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Original Article

Effect of Antioxidant Co-supplementation on Oxidative Stress in Leprosy: A Randomized Open Labelled Hospital Based Study

I Raka[1], P Gahalaut[2], N Mishra[3], M K Rastogi[4], AP Varshney[5], N Agarwal[6]

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Biochemical damage produced by reactive oxygen species (ROS) has emerged as an important mechanism influencing the pathogenesis of various diseases including leprosy. Oxidative stress increases in leprosy and studies have advocated use of antioxidants in leprosy treatment. This study examines the effect of co-administration of exogenous antioxidants on various oxidative stress parameters in patients having leprosy. This is an open labelled prospective randomized hospital based study to evaluate the effect of antioxidant supplementation on various oxidative stress parameters in patients having leprosy. Total 65 patients of leprosy were randomized to 2 groups. Group A (intervention group) was given exogenous antioxidants along with MDT whereas Group B (controls) received only MDT. Various oxidative parameters were recorded at baseline and at an end point of 3 months' time period. 40 patients completed the study, 20 in each group. There were statistically significant differences between the two study groups for the total oxidant status and oxidative stress index at the study end point of 3 months. A statistically significant decrease in serum Total oxidant status (TOS) and oxidative stress index(OSI) was seen when non-enzymatic antioxidants were co-administered for 3 months along with standard MDT in patients of leprosy.

Key words : Oxidative stress, Leprosy, Hansen Disease, Redox Balance, Anti-oxidant, Co-supplementation

Introduction

Leprosy (Hansen's disease) is a chronic, granulomatous infective disease that is associated with hypopigmented and/or hypoanesthetic skin lesions and thickened peripheral nerves. The global registered prevalence rate of leprosy was 0.25 per 10000 populations at the end of 2017. India is still the largest home for leprosy in terms of absolute number of cases, prevalence and the incidence (WHO 2018).

Biochemical damage produced by reactive oxygen species (ROS), especially oxygen free radicals has emerged as an important mechanism influencing the pathogenesis of leprosy (Abdel

[1] I Raka, MD, Senior Resident
[2] P Gahalaut, MD, Professor
[3] N Mishra, MD, Professor
[4] M K Rastogi, MD, Associate Professor
[5] A P Varshney, MD, Senior Resident
[6] N Agarwal, MD, Senior Resident
Department of Dermatology, Shri Ram Murti Smarak Institute of Medical Sciences (SRMSIMS), Bareilly, Uttar Pradesh, India
Corresponding author : Dr Pratik Gahalaut, ORCID: 0000-0002-1566-6871; **Email:** drpratikg.srmsims@gmail.com

Hafez et al 2010, Marolia & Mahadevan1989).

The 'antioxidant defence system', comprises various enzymatic and non-enzymatic antioxidants which protects the cells and biomolecules from the oxidative stress (Cotgreave et al 1988). Biologically natural antioxidants include enzyme systems such as superoxide dismutase, catalase, and the glutathione system; non-enzymatic macromolecules like ceruloplasmin, albumin and transferrin; and an array of small molecules including glutathione, bilirubin, uric acid and Vitamin E, A and C (Baumann & Alemann 2009).

Past studies have reported increased oxidative stress in various types of leprosy and advocated use of antioxidants in leprosy treatment (Swathi & Tagore 2015, Raka et al 2018). However, no data is available for effect of antioxidant therapy per se on oxidative stress in leprosy. The present study aims to examine the effect of co-administration of exogenous antioxidants to standard anti-leprosy multidrug therapy (MDT) by measuring the various oxidative stress parameters.

Patients and Methods

This randomized open labelled hospital based clinical pilot study was conducted in the Dermatology Department of Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly in northern India, from December 2014 to July 2016 and registered retrospectively in the Clinical Trials Registry – India. This study was approved by Institutional Ethics Committee before starting the recruitment of participants. New clinically diagnosed or biopsy proven cases of leprosy presenting to the leprosy clinic of the Dermatology Outpatient department were screened and enrolled in the study after taking an informed written consent. The patients were categorized according to the WHO (World Health Organization) classification based on clinical and bacteriological criteria (WHO 2018).

Inclusion criterion were age $\geq$ 18 years; not on any antioxidants during last 3 months; willing for investigation, treatment and regular follow-up visits. Patients with hepatic or renal impairments, having leprosy reactions at the time of presentation or developing them during the course of study, past or present history of any malignancy or immune-bullous disorder, any chronic systemic disorder, patients concurrently taking any antioxidant or giving past history of hypersensitivity to any antioxidants, patients who took treatment irregularly and female patients who were pregnant or lactating were excluded from the study. Chronic alcoholics, smokers and hypertensives were also excluded from the study.

As per the study design (Fig. 1), 151 newly diagnosed patients of leprosy presented in Dermatology out-patient department from December 2014 to July 2016. 90 patients were considered fit for inclusion in this study after initial screening according to the inclusion and exclusion criterion given above. 65 patients gave the consent and were enrolled in this study with an intention to treat. These patients were randomly allocated to group A & B as per study design by computer generated randomization freely available from www.randomizer.org. Patients in Group B were given standard MDT (Multidrug therapy) as per WHO regimen, i.e multibacillary patients were treated with MB-MDT and paucibacillary patients were treated with PB_MDT. In group A patients were co-prescribed following antioxidants daily along with the standard WHO MDT regimen of group A: Vitamin E 268mg, vitamin C 150mg, zinc sulphate 27.5mg, selenium 200µg, manganese 2mg, beta carotene 30mg and copper 1 mg in form of commercial capsules by brand names CUTE-E (Talent India Ltd, Ahmedabad, India) and ANTOXID-HC (Dr Reddy's Pharma, Hyderabad, India). These patients were followed up for the

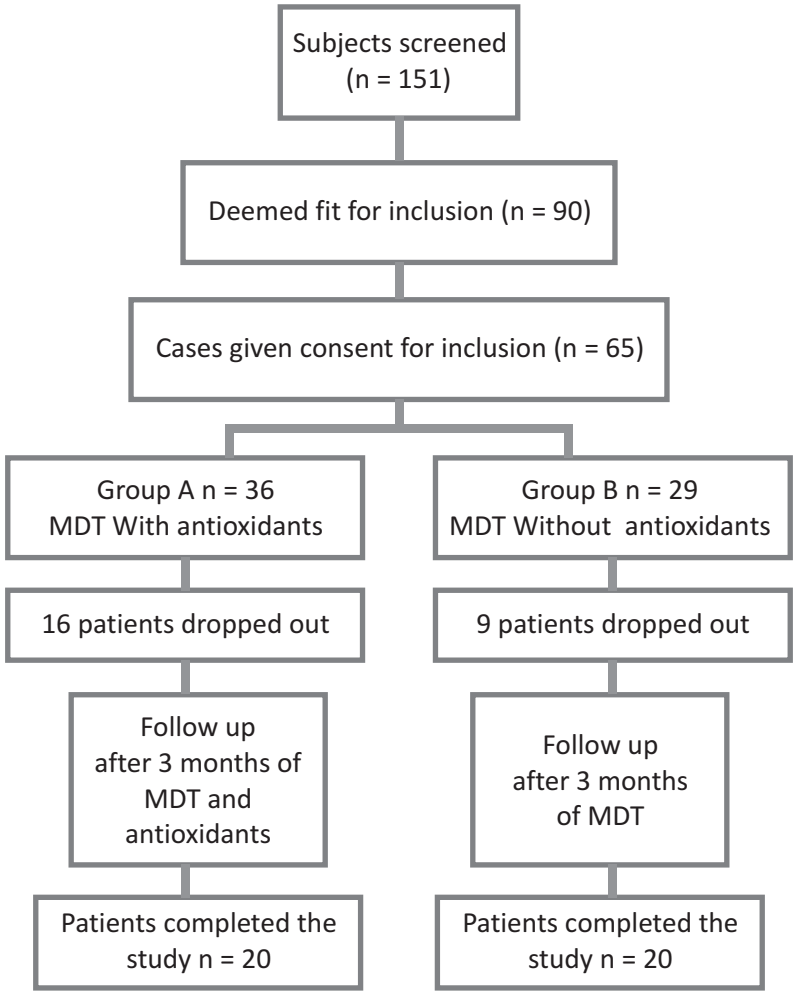

Fig. 1 : Study design of clinical trial in leprosy cases with and without co-supplementation with anti-oxidants

total study period of 3 months. All together 25 patients dropped out from study. Among these 16 patients were from group A and 9 from group B. In group A, 2/16 patients left the treatment due to cost of treatment, 4/16 due to recurrent reactions, 7/16 due to no clinical improvement and 3/16 due to logistical reasons. In group B 2/9 patients left the treatment due to no clinical improvement, 3/9 due to recurrent reactions and 4/9 due to logistical reasons. Final analysis (response to treatment) was done on 40 patients i.e 20 in each group A & B who completed the whole study.

For the blood samples, five ml blood was drawn from median cubital vein of the patients into plane tubes at baseline and after completing 3 months of study time period. 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 -30°C until time of processing.

Total oxidant status (TOS) of plasma was measured using colorimetric measurement method for TOS developed by Erel (2005) In this method oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity was measured with the help of a spectrophotometer (Analytic Technologies Limited, Vadodara, Gujarat, India; batch no: LO-1/01)and it is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H2O2 Eq/l). The test parameters were as follows: end-point measurement, serum 35 µl, R_1 225 µl, R_2 11 µl, primary wavelength 560 nm and secondary wavelength 800 nm.

Total antioxidant status (TAS) was measured with the help of Caymans antioxidant assay kit, (Item number 709001 from Caymen chemical company, 1180 East Ellsworth Road, Ann Arbor, Michigan 48108, USA). Lipid peroxidation was measured by the quantification of MDA (Malondialdehyde) in blood samples of patients using the thiobarbituric acid-reactive substances (TBARS) assay (Devasagayam et al 2003). Caymans superoxide dismutase (SOD) assay kit, (item number 706002 from Caymen chemical company, 1180 East Ellsworth Road, Ann Arbor, Michigan 48108, USA) was used for estimating SOD levels in the sample. The NO (nitric oxide) was evaluated as per Griess method (Granger et al 1999).

The oxidative stress index (OSI) was calculated as a percentage ratio of TOS to the TAS as explained elsewhere (Bakir et al 2012).

OSI= TOS (µmol H2O2 equivalent/L) / TAS (µmol Trolox equivalent/L) X100

Statistical analysis : Statistical analysis was done using SPSS version 20.00. Results were expressed as mean±SD and percentages. Continuous variables were evaluated using student paired t test and categorical data was analysed by χ2-test. A P value of < 0.05 (at confidence level of 95%) was taken as statistically significant.

Results

In response to treatment analysis, both the study

Table 1 : Baseline characteristics among study groups

Parameters	Group A (n=20)	Group B (n=20)	P value
Age in years (SD)	33(12.2)	34(13.1)	0.8041
Gender n(%)			
Male	16(80%)	16(80%)	1.000
Female	4(20%)	4(20%)	
Classification of leprosy			
Paucibacillary (%)	7(35%)	8(40%)	1.000
Multibacillary (%)	13(65%)	12(60%)	
Bacterial index			
BI = 0	10(50%)	11(55%)	1.000
BI $\geq$ 1	10(50%)	9(45%)	

Table 2 : Details of various oxidative parameters in the study groups

S No	Test category	Group A			Group B			P Value (Intergroup between group A & B)	
		Baseline	Post treatment	P value (Intra-group)	Baseline	Post treatment	P value (Intra-group)	At base-line	Post treatment
1	MDA	0.86 ± 0.33	0.45± 0.86	0.0001	0.94±0.34	0.45±0.25	00.0001	0.5180	1.000
2	NO	157.41±8.10	162.5±14.09	0.150	162.01±9.94	161.53±13.10	0.872	0.116	0.822
3	SOD	2.08±0.48	3.30±1.47	0.0012	2.47±2.16	2.90±1.04	0.4585	0.435	0.326
4	TOS	161.3±44.01	86.70±14.81	0.0001	157.1±50.23	150.80±48.33	0.26	0.782	0.0001
5	TAS	0.40±0.31	0.94±0.67	0.006	0.43±0.32	0.72±0.57	0.054	0.765	0.746
6	OSI	75.85±86.30	15.60±15.0	0.007	74.0±73.83	58.07±71.15	0.377	0.9432	0.012

Abbreviations : MDA - Malondialdehyde; NO - Nitric oxide; SOD - Superoxide Dismutase; TOS - Total oxidant status; TAS - Total antioxidant status; OSI - Oxidative stress index

groups were comparable in terms of age, gender, type of leprosy, bacterial index and various oxidative parameters at baseline (Table 1 and Table 2). Majority of patients were middle aged and 80% of study patients in both study groups were males. At least 60% of study patients were multibacillary. After 3 months of study time period, when both the study groups were compared, there was a statistically significant decrease in TOS and OSI levels among group A only (Table 2). Intra-group comparison of the oxidative parameters before and after completing 3 months of treatment revealed a statistically significant difference in levels of MDA, SOD, TOS, TAS and OSI in group A compared to group B where a significant difference was seen in pre and post treatment levels of MDA only.

Discussion

This prospective study determines the changing trends of various oxidative stress parameters after co-administration of anti-oxidants to standard MDT for leprosy. Hence, the trend of change in these parameters was compared with baseline values for each individual.

Malondialdehyde (MDA) :

In the intra-group comparison, both the study groups showed a significant decline in the levels of MDA from their respective baseline scores. However, inter-group comparison of the 2 study groups showed no significant difference in level of MDA between group A and B at completion of study (P=1.000). In other words antioxidant supplementation had no additive effect on MDA level. Levels of MDA alone may not be true indicator of oxidative stress in leprosy (Ozan et al 2006). However, several past studies advocated antioxidant supplementation to counteract the high levels of lipid peroxidation (Abdel Hafez et al 2010, Swathi & Tagore 2015).

Nitric Oxide (NO) :

Microbial killing by macrophages is associated with a burst of respiratory activity that leads to the production of reactive oxygen species which includes NO (Valko et al 2007). In the present

study no significant difference was observed when NO levels were compared between both the study groups. Antioxidant supplementation didn't have any effect on nitric oxide levels. No inference can be drawn from these observations because NO exerts heterogeneous and diverse phenotypic effects (Bogdan 2001).

Superoxide dismutase (SOD) :

Following MDT, levels of serum enzymatic antioxidant superoxide dismutase (SOD) increased in both the study groups respectively, but this increase was significant in group A only. These observations are supported by past studies which have shown beneficial effects of dietary antioxidant supplementation on serum SOD levels among human beings and animals (Chen et al 2011, Bose et al 2007). Schalcher et al (2014) also found an increase in SOD among leprosy patient after three months of MDT.

Intergroup comparison among study groups failed to reveal any significant difference in SOD values. Primary reason for these findings might be less number of subjects in the present study. Also we had given only non-enzymatic antioxidant supplementation in the form of vitamin E, vitamin C, beta carotene, zinc, selenium, manganese and copper. It should be interesting to study the effect of exogenous enzymatic antioxidant supplementation on SOD levels in serum in future.

Total Oxidant Status (TOS) :

After treatment, the total oxidant status significantly decreased in the group A compared to group B during intra and inter group analysis. Noteworthy, this change in TOS level was not mirrored by a corresponding decline of MDA or increase of SOD and NO levels between the two study groups after treatment as discussed above. Non-enzymatic anti-oxidant supplementation along with MDT can reduce the TOS produced during leprosy without any effect on the enzymatic oxidative markers. Only elemental anti-oxidants were administered in the present study. Vijayraghvan et al (2009) also showed decreased oxidative stress when vitamin E was co-administered along with MDT.

Total Antioxidant Status (TAS) :

Few earlier studies have studied one or the other antioxidant component, there is hardly any past literature or data regarding total antioxidant status in leprosy. Compared to group B, group A showed significantly increased TAS levels from baseline after 3 months of co-administration of antioxidants with MDT. Schalcher et al (2014) observed that treatment with MDT alone did not alter total antioxidant capacity level in plasma of leprosy patients. Kusuma et al (2019) have shown a decrease in serum TAS of leprosy patients even after 3 months of regular WHO MDT among patients having higher bacterial index. Addition of non-enzymatic antioxidants in the present study significantly decreased the TAS among study patients. Our endogenous antioxidant defence systems are incomplete without exogenous originating reducing compounds such as vitamin C, vitamin E, carotenoids and polyphenols which play an essential role in many antioxidant mechanisms in living organisms.

Oxidative Stress Index (OSI):

OSI decreased in both the study groups at the end point. Group A showed statistically significant decrease in OSI among leprosy patients compared to group B. This may be attributed to the antioxidant co-administration in group A. The present study confirms that antioxidant co-administration has a beneficial effect on TOS and OSI in the patient of leprosy. Future studies should examine various oxidative stress parameters after supplementing both enzymatic and non-enzymatic antioxidant components during the treatment of leprosy. Estimating the appropriate dosage of various antioxidants to alleviate total oxidative stress among leprosy patients would be

another area of further research. Ultimately introduction of such co-supplementation of antioxidants in the management will depend upon clinical and therapeutic benefits in these patients which needs to be investigated in depth by well-planned studies.

A small sample size, no comparison of diet, lack of long-term follow-up, no consensus for complete ROS working model etc. are few limitations of the present study. Further research is warranted to increase the understanding and develop mechanisms to monitor the effects of various antioxidant interventions.

Conclusion

TOS and OSI decreased significantly when patients of leprosy were given exogenous non-enzymatic antioxidant supplementation for 3 months duration.

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