



Effect of Relationship between Protein Oxidation and Total Thiols in Progression of Leprosy

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objective: In several countries including India, leprosy is an older disease & until now continuous to be an important health issue. Leprosy is a chronic granulomatous disease, the causative agent for which is mycobacterium leprae. Due to imbalance between natural antioxidants and oxidative stress is significant event occurs that influences the pathogenesis of leprosy. Considering this the study was carried out to find, what type of relationship between MDA, Protein carbonyl and thiols as disease advances.

Methods: 50 diagnosed leprosy patients & 50 healthy controls were included in this study. In leprosy group, 16 were Paucibacillary (PB) and 34 were Multibacillary (MB) type leprosy patients.

Results: Serum Protein carbonyl and serum Malondialdehyde increased in leprosy patients than controlling. Further analysis reveals that the serum malondialdehyde was significantly increased in MB leprosy patients than PB leprosy patients. On the other hand, our proposed system significantly lowers the total thiols in leprosy patients when compared with controls. Serum total thiols were lower in MB leprosy than PB leprosy patients. Among leprosy patients the negative correlation of Malondialdehyde with total thiol and Protein carbonyl with total thiols was observed.

Conclusion: Thus, as protein oxidation and lipid per oxidation in leprosy increases, the total thiols decreases which may lead to modifications of protein and lipids that are responsible for severity of disease hence therapy aimed at reducing generation of free radicals that can result in oxidative stress and as a result a modification of proteins and lipids.

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1. INTRODUCTION

Granulomatous, the causative agent for which is *Mycobacterium leprae*, the prevalence of leprosy has decreased over the past 20 years, and the incidence of newly detected cases is still high. India has the maximum new leprosy cases in the world. As per skin lesions and nerves involved, WHO classified leprosy into paucibacillary or multibacillary type. The pathogenesis of leprosy has been found to be influenced by several factors including oxidative stress (OS) [1]. The infected macrophages show more oxygen consumption and increased phagocytosis known as "respiratory burst," associated with production of oxidants or free radicals. These free radicals apart from destroying the bacteria, also damages the host tissues mainly nucleic acids, proteins and lipids. In leprosy, this well controlled physiological balance is turned in favour of ROS from phagocytes [2]. Normally the oxidative stress produced is compensated by the antioxidant system in the body. But in diseased condition the balance between oxidants and antioxidants are disturbed. Prime targets of peroxidation by ROS are, as a result malondialdehyde (MDA) is formed [3]. The marker of protein oxidation is protein carbonyl. In addition to this event the thiol groups of amino acids which contains sulfhydryl group are also oxidised. Antioxidants which are available in the body and thiols form the crucial part of them and in defence against ROS thiols have important roles [4]. Modifications of SH groups into disulphides and more oxidized species are the initial observable event during the radical mediated protein oxidation [5]. The diagnostic alarm of various pathological states can be the redox status of plasma total thiols. Total thiols contain intracellular and extracellular thiols together either in the thiols bound to proteins or reduced glutathione or in the free form as oxidized [6].

In Oxidative stress (OS), various deleterious processes occurs because of the disequilibrium between free radical generating and scavenging systems. It occurs if antioxidants are not available for scavenging ROS. As a result of this metabolic impairment and cell death occurs [7]. Keeping all these facts in mind, this study was undertaken to find out the type of relationship between MDA, protein carbonyl and thiols as advance diseases.

2. MATERIALS AND METHODS

In the department of biochemistry, KIMS Karad given the study that was carried out (Maharashtra). 50 healthy controls and 50 are clinically diagnosed leprosy patients were included in this study [8].

From all subjects' venous blood was collected by venepuncture using a 5ml sterile syringe under aseptic condition. After clotting, the blood serum was separated and stored at -20°C till analysis.

2.1 Inclusion Criteria

Clinically diagnosed leprosy patients without any other complications were included in this study. As per WHOM formula, skin specialist diagnosis and classification of leprosy was done [9].

2.2 Exclusion Criteria

The controls as well as leprosy patients who had disorders associated with kidney, lung, heart and other organs were excluded.

Kei Satoh method is used for determination of serum Malondialdehyde (MDA), serum protein carbonyl by Lewin method [10] serum total thiol. All values of biochemical parameters were expressed as mean \pm SD in leprosy patients and in healthy controls. Statistical analysis was done by applying z test and Turkeys' test. By using Pearson's correlation coefficient correlations between the variables were estimated.

3. RESULTS

The total thiol was determined as a measure of antioxidant in leprosy and their correlation.

When compared with controls, paucibacillary and multibacillary leprosy patients, serum MDA & protein carbonyl levels are significantly higher ($P < 0.01$) (Table 1). Furthermore, in MB leprosy patient's serum Malondialdehyde level is higher than in PB leprosy patients (Table 1). In both types of leprosy patients, we observed that serum total thiols is decreased ($p < 0.01$) which is statistically significant as shown in Table 1 than controls. Furthermore, observed in total thiols in MB leprosy (more severe condition than PB)

Table 1. Serum MDA, protein carbonyl & thiol in healthy controls & PB and MB leprosy patients

GROUP	MDA ($\mu\text{mol/L}$) mean \pm SD	Protein carbonyl($\mu\text{mol/L}$) mean \pm SD	Thiol($\mu\text{mol/L}$) mean \pm SD
Controls (n=50)	2.96 \pm 1.30	13.07 \pm 5.65	16.37 \pm 2.91
Paucibacillary leprosy (PB)(n=16)	6.20 \pm 1.36	26.18 \pm 12.86	13.24 \pm 2.63
Multibacillary leprosy (MB)(n=34)	8.27 \pm 1.60	33.83 \pm 13.87	10.52 \pm 3.65

Table 2. Correlation (Rvalue) between lipid and protein modifications and total thiols in MB leprosy patients

	MDA	Protein carbonyl	Thiol
MDA	--	+0.946	-0.907
Protein carbonyl	+0.946	--	-0.907
Total Thiols	-0.907	-0.907	--

Table 3. Correlation (R value) between Lipid and Protein modifications and total thiols in PB Leprosy patients

	MDA	Protein carbonyl	Total Thiols
MDA	--	+0.957	-0.984
Protein carbonyl	+0.957	--	-0.950
Total Thiols	-0.984	-0.950	--

patients are than in PB leprosy patients as shown in Table 1. In MB and PB leprosy patients negative correlation between MDA and thiols as well as in between protein carbonyl & thiol was observed [11]. Furthermore, in MB and PB leprosy patient's positive correlation between MDA and protein carbonyl was observed in Table 2 & Table 3.

4. DISCUSSION

The concept of lipid peroxidation has attracted appreciable recognition in various pathologic situations in recent years. As a part of defence system macrophages plays a significant role to kill bacteria. And it is related to respiratory burst and activates ROS [12]. Various drugs used in multidrug therapy (MDT) also generate ROS and may further increase the damage to host tissues. The membrane lipids polyunsaturated fatty acids (PUFA) are main prey of ROS for peroxidation which leads to the formation of malondialdehyde (MDA) [13]. MDA in serum is one of the markers of cellular damage. To see the extent of peroxidation of lipids MDA was evaluated in leprosy patients as it is the lipid peroxidation index. In this study, serum MDA were significantly increased ($p < 0.01$) in both PB (26.18 \pm 12.86 $\mu\text{mol/L}$) and MB (33.83 \pm 13.87

$\mu\text{mol/L}$) leprosy patients compared to those in healthy controls (2.96 \pm 1.30 $\mu\text{mol/L}$).

In leprosy patients, this suggests that elevated lipid peroxidation of free radical mediated injury occurs and reported similar results. In addition to this carbonylation of proteins also occurs due to oxidative stress. We observe significantly increased ($p < 0.01$) protein carbonylation in both types that is, in PB and in MB leprosy patients than healthy controls.

Aldehydes of 4-hydroxy-2-nonenal Malondialdehyde generated during lipid peroxidation that is responsible for the introduction into proteins of carbonyl groups. These reactive derivatives (ketoamines, deoxyosones, ketoaldehydes) are also generated as a consequence of the interaction of sugar reduction or their oxidative products with protein lysine residues (glycoxidation and glycation reactions) [14].

Cellular damage due to ROS can be assessed by Protein carbonyl (PC). An increase in PC is a sign of oxidative stress which causes altered protein functions & oxidative damage may lead to entangling the pathophysiology of leprosy. The observed increase in protein carbonyl with

severity makes it a good marker of oxidative stress [15].

There are different scavengers to fight with the oxidative stress produced. Body thiols are big scavengers. In this study, we observed a significant decreased ($p < 0.01$) thiol in both PB and MB leprosy patients) leprosy groups are compared to controls. This decrease may be due to the utilization of thiols in order to alleviate the oxidative stress [3]. Present study supports the oxidative tension in leprosy exists which is high in MB leprosy which may be blameworthy for disease continuation in leprosy.

Present study supports the existence of oxidative tension in leprosy which is high in MB type of leprosy, which may be blameworthy for disease continuation in leprosy.

To support these findings, we have correlated the MDA, Protein Carbonyl and thiols in both groups. We observed a positive correlation between MDA & Protein carbonyl (Table 2 and Table 3) with the severity of the disease. There were negative correlations among the oxidative stress markers (MDA & PC) and the total thiols. (Table 2 and Table 3)

5. CONCLUSION

The above results indicate that oxidative stress increases with increased severity. The decrease in thiol level indicates that thiols are depleted to alleviate the oxidative stress produced in leprosy.

In addition, several detoxifying enzymes help in the removal of lipid peroxidation products that use thiols as reducing equivalent. Thus, the protein oxidation as well as both enzymatic and nonenzymatic reactions of lipid peroxidation product, leads to consumption and often decrease in intracellular thiols.

Thus, thiols represent the most attackable targets of ROS and related free radicals, represents a flexible and sturdy defense system against biochemical excitement caused by oxidative stress.

Thus we conclude that oxidative stress successively increases in PB & MB leprosy. The negative correlation between oxidants and thiols indicates the alleviating effects of thiols, which is insufficient. This fact led us to think that the supplementation of antioxidants might be useful along with the anti-leprosy drugs therapy

to get better results. Thus, the therapy succeeds in preventing lipid and protein leprosy oxidative modification.

CONSENT AND ETHICAL APPROVAL

Institutional ethics committee of Krishna Institute of Medical Sciences, Karad approved the protocol.

From patients the consent form was obtained.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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