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Research Article

Effects of administration of oral n-acetylcysteine on oxidative stress in chronic obstructive pulmonary disease patients in rural population

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) is a common pulmonary disease and the fourth leading cause of death globally. Oxidative stress is an important attribute in the pathogenesis of COPD. Targeting oxidative stress would be a logical therapeutic approach for COPD and glutathione precursors like N-acetylcysteine (NAC) have shown therapeutic promise in the treatment of this chronic pathology. This study attempts to determine the dose related effects of NAC on the oxidative stress, its safety and efficacy in COPD patients.
Methods: A randomized, double blind, placebo controlled, parallel group, single centred study, and was carried out in rural set-up. Sixty eight diagnosed cases of COPD according to GOLD criteria (global initiative for chronic obstructive lung disease), were recruited in the study, following approval from the ethics committee. The patients were randomized to three treatment arms (placebo, NAC 600 mg once a day (OD) and NAC 600 mg bis a day (BID). The patients were monitored for incidence and severity of adverse effects as a measure of safety. Efficacy of NAC was determined based on symptom improvement, pulmonary function, oxygen saturation and serum malondialdehyde (MDA) concentration.
Results: Results indicate a significant improvement in the efficacy parameters in the group that received higher dose of NAC. NAC was well tolerated by all the study subjects. Addition of NAC to the standard treatment of COPD exhibits beneficial effects in disease exacerbations, symptom improvement and a decline in oxidative stress parameters, reinforcing the antioxidant, anti-inflammatory and mucolytic properties of NAC.
Conclusions: This approach opens possibilities for a novel therapeutic approach in COPD.

Keywords: COPD, N-acetylcysteine, Oxidative stress

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common respiratory disease and is the fourth leading cause of death globally.[1] 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. It is associated with long-term exposure to cigarette smoke, toxic gases and combustion of biomass fuel. The prevalence of COPD in India is approximately 5% in males and 2.7% in females of age above 30 years.[2] 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.[3] Although there have been significant advances in the understanding of etiopathogenesis and treatment options for COPD, unmet challenges still exist.[2]

Oxidative stress is an important attribute in the pathogenesis of chronic obstructive pulmonary disease (COPD). The lungs are continuously exposed to oxidants generated either endogenously from intracellular oxidants or exogenously from air pollutants or cigarette smoke.[4] There is considerable evidence that oxidants can not 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 considerable magnitude and progression of the disease.[5]

Thus, targeting oxidative stress would be a logical therapeutic approach for COPD. Glutathione (GSH) is an effective intracellular antioxidant that counters excess oxidant production in COPD. The role of several antioxidant agents such as vitamin C and vitamin E have been evaluated in COPD, but the evidence is still not conclusive.

N-acetylcysteine (NAC) acts as a precursor for the substrate cysteine in synthesis of GSH and also has a mucolytic and anti-inflammatory activity.[6] NAC has been widely investigated in both animal and clinical studies and has shown benefit in terms of reducing exacerbations, symptomatic improvement and improved lung function.

A clinical study demonstrated the efficacy of oral NAC at a dose of 600 mg/day for a period of two months in reducing the viscosity of expectoration and reducing the severity of cough.[7] Other studies have documented that NAC prevents COPD exacerbations at high dosage (≥1200 mg daily).[6]

In view of this, the present study explores the effect of oral administration of NAC in patients with COPD. The objectives of this study were to determine the dose related effects of N-acetylcysteine (NAC) and to investigate the efficacy and tolerability of high-dose N-acetylcysteine (NAC) in the treatment of patients of chronic obstructive pulmonary disease.

METHODS

This was a randomized, double blind, placebo controlled, parallel group, single centre, prospective study, carried out at Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, Wardha, India. Patients diagnosed as cases of COPD according to GOLD criteria (global initiative for chronic obstructive lung disease), were recruited, following approval from the institutional ethical committee (IEC).

Patients diagnosed as COPD, between ages 35-80 years were included in the study after obtaining written informed consent.

Key exclusion criteria

- Decompensated cardiovascular, endocrine, hepatic or renal function.
- Parenchymal lung pathology or active infection due to Mycobacterium tuberculosis.
- History of gastric or duodenal ulcer in the past six months.

Study population

A total of 75 patients were screened to recruit 68 of them in the study and assigned into the following groups randomly, using random allocation software (Rando: 8)

Study arm I: Control (n=23) / Receiving standard treatment for COPD.

Study arm II: NAC 600 mg once daily (n=22) (In addition to the standard treatment)

*Study arm III:*NAC 600 mg twice daily (n=23) (In addition to the standard treatment)

After screening and obtaining consent for participation, a pre-validated questionnaire was given to the study population to obtain data on demographic details, occupational status, history of respiratory complaints and treatment history. At baseline (day 0), the patients were evaluated for respiratory symptoms, oxygen saturation, chest X- ray, and spirometry was done. Serum samples were collected for malondialdehyde (MDA) estimation and treatment was give according to the randomization plan. The patients were followed up for two months and were assessed for symptomatic improvement and adverse effects to medications at 15, 30 and 60 days. Spirometry and estimation of serum MDA were done at baseline (day 0), end of one month and end of two months.

Spirometry was performed using Medspiror spirometer, recorders and medicare system, RMS, Chandigarh, India, as per the American thoracic society (ATS) Guidelines. Reversibility of airflow obstruction was also assessed by administering Salbutamol 200 micro grams by a metered dose inhaler (MDI), and values were recorded before and after administration of the bronchodilator. Baseline investigations including chest X-ray was performed prior to spirometry. Oxygen saturation was recorded at baseline (day 0), day 30 and day 60 using mediaid pulse-oximeter (M340 DT) with a finger clip sensor.

Malondialdehyde, a highly reactive three carbon dialdehyde, is produced from lipid hydro peroxides. Serum malondialdehyde 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. Lipid peroxidation end product malondialdehyde was estimated by thiobarbituric acid reactive substance assay method (TBRAS), as described by Ohkawa et al.[9]

The drug (N-acetylcysteine) was provided by Cipla pharmaceuticals private limited, Mumbai, India. Identical placebos were also provided so as to facilitate blinding. Drugs and placebos were in the form of effervescent tablets to be taken orally twice a day, for a period of two months. Study arm I received placebo twice daily, Study arm II received NAC 600 mg and placebo both once daily

whereas, Study arm III received NAC 600 mg twice daily.

The data was analysed statistically by one-way ANOVA, paired t-test and McNemar's test, using SPSS version 13.0.

RESULTS

Demographics

Socio-demographic details of the three study arms are represented in Table 1. The patients were given a respiratory questionnaire, which consisted of questions designed to assess the duration and type of respiratory complaints, relationship to weather or ambience, treatment status and loss of work days (Table 2).

Table 1: Demographic data.

Particular	Group I (n=23)	Group II (n=22)	Group III (n=23)
Gender			
Male	69.57%	63.64%	78.26%
Female	30.43%	27.27%	21.74%
Age (in years)			
35-50	30.43%	27.27%	8.70%
51-65	21.74%	45.45%	39.13%
66-80	47.83%	27.27%	52.17%
Height(meters)*	1.58	1.58	1.60
Weight (Kg)*	62.82	62.77	64.69
BMI^ (Kg/m^2)*	19.89	19.90	20.17
Smoking	65.22%	63.64%	60.87%
Biomass fuel exposure	26.09%	22.73%	34.78%

*^BMI – Body mass index, *Height, weight, BMI- Mean values indicated.*

Table 2: Descriptive statistics for respiratory questionnaire.

Item		Group I (%)	Group II (%)	Group III (%)
Duration of respiratory complaints	< 1year	13.04	22.73	8.70
	1-5 years	69.57	40.91	65.22
	>5 years	17.39	36.36	26.09
Difficulty in expectoration		69.57	81.82	78.26
Nocturnal attacks (past 6 months)		8.70	0.00	0.00
Relationship to weather/ambience	Winter	43.48	59.09	52.17
	Summer	8.70	0.00	17.39
	None	47.83	50.0	30.43
Inhaled medication use in past		65.22	63.64	69.57
Loss of work days	Yes	8.70	0.00	4.35
Routine activities without dyspnoea	Yes	86.96	95.45	82.61
Frequency of consultation	<2 months	21.74	18.18	26.09
	2-6 months	69.57	59.09	56.52
	>6 months	8.70	22.73	17.39
Treatment in past	Yes	73.91	86.36	73.91
Influenza vaccine		30.43	18.18	17.39
Pneumococcal vaccine		30.43	18.18	17.39

Serum malondialdehyde (MDA)

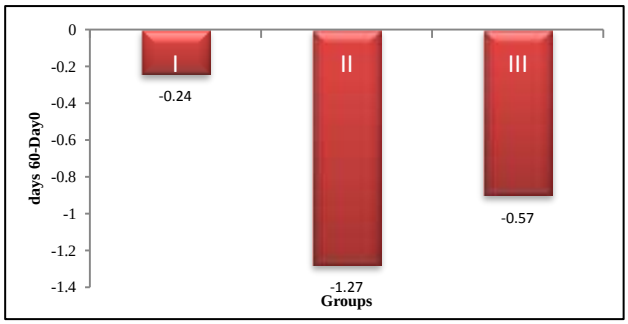

Figure 1: Serum malondialdehyde (MDA): change from baseline (day 0) to post-treatment (day 60)

A significant reduction of serum MDA after two months was seen in study arm II (p=0.001) and study arm III (0.023), as depicted in Figure 1.

Oxygen saturation

Figure 2 illustrates the effect on SpO_2 (oxygen saturation) in the study population over the treatment period of 2 months. A significant increase in SpO_2 from baseline (day 0) was observed at post-treatment (day 60) in study arm II ($p < 0.05$) and III ($p < 0.05$); however there was no significant difference in the placebo controlled group i.e. study arm I.

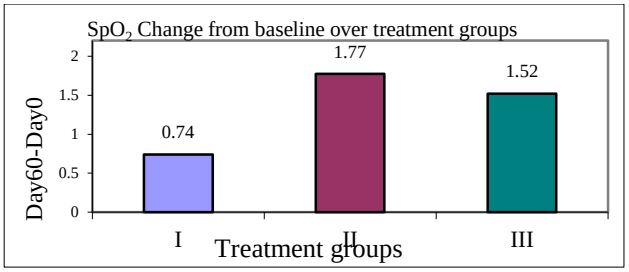

Figure 2: Comparison of SpO_2 in the study groups.

Pulmonary function test

Table 3 shows the spirometry values, expressed as the change from baseline to post-treatment levels. Group III showed a significant increase in FVC (%) (p=0.02) as compared to pre-treatment values. There was no significant change in the mean values of FVC (%) in group I and II. Other parameters i.e. FEV1 and PEFR were not significantly improved in any of the study arm.

Table 3: Spirometry; difference in values from baseline (Day 0) to post-treatment (Day 60) (%).

Parameter	Groups	Day 0 Mean±SD(%)	Day 60 Mean±SD(%)	p-value
FEV_1	I	47.70±13.10	48.49±13.19	0.840
	II	39.44±11.20	41.50±10.68	0.535
	III	36.01±10.54	38.86±11.58	0.390
FVC	I	48.29±16.82	50.73±15.69	0.616
	II	41.28±11.87	42.45±11.59	0.742
	III	37.34±4.61	40.50±4.56	0.020*
PEFR	I	65.13±25.86	66.99±23.97	0.800
	II	45.21±16.16	46.31±15.82	0.820
	III	39.63±12.21	40.96±11.97	0.711

* Significant at $p < 0.05$, Analysis was done by using Paired t- test and significance was tested at 5% and 1% level. *Key:* FEV_1 – Forced expiratory volume, FVC – Forced vital capacity, PEFR – Peak expiratory flow rate.

Symptomatic improvement

Patients were assessed for respiratory symptoms at the baseline and post-treatment, for improvement.

It was observed that all the study arms showed significant reduction in symptoms like cough, sputum production and expectoration, throat irritation and chest tightness. Significance was tested using McNemar's test. The results are depicted in (Figure 3A, 3B and 3C).

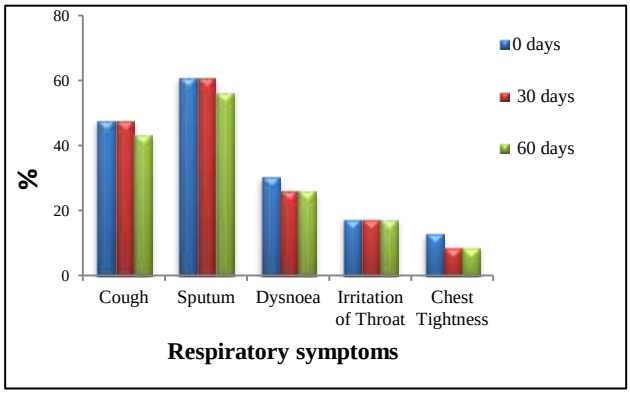

Figure 3: (A) Improvement in symptoms in study arm I (placebo).

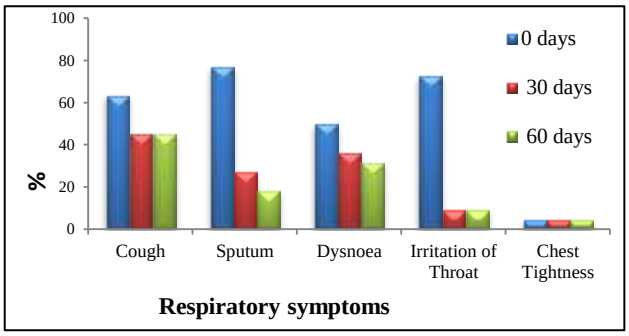

Figure 3: (B) Improvement in symptoms in study arm II (NAC 600 mg OD).

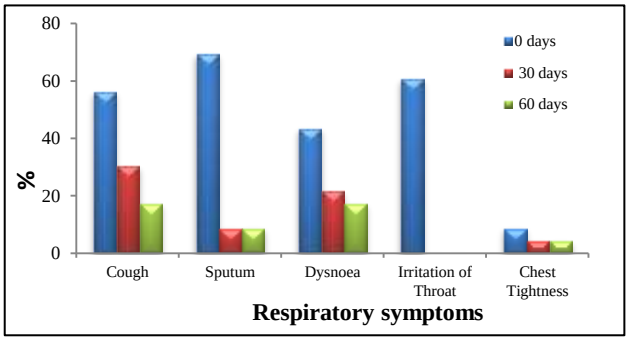

Figure 3: (C) Improvement in symptoms in study arm III (NAC 600 mg BID).

Safety profile

In study arm I, 17.39% and 8.70% patients complained of nausea and stomatitis respectively, which declined to 13.04% and 4.35% after 60 days i.e. post-treatment. Other adverse effects experienced were fever and drowsiness. In study arm II, 13.64% patients complained of nausea, which persisted throughout the study duration. 9.09% patients from the group experienced diarrhea, while 9.09% and 4.55% patients experienced stomatitis and rhinorrhea respectively. In the third study arm, 8.70% patients reported nausea at day 15 and 17.39% at day 60. Stomatitis and rhinorrhea was complained of by 13.04% patients. Only 4.35% patients complained of diarrhea at day 15, which gradually diminished after day 30.

Nausea and stomatitis were the most common reported adverse effects in all the study subjects.

DISCUSSION

Underlying local and systemic oxidative stress is recognized as the main pathogenic factor implicated in disease progression and increasing the severity of COPD. Sources of oxidative stress arise from the increased burden of inhaled oxidants, as well as elevated amounts of reactive oxygen species from the inflammatory cells. Increased levels of ROS (reactive oxygen species), either directly or by formation of lipid peroxidation products, may play a role in enhancing the inflammatory response in COPD.[10] Smoking in males, and exposure to solid biomass fuel in females has been attributed to the development of COPD.[11,12]

In our study, the percentage of male patients was higher than their female counterparts in all the three groups. Smokers from study arm I showed an average smoking pack year status of 19.26, while those in arms II and III had an average of 27.87 and 29.71 respectively. These findings were supportive to a dose response relationship observed by Mahesh et al which suggested that 9.6% smokers who smoked for less than 20 pack years and 18% of those who smoked more than 20 pack years developed COPD.[12]

Twenty six patients in our study had a lower than normal (<19) body mass index (BMI). A study done to evaluate the determinants of BMI in patients with COPD reported a mean BMI of 24.82±3.46 and significantly lowers values in current smokers. Thus reflecting that, BMI is related to smoking status (pack years and continuance of smoking) in COPD. Low BMI is an independent predictor of mortality in COPD.

It is known that daytime SpO_2 is an important predictor of nocturnal oxygen desaturation in COPD patients.[13] The effect of NAC on daytime oxygen saturation was found to be positive in our study. A significant increase in mean SpO_2% was observed in study arm II and III, from baseline to post-treatment levels; the highest being in the study arm treated with NAC 600 mg BID.

There was a significant decrease in serum malondialdehyde (MDA) levels from baseline to post-treatment levels in the study arms II and III. Pre-treatment levels of MDA (nmol/ml) in all the study arms (4.25±0.6, 5.43±1.04 and 5.30±0.97) were high, which is an indicator of oxidative stress in these patients. Apart from MDA, NAC has also shown to increase the protective markers for oxidative stress such as glutathione peroxidase and total antioxidant capacity, as demonstrated in a study comparing the effects of inhaled corticosteroids and NAC in COPD patients.[14]

The direct antioxidant properties of NAC are attributed to the free thiol group that interacts with the electrophile groups of reactive oxygen species (ROS). NAC being a glutathione precursor acts as an indirect antioxidant. 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.[15,16]

Study arm treated with NAC 600 mg BD exhibited a significant improvement in FVC and ($p < 0.05$). Improvement of FEV1 and FVC have been demonstrated by Decramer et al who studied the effect of NAC 600 mg once daily in 523 patients of COPD.[17] Another long term study concluded that, after 5 years, the reduction in FEV1 in the NAC treated group was less than that in the reference group.[18]

Symptomatic improvement at the end of the study period depicted clinical efficacy of NAC in COPD in our study. All the study arms showed a significant reduction in symptoms like cough, sputum production and expectoration, dyspnoea, throat irritation and chest tightness. It has been demonstrated that NAC decreases the sputum viscosity, improves sputum expectoration, and reduces exacerbations and sick-leave days. Parenthetically, NAC has also shown to decrease the number of viral infections and airway bacterial colonization in patients with COPD.[19] Thus, NAC has shown to improve symptoms, reduce exacerbations, improve lung function, thereby reducing the morbidity and healthcare cost associated with COPD. Consequently, long-term administration of oral NAC during stable condition of the patient or at least in winter months on a preventive basis is advisable.[20]

Evaluation of the safety profile was done by monitoring the adverse effects of NAC. Nausea and stomatitis were the most commonly observed adverse effects; however drowsiness, diarrhoea and rhinorrhoea were also experienced by a small set of patients. It is documented that NAC is well tolerated, mild effects like nausea and vomiting may be observed, urticaria and bronchospasm being extremely rare. Anaphylactoid reactions, including rash, flushing, urticaria, bronchospasm and angioedema,

mostly associated with very high doses of NAC have been described in adults by Prescott et al.[21]

Effects of NAC can be attributed to multiple protective mechanisms, such as antioxidant activity, ability to act as a precursor of intracellular reduced glutathione (GSH), detoxification of oxidants and modulation of DNA repair process. Also various studies have established the clinical efficacy of NAC in COPD as a mucolytic as well as an antioxidant. The findings in our study indicate the important role played by N-acetylcysteine in combating the oxidative stress in COPD and improvement of various clinical parameters, at well tolerated doses.

CONCLUSION

Considering the chronic nature of treatment for COPD, 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. It would also be worthwhile to evaluate other parameters of oxidative stress like exhaled carbon monoxide, superoxide dismutase and glutathione levels in the future. Pharmaco-economic analysis considering cost of therapy, duration of disease free intervals and disability-adjusted life years is suggested. Thus, it would be relevant to consider NAC as a novel therapeutic approach in the management of COPD, in addition to the standard line of treatment.

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