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Research Article

Evaluation of Oxidative Stress among Treated Multibacillary and Paucibacillary Leprosy in Pakistan

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ABSTRACT

Leprosy is a chronic infectious disease associated with immune dysregulation and oxidative stress. Even after treatment, biochemical alterations may persist. This study aimed to evaluate oxidative stress and antioxidant status in treated multibacillary and paucibacillary leprosy patients compared with healthy controls. A comparative cross-sectional analysis was conducted including treated MB (n=73), treated PB (n=37) leprosy patients, and healthy controls (n=76). Biochemical parameters assessed included malondialdehyde (MDA) as a marker of lipid peroxidation and superoxide dismutase (SOD) as an antioxidant enzyme. Data were analyzed using appropriate statistical tests, with $p < 0.01$ considered significant. MDA levels were significantly elevated in both treated MB (99.3 ± 4.26 nmol/100 ml) and PB (98.4 ± 2.42 nmol/100 ml) groups compared to controls (77.5 ± 3.48 nmol/100 ml) ($p < 0.01$), indicating increased oxidative stress. Among leprosy groups, MB patients showed slightly higher MDA levels than PB cases. SOD activity was significantly reduced in both treated MB (4.6 ± 0.69 U/ml) and PB (4.4 ± 0.87 U/ml) groups compared to controls (5.4 ± 0.39 U/ml) ($p < 0.01$), reflecting decreased antioxidant defense capacity. No major difference was observed between MB and PB groups in SOD levels. Both treated multibacillary and paucibacillary leprosy patients exhibit persistent oxidative stress characterized by elevated MDA levels and reduced SOD activity compared to healthy individuals. These findings suggest ongoing imbalance between oxidant and antioxidant systems even after treatment, highlighting the need for monitoring oxidative stress and considering antioxidant support in leprosy management.

Keywords: Leprosy, Oxidative Stress, Multibacillary, Paucibacillary, Antioxidant, Lipid Peroxidation, Reactive Oxygen Species.

INTRODUCTION

Leprosy, also known as Hansen's disease, is a chronic and debilitating condition caused by Mycobacterium leprae. It affects the skin, peripheral nerves, mucosal surfaces of the upper respiratory tract, and the eyes, leading to progressive and permanent disabilities if left untreated [1]. Despite leprosy being eliminated as a public health problem globally in 2000 (defined as a disease burden of less than 1 case per 10,000 persons), it continues to be a global public health issue [2]. Approximately 200,000 to 300,000 individuals are diagnosed with leprosy annually, and around 2 to 3 million people suffer from disabilities due to this condition [3]. According to official data from 150 countries across 6 WHO regions, there are globally 7,607,837 individuals infected with leprosy [4]. The global registered prevalence reached 192,713 cases in 2017, marking an increase of 20,765 cases compared to 2016, with 210,671 new cases identified that year [5]. The process of microbial killing by macrophages is linked to a surge in respiratory activity that produces various molecules and free radicals known as reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and hydroxyl radicals [6]. These ROS can harm lipids, proteins, and nucleic acids, with polyunsaturated fatty acids in membrane lipids being prime targets for peroxidation. Free radicals break down polyunsaturated fatty

acids, resulting in the formation of malondialdehyde (MDA). MDA concentration indicates the extent of cell damage caused by free radicals [7]. Cells utilize various methods to eliminate free radicals, thus reducing tissue damage. Antioxidants (AO), including superoxide dismutase (SOD), catalase, and nutritional AOs, capture free radicals and function as free radical-scavenging systems [8]. The term oxidative stress (OS) refers to a range of harmful processes that occur when there is an imbalance between systems that generate free radicals and those that scavenge them. OS causes metabolic dysfunction and cell death. OS happens when ROS are not sufficiently neutralized by AOs [9]. The MDA/SOD ratio can be regarded as an indicator of OS [10].

MATERIAL AND METHOD

Sample Collection

Untreated and treated leprosy patients were collected from leprosy centre (National Training Centre), Karachi. A total of 220 leprosy cases were included in this study, among them 110 were untreated newly diagnosed and 110 treated leprosy patients. Clinical and bacterial based skin smear test for diagnostic purpose performed, control samples were taken from general population for comparison.

Grouping of Subjects

Subjects included for study were divided into three groups:

Group A: Control group.

For control group 76 samples were collected which include male 55 (72.4%) female 21 (27.6%), taken from general population.

Group B: (Untreated Multiibacillary Leprosy Cases) Test Group. This group was comprised of untreated multiibacillary leprosy patients. Total number of patients in this group was 76 among them male 55 (72.4%) and female 21 (27.6%).

Group C: (Treated Multibacillary Leprosy Cases) Test Group

This group was comprised of untreated multiibacillary leprosy patients. Total number of patients in this group was 73 which comprised of male 55 (75.3%) and female 18 (24.7%).

Collection of Blood Samples

The blood samples of patients who fulfilled the inclusion criteria were collected after an overnight fasting of 14 hours. About 5 ml of blood drawn from antecubital vein after all aseptic measures and collected into gel tubes for , lipid profile. Serum was taken after centrifugation at 3000 rpm for 10 minutes and stored at -80°C till analyzed.

Estimation of Malondialdehyde (MDA)

Assay was performed by using method of OKHAWA et al. Absorbance was read at 530 nm. Malondialdehyde formed with thiobarbituric acid and produced MDA-TBARs. 200 uL of samples were taken, carefully added with added trichloroacetic acid 1cc, TRIS-HCL 0.9 cc then put on100°C for 1 hour.

Estimation of Superoxide Dismutase (SOD)

The SOD were calculated used Ransod kits model AE-350, Erma Inc. Absorbance was measured on spectrophotometric method. Absorbance was read at 530 nm. Superoxide redicals (SR) were formed by xanthine and xanthine oxidase. For the production of formazan dye SR react with Iodophenyl nitrophenol phenyltetrazolium chloride then inhibition is calculated to see the SOD activity [11].

Statistical Analysis

Data analysis was on SPSS version 19. Clinical characteristics were summarized as mean ± S.D. for biochemical (total cholesterol, triglyceride, HDL, LDL, Student t-test/ANOVA were used for comparison between cases Multibacillary Vs. controls. In statistical evaluation only p-value <0.05 will be considered significant.

RESULT

The present result indicates that both MB and PB treated leprosy patients continue to experience significant oxidative stress with compromised antioxidant defense compared to healthy individuals. These results suggest the potential benefit of adjunct antioxidant therapy and regular biochemical monitoring to improve long-term outcomes in leprosy management.

Table 1: Comparison of Biochemical Parameters of Treated Multibacillary Leprosy and Controls

Biochemical Parameters	Treated Leprosy Multibacillary (MB) (n=73)	Controls (n=76)	P-value
	Mean ± S.D	Mean ± S.D	
MDA (nmol/100 ml)	99.3 ± 4.26 **	77.5 ± 3.48	0.001
Superoxide Dismutase (u/ml)	4.6 ± 0.69 **	5.4 ± 0.39	0.001

** Statistically Significant (p<0.01)

This table presents a comparison of biochemical parameters between treated Multibacillary leprosy cases (n=73) and healthy controls (n=76), focusing on malondialdehyde (MDA) levels as a marker of oxidative stress. The mean MDA level in treated leprosy patients was significantly higher (99.3 ± 4.26 nmol/100 ml) compared to controls (77.5 ± 3.48 nmol/100 ml), with a highly significant difference (p = 0.001). This indicates that despite treatment, Multibacillary leprosy patients continue to exhibit elevated lipid peroxidation, suggesting persistent oxidative stress. The higher MDA levels in the treated group reflect ongoing cellular membrane damage and an imbalance between oxidant and antioxidant systems. In contrast, the control group shows comparatively lower MDA levels, indicating normal oxidative status. Overall, these findings suggest that oxidative stress may persist even after treatment in Multibacillary leprosy, potentially contributing to residual tissue damage or long-term metabolic alterations.

Table 2: Comparison of Biochemical Parameters of Treated Paucibacillary Leprosy and Controls

Biochemical Parameters	**Treated Leprosy Paucibacillary (PB) (n=37)**	**Controls (n=76)**	**P-value**
	Mean ± S.D	**Mean ± S.D**	
MDA (nmol/100 ml)	98.4 ± 2.42 **	77.5 ± 3.48	0.001
Superoxide Dismutase (u/ml)	4.4 ± 0.87 **	5.4 ± 0.39	0.001

** Statistically Significant (p<0.01)

This table represents a comparison of biochemical parameters between treated Paucibacillary leprosy patients (n=37) and healthy controls (n=76), focusing on oxidative stress markers, including malondialdehyde (MDA) and superoxide dismutase (SOD). The mean MDA level was significantly elevated in treated Paucibacillary patients (98.4 ± 2.42 nmol/100 ml) compared to controls (77.5 ± 3.48 nmol/100 ml), with a statistically significant difference (p = 0.001). This indicates increased lipid peroxidation and persistent oxidative stress even after treatment. In contrast, the antioxidant enzyme superoxide dismutase (SOD) was significantly reduced in treated paucibacillary cases (4.4 ± 0.87 U/ml) compared to controls (5.4 ± 0.39 U/ml), also showing a highly significant difference (p = 0.001). This reduction reflects a diminished antioxidant defense mechanism in treated patients.Overall, these findings suggest that even after treatment, paucibacillary leprosy patients exhibit an imbalance between oxidative stress and antioxidant defense, characterized by increased oxidative damage (elevated MDA) and reduced enzymatic protection (lower SOD) compared to healthy individuals.

Table 3: Comparison of Biochemical Parameters of Treated Multibacillary, Paucibacillary Leprosy and Controls

Biochemical Parameters	**Treated Leprosy Cases**		**Controls (n=76)**
	Multibacillary (MB) (n=73)	**Paucibacillary (PB) (n=37)**	
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**
MDA (nmol/100 ml)	** 99.3 ± 4.26	** 98.4 ± 2.42	77.5 ± 3.48
Superoxide Dismutase (u/ml)	** 4.6 ± 0.69	** 4.4 ± 0.87	5.4 ± 0.39

Significant as compared to Controls ** p<0.01, Significant as compared to Paucibacillary (PB) ▲▲ p<0.01

This table represents a comparative analysis of biochemical parameters among treated Multibacillary (MB) leprosy cases (n=73), treated Paucibacillary (PB) cases (n=37), and healthy controls (n=76), focusing on oxidative stress markers including malondialdehyde (MDA) and superoxide dismutase (SOD).The mean MDA levels were markedly elevated in both treated MB (99.3 ± 4.26 nmol/100 ml) and PB (98.4 ± 2.42 nmol/100 ml) groups compared to controls (77.5 ± 3.48 nmol/100 ml), with statistically significant differences (p

< 0.01). Although both leprosy groups showed similarly high MDA levels, the Multibacillary group demonstrated slightly higher values than the Paucibacillary group, indicating relatively greater lipid peroxidation and oxidative damage in MB cases. Regarding antioxidant status, superoxide dismutase (SOD) activity was significantly reduced in both treated MB (4.6 ± 0.69 U/ml) and PB (4.4 ± 0.87 U/ml) patients compared to controls (5.4 ± 0.39 U/ml) ($p < 0.01$). The PB group showed a marginally lower SOD level than the MB group, suggesting a slightly more pronounced reduction in enzymatic antioxidant defense. Overall, the findings demonstrate that both treated Multibacillary and Paucibacillary leprosy patients continue to exhibit a persistent imbalance between oxidative stress and antioxidant defense when compared with healthy controls. Elevated MDA levels alongside decreased SOD activity indicate ongoing oxidative damage even after treatment, with relatively comparable biochemical alterations in both disease types.

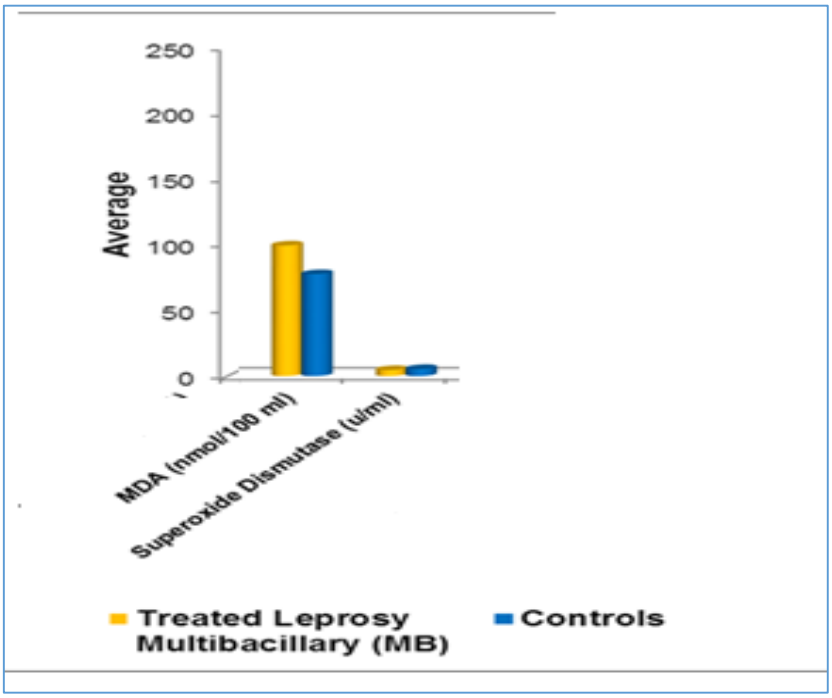

Figure 1: Comparison of Biochemical Parameters of Treated Multibacillary Leprosy and Controls

For MDA (nmol/ml), the treated MB leprosy group shows a clearly higher level, approximately 105 nmol/ml, compared to controls, which are around 80 nmol/ml. This indicates increased lipid peroxidation and ongoing oxidative stress even after treatment. For Superoxide Dismutase (SOD) (U/ml), the treated MB group shows lower activity, approximately 6 U/ml, while controls show slightly higher activity, around 8 U/ml. This suggests a relatively reduced antioxidant defense in treated leprosy patients compared to healthy individuals. The figure demonstrates an imbalance between oxidative stress and antioxidant defense in treated MB leprosy cases, characterized by elevated MDA levels and decreased SOD activity when compared with controls.

DISCUSSION

The present study demonstrates a persistent imbalance between oxidative stress and antioxidant defense systems in both treated Multibacillary and Paucibacillary leprosy patients compared with healthy controls. The findings are based on significantly elevated malondialdehyde levels and reduced superoxide dismutase activity, indicating ongoing oxidative damage even after completion of treatment. In the literature regarding the evaluation of OS in the lesional skin of leprosy patients, only one study by Abdel-Hafez et al. [12] could be identified. The present study found that tissue levels of MDA and OS were significantly higher, while tissue levels of SOD were significantly lower in MB leprosy compared to PB leprosy. While numerous studies have indicated a considerable rise in MDA among MB leprosy patients, the comparison of its levels in PB leprosy patients with those in the control group showed no

significance in blood samples, although it was significantly elevated in the lesional skin of PB leprosy patients. Based on these findings, a study conducted by Abdel-Hafez et al. [12] determined that the OS index was considerably elevated in the tissue and serum of MB patients, as well as in the tissue of PB patients, in comparison to the controls. Compared to the controls, the average MDA levels in skin lesions of PB and MB patients were significantly elevated. Furthermore, SOD activity in the skin lesions of PB and MB patients was considerably reduced. Moreover, research conducted by Jyothi et al. [17] found that in the serum of individuals with MB leprosy, SOD levels were low while MDA and the MDA/SOD ratio were increased, with all three differences being statistically significant when compared to the control group. The occurrence of increased oxidative stress in MB leprosy may be due to insufficient immunological activation of monocyte-macrophages and a reduction in SOD activity. According to Sengupta [20], cell-mediated immunity is robust in PB leprosy, which accounts for the greater severity of the OS in MB compared to PB leprosy, as noted in the current study. Conversely, the studies by AbdelHafez et al. [12], Jyothi et al. [17], Reddy et al. [21], and Prasad et al. [22] found that the changes in MDA serum levels of PB leprosy patients were not significant in comparison to those in the control group. This may be due to the fact that, in the current study, MDA tissue levels were assessed in skin biopsies from new lesions indicating disease activity, particularly in PB, resulting in higher tissue MDA levels in PB leprosy compared to the control group.

CONCLUSION

It is concluded that significantly elevated malondialdehyde (MDA) levels and reduced superoxide dismutase (SOD) activity in both patient groups. Although antimicrobial therapy effectively manages the infectious component of leprosy, it does not appear to fully restore the oxidative-antioxidant balance. The persistence of increased lipid peroxidation and decreased antioxidant enzyme activity suggests ongoing subclinical oxidative damage even after treatment completion. Both Multibacillary and Paucibacillary groups showed similar biochemical disturbances, with only minor variations between them, indicating that oxidative stress is a common feature across the spectrum of treated leprosy. These findings highlight the importance of considering oxidative stress as a contributing factor in post-treatment biochemical alterations. In conclusion, monitoring of oxidative stress markers and the potential use of antioxidant supplementation may be beneficial as adjunct strategies in the long-term management of leprosy patients to reduce residual biochemical imbalance and improve recovery outcomes.

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