# Study Of Malondialdehyde As An Indicator Of Oxidative Stress And Its Modulation By N-Acetylcysteine In Chronic Obstructive Pulmonary Disease

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## ABSTRACT

**Background & objectives:** Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease characterized by systemic and local inflammation and oxidative stress. In view of the lack of therapy that might inhibit the progress of the disease, there is an urgent need for a successful therapeutic approach. This study aims to evaluate the effects of administration of oral N-acetylcysteine (NAC) on the oxidative stress and respiratory symptoms and also to check the safety of oral NAC. **Methods:** This randomized, double blind study was conducted in sixty-eight adult patients diagnosed as having COPD. All patients received standard treatment for COPD and were divided into three groups. Group I received placebo, Group II and Group III received NAC 600 mg once daily (OD) and twice daily (BID) dose respectively for two months. Efficacy parameters included assessment of serum malondialdehyde (MDA) and respiratory symptoms. Adverse drug reactions were monitored as a safety measure. **Results:** Mean serum MDA levels showed significant fall in group-II (p=0.001) and group-III (P=0.023) as compared to group-I (P=0.147) and improvement in respiratory symptoms was noticeable in group-III followed by group-II from day 0 to day 60. Nausea and stomatitis were the most common adverse drug reactions experienced by the patients in all the three groups. **Interpretation & conclusions:** Maximum decline in oxidative stress was with OD dose followed by BID dose which indicates that antioxidants above a certain limit may not be much beneficial. Improvement in symptoms was seen maximum with BID dose followed by OD dose. This was due to mucolytic and immunomodulating properties of NAC besides its antioxidant role. Minor adverse drug reactions confirm the safety of NAC with both the doses. This confers a new facet in management of COPD and an attempt to impede the disease progression.

**Key Words:** Chronic obstructive pulmonary disease, N-acetylcysteine, Oxidative stress

**Key Messages:**
- N-acetylcysteine has a definite role in reducing oxidative stress in patients of COPD
- N-acetylcysteine also plays important role in reducing respiratory symptoms in COPD patients.
- N-acetylcysteine is a very safe drug with negligible adverse reactions and can be used in COPD patients.

## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the most common respiratory illness in the elderly and is the fourth leading cause of death globally [1]. The prevalence of COPD is higher in countries where smoking is highly prevalent. In India, there is an increasing tendency to abuse tobacco and COPD is emerging to be a major public health problem with significant mortality and morbidity. According to the estimate of global burden of disease study, by the year 2020, COPD is likely to become the fifth leading cause of disability-adjusted life years (DALYs), moving ahead from twelfth position it occupied in 1990 and third most common cause of death worldwide. Its prevalence is estimated to increase in years to come in India [2].

COPD has been defined by global initiative of chronic obstructive lung disease (GOLD), as a state characterized by airflow limitation that is not fully reversible.

There is considerable evidence that an increased oxidative burden occurs in the lungs of patients with COPD which plays a role in its pathogenesis. The lungs are continuously exposed to oxidants generated either endogenously or exogenously. Cigarette smoke, consisting of the gas phase and particulate phase (tar phase) is thought to contain 1017 oxidant molecules per puff [3]. The inflammation in stable COPD patients is dominated by macrophages, CD8+ T lymphocytes and neutrophils. Chemical mediators like interleukins, leukotrienes and tumor necrosis factor attract the inflammatory cells and increase the oxidant production. Oxidants cannot only damage DNA, lipids and proteins, but also mediate a variety of processes that could foster the development of COPD e.g. increased production of mucus and impairment of cilia function. This might lead to pulmonary damage of greater magnitude and progression of the disease [4].

Antioxidant therapy therefore would seem to be a logical therapeutic approach in COPD, apart from standard pharmacological treatment to combat the oxidative stress [5].

The challenges in developing novel therapies include limited understanding of the molecular pathogenesis of COPD [6]. Several agents with antioxidant properties such as Vitamin C, Vitamin E and beta-carotene have been tried in patients with COPD, but the evidence is still not consistent. N-acetylcysteine (NAC) is the most widely investigated drug with antioxidant properties that has been used in both experimental and clinical settings which are relevant to COPD [4]. NAC is the acetylated precursor of both amino acid L-cysteine and reduced glutathione (GSH). This antioxidant has been applied in patients with COPD in order to reduce symptoms, exacerbations and the accelerated decline in lung function. In addition to this antioxidant action, NAC exhibits mucolytic

properties by destroying the disulphide bridges of the mucoprotein macromolecules.

There is need for a successful therapeutic approach that might inhibit the progression of the disease. Considering these facts, this study was conducted to throw light on the effects of oral administration of NAC in patients with COPD in relation to oxidative stress and respiratory health hazards.

## MATERIALS AND METHODS

This randomized, double blind, placebo controlled, parallel group, single centered study was carried out at Jawaharlal Nehru Medical College, Sawangi, Wardha. Recruitment of patients was started in the month of November 2008 and study was completed by the end of May 2009. Clearance from institutional ethical committee was obtained.

A questionnaire was designed to obtain information on demographic data, occupational status and respiratory complaints. Consent was taken. The smoking history was measured by the unit of "pack-year" which was defined as smoking of a pack of cigarettes (20 pieces) per day for one year. The number of pack years was calculated as: [(the number of cigarettes smoked per day/20) × (the number of years smoked)] [2]. Randomization was done by a randomization plan obtained from Random Allocation software, Rando [7].

At baseline, patients were evaluated for respiratory symptoms, chest X- Ray, and serum malondialdehyde (MDA). Patients were then divided into three groups, and respective treatment was started after which, they were followed up to obtain information on symptomatic improvement (after 60 days) and monitoring of adverse drug effects (after 15, 30 and 60 days) and serum MDA estimation was done again at the end of 30 and 60 days.

Total of 68 patients diagnosed as COPD were divided into three groups and received following drugs.

**Group I:**    Control n=23
            (Receiving standard treatment for COPD)

**Group II:**   NAC 600 mg OD n=22
            (In addition to the standard treatment for COPD)

**Group III:**   NAC 600 mg BID n=23
            (In addition to the standard treatment for COPD)

Drugs and placebos were provided by Cipla Limited, Mumbai and were in the form of effervescent tablets to be taken orally twice a day, for a period of two months. Group I received placebo in BID dose, Group II received NAC 600 mg & placebo both in OD doses, whereas Group III received NAC 600 mg in BID dose.

Drug (NAC) / Placebo was randomly given to each group from boxes containing either, unknown to the investigator and to the patients.

## INCLUSION CRITERIA:

1. Patients diagnosed as COPD, between ages 35-80 yrs of either sex.
2. Patients who understand and are willing to fill the questionnaire regularly.
3. Informed written consent taken prior to the start of the study.

## EXCLUSION CRITERIA:

1. Any decompensated cardiovascular, endocrine, hepatic or renal function.
2. Parenchymal lung pathology or active infection due to *Mycobacterium tuberculosis.*
3. H/O allergy to NAC.

4. Clinically proven gastric or duodenal ulcer in the previous six months.
5. Participation in any clinical trial in the past six months.
6. Pregnancy / Lactation.

## SERUM MDA ESTIMATION

Serum MDA estimation was done on day 0, 30 and 60 i.e. prior to the administration of drug (NAC or Placebo), and subsequently repeated twice at one-monthly intervals after initiating the drug treatment. Blood samples were collected under all aseptic precautions using disposable syringes, in non-heparinized bulbs and serum was separated.

Lipid peroxidation end product MDA was estimated by Thiobarbituric Acid Reactive Substances (TBARS) method, as described by Ohkawa et al [8].

The detailed data was analyzed statistically by One-way ANOVA and Paired t-test, using SPSS version 13.0.

### Results:

Serum MDA levels showed significant difference in mean values from baseline (0 day) to post-treatment (60 days), in group II (p=0.001) and group III (p=0.023). No significant decrease in mean serum MDA levels was demonstrated in group I (p=0.147). [Table/Fig 1]. The observations are also depicted in [Table/Fig 2].

| Group | Day 0 Mean ± SD (nmol/ml) | Day 30 Mean ± SD (nmol/ml) | Day 60 Mean ± SD (nmol/ml) | Change from Baseline Day60 – Day 0 Mean Diff (95%CI) | p value |
|---|---|---|---|---|---|
| I | 4.25 ± 0.6 | 3.93 ± 0.57 | 4.01 ± 0.52 | -0.241 (-0.51, 0.03) | 0.147 |
| II | 5.43 ± 1.04 | 4.58 ± 1.27 | 4.15 ± 0.86 | -1.277 (-1.84, -0.70) | 0.001† |
| III | 5.30 ± 0.97 | 4.69 ± 1.19 | 4.40 ± 1.14 | -0.9 (-1.26, 0.13) | 0.023* |

**Table 1:** Effect of different treatment modules on serum MDA (nmol/ml) levels from baseline (Day 0) to post-treatment (Day 60).

\* Significant at $p < 0.05$, † Significant at $p < 0.001$
p value was calculated by using One-way ANOVA
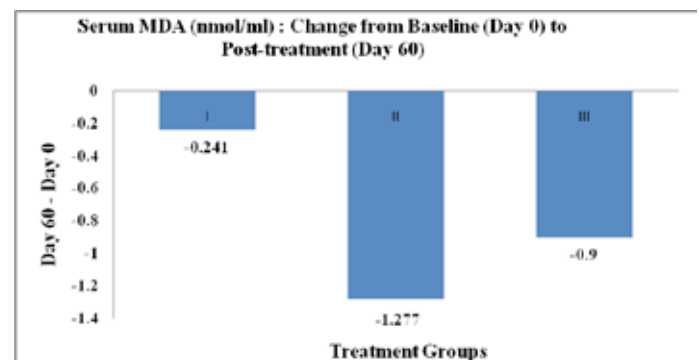


[Table/Fig 2]: Change in serum MDA levels (nmol/ml) from day 0 to day 60 in group I, II and III

Patients were assessed for respiratory symptoms at the baseline and their improvement, if any, post-treatment. It was observed that all the groups showed improvement in symptoms like cough, sputum production and expectoration, dyspnoea, throat irritation and chest tightness. In group II and group III there was noticeable improvement in all the respiratory symptoms from day 0 to day 60. Maximum improvement in respiratory symptoms was seen in group III. In group I there was no improvement in cough, throat irritation

| Symptom | Group I (n=23) | | Group II (n=22) | | Group III (n=23) | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 60 | Day 0 | Day 60 | Day 0 | Day 60 |
| Cough | 11(48) | 11(48) | 14(64) | 10(45) | 13(56) | 4(17) |
| Sputum production | 14(61) | 13(57) | 17(77) | 4(18) | 16(70) | 2(9) |
| Dyspnoea | 7(30) | 6(26) | 11(50) | 8(36) | 10(43) | 4(17) |
| Throat Irritation | 4 (17) | 4(17) | 16(73) | 2(9) | 14(61) | 0(0) |
| Chest Tightness | 3(13) | 3(13) | 1(5) | 1(5) | 2(9) | 1(4) |

[Table/Fig 3]: Symptom score in group I, II and III.

Values in the parentheses indicate the percentage of patients
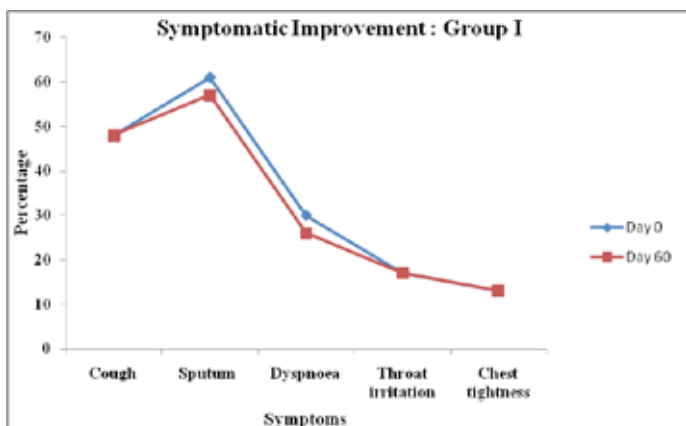


[Table/Fig 4]: Symptomatic improvement in group I (Placebo controlled group).

and chest tightness from day 0 to day 60, while little improvement was seen in sputum production and dyspnoea. The observations are illustrated in [Table/Fig 3]. The results are also depicted in [Table/Fig 4, 5 and 6] for group I, II and III respectively.
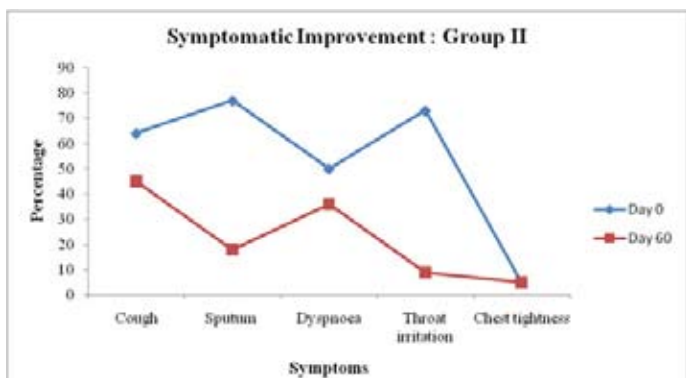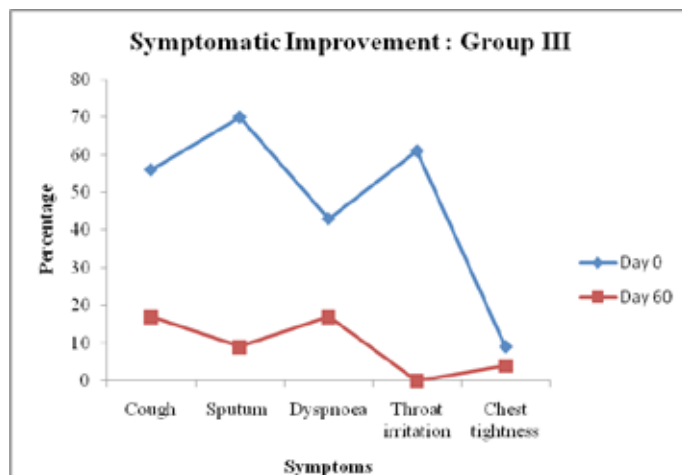


[Table/Fig 5]: Symptomatic improvement in group II (NAC OD group)

Adverse drug reactions were monitored as a safety measure. Nausea and stomatitis were the most common adverse reactions experienced by the patients in all the three groups.

## DISCUSSION

Increased oxidative stress may play a role in enhancing the inflammatory response and it is now recognized as the main pathogenic factor for progression and increasing the severity of COPD [9]. A significant amount of oxidative stress is caused by the household use of solid biomass fuels, especially in rural settings in developing countries.

Domestic pollutants like particulate matter, carbon monoxide, polycyclic aromatic hydrocarbons cause bronchial irritation and



[Table/Fig 6]: Symptomatic improvement in group III (NAC BID group)

decreased mucociliary clearance. Around 50% of women suffer from COPD who are non-smokers and can be attributed predominantly to biomass fuel [10]. The importance of the underlying local and systemic oxidative stress and inflammation in COPD has long been established.

The study groups treated with NAC 600 mg OD and 600 mg BID showed a significant decrease in serum MDA levels from baseline to post-treatment levels with maximum decline in group II. [Table/Fig 1, 2]. High pre-treatment levels of MDA (nmol/ml) in all the groups (4.25±0.6, 5.43±1.04 and 5.30±0.97) [Table/Fig 1] indicate the presence of oxidative stress in these patients, and serves as a biomarker of lipid peroxidation.

NAC exhibits direct and indirect antioxidant properties. Its free thiol group is capable of interacting with the electrophile groups of reactive oxygen species (ROS). Its indirect effect is attributed to its role as a glutathione precursor. NAC has also been shown to increase the protective markers for oxidative stress such as glutathione peroxidase and total antioxidant capacity and thus reducing the MDA levels [11]-[12].

The clinical efficacy of NAC in COPD patients in our study was also evaluated by studying the symptomatic improvement at the end of two months. All the three groups showed improvement in symptoms like cough, sputum production and expectoration, dyspnoea, throat irritation and chest tightness at the end of two months [Table / Fig 3]. Improvement in symptoms in group II (NAC 600 mg OD) and group III (NAC 600 mg BID) were noticeable as compared to group I. Treatment with NAC causes symptomatic improvement in COPD patients, reflected by the decrease in sputum viscosity and purulence and improved sputum expectoration. NAC also has a mucolytic effect which helps in reducing the exacerbations and the days of illness [13]. In animal models of chronic bronchitis, oral NAC inhibits smoke induced goblet cell hyperplasia and also improves mucociliary clearance [14]. Thus NAC has been shown to improve clinical symptoms, decrease exacerbations and hospital admissions and improve the lung function parameters in COPD. Thus reducing exacerbations indirectly reduced the morbidity and healthcare costs associated with progressive disease.

The efficacy and tolerability of high dose NAC in treatment of patients with COPD was well studied by Zuin et al [13]. In our study both the doses (600 mg OD and 600 mg BID) of NAC had a good outcome on the oxidative stress, and other clinical symptoms like cough intensity and frequency.

NAC also reduced the bacterial adherence to mucosal and epithelial cell structures of the airways. This action contributes to the reduction in infectious exacerbations in patients with chronic bronchitis [15].

NAC is also effective regarding the parameters related to bronchial hypersecretion [16]. These patterns suggest that the protective effects of this thiol do not merely depend on its mucolytic action but are to be additionally ascribed to other mechanisms such as antioxidant and immuno-modulating, comprehensively termed as broad spectrum protection. Due to these multiple properties of NAC, group III shows maximum decline in respiratory symptoms.

Adverse drug reactions were monitored in our study as a safety measure. It was observed that, nausea and stomatitis were the most common adverse reactions experienced by the patients in all the three groups. Fever, drowsiness, diarrhea and rhinorrhea were other minor adverse effects noted. The adverse drug reactions of NAC reported in literature have been related to high doses or polypharmacy [17]. It is documented that NAC is well tolerated. Mild effects like nausea and vomiting may be observed, urticaria and bronchospasm being extremely rare.

Intravenous infusion of NAC has caused hypotension by decreasing the systemic vascular resistance, but can be treated symptomatically [18]. Anaphylactoid reactions, including rash, flushing, urticaria, bronchospasm and angioedema, mostly associated with very high doses of NAC (more than ten times the dose used in this study) have been described in adults [19]. Thus, the use of intravenous NAC is reserved for grave conditions like paracetamol intoxication and acute myocardial infarction, to list a few.

The findings in our study indicate the important role played by NAC in combating the oxidative stress in COPD and improvement of various respiratory symptoms, at well tolerated doses. Thus, it would be relevant clinically to consider NAC as a novel therapeutic approach in the management of COPD, as add-on therapy to the standard treatment.

Nevertheless, the treatment duration in our study was 2 months and since COPD requires treatment for long duration, it would be worthwhile to conduct a study with prolonged treatment with NAC, involving a larger sample size. This approach would help to further evaluate the clinical efficacy and safety of NAC. Cost-effectiveness analysis also may be proposed by monitoring duration of disease free intervals, saved days of absence from work and cost of concomitant medications. It would be worthwhile to evaluate other parameters of oxidative stress like exhaled CO, spirometry, superoxide dismutase and glutathione levels in the future.

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