatment by serum collection.

Results The mean OSI decreased significantly in both the study groups individually (P = 0.037 & 0.040 for Group A and B respectively). However inter-group comparison between the 2 study groups showed no statistically significant difference (P = 0.052).

Conclusion Addition of antioxidants to PUVASol in treatment of unstable vitiligo has no effect on oxidative stress index.

Key words
Vitiligo, PUVASol, total oxidant status, total antioxidant status, oxidative stress index.

Introduction

Vitiligo is a common and often heritable, acquired pigmentation disorder in which melanocytes in the skin, mucous membranes and the retina are destroyed.[1] Upto 8.8 percent of the world population is believed to be afflicted with it.[2,3] It is one of the commonest dermatological problems in India.[2,3] It has a special significance to patients here, because depigmentation is obvious on dark skin.[4]

Although the precise etiology of vitiligo is not known, it has become quite clear that in recent times complex genetic, immunological, neural

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and self-destructive mechanisms interplay in the pathogenesis of vitiligo.[5,6] According to the auto cytotoxic hypothesis, oxidative stress has been suggested to be the initial pathogenic event in melanocyte degeneration with H_2O_2 accumulation in the epidermis of patients with active disease.[7]

In recent years, interest has grown in studying the role played by oxidative stress in vitiligo by investigating one or more of the antioxidant markers, including Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), vitamin E, vitamin C.[8-10] However, the measurement of different antioxidant molecules separately is impractical and has no clinical significance because the effects of antioxidants can be additive and measuring individual antioxidants separately is time consuming and labor intensive. Hence a measurement of the combined activities of all antioxidants or the Total Antioxidant Status (TAS) is often used to estimate the overall antioxidant status.[11] Likewise, Total Oxidant Status (TOS) is measured to determine a patient's overall oxidation state.[12] Furthermore, the Oxidative Stress index (OSI), which is calculated as the ratio of TOS to TAS, may be a more accurate index of oxidative stress in the body because it is a comprehensive measurement of TAS and TOS.[13]

Taken together, this study aimed to measure the effect of oral antioxidant supplementation therapy for 1 month on the Oxidative Stress Index in patients of vitiligo.

Methods

Study population

This randomized open clinical trial was registered in the Clinical Trial Registry-India (CTRI/2014/11/005223) and conducted in the Department of Dermatology and Department of Biochemistry at Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly (India). This study was approved by the Institutional Ethics Committee. The study duration was from April 2013 to April 2014. In this pilot study 40 consecutive patients presenting in departmental vitiligo clinic were screened and enrolled based on inclusion and exclusion criteria. Inclusion criteria for the study were patients of unstable vitiligo vulgaris; aged ≥18 years; literate; willing for investigation, treatment, and regular follow-up and had not been under any therapeutic regimen for the previous two months and had not received drugs containing any antioxidants. Patients with hepatic or renal impairments, photodermatoses, past or present history of any malignancy or immunobullous disorder, any chronic systemic disorder, patients who took treatment irregularly, chronic alcoholics and/or smokers and pregnant or lactating females were excluded from the study. A washout period of two months was given for topical and systemic therapies, respectively, before including the patients in study.

Written informed consent from all the subjects was taken before recruitment in this study. History, Examination, Vitiligo Disease Activity Score (VIDA)[14], vitiligo European task force (VETF) for disease activity[15], point counting method of area estimation16 and other relevant investigations were recorded in a specially designed proforma.

These 40 patients were randomly allocated into 2 groups of 20 patients each, viz group A and B. Patients in group A were prescribed oral prednisolone at a dosage of 1mg/kg for two consecutive days/week along with oral 8-methoxypsoralen on three alternate days in a week at a dose of 0.6mg/kg body weight. The psoralen was administered orally in the morning with breakfast followed by sunlight exposure

after an interval of 2 hours preferably between 10am to 3pm. The sunlight exposure was for 5 minutes initially, and then exposure time was increased by 5 minutes to a maximum of 30 minutes at every alternate. Topical calcineurin inhibitor, tacrolimus 0.1% was applied topically at night for lesion on the face and neck. Patients in group B received the following antioxidants orally along with treatment of group A: Vitamin C 300mg, Vitamin E: 400 IU, Beta Carotene: 30mg, Zinc oxide: 40mg, Sodium selenate: 200µg, Cupric oxide: 2mg, Manganese sulphate: 5mg. End point of the study was 1 month of continuous therapy in the respective groups. OSI was calculated in serum samples before initiating the treatment.

Sample collection

Five ml blood was drawn from median cubital vein of the patients into plane tubes. To separate the serum from the plasma sample, it was centrifuged at 5000 × g for 5 min at room temperature. All serum samples were stored at -20°C until time of processing.

Evaluation of total oxidant status (TOS) in all the study subjects

Oxidant present in the sample oxidizes the ferrous ion-o-dianisidine complex to ferric ions. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ions make a colored complex with Xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecule present in the sample. The assay is calibrated with hydrogen peroxide and results are expressed in terms of micro molar hydrogen peroxide equivalent per litre.[12]

Estimation of total antioxidant status (TAS)

This was measured with help of Quantichrom™ Antioxidant Assay Kit, CAT# DTAC – 100, Lot: BD06A17 from BioAssay Systems. 1180 East Ellsworth Road Ann Arbor, Michigan 48108· USA. (www.Bioassaysys.com)

Oxidative stress index (OSI)

It was calculated from a percent ratio of total peroxide level to the TAS level.[13]

OSI=[TOS (µmol H2O2 equivalent /L) / TAS (µmolTrolox equivalent /L) X100.

Clinical response to treatment was assessed by VIDA score, VETF and point-counting method of area estimation.

Statistical analysis

SPSS version 20.0 was used for all statistical analysis. $P<0.05$ was taken as significant in all cases. t-test was done to determine the statistical significance among two groups of data. All data was represented as mean ±standard deviation.

Results

Both the study groups were comparable at baseline as per **Table 1**. In the present study majority of the patients presented from 21 to 40 years of age. There was no predilection towards sex and religion. More than half of them were married, having no family history of the disease, intermediate education qualification and were either students or involved in some skilled work. In most number of them, the onset of disease was at the age of 19 to 23 years and they were suffering from it for less than or equal to 10 years. According to VIDA scoring, majority had active disease for at least 6 months. Vitiligo was classified as mixed and generalized in majority and almost half of them had leucotrichia. All the patients had past treatment

Table 1 Baseline profile of study groups

Variables	*Group A*	*Group B*	*P value*
Age			
Range	18-50	18-70	
Mean±SD	29.40±8.28	32.0±13.65	0.471
Gender (Male/Female)	12/8	9/11	0.342
Education			
Illiterate/Primary	0	0	
Middle school	2	5	
High school	6	5	0.548
Intermediate	9	6	
Graduate	3	4	
Occupation			
Housewife	4	5	
Student	6	5	
Farmer	1	3	
Skilled	6	4	
Semi-skilled	3	3	
Marital status			
Married/Unmarried	13/7	14/7	1.000
Duration of disease (yrs)			
Mean±SD	9.1±3.91	7.4±4.74	0.309
Age of onset (yrs)			
Mean±SD	19.4±10.72	23.95±15.46	0.175
Family history (Present/Absent)	4/16	4/16	1.000
Past treatment history (Present/Absent)	17/3	19/1	0.605
Body surface area involved (%)			
≤3	9	7	0.748
>3	11	13	
Type of vitiligo			
Focal	6	3	
Mucosal	2	3	0.414
Acrofacial	0	1	
Segmental	0	0	
Mixed	10	7	
Generalised	2	5	
Universal	0	1	
Leukotrichia present	8	8	1.000
VIDA score (Disease activity)			
6 weeks	1	3	
3 months	4	3	0.267
6 months	4	8	
1 year	11	6	
Point counting method			
Baseline Pre treatment	16.21±14.23	16.86±21.44	0.351
After treatment	15.64±14.83	15.74±22.27	

history and majority of them had tried both topical and systemic treatment.

After four weeks of treatment the point counting method of area estimation showed a decrease in the mean size of the lesion from 16.21 cm^2 to 15.64 cm^2 in group A and 16.86 cm^2 to 15.74 cm2 in group B but the decrease was statistically not significant (P = 0.351) **(Table 1)**.

VETF scoring system **(Figure 1)** showed no significant changes in the lesions/ body surface area (BSA) and progression of the disease

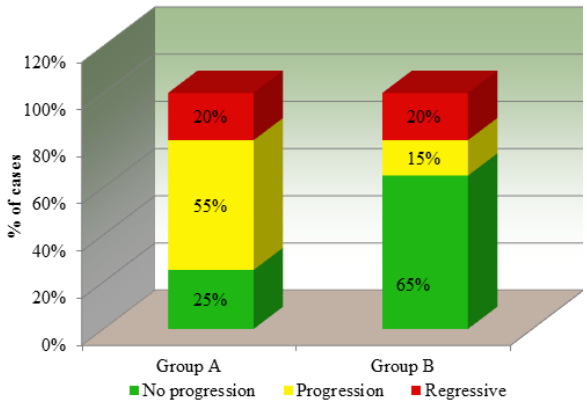

Figure 1 Comparison of VFTF System to assess spreading after treatment between group A and group B

among cases and controls after four weeks of respective therapies (P = 0.017).

As per **Table 2 & 3**, after four weeks of treatment, mean TOS decreased insignificantly in both the study groups (P = 0.263 & 0.398 for Group A and B respectively). However this difference between the 2 study groups was not statistically significant (P = 0.076). Mean TAS also showed a statistically insignificant drop in group A & B from baseline (P = 0.911 & 0.575 respectively).

Noteworthy, the mean oxidative stress index (OSI) decreased significantly in both the study groups individually (P = 0.037 & 0.040 for Group A and B respectively). However inter-group comparison between the 2 study groups showed no statistically significant difference (P = 0.052).

None of the patients observed any side effects warranting withdrawal of any patient in either of the groups during study duration.

Discussion

The balance between oxidation and antioxidation is believed to be critical in maintaining healthy biological systems. Under physiological conditions, the human antioxidative defense system including, e.g.- SOD, CAT, GPx, glutathione (GSH) and others, allow the elimination of excess ROS such as superoxide anions ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$), alkoxyl radicals ($RO^{\cdot}$) and peroxyradicals ($ROO^{\cdot}$).[8] However, our endogenous antioxidant defense systems is incomplete without exogenous originating reducing compounds such as vitamin C, vitamin E carotenoids and

Table 2 Baseline stress indices in study groups

	Group A (n=20)		*Group B (n=20)*		*P Value*
	Mean ± SD	*Min - Max*	*Mean ± SD*	*Min - Max*	
Total oxidative stress before treatment	91.85± 47.50	13.50- 198.20	107.13± 38.22	48.20- 180.30	0.250
Total antioxidant stress before treatment	528.82± 340.24	237 - 1692	525.40± 226.81	258 - 1078	0.552
Oxidative stress index before treatment	19.34± 9.15	4.80 - 36.32	22.46 ± 8.81	9.65 - 40.70	0.267

Table 3 Stress indices in study groups after treatment

	Group A		*Group B*		*P Value*
	Mean± SD	*Min - Max*	*Mean ± SD*	*Min - Max*	
Total Oxidative stress after treatment	75.85± 63.97	13.50- 282.20	95.79± 43.79	29.10- 201.60	0.076
Total antioxidant stress after treatment	510.18± 384.56	220 - 1910	513.46± 209.68	313 - 1068	0.974
Oxidative stress index after treatment	15.68± 10.36	4 - 52	19.18 ± 6.73	8 - 33	0.052

polyphenols that play an essential role in many antioxidant mechanisms in living organisms. Therefore, there is continuous demand for exogenous antioxidants in order to prevent oxidative stress, representing a disequilibrium redox state in favor of oxidation.[8]

Increased oxidative stress is observed in the active vitiligo patient.[17] According to autocytotoxic hypothesis in active disease, oxidative stress is the initial pathogenic event in melanocyte degeneration with H2O2 accumulation in the epidermis of patients with active disease.[18]

The oxidative stress can either be from increased generation of free radicals or decreased destruction of these. It is not a localized phenomenon but a more generalized process. This may be one of the explanations for developing newer lesions in vitiligo patients in the course of the disease.[19]

In recent years, interest has grown in studying the role played by oxidative stress in vitiligo by investigating one or more of the antioxidant markers, including SOD, CAT, GPx, vitamin E, vitamin C.[8-10] However there are conflicting evidence for the same. Some researchers report increased total antioxidant levels, others report no change or even decreased levels of these markers like SOD, GPx, malondialdehyde (MDA), Nitric Oxide (NO), and CAT.[9,10,20,21] But these studies didn't measure the TOS and TAS. Because the effects of antioxidants and oxidants could be additive and measuring individual oxidants and antioxidants separately is time consuming and labor intensive, a measurement of the combined activities of all oxidants and antioxidants is often used to estimate the overall antioxidant status.[22] In the present study after 4 weeks of treatment a non significant total oxidative stress and total antioxidant stress decreased was observed in both the groups. Noteworthy the difference between the two was statistically not significant even after addition of exogenous antioxidants to the study patients in group B.

Furthermore, the OSI, which is calculated as the ratio of TOS to TAS, is a more accurate index of oxidative stress in the body because it is a comprehensive measurement of TAS and TOS.[22]

Significant decrease in the OSI was observed within group A & B individually. But when this decrease was compared between the two study groups this came out to be statistically not significant. This shows that addition of antioxidants had no added advantage. These results are similar to the finding observed by *Jayanth et al.* who observed no distinct advantage of adding antioxidants to the standard regimen of photochemotherapy for treating patients of vitiligo.[23]

The VETF proposed a system that combines analysis of extent, stage of disease (staging), and disease progression (spreading).[15] In the present study majority of the patients had complete depigmentation and all of them were spreading. After 4 weeks of treatment the BSA and the type of lesion remained unchanged however addition of antioxidant had no effect on spreading of active vitiligo.

In conclusion, the mean Oxidative Stress Index of all the patients in the study before and after treatment was 41.79 & 34.86 (arbitrary unit) respectively. Addition of antioxidants (vitamin E, vitamin C, beta carotene, zinc, selenium, manganese and copper) to PUVASol in treatment of unstable vitiligo had no effect on OSI. Further large scale and long term studies are warranted to substantiate these results and study the clinical effect of adding the antioxidants to the standard regime for treating a patient of vitiligo.

Limitations of the study

The present study should be performed with more patients and treatment needs to be continued for a longer duration along with a longer follow-up.

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