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**Original Research Article**

# Assessment of nitric oxide and uric acid in patients of leprosy

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## ABSTRACT

**Background:** Leprosy is an old, dreaded infectious disease caused by the obligate intracellular bacterium mycobacterium leprae. Leprosy still continues to be a significant public health problem in few countries including India. Oxidative stress caused by derangement in the balance between ROS and natural antioxidants plays a crucial role in the pathogenesis of leprosy. Hence this study attempts to assess the oxidative stress and antioxidant status in terms of Nitric oxide and uric acid.
**Methods:** A case control observational study was carried out in100 untreated leprosy patients and compared with 50 healthy controls. Leprosy patients were divided as paucibacillary and multibacillary. Serum Nitric oxide and uric acid levels were estimated in both groups to find out correlation of Nitric Oxide with uric acid.
**Results:** There was a significant rise in serum NO in both PB and MB leprosy as compared to controls. The uric acid level was significantly decreased in both PB and MB leprosy patients as compared to controls.
**Conclusions:** Elevated NO levels indicate oxidative stress in leprosy patients, denoting its crucial involvement in the pathogenesis and nerve damage in leprosy. Low uric acid indicates decrease defence of antioxidants in leprosy.

**Keywords:** Leprosy, Nitric oxide, Oxidative stress, Uric acid

## INTRODUCTION

Leprosy is one of the oldest diseases known to man. Despite advances in all spheres of medical science, it continues to be a public health problem in countries like India. India continues to account for 60% of new cases reported globally each year and is among the 22 global priority countries that contribute 95% of world numbers of leprosy warranting a sustained effort to bring the numbers down.[1]

Leprosy is a chronic infectious disease caused by mycobacterium leprae.[2] It affects mainly the peripheral nerves and also muscles, eyes, bones, internal organs and testes.[2] Oxidative Stress might play an indirect role in the etiology, complications and development of inflammatory episodes in leprosy. The current concept of "oxidative stress" include the pathways related to the "nitrosative stress" for their implication in cellular and extracellular metabolic events. In leprosy, the defense mechanism against mycobacterium bacilli has primordial participation of the macrophages, lymphocytes and its cytokines, regulating the production, release and modulation of diverse and important cellular immunity reactions.[3] NO, a product of macrophages activated by cytokines , microbial compounds or both, is derived from the amino acid L-arginine by the enzymatic activity of inducible nitric oxide synthase (iNOS or NOS2) and functions as an antimicrobial molecule.[4]

The NO and peroxynitrite produced by macrophages in skin lesions are shown to be involved in nerve damage in borderline leprosy patients.[5] Oxidation of proteins due to NO can lead to diverse functional consequences such as inhibition of enzymatic activities, proteolysis, and altered immunogenicity.[6]

Humans are well endowed with antioxidant defense mechanism against reactive oxygen species; among the antioxidants are vitamins A, C and E and enzymes such as superoxide dismutase and catalase. Recently Uric acid has also been added to this list.[7] Uric acid, the end product of purine catabolism is considered as the most abundant aqueous phase antioxidant in humans. Uric acid contributes to two thirds of the free radical scavenging capacity in plasma. In previous studies alterations in renal functional status in leprosy patients have been studied.[8,9] However reports on serum urate levels as an antioxidant are few and they are inconsistent.[10,11]

The objective of the present study was hence to assess the oxidative stress in leprosy patients by estimating levels of nitric oxide and uric acid in them.

## METHODS

The study was designed as a case control study comprising of 100 newly diagnosed cases of various types of leprosy and the control group consisted of 50 age and sex matched healthy individuals. The present study was carried out in the Department of Biochemistry in collaboration with Department of Skin - V.D. Department at Bhausaheb Sardesai Rural Hospital and MIMER Medical College Talegaon, Dabhade Pune from January 2011 to March 2013. The study was approved by Institutional Ethical Committee. The diagnosis was done on clinical grounds and bacterial examination by skin clip method. Patients were classified into two groups Paucibaillary (PB) and Multibacillary (MB) based on the WHO guidelines.[12] The patients were not taking any vitamins like tocopherol, ascorbic acid etc. Written Informed consent was taken from both patients as well as controls.

### *Inclusion criteria*

- *Cases:* Newly diagnosed leprosy patients in the age group 21-60 years.
- *Control:* Healthy controls in the age group 21-60 years.

### *Exclusion criteria*

The patients with history of smoking, diabetes mellitus, rheumatoid arthritis and other concomitant bacterial infections and major illness were excluded.

About 5 ml of fasting venous blood was collected with all aseptic precautions in plain bulb. Separated serum was used for measurement of Nitric Oxide and uric acid. Serum nitric oxide levels were assessed by kinetic cadmium granule reduction method.[13] Uric acid level was estimated by kit method.[14]

### *Statistical analysis*

The data was subjected to statistical Analysis of Variance (ANOVA). The biochemical data was expressed as mean±standard deviation. Significance was analyzed using student 't' test. Correlations between NO and uric acid were also calculated by Pearson Correlation test.

## RESULTS

Table 1 illustrates comparison of serum Nitric oxide in study groups. The present study shows significant rise in nitric oxide levels in both PB and MB groups of leprosy patients than in control individuals ($p<0.001$). It is observed that serum Nitric oxide in PB leprosy patients were significantly increased as compared to controls (59.22±11.14 vs 46.99±8.7 5 µmol/L, $p<0.0001$). Also serum Nitric oxide was significantly higher in MB leprosy patients as compared to controls (68.70±15.71 vs 46.99±8.7 5 µmol/L, $p<0.0001$). Multibacillary leprosy shows higher nitric oxide levels as compared to paucibacillary leprosy (68.70±15.71 vs59.22±11.1471µmol/L, $p <0.0001$).

There was significant decrease in uric acid levels in both groups PB and MB of leprosy patients than controls. It is observed that serum uric acid levels in PB leprosy patients were significantly decreased as compared to controls (4.53±0.69 vs 5.02±1.06mg/dl, $p<0.05$). Uric acid level was also significantly lower in MB leprosy patients as compared to controls (4.01±0.87 vs 5.02±1.06mg/dl, $p<0.0001$). There was statistically significant difference was seen in MB leprosy group as compared to PB leprosy patients (4.01 ±0.87 vs 4.53±0.69 mg/dl, $p<0.05$).

Table 2 shows Pearsons coefficient correlation (r) between Nitric oxide and Uric acid in leprosy. This study observed non significant correlation between NO and uric acid in PB leprosy patients. In MB leprosy patients there is significant negative correlation between NO and uric acid levels.

**Table 1: Illustrating Nitric Oxide and Uric acid levels in different types of leprosy and controls.**

| | (PB) (n=38) (Mean±SD) | (MB) (n =62) (Mean±SD) | Controls (50) (Mean±SD) |
|---|---|---|---|
| Nitric Oxide (µmol/L) | 59.22±11.14** | 68.70±15.71** | 46.99±8.75 |
| Uric acid (mg/dl) | 4.53±0.69* | 4.01 ±0.87** | 5.02±1.06 |

n = number of subjects
*=significant ($p <0.05$),
**= significant ($p <0.0001$)

**Table 2: Illustrating Pearson's Correlation coefficient (r) values between NO and Uric acid in leprosy patients group.**

| Correlation between NO (µmol/L) | (PB) | | (MB) | |
|---|---|---|---|---|
| | r value | p value | r value | p value |
| Uric acid (mg/dl) | -0.07 | 0.66 | -0.63* | <0.0001 |

*p<0.0001-significant

## DISCUSSION

Leprosy is a chronic infectious disease caused by mycobacterium leprae. Activated macrophages play an important role in host resistance to the development of clinical leprosy and limitation of growth of mycobacterium leprae.[15] Macrophages activated with bacterial stimuli can secrete a variety of cytokines including TNFα, IFN-β.[16] Activation of macrophages by proinflammatory cytokines such as IFN-γ is known to induce the production of NO through iNOS.[17] NO plays an important role in vasodilation, bacterial challenges and cytokine stimulation, neurotransmission and platelet aggregation. However, under pathological conditions Nitric Oxide has damaging effects. iNOS is closely related to pathophysiological characteristics of inflammatory diseases.[16]

The present study has estimated the NO metabolite levels in leprosy patients clinically classified in to PB and MB. There is a significant rise in NO levels in both groups as compared to controls. The rise is significant in MB leprosy patients than PB patients.

Though iNOS is induced during inflammatory responses, its increased expression is in localized TT skin lesions. The serum value also reflects metabolic stress of nitric oxide in the entire body and hence NO levels are presumably higher in multibacillary patients with chronic multiple lesions.[18] The study shows statistically significant rise in serum NO levels in paucibacillary leprosy patients as compared to controls which is in agreement with similar study by Garad et al. Highest rise was observed in MB group which is in agreement with other studies.[15,18-20] This is in contrast to the maximum localization of the iNOS enzyme in skin lesions of tuberculoid leprosy patients.[21]

Uric acid is particularly effective in scavenging hydroxyl, superoxide and peroxynitrite radicals, and it inhibits oxidation of protein, DNA and lipids.[22] Serum uric acid levels were significantly decreased in all leprosy groups compared to control group. The study observed statistically significant difference in uric acid levels in PB and MB leprosy patients. These finding corroborate with other authors who have seen decrease in uric acid.[10,11] The significant decreased uric acid levels in all leprosy groups (PB, MB) may be due to increase in nitric oxide formation which cannot be handled by the uric acid level. Uric acid is a strong peroxynitrite (ONOO-) scavenger but in leprosy patient's nitric oxide production is far more increased.

The findings of the previous study demonstrated low levels of Vitamin E and Vitamin C in leprosy patients.[23] These vitamins are known for their immune system stimulant action. MB leprosy patients have severely impaired cell mediated immunity which may be responsible for reduction in the concentration of non-enzymatic antioxidant vitamin E and C. This is further favored by finding of this study where uric acid is also low in serum leading to immunocompromised state found in MB leprosy. The low levels of antioxidants may expose the tissues to oxidative stress and could mediate inflammatory episodes, organ damage, depressed cell mediated immunity response and degeneration of nerves in leprosy patients.[2]

## CONCLUSION

In conclusion this study reports increased levels of nitric oxide in both types of leprosy group i.e. PB and MB. These findings partially correlate with the clinical status of patients with values being higher in MB leprosy patients. The decreased levels of serum uric acid may be due to inability to scavenge the increase production of peroxynitrite (ONOO-). Low antioxidant uric acid may support the involvement of oxidative stress in leprosy.

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