N-acetylcysteine as an add-on to Directly Observed Therapy Short-I therapy in fresh pulmonary tuberculosis patients: A randomized, placebo-controlled, double-blinded study

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Abstract

Purpose: Pulmonary tuberculosis is associated with increased oxidative stress, enhanced lipid peroxidation, and decreased glutathione (GSH) levels. N-acetylcysteine (NAC) effectively increases GSH levels, improves lipid peroxidation, and decreases reactive oxygen species levels as reported by earlier studies. Hence, we planned to clinically evaluate the effect of NAC as add-on to Directly Observed Therapy Short-I (DOTS-I) regimen on treatment outcome in PTB with the objectives to study the effect of NAC as an add-on to intensive phase of DOTS-I (2 months) on sputum conversion, radiological improvement, GSH peroxidase (GPx) level, and weight and immunological response compared to placebo add-on at the end of 2 and 6 months.

Materials and Methods: This was a design-prospective, randomized, parallel group, add-on design, placebo-controlled, double-blinded, 24-week study. Parameters studied were sputum acid-fast bacillus examination, radiological improvements, GPx level, weight, and Mantoux response. NAC/placebo was added to DOTS Category I in intensive phase.

Results: Totally 48 patients completed the study. In NAC group, 23 patients achieved sputum negativity in 3 weeks while 14 patients in PLACEBO group. There was a significant clearing of infiltration and reduction in cavity size in NAC group compared to placebo at 2 months. At 2 and 6 months, NAC significantly raised GPx level and body weight. In 2 months, the patients with Mix ≤5 became Mx positive (100%) in NAC group while none in placebo group.

Conclusion: NAC addition to DOTS-I significantly brings about faster sputum negativity, improves radiological response, weight, raises serum GPx level, and rectifies the deregulated immune response. Thus, NAC may be a useful adjuvant to DOTS in PTB.

Keywords: Glutathione peroxidase, n-acetylcysteine, pulmonary tuberculosis, sputum conversion

INTRODUCTION

Pulmonary tuberculosis (PTB) is associated with increased oxidative stress (OS), enhanced lipid peroxidation, and decreased glutathione (GSH) levels.1-4 There is substantial evidence which demonstrates the role of...
GSH in T-cell activation, phagocytosis, and Th1/Th2 response balancing. Previous studies documented that N-acetylcysteine (NAC) effectively increases GSH levels. Very few clinical studies demonstrated the antioxidant and immunoregulatory effects of NAC. Hence, the present study was planned to evaluate the effect of NAC as add-on to Directly Observed Therapy Short (DOTS) Category I regimen on treatment outcome in PTB with the following objectives.

- To study the effect of NAC (add-on to DOTS Category I regimen) on sputum conversion, radiological improvement, and glutathione peroxidase (GPx) level compared to placebo (add-on to DOTS Category I regimen) at the end of 2 and 6 months
- To study the effect of NAC (add-on to DOTS Category I regimen) on weight and immunological response compared to placebo.

MATERIALS AND METHODS

Study design
A prospective, randomized, parallel group, add-on design, placebo-controlled, double-blinded study was conducted in the Department of Pharmacology in collaboration with the Department of TB and Chest, at a tertiary care teaching institute for 1½ years from July 2007 to December 2008. Totally 67 participants with newly diagnosed sputum-positive PTB were enrolled in the study after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from all participants after explaining detailed information about the study protocol. Patients were randomized into Group A or Group B using a computer-generated randomization table. Either gender between 18 and 60 years with newly diagnosed PTB with 3 sputum specimen-positive for acid-fast bacilli by direct microscopy were included in study. Patients with miliary TB and extra-PTB, allergic diathesis, hepatic and renal dysfunction, pregnant and lactating women, and patients exposed to antioxidant supplementation in the last 2 months were excluded from the study. Both the groups received standard anti-TB treatment as per DOTS Category I. In addition, Group A received two tablets from packet X daily and Group B received two tablets from packet Y for 2 months. Patients were asked to take the study drugs for the first 2 months only and then follow-up was done for the next 4 months. During the first 2 months of the study, the patients were asked to follow up every week for clinical examination, investigations, and to collect drugs. Unblinding of the study drugs was done at the end of the last follow-up of the last patient. It was identified that packets labeled as “X” were containing N-acetylcysteine 600 mg supplied by Healers Nutraceuticals Pvt. Limited, Chennai (India) whereas packets labeled as “Y” were having placebo. The mandatory registration of the Clinical Trial Registry of India was started from December 15, 2009. Hence, the study was not registered.

Methods
- Bacteriological examination - This was a primary outcome measure done by Ziehl–Neelsen staining technique. Sputum examination was done weekly for 2 months and then monthly until the completion of treatment
- Radiological examination - This was a secondary outcome measure by X-ray chest posteroanterior view. Patients were classified as cases of minimal (M), moderate advanced, and far advanced (FA) disease, according to radiological classification given by the American Thoracic Society. In patients with cavity, the total area of exposed cavity wall was calculated from the radii of visible cavities (πr²)
- Immunological examination - This was a secondary outcome interest done by tuberculin skin test (Mantoux test [Mx]) for illumining delayed hypersensitivity. Purified protein derivative RT-23 Tween-80 with a dose of 1TU (0.1 ml) was injected intradermally in volar area of the forearm. Reactions were read after 72 h by measuring the transverse diameter of the induration to the nearest millimeter. Erythema is not taken into account. Induration of diameter ≥10 mm – positive, ≤5 mm – negative, and 6–9 mm – equivocal.
- Blood glutathione peroxidase level - It was determined by a commercially available kit (Randox Labs Ltd., Antrim, UK), based on the method of Paglia and Valentine on Semi-Auto analyzer (TRANSASIA, ERBA, CHEM-5-PLUS). Normal range obtained from laboratory was 2000–3000 U/L.

Sample size calculation
Considering the conventional antitubercular therapy to be causing bacteriological conversion at 3 weeks approximately 21%, the newer add-on therapy of NAC causing the same conversion of the magnitude of 45% (clinically relevant) sample size turned out to be 62 (α = 0.05, power = 80%).

Statistical analysis
Data were analyzed using GraphPad prism version 5.1. Mean values of change in radiological lesions and blood GPx levels were compared between two groups by using unpaired t-test and in the groups by Paired t-test. Chi-square test was applied to find significant improvement
in sputum conversion and delayed type hypersensitivity response by Mx test. Correlation test was used to identify any correlation between radiological extent of disease and GPx level at 2 months. $P < 0.05$ was considered statistically significant in all analyses.

### RESULTS

Out of 67 patients enrolled, 24 patients completed the study in each group [Figure 1]. After unblinding, Group A was NAC add-on whereas Group B was placebo add-on. In Group A, M:F ratio was 13:11. While in Group B, M:F ratio was 14:10. Mean age of the patients in Groups A and B was 31.96 ± 13.14 and 29.79 ± 11.12 years, respectively. There was statistically significant weight gain ($P < 0.001$) in Group A compared to Group B at 2 months and 6 months [Table 1].

- **Bacteriological response** - In Group A, 23 (95.83%) patients achieved sputum negativity in 3 weeks while 14 (58.35%) patients in Group B ($P = 0.0019$) [Figure 2]

- **Radiological response** - There was a statistically significant clearing of infiltration in Group A (21 [87.5%]) compared to Group B (8 [33.33%]) at 2 months ($P < 0.05$) [Table 2]. Reduction in cavity size was observed at the end of 2 ($P < 0.001$) and 6 months ($P < 0.01$) compared to baseline in Group A and at 6 months in Group B ($P < 0.01$). Improvement in Group A was more ($P < 0.05$) than in Group B [Table 3]

- **Glutathione peroxidase levels** - At the end of 2 and 6 months [Table 3], Group A showed raised GPx level (U/L) ($P < 0.001$) compared to Group B. No significant positive correlation was found between GPx level and radiological improvement. Delayed type hypersensitivity response - In 2 months, all five patients with Mx ≤5 became Mx positive in Group A while none with Mx ≤5 converted to Mx positive in Group B ($P = 0.003$) [Table 4].

### DISCUSSION

Clinical study of NAC add-on in tuberculosis patients is lacking and hence no comparator is available. However, supportive studies are available confirming the role of NAC in various cellular defense mechanisms. The early sputum negativity seen in the NAC supplemented patients (95.83%)...
at the end of 3 weeks is a highly significant finding compared to (58.33%) placebo add-on to DOTS Category I regimen. Previously, Bawri et al. found 71% sputum negativity after 4 weeks and 84% in 2 months with DOTS I therapy.[16] Parikh et al. reported 84.5% conversion at 35 days with DOTS I.[17] Thus, NAC has accentuated the sputum conversion as add-on compared to placebo as well as previous reports. Karyadi et al. reported early sputum conversion (P < 0.05) with supplementation of Vitamin A and zinc to antituberculosis treatment.[18] Morris et al. demonstrated a pattern of OS brought on by Mycobacterium tuberculosis infection, which decreases GSH and impairs the intracellular killing of \textit{M. tuberculosis} in neutrophils. However, NAC addition to the culture of \textit{M. tuberculosis} infected neutrophils caused their killing with an elevation of GSH levels.[19] Thus, early sputum negativity achieved by NAC could be because of augmented GSH level in macrophages forming S-nitrosoglutathione which may have produced its direct toxicity on bacteria and also decrease in TH2 cytokine response favoring the host immune cells to control the replication of bacteria. Dalvi et al. showed that in tuberculosis patients, the marked reduction in GSH concentration and the greater susceptibility of erythrocytes for lipid peroxidation and hemolysis suggest the occurrence of oxidant stress.[20] Thus, NAC appears to be helpful by reversing antioxidant stress by elevation of GSH level.

In our study, the radiological improvement (87.5% in NAC group compared to 33.33% in placebo group) in clearing of infiltration and also reduction in cavity size till the end of 2 months showed effective add-on response to NAC. This observation is in accordance with Karyadi et al. who used Vitamin A and zinc supplementation to antitubercular therapy.[18] This improvement can be attributed to the suppression of the TH2 response, i.e., increased production of interleukin 4 (IL-4), IL-6, tumor necrosis factor-alpha (TNF-\(\alpha\)), IL-4-induced immunoglobulin IgE, and downregulation of interferon \(\gamma\). NAC by reversing this TH2 overactivity causes downregulation of IL-4 and upregulation of interferon \(\gamma\), leading to T-helper cell Type 1 polarization. This arrests the destructive phase of cavitation and also infiltration. In our study, we found one interesting finding that two patients in NAC add-on group had 24 mm and 22 mm Mx response, respectively, with FA cavitation tuberculosis. At the end of 2 months, they showed Mx response of 13 mm and 8 mm, respectively, with reduction in the cavitation.

We also observed elevated GPx level in the NAC-treated patients. Similar rise in GPx level along with decreased blood levels of reactive oxygen species (ROS), serum level of IL6, and TNF-\(\alpha\) is reported with a dose of 1800 mg/day i.v. for 10 days by Mantovani et al.[21] Lubos et al. have proved that increased expression of GPx-1 is protective against many apoptotic stimuli.[22] Enhancement of GPx activity in \textit{MTb} infected monocyte culture after the addition of NAC indicates reversal of impaired neutralizing mechanisms. In addition, enhanced GPx activity is correlated inversely with the downregulation of TNF-\(\alpha\) mRNA expression and ROS in monocyte infected with \textit{MTb} as reported by Hasan et al.[23] This mechanism can explain the underlying cause for early sputum conversion in the NAC-treated patients. However, we could not establish the definite correlation between patients’ GPx level and their radiological extent of disease. Kaur et al. observed that decreased antioxidant activity in patients of PTb correlates with radiological extent, sputum grading, and cavity status.[24]

The improvement in the weight gain and correction in other associated symptoms such as fever, loss of appetite, and fatigability observed in our study can be correlated with reduction in the level of TNF-\(\alpha\) and IL-6 by NAC.

The immune-competence status of patients in our study was assessed by Mx test. It is a classical delayed type hypersensitivity (DTH) response, which is a marker of TH1 immunity. A loss of DTH response serves as an indicator of deterioration in cell-mediated immune function.[25-27] In our study, the NAC-treated patients showed a significant improvement in the DTH response by conversion of anergy to Mx positive status, thus improved the cellular immunity in tuberculosis patients.
Strength of the study
• This is a double-blinded study
• This is the only study with NAC where sputum conversion parameter is applied.

Limitations of the study
• This is a single-center study
• Glutathione level could not be estimated which is a direct parameter of NAC effect.

Future prospects
A multicentric study with larger population sample can reveal better idea about the efficacy of NAC as an adjuvant to antituberculosis regimen.

CONCLUSION
Significant early negativity of sputum with complementing radiological clearing and immunological response along with weight gain and improved antioxidant status was observed in NAC-supplemented patients. Thus, N-acetylcysteine may be useful as add-on to DOTS Category I regimen in newly diagnosed sputum-positive PTB patients.

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Conflicts of interest
There are no conflicts of interest.

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