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"OXIDATIVE STRESS AND ROLE OF ANTIOXIDANTS IN ACNE: A NON RANDOMIZED CASE CONTROL STUDY."

Dermatology

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ABSTRACT

Introduction: As acne is associated with oxidative stresses and the role of antioxidants is of much importance in treatment of acne as antioxidant defense systems, such as super oxide dismutase (SOD) and glutathione (GSH) keep ROS production in check, thereby maintaining an appropriate cellular redox balance thus this study is aimed at assessing the role of antioxidants in acne which shows significant improvement in acne lesions with oral anti oxidant supplementation.

Objective:

- To assess the oxidative stress in acne patients.
- To compare the results of anti-acne therapy versus anti-acne therapy with anti-oxidants.

Material & Methods: This non-randomized, case-control study was conducted over 100 acne patients between 16 to 25 years and divided into 2 groups. Assessment of oxidative stress was done using Fridovich and malonyl dialdehyde by Ohkawa method. Group A patients received anti-acne therapy along with anti-oxidant while group B received only anti-acne therapy, assessment using clinical photographs, measurement of SOD level, MDA level, global acne grading scale and reduction in number of lesion was done on 1st follow-up(1 month) and at the end of study i.e. 2nd month.

Results: Oral antioxidants reduce oxidative stress by increasing SOD level and by decreasing MDA level. Beneficial clinical and biochemical effects are seen, also subjects taking oral antioxidants showed significant reduction in clinical parameters such as mean GAG score, number of lesions and pigmentation as compared to other group. **Conclusion:** Oral supplementation in the form of antioxidants improves clinical outcome of anti acne therapy.

KEYWORDS

INTRODUCTION:

Acne vulgaris is a common dermatological condition characterized by hormonally-mediated sebum overproduction, follicular hyperkeratinization and chronic inflammation of the pilo-sebaceous unit [1]. Excessive generation of reactive oxygen species (ROS) has been known to initiate damage to proteins, lipids and nucleic acids [2] [3]. Antioxidant defense systems, such as super oxide dismutase (SOD) and glutathione (GSH) keep ROS production in check, thereby maintaining an appropriate cellular redox balance. Alterations in this redox balance resulting from elevated ROS levels and/or decreased antioxidant levels can lead to oxidative stress [4]. Lipid peroxidation, a process of oxidative degeneration of polyunsaturated fatty acids set into motion by ROS, leads to formation of highly reactive aldehydes, such as malondialdehyde (MDA) that can bind covalently to proteins and thus causes structural protein modifications and affects biologic function [5]. Free radical mediated damage has been implicated in the pathogenesis of acne vulgaris [6, 7, 8]. Overproduction of reactive oxygen and nitrogen species (RONS) leads to a variety of RONS-mediated modifications of the endogenous proteins, such as increased formation of protein oxidative biomarker carbonyl contents, lipid peroxidative byproducts MDA, and nitrosative biomarker NO, which thus leads to oxidative and nitrosative stress in acne patients. Reactive oxygen species, a marker of oxidative stress is involved in the irritation and destruction of the follicular wall and causes inflammatory progression of acne. In comedones, the proportion of linoleic acid to other lipids is markedly decreased. Linoleic acid was found to have inhibitory effects on the action of several types of Reactive Oxygen Species (ROS) released by neutrophils (superoxide radical, hydrogen peroxide and hydroxyl radical).

As a consequence, in comedonal lesions, ROS become excessive due to a lack of scavengers. Among skin superficial lipids, squalene which is specific to human sebum appears to act as a quencher of singlet oxygen in vitro protecting skin surface from lipid peroxidation [9,10,11,12]. However, its oxidation generates squalene peroxides, which exert comedogenic effects [13]. Antioxidants, targeting decrease of these free radicals are thus thought to reduce the disease process and thus helps fight acne lesion. Few studies are available regards to the role of antioxidants in acne. However, to the best of your knowledge no study has yet been done to assess the utility of antioxidants as oral supplementation in anti acne therapy. We wanted to perform a head on comparison of antioxidants versus conventional acne therapy. However there are no study to justify the effect of antioxidants in acne .Thus on ethical ground we had compared the role of addition of antioxidants to conventional anti acne therapy .Also studying the addition of antioxidants to conventional anti acne therapy is more close to current real world treatment regimen.

RATIONALE OF STUDY:

We don't know the level of redox markers in mild to moderate acne. Neither we know whether giving antioxidants will give a profit or not in the treatment of acne along with conventional anti-acne therapy. To know this we are doing this study to assess the role of antioxidants in decreasing redox markers at the same time assess its beneficial role in clinical profile of patients.

AIM & OBJECTIVE:

AIM:

To assess the role of antioxidants as adjuvant to anti- acne therapy

1. To assess clinical improvement in acne control.
2. To assess changes in biochemical markers in acne control.
3. To assess clinical improvement in acne cases.
4. To assess in biochemical markers in acne cases.

MATERIAL AND METHODS:

This study was conducted over a period of one year at Department of Dermatology, Venereology & Leprosy in a tertiary health care centre of central India, as a single centre, non-randomized, and case-control study. Permission from Institutional Ethics Committee was obtained. 50 mild to moderate acne patients between 16 to 25 years of age, meeting the inclusion and exclusion criteria of study were recruited in each study arm and written informed consent was obtained.

Recruited patients were divided in group A and group B by non random allocation. Patients in both groups were matched by age, gender and grade (according to global assessment scale) of acne. Equal numbers of patients were allocated to both the case and control arm. All patients in study underwent a detailed history taking including general, physical, dermatological examination. Group A consisting of 50 patient were given normal conventional anti acne therapy which mostly included topical such as clindamycin 1% gel Adapalene gel and Benzoyl peroxide 2.5 % gel .Systemically doxycycline and

isotretinoin around .25 mg per kg was given alone for 2 month while group B patients were given conventional anti acne therapy along with antioxidant supplement containing Omega fatty acid , copper ,zinc ,selenium, beta carotene ,cryptoxanthin,vitamin C and E were given once per day. Baseline blood investigations (complete blood count, liver function test, renal function test, lipid profile) along with investigations for assessing oxidative stress and the effect of antioxidants on oxidative stress in both case and control group was checked by assessing the level of superoxide dismutase by Fridovich and malonyl dialdehyde by Ohkawa method respectively. All the investigations were done on both the cases and controls at the start of study, at 1st follow up and at the end of the study (within 2 months). Photographs were taken at first visit, then at 4 and 8 weeks respectively. Disease activity was monitored during the study period on the parameters of appearance of new lesions, changes in lesion morphology, repigmentation and according to global assessment scale. Record analysis was done at the end of study period. All adverse effects were recorded.

Enzyme-linked Immunosorbent Assay for Quantitative Detection of SOD & MDA

1. Intended Use:

The Human Superoxide Dismutase (SOD) ELISA Kit is to be used for the vitro quantitative determination of Human SOD in Serum, Plasma and other biological fluids.

2. Principle Of The Assay

The kit measures human SOD levels in the samples like serum and plasma. It uses purified human SOD antibody to coat micro titer plate wells which makes a solid-phase antibody, then samples (Containing Human SOD) from cases and controls were added to wells which leads to antigen-antibody complex formation, to which HRP conjugate reagent was added leading to antibody - antigen – enzyme - antibody complex formation, add TMB substrate solution, on addition of TMB substrate the color of sample becomes blue due to HRP enzyme mediated catalysis of reaction. Reaction is terminated by the addition of a sulphuric acid solution and the color of sample changes to yellow which is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Human SOD in the samples is then determined by comparing the O.D. of the samples to the standard curve.

CALCULATION:

Take the Standard density as the horizontal axis and the OD value for vertical axis, obtain the Standard Curve, then find out the corresponding density according to the sample OD value, and then it is multiplied by the dilution multiple. Or calculate the straight line regression equation of the standard curve with the standard density and the OD value, with the sample OD value in the equation, calculate the sample density, multiplied by the dilution factor, the result is the sample actual density.

Statistical Analysis

All subjects who received conventional anti acne therapy as well as antioxidants and completed the study period were included for analysis The data collected was entered in MS excel sheet and thereby analyzed using the software SPSS version 20.0 . For the continuous variables, mean and standard deviation were calculated the tests applied were as follows:

1. Unpaired student t –test –For inter group comparison of group a (taking conventional plus antioxidants) and group b (taking only conventional anti acne therapy)
2. Repeated measure Annova
3. Chi Square Test (Qualitative test)

OBSERVATION AND RESULTS:

Table 1: Baseline characteristics of study population

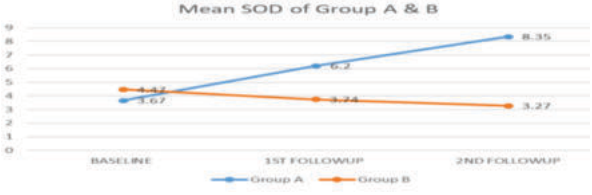
	Group A	Group B	Total	P-value
Male	28	28	56	1
Female	22	22	44	
Age range	18-25	18-25		
Mean age (+SD)	23.2	23.6	23.1	0.64
Mean Weight	58.1	57.5	57.3	0.070
Mean Global assessment scale at onset	2.5	2.3	2.36	0.23
Mean number lesions at onset	14.6	14.18	14.07	0.28

Both groups were matched according to age, gender, weight and Global Assessment Scale score at start of study.
Statistically significant P values were not found.

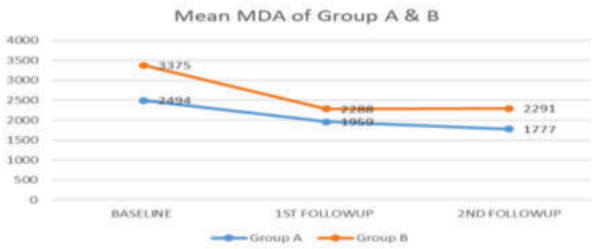
Table 2: Comparison of mean SOD, MDA level and clinical parameter including GAG score and number of lesion with duration of treatment in cases and control

	GROUP A(CASES)	GROUP B(CONTROL)	P-VALUE
SOD LEVELS			
BASELINE (MEAN ± SD)	3.6722 ± 2.49	4.4752 ± 2.3	0.10
1ST FOLLOWUP (MEAN ± SD)	6.2040±3.2	3.7460±2.6	0.00
2ND FOLLOWUP (MEAN ± SD)	8.3500±3.9	3.2735±2.8	0.00
MDA LEVEL			
BASELINE (MEAN ± SD)	2494.5400±774	3375.2800±532	0.25
1ST FOLLOWUP (MEAN± SD)	1959.9000±566	2388.5000±590	0.00
2ND FOLLOWUP (MEAN ± SD)	1777.6800±1243	2291.8400±605	0.10
GAG SCORE			
BASELINE (MEAN+SD)	2.4800±.50	2.3400±.65	0.23
1ST FOLLOWUP (MEAN ± SD)	.7800±.50	2.0800±.75	0.00
2ND FOLLOWUP (MEAN ± SD)	.2000±.40	1.2200±.61	0.00
NUMBER OF LESION			
BASELINE (MEAN ± SD)	14.8000±2.9	14.1800±2.7	0.28
1ST FOLLOWUP (MEAN ± SD)	7.0600±2.0	10.2200±2.6	0.00
2ND FOLLOWUP (MEAN ± SD)	2.4000±1.3	7.0000±2.6	0.00

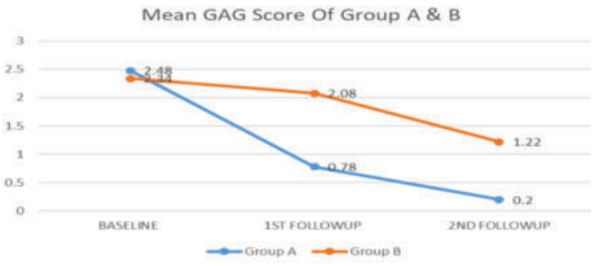
In our study there were 28 male and 22 females in both the groups, mean age was 23.2 and 23.6 years, mean weight was 58.1 & 57.5, mean GAG at onset was 2.5 & 2.3, mean no of lesion at onset was 14.6 & 14.18 for group A and group B respectively. Statistically significant P values were not found that is both groups were matched according to age, gender, weight and Global Assessment Scale score at start of study. Mean SOD level of group a and group b at baseline were in nearly normal range (0.42 to 10 ng/ml) but with duration of treatment mean SOD of subjects of group A (cases) showed significant increase (pvalue-0.00) compared to group B (controls), which rather showed decreasing trend with duration of treatment [Figure A]. Mean MDA level of both groups at baseline were higher than normal range (24.5ng/ml to 2000) but with duration of treatment there occurs a significant reduction in mean MDA level in both the groups but the reduction was much more in group A (p value- 0.00) [Figure B]. Mean GAG score at baseline in both groups were almost equal (p value-0.23) but during treatment period there was reduction in GAG score in both the groups but more significant reduction was seen in subjects taking antioxidants [Figure C]. Similarly clinical parameter including no. of lesions of both groups at baseline were almost equal (p value at baseline 0.28), with duration of treatment there occurs a decrease in number of lesions in both groups but the reduction in number of lesion was significantly more in group A taking antioxidants [Figure D]. Other clinical parameter such as pigment reduction was found more with group A subjects taking antioxidants as adjuvant [Figure E] as compared to group B [Figure F].



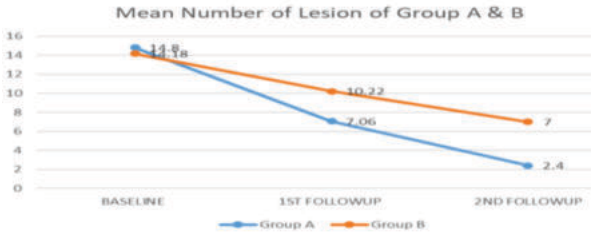
A)Comparative assessment of mean SOD levels in group A & B with time



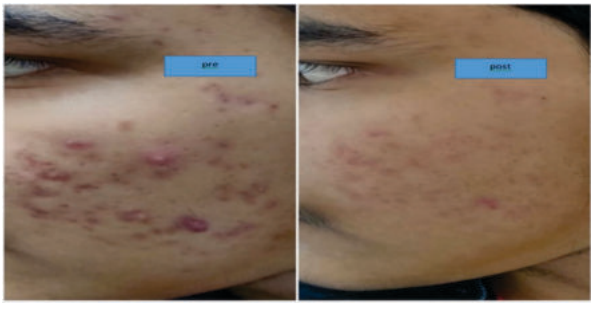
B) Comparative assessment of mean MDA levels in group A & B with time



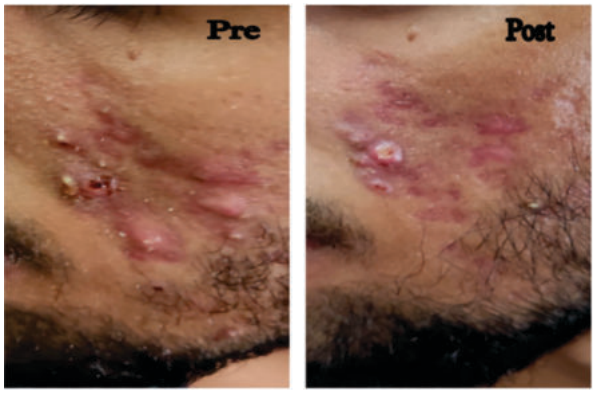
C) Comparative assessment of mean GAG score of group A & B with time



D) Comparative assessment of mean number of lesions of both the group with time



E) Pre & Post Treatment Photograph Of Group A



F) Pre & Post Treatment Photograph Of Group B

DISCUSSION:

In this study, we found that oral antioxidants given as adjuvant to conventional anti acne therapy reduce oxidative stress by increasing SOD level and by decreasing MDA level. Beneficial clinical and biochemical effects of anti-oxidants are seen when added to conventional anti acne therapy. We also found that subjects taking oral antioxidants showed much significant and faster reduction in clinical parameters such as mean GAG score, number of lesions and post inflammatory hyperpigmentation as compared to other group. The generation of reactive oxygen species by neutrophils which causes tissue injury is proposed to play a role in pathogenesis of acne. MDA, an end product of lipid peroxidation induced by ROS is considered as marker of lipid peroxidation, which is found to be increased in acne subjects. SOD, a major enzyme and first line of defense against oxygen free radical catalyses conversion of oxygen free radicals to H2O2, thereby maintaining proper redox balance. So decreased SOD level can lead to oxidative stress. In our study at baseline mean SOD level was reduced in both groups. Also MDA level was more than normal range in both groups. In subjects taking oral antioxidants there was a significant increase in mean SOD level and decrease in mean MDA level compared to subjects taking only conventional anti acne therapy. Clinical parameters were significantly improved in subjects taking anti-oxidants. In this study we measured the treatment response by measuring clinical improvement along with measurement of biochemical markers of oxidative stress .Two test were taken in form of SOD(marker of antioxidant system) and MDA (marker oxidative stress)which gave better strength to our study. No such study have been performed to assess the utility of antioxidants in conventional anti acne therapy. Although various studies are available to assess oxidative stress in acne subjects by measuring SOD and MDA level but none assess the effect of anti oxidants on outcome if treatment. Study on Biochemical markers of oxidative and nitrosative stress in acne vulgaris by AL-Shobaili et al [14] showed significantly higher MDA level and lower level of SOD and GSH in acne patients compared to normal healthy population. Study by Mayumi Nomoto et al [15] showed the relationship between Antioxidative effect of Jumihaidokuto (Japanese medicine) and acne improvement .This study showed reduction in inflammatory and non inflammatory lesions with reduction of d-ROMs (diacron reactive oxygen metabolites) value, a measure of antioxidant potency. In this study no control group (without antioxidant) was taken. Subjects having acne were divided in two groups based on high d-ROMs and normal d-ROMs and were evaluated before and after JHT (Japanese herbal medicine) administration. All grades of acne were included in this study. Study by OTTO H. MILLS et al [16] addressing free radical oxidation in acne vulgaris showed that application of topical vitamin E in sunflower seed oil along with conventional topical anti acne therapy significantly reduces number of lesion and GAG score .No biochemical markers were assessed in this study. No control group was there all subjects were given topical vitamin E. Arican O et al [17] in their research paper "Oxidative stress in patients with acne vulgaris" Aimed to determine the effects of oxidative stress in acne vulgaris .Their findings showed that oxidative stress exists in the acne patients therefore, it will be useful to apply at least one antioxidant featured drug along with the combined acne treatment. In Early therapy antioxidants have beneficial effect as adjuvant to conventional anti acne therapy. So we might have to increase the cost of therapy for better and faster results. Oral antioxidants decrease the duration of disease thus helping decrease the duration of intake of conventional anti acne therapy thus helping combat drug resistance.

The oxidative modification of proteins and lipids has been implicated in etiology of acne.

CONCLUSION:

This study biochemically proves the oxidative stress seen in acne vulgaris and also shows the beneficial effects of oral anti-oxidants clinically and biochemically.

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