Increased level of urinary nitric oxide metabolites in leprosy patients during Type 2 reactions and decreased after antireactional therapy

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Summary

Objectives To assess the urinary nitric oxide metabolites in lepromatous patients in ENL (type 2 reactions) and to compare these metabolites after subsidence of reactions following antireactional therapy. Further to compare the levels in a group of lepromatous leprosy patients without reactions.

Design The initial urine samples were collected from lepromatous leprosy patients when they came with ENL before commencing antireactional therapy and repeat samples were taken after resolution of ENL. Morning urine samples were collected from LL patients without reactions. Nitrites and nitrates in urine were measured using commercially available kit. Mean levels of nitric oxide metabolites of LL patients with ENL and without ENL were compared by student’s ‘t’ test. The level during ENL and after resolution was compared by paired ‘t’ test.

Results The nitric oxide metabolites were analyzed in 14 LL patients with ENL and after resolution of ENL and in 5 LL patients without reaction. The level of urinary nitric oxide metabolite is higher in LL patients in ENL reaction compared to LL patients without reaction (P < 0.04). These levels were reduced significantly with resolution of reaction following antireactional therapy (P < 0.004).

Conclusion The findings of this study suggested that the NO/NOM excretion is increased in leprosy patients during ENL episodes. With antireactional therapy (steroids) and clinical improvement the levels are reduced.

Introduction

Leprosy patients often develop acute inflammatory responses. The overall prevalence of reaction has been reported to be around 30% from hospital-based data in India.1,2 Reactions may be responsible for nerve damage, disability and deformity in these patients. Type 2
reaction is a complication of borderline lepromatous (BL) and lepromatous leprosy (LL) patients and it appears to be associated with the formation of immune complexes. Therefore, exploring the underlying mechanism/mediators causing acute inflammation in leprosy reaction is essential for the effective management of patients in reaction.

Upon activation by cytokines following interaction with pathogens, phagocytic cells such as macrophages, neutrophils etc. produce nitric oxide (NO) and other reactive nitrogen intermediates (RNI) by the induction of nitric oxide synthase (iNOS). These RNIs modulate the immune response and are known to control inflammation.\(^3\) NO is highly unstable and is rapidly converted into nitrates (NO\(_3\)) and nitrites (NO\(_2\)).\(^4\) Association of nitric oxide metabolites (NOM) with type 1 reactions (RR) has been reported earlier.\(^5\) However, there is little information on NOM in ENL (type 2) reactions.

Patients with moderate to severe ENL reaction are treated with corticosteroids or thalidomide. Prednisolone treatment was found to be associated with the rapid decrease in urinary nitric oxide metabolites and clinical improvement in leprosy reactional patients mainly with RR.\(^6\) There is little information on urinary nitric oxide metabolites in patients with ENL reactions who are also treated with corticosteroids.

This study has been undertaken to estimate the urinary nitric oxide metabolites in leprosy patients in ENL reaction and after the subsidence of reaction.

**Materials and Methods**

Ethical permission for this study was taken from the Institute’s ethical committee, constituted as per the guidelines laid down by the Indian Council of Medical Research, New Delhi. Informed consent was taken from all patients before their inclusion in the study.

**PATIENTS**

Leprosy patients recruited for this study were classified according to the Ridley-Jopling classification\(^7\) with histopathological confirmation.

The case definition of ENL was: fever, crops of painful papulo nodular skin eruptions with or without other manifestations such as uveitis, arthritis, lymphadenitis, dactylitis.

Resolution of ENL was defined as patients being Afebrile, stoppage of appearance of new lesions, regression of older ones, absence of pain in the nerves and regression of other manifestations.

**Collection of urine samples from patients** The initial urine sample was collected when patients came with ENL (type 2 reaction) before commencing anti-reactional therapy. Repeat urine samples were taken after the resolution of ENL. Morning urine samples were collected from other leprosy patients without reactions. These samples were stored in a deep freezer at −20°C till further processing.

**Assay for estimation of nitric oxide metabolites in urine** Nitrites and nitrates in the urine were measured using a commercially available kit (Roche, Germany) and following the instructions provided in this. The standard curve was plotted for various concentrations of nitric oxide standards. Concentration of nitrites and nitrates were extrapolated from this
standard curve. Then the final concentration was calculated by multiplying with the dilution factor.

Statistical analysis A paired ‘t’ test was performed to compare the mean concentration of nitric oxide metabolites in the urine of leprosy patients with ENL reaction and after subsidence of that reaction. The mean concentration of nitric oxide metabolites in these groups were compared by student’s ‘t’ test.

Results

Fourteen leprosy patients with type 2 reaction (ENL) and five LL patients who were free from any reaction, were included in this study. All patients were receiving WHO multi-drug therapy i.e. multibacillary (MB) combination consisting of monthly rifampicin and clofazimine and daily clofazimine and dapsone.

All the patients with ENL had active lepromatous leprosy with nerve involvement. All were skin-smear positive for acid-fast bacilli, having bacillary index (BI) either 3+ or 4+. These patients had fever, crops of painful papulo nodular skin eruptions with or without other manifestations such as uveitis, arthritis, lymphadenitis, dactylitis etc. All reactional patients were admitted to the Institute-Hospital and treated with anti-reactional therapy mainly corticosteroids in doses of 20–40 mg prednisolone equivalent/day with the addition of aspirin, as and when required in varied dosages and duration. It took 3 to 24 (mean 10) days for the reaction to subside.

CONCENTRATION OF URINARY NITRIC OXIDE METABOLITES IN LEPROSY PATIENTS

The mean level of urinary nitric oxide metabolites is much higher in patients with ENL reaction (Mean±SD=15 620µM ± 8464) compared to leprosy patients without reaction (Mean±SD=6471µM ± 4476), (P < 0.04). The level of urinary nitric oxide metabolites in each patient and their mean levels are shown in Figure 1.

Following the subsidence of reaction (with mean 10 days of corticosteroid treatment) the levels of NOM (Mean±SD=5586µM ± 6992) were found to have reduced significantly as compared to the active reaction state (P < 0.004). The level in each patient during ENL and after resolution is shown in Figure 2.

Discussion

We observed a higher level of urinary nitric oxide metabolites in leprosy patients while they were in ENL reactions. These levels came down with resolution of the reaction similar to non-reactional leprosy patients. This finding was based on 14 leprosy patients who were suffering from moderate to severe ENL needing prednisolone and, for initial comfort, aspirin. The time taken for the reaction to subside varied from 3–24 days (mean = 10 days). Higher NOM level observed during ENL are probably due to reactional pathology. Similar findings of a higher level of urinary nitric oxide metabolites during reversal reaction (type 1) which went down with a high dose of prednisolone therapy with clinical improvement supports reaction pathology being related to raised NOM levels. The above group of workers also...
looked at NOM levels in four ENL patients. Their observation in three patients likewise indicates not only higher levels in ENL, but also a slight decrease in the level of nitric oxide metabolites with the resolution of reactions with prednisolone therapy. Our observation in 14 patients with ENL reaction supports their observations. In addition, Rada et al. have reported an increased level of nitric oxide concentrations in serum and mononuclear cell culture from patients with type 2 reactions compared to stable polar forms of leprosy patients. One of the reasons for this increased level could be due to up regulation or increase of the proinflammatory cytokines as has been observed in both ENL and RR reactions. These cytokines are reported to increase the expression of NOS 2 thus increasing the nitric oxide metabolites.

Figure 1. Nitric oxide metabolites in µM measured by colorimetric method. Horizontal bars show the mean NOM level in each group. LL patients with ENL reactions {ENL (+)} (n = 14), LL patients after regression of reaction {ENL (−)}, Lepromatous leprosy patients without reaction (LC) (n = 5).

Figure 2. Nitric oxide metabolites in µM measured by colorimetric method. The level of urinary NOM in patients in ENL is shown as ENL (+) (n = 14) and after resolution of reaction ENL (−) (n = 14).
Our observations indicate that the nitric oxide level comes down with the subsidence of reaction. Whether this is due to the drug (prednisolone) or the control of the reaction (ENL) in most patients is not clear. Glucocorticoids are known to exert anti-inflammatory actions partially by inhibiting the activation of transcription factors such as NF-κB which regulate many genes including genes for cytokines and iNOS. Following prednisolone treatment a decrease in expression of cytokines and iNOS was observed in leprosy skin lesions. Further, it was investigated that the drug prednisolone does not ‘switch off’ the cytokine response effectively in leprosy patients with type 1 reaction. Thus the drug (prednisolone) may be directly responsible for the return of nitric oxide metabolites to normal levels, apparently by inhibiting iNOS production and not through suppression of pro-inflammatory cytokines.

The findings from the present study suggested that the NO/NOM excretion is increased in leprosy patients during ENL episodes. With antireactional therapy (steroids) and clinical improvement the levels are reduced.

Many important questions remain to be addressed as to why and when this nitric oxide level increases during the course of the disease. A careful follow-up study is required to explore if this level can be used as a prognostic marker for ENL reaction.

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References

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