Study of Effect of Nitrosamine Stress 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 & till now continues to be an important health issue. Leprosy is a chronic granulomatous disease, the contributing agent for this is mycobacterium leprae. Nitric oxide (NO) plays a significant role in immunity to fight against bacteria. But increased NO can harm host tissue by causing altered structure of thiol-containing compounds, which is a significant event that influences the pathogenesis of leprosy. Considering this, the study was planned to determine the type of relationship between NO, nitrothiols, and thiols as disease advances.

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

Results: Serum NO and serum nitrothiol significantly increased (p<0.01) in leprosy patients than controls. Further, Serum NO and serum nitrothiol significantly increased (p<0.01) in MB leprosy patients than PB leprosy patients. On the other hand, we found a significantly decreased (p<0.01) level of total thiols in leprosy patients compared with controls. Serum thiols significantly decreased (p<0.01) in MB leprosy patients than PB leprosy patients. Among Both leprosy patients, the negative correlation of NO with thiol and nitrothiol with thiols was observed.
Conclusion: Thus, in leprosy, increased nitric oxide causes modification of thiol groups of proteins and impaired their activity which may be responsible for the severity of disease; hence therapy aimed to reduce nitrosative modification of proteins leprosy.

Keywords: Leprosy; nitric Oxide; thiol, Nitrohiol; Mycobacterium Leprae.

1. INTRODUCTION

Leprosy /Hansen's disease is a long-term infectious disease. Myco-bacterium leprae is responsible for this type of infection. Leprosy can occur at any stage of age in life. The peripheral nerves, eyes, skin, upper respiratory tract mucosal surfaces are mainly affected in this. Approximately a quarter of a million people worldwide are affected by leprosy currently, India reported the majority of these cases [1]. Currently, more than 30 years through the availability and implementation of effective multidrug therapy (MDT) for riddance of leprosy exists as a significant healthiness issues. The nerve damage in leprosy results in a lack of ability to feel pain [2]. The frequent injuries or illness sowing to ignored wounds occur because of loss of parts of a person's extremities [3]. Leprosy patients also experience muscle weakness and poor eyesight. The Leprosy symptoms might appear in a year, and in some cases, it takes 20 years or more [4].

As per the WHO directions (2012), leprosy is classified into paucibacillary and multibacillary. Single lesion or less than five lessons with negative skin smear of myco-bacterium leprae is paucibacillary leprosy, while positive skin smears with more than six lesions are multi-bacillary leprosy [5].

The clinical signs of leprosy are affected by the host's immune system. Nitric oxide (NO) has been principally connected with anti-bacterial defenses exerted through nitrosative, oxidative, and nitrative stress and cyclic GMP-dependent mechanisms signal transduction in the immune system. For nonspecific host defense, NO's biological production is also significant, but NO itself acts directly to destroy intracellular pathogens and tumors [6].

L-arginine undergoes oxidative deamination to form NO. The enzyme responsible for this is nitric oxide synthases (NOS). There are three isoforms of this enzyme [7]. These are inducible NOS (iNOS), endothelial NOS (eNOS) & Neuronal NOS (nNOS). The iNOS is absent in resting cells [8]. In response to stimuli such as pro inflammatory cytokines, the gene of iNOS is rapidly expressed [9]. Thus the high concentration of nitric oxide species (NOx) can inhibit the activity of a various microbes and besides potentially injure the host, thus put up to pathology. The antimicrobial consequence arises not from NO itself other than reactive nitrogen intermediates (RNIs). The reactive nitrogen species formed by the oxidation of NOx like peroxynitrite, nitrothiols, and nitrotyrosine etc [10]. RNIs inactivates aconitase and ribonucleotide reductase. These are significant microbial enzymes. These enzymes contain iron groups that are susceptible to RNIs attack. The intracellular thiols, low molecular weight thiols like glutathione & cysteine & metal containing proteins are the target molecule for RNIs [11]. The nitrosylated thiols products are nitrothiols that are investigated as a significant intermediate in NOx mediated biological reactions [12]. Hence NOx may destroys proteins in leprosy [13]. No study was done on nitrothiol in leprosy as per our knowledge. Nobody correlates the role of the relationship of nitrothiol with thiol and NO with thiol in disease progression [14]. By considering these facts, this study was taken to see the activity of NOx synthesis and its relation with nitrothiol formation and thiol and its toxic effects on protein in patients with leprosy and to find out its role in disease progression [15].

2. PROPOSED METHODOLOGY

In bio-chemistry, VMGMC, Solapur (Maharashtra), the present study was carried out. From patients, the consent form was obtained [16].

In this study, 50 were healthy controls, and 50 newly diagnosed leprosy included. In 50 leprosy patients, 34 belong to PB & 16 belong to MB leprosy [17].

The venous blood was collected by venipuncture under the aseptic condition from all the subjects. Serum was stored after clotting, and serum was stored at -20°C till analysis.

Inclusion criteria: Leprosy patients were taken from the 21-60 years age group, without any
other complications. Skin specialists diagnosed leprosy patients and classified them into PB and MB leprosy by using the WHO formula. Exclusion criteria: Those having disorders related to important organs like the heart, lung, kidney were excluded from the present study [18].

The serum nitric oxide species (NOx) as an indicator of NO synthesis (nitrate + nitrite) by the method of kinetic cadmium granule reduction [19], serum nitro thiol by Cook method, and Total serum thiol by Habeeb method [20] estimated. Result values of all parameters of the present study were expressed as mean ± SD. Statistical analysis was done by applying the z test and Tukeys test. By using Pearson’s correlation coefficient, correlations between the variables were estimated.

### 3. RESULTS AND DISCUSSION

NOx, nitrothiol, and total thiol were determined as a marker of nitric oxide toxicity in leprosy, and their correlation was the aim of the present study. When compared with controls, serum NOx and nitrothiol levels increased in both PB & MB leprosy patients, which is statistically highly significant (Table1). We observed significantly higher Nitrothiol and NOx levels in MB leprosy patients (P<0.01) when compared with PB leprosy patients (Table 1). Thiol level decreases in PB & MB leprosy patients when compared to highly significant controls (P<0.01) in this study significantly decreased (p<0.01) thiol levels in MB leprosy patients than in PB leprosy patients (Table 1) was found. We observed a negative correlation between NOx and thiols in MB and PB leprosy patients. Also, a negative correlation between nitrothiol & thiol was observed in both groups of leprosy. We found a positive correlation between NOx and nitrothiol in PB and MB leprosy patients, shown in Table 1, Table 2, Table 3.

Nitric oxide (NOx) is an intercellular messenger that has an important role in blood flow modulating, thrombosis, and neural activity. nNOS is constitutively available in the inner most and marginal nervous systems NOx serves as a neurotransmitter. eNOS is constitutively articulated by endothelium as well as another cell types. SIT plays an important role in cardiovascular homeostasis [10]. In contrast, iNOS is not present in resting cells, but the gene responsible for information is expressed rapidly in recirculation to stimuli like proinflammatory cytokines. At this time, iNOS produces the NOx is 100–1000 times greater than the constitutive enzymes. It do so for longer periods. The manufacture of NO by eNOS in addition to nNOS have been liken to a dripping tap that by iNOS to a fire horse [21]. To see the extent of NOx synthesis, we evaluated serum nitric oxide species in leprosy patients as it plays

| GROUP       | nitric oxide (NOx) (µmol/L) | nitrothiol (µmol/L) | Thiol (µmol/L) |
|-------------|-----------------------------|---------------------|----------------|
| Controls    | 59.67±19.24                 | 2.4±0.91            | 16.37±2.91     |
| PB leprosy  | 70.87±19.21                 | 5.12±2.95           | 13.24±2.63     |
| MB leprosy  | 144.78±92.57                | 9.68±5.76           | 10.52±3.65     |

| nitric oxide (NOx) | nitrothiol | Thiol  |
|--------------------|------------|--------|
| nitric oxide (NOx) | +0.845     | -0.954 |
| nitrothiol         | -0.842     |        |
| Thiol              | -0.884     |        |

| nitric oxide (NOx) | nitrothiol | Thiol  |
|--------------------|------------|--------|
| nitric oxide (NOx) | +0.914     | -0.839 |
| nitrothiol         | -0.884     |        |
| Thiol              | -0.884     |        |
an important role in immunity to fight against bacteria. In our study serum NOx were increased (p<0.01) in both PB (70.87±19.21 µmol/L)) and MB (144.78±92.57 µmol/L) leprosy patients compared to those in healthy controls (59.67±19.24 µmol/L), which is highly significant. Further, we also observed increased serum nitric oxide levels in MB than PB leprosy patients, which is also highly significant (p<0.01).

The first-line defense against microbial organisms may be NOx formation. The toxic effects of NOx for cells occurred when it provide nonspecific immunity for foreign organisms or invading organisms like bacteria, viruses, etc. The induced activity of iNOS is a non specific event. It comes into the scene when excessive cytokine responses occur across a large variety of cells. Macrophages that are activated have a significant role in the host's resistance for the growth of a clinical type of leprosy. Macrophages have an important role in the limitation of the widening of mycobacterium leprae.

These activated macrophages can secrete various cytokines like TNFα, IFN-β. Infected macrophages showed the production of nitrite with intracellular pathogen who played an important role in the destruction of bacterial particles in the presence of interferon β (IFN-β). Macrophages play this role in combination with IFN-γ or alone or whole for the destruction of bacterial particles. NOS activated in cells, primarily in lesions, due to the rush of iNOS-positive cells during the reactive events, can destroy the host tissues and invade microorganisms. Hence, serum NOx concentration may be used as a marker of inflammation for the progression of disease status. However, under pathological conditions, NOx has damaging effects.

Potentially molecules like transition metals, thiols, lipids, and DNA are the targets of NOx include. Peroxynitrite (ONOO−) is the unstable molecule formed by free radical superoxide (O2−) and NOx, while nitroso thiol by NOx and thiol groups.

In this study, serum nitro hial was significantly increased (p<0.01) in both PB (5.12±2.95 µmol/L)) and MB (9.68±5.76 µmol/L) leprosy patients compared to those in healthy controls (2.4±0.91 µmol/L). Further serum nitrothiol increased in MB leprosy patients than PB leprosy patients, significantly higher (p<0.01).

Glutathione (GSH), like compounds that contain thiol as a structural component, is easily susceptible to the attack of NOx under the physiological condition to form S-nitrosothiols. S-nitrosothiols came into the picture with high concentrations of plasma, human Broncho alveolar lavage fluid, platelets, and polymorphonuclear neutrophils in the condition of inflammation. S-nitroso thiols have been recognized for their activity against microbial organisms. Nitro thiols have also role in mediating the transfer of NOx to thiols of the outer membrane in the bacillus and inhibits the spouse's outgrowth. The significant physiologic redox forms of NO are S-nitrosothiols.

S-nitrosothiols are formed when the addition of nitrosium equivalent (NO+) with thiol takes place & similarly, N-nitrosamine formed when combining with amines. As per our knowledge, in leprosy, no study was done on nitrothiols. Thus nitrothiols are formed from the attack of NO on thiols & acts as a carrier. This increase in serum NOx may increase serum nitro thiolin leprosy. We observed positive correlation between nitrothiol and NOx in MB (r=+0.845) and PB (r = +0.914) leprosy patients. It is specified that elevated NOx may increase nitrothiols & affect the protein molecules, which leads to oxidation of proteins. Thus in leprosy, protein structure and activity get disturbed, which will be responsible for disease progression.

The redox status of thiol is intracellular and its compartments is crucial for protein structure, enzyme activity regulation, transcription factor activity. The antioxidant property of thiol showed by the various mechanism. These include parts of redox buffer of thiol/disulfide, reduced glutathione, metal chelators,bonds of protein disulfate (thioredoxin), etc. -SH groups modifications to disulfides and more oxidized species is initial, observable events during the radical-mediated proteins oxidation. Diagnostic indicators of different 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 in the free form as oxidized. This play a significant role in protecting oxidant stress from adverse effects. The outstanding thiols in plasma are albumin thiols, γ-glutamylcysteine, protein, thiol, cysteine, cysteinylglycine, glutathione, and homocysteine [19].

In this study serum thiols were significantly decreased (p<0.01) in both PB (13.24±2.63 µmol/L)) and MB (10.52±3.65 µmol/L) leprosy
patients compared to those in healthy controls (16.37±2.91 µmol/L). A significant decrease was observed in serum thiols (p<0.01) in MB leprosy patients compared to PB leprosy patients.

4. CONCLUSION

This paper presents the negative correlation between thiol and nitric oxide and also in between thiols and nitrothiols leprosy. Thus, increased NOx kill bacteria in leprosy affects the host tissue [6]. In leprosy, increased NO forms other reactive nitrogen species like peroxynitrite, which consumes free thiol groups and thiol groups of proteins during oxidation. Thiol modification leads to altered protein structure and impairs its functions [3]. Thus, in leprosy, increased nitric oxide caused a modification of thiol groups by oxidation and nitration of key protein moieties and impaired their activity. These causes can cause various functional consequences like inhibition of enzymatic activities and breakdown of proteins & changed immunogenicity, which may be responsible for the severity of disease; hence, therapy aimed to reduce nitrosative modification of proteins leprosy. Result values of all parameters of the present study were expressed as mean ± SD and the statistical analysis was done by applying the z test and Tukeys test by Pearson’s correlation coefficient, correlations between the variables were estimated.

CONSENT

As per international standard or university standard, patient’s written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical clearance taken from Symbiosis Medical College for Women, Symbiosis International (Deemed University), Pune, Maharashtra, India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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