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Original Research Article

Effect of N-acetylcysteine in treatment of COPD with pulmonary interstitial fibrosis, inflammatory factors and VEGF levels

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Abstract

Purpose: *To investigate the efficacy of N-acetylcysteine as an adjuvant therapy in chronic obstructive pulmonary disease (COPD) with pulmonary interstitial fibrosis (PIF), and its effect on inflammatory factors and serum vascular endothelial growth factor (VEGF) levels.*

Methods: *A total of 94 patients with COPD-PIF from May 2020 to May 2022 in Wuhan Fourth Hospital, China were equally randomized into study and control groups. Control group was administered conventional treatment while the study group was given N-acetylcysteine in addition to conventional treatment. Therapeutic efficacy, forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), FEV1/FVC, blood gas, pulmonary fibrosis, and oxidative stress were evaluated. Changes in inflammatory factors, serum vascular endothelial growth factor (VEGF), and incidence of adverse drug reactions were compared.*

Results: *The study group showed significantly higher efficacy, FEV1, FVC, FEV1/FVC compared to control group ($p < 0.05$). Also, study group showed significantly higher partial pressure of oxygen (PaO_2), blood oxygen saturation, superoxide dismutase (SOD), and glutathione (GSH) levels after treatment compared to control group ($p < 0.05$). Platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), vascular cell adhesion molecule (VCAM), tumor necrosis factor (TNF), interleukin-6 (IL-6), and vascular endothelial growth factor (VEGF) levels after treatment were significantly lower in the study group than in the control group ($p < 0.05$).*

Conclusion: *N-acetylcysteine as an adjuvant therapy for COPD-IF is highly effective, improves lung function and oxidative stress, reduces airway inflammation, and VEGF, and attenuates the degree of hypoxia and pulmonary fibrosis with no serious adverse effects. A larger sample size, multicenter, randomized trials to validate these findings and evaluate the downstream regulatory mechanism of N-acetylcysteine in COPD-PIF.*

Keywords: *Chronic obstructive pulmonary disease, N-acetylcysteine, Pulmonary interstitial fibrosis, Clinical effect, Inflammatory factors, Vascular endothelial growth factor*

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) manifests as a long-term pulmonary condition marked by partially reversible restriction of airflow, which has a high rate of disability and mortality, thus affecting the survival rate. Pulmonary interstitial fibrosis (PIF) occurs after

COPD, and respiratory failure ensues with the progression of the disease [1]. Low-flow oxygen and glucocorticoid are the main treatment methods for COPD-PIF, but clinical efficacy is poor. N-acetylcysteine has good therapeutic efficacy in COPD in effectively reducing lung damage caused by oxygen free radicals, alleviates the process of pulmonary fibrosis, and reduces clinical symptoms [2,3]. N-acetylcysteine is a precursor of glutathione which effectively scavenges oxygen free radicals and lowers lung damage. This study investigated N-acetylcysteine as an adjuvant treatment following conventional treatment for COPD-PIF, focusing on its effect on lung function, inflammatory indices, and VEGF.

METHODS

Participants

A total of 94 patients with COPD-PIF from May 2020 to May 2022 in Wuhan Fourth Hospital, Wuhan City, China were randomized equally into study and control groups (n = 47 in each group). Clinical data showed no significant difference between the two groups ($p > 0.05$). The present study was approved by the Ethics Committee of Wuhan Fourth Hospital (approval no. 201906WH031) and conducted in accordance with the Declaration of Helsinki [4]. Written informed consent was provided by all patients before the commencement of the study.

Inclusion criteria

Patients who met the diagnostic criteria for COPD and PIF (high-pitched fine bursting sounds can be heard in the lungs) [5], disease aggravated by mixed dyspnea, x-ray chest film reveals nodular or diffuse punctate, honeycomb, or reticular shadows), presence of ventilatory and diffusion dysfunction, and signed the informed consent form.

Exclusion criteria

Patients with severe cardiac, liver, and renal dysfunction, presence of combined pulmonary tuberculosis and other pulmonary diseases, hypertension and other chronic diseases, presence of multiple organ dysfunction, and drug history of glucocorticoid and nintedanib during treatment.

Treatments

Control group received conventional treatment (intravenous infusion of methylprednisolone sodium succinate (Pfizer Manufacturing Belgium NV, batch no. H20130301)) at 80 mg daily.

Oral methylprednisolone tablet (Pfizer, batch no. H20100730) was administered depending on the patient's situation after 14 days of treatment at 20 mg daily. The study group received nebulized N-acetylcysteine (Zambon, Italy, specifications: 0.1 g/mL, batch no. 19837001) (3 mL per day by inhalation) in addition to conventional treatment. Both groups underwent treatment for three months.

Evaluation of parameters/indices

Efficacy

Clinical efficacy was classified as markedly effective (characterized by increase in diffusing lung capacity for carbon monoxide (DLCO) exceeding 15 %, absorption of over 50 % of the area of pulmonary infiltrates on imaging examination, daily cough frequency below 10, resolution of symptoms such as shortness of breath and inspiratory bursts); moderately effective (characterized by DLCO increase of 6 - 15 %, absorption of 20 - 50 % of the area of pulmonary infiltrates on imaging examination, daily cough frequency of 10 - 20, and persistence of symptoms such as shortness of breath or inspiratory burst sound after mild exercise); and invalid outcomes (characterized by failure to meet the above criteria).

Biochemical indices

Lung function indices (FEV1, FVC, and FEV1/FVC values) were measured using chest hi-801 lung function instrument. Also, PaO_2 and blood oxygen saturation of patients were detected using the Kangli BG-800 blood gas analyzer. Serum PDGF, TGF-β, VCAM-1, TGF-β1, IL-6, and VEGF were analyzed using ELISA kits (Shanghai Tongwei Biotechnology Co., Ltd.). Furthermore, oxidative stress indices (SOD, MDA, and GSH) were measured using chemical colorimetry method.

Statistical analysis

Data was analyzed using Statistical Packages for Social Sciences 22.0 software (SPSS, IBM, Armonk, NY, USA). Categorical data were presented in frequency and percentages and analyzed using Chi-square test. Measurement data are presented in mean ± standard deviation (SD) following a normality test, while the student t-test was used for comparison. $P < 0.05$ was considered statistically significant.

RESULTS

Efficacy

There was significant difference in clinical efficacy between the study and control groups ($p < 0.05$, Table 1).

Lung function

There was no significant difference in lung function before treatment ($p > 0.05$). However, study group showed significantly higher FEV1 (Figure 1 A), FVC (Figure 1 B) and FEV1/FVC (Figure 1 C) compared to control group after treatment ($p < 0.05$).

Partial pressure of oxygen and oxygen saturation

There was no significant difference in PaO_2 and oxygen saturation in both groups before treatment ($p > 0.05$). After treatment, PaO_2 (Figure 2 A) and oxygen saturation (Figure 2 B) were significantly higher in study group compared to control group ($p < 0.05$).

Table 1: Clinical efficacy (N = 47 in each group)

Group	Markedly effective	Effective	Invalid	Total effective rate (%)
Study	18	25	4	91.49
Control	10	26	11	76.6
χ^2				3.887
P-value				0.048

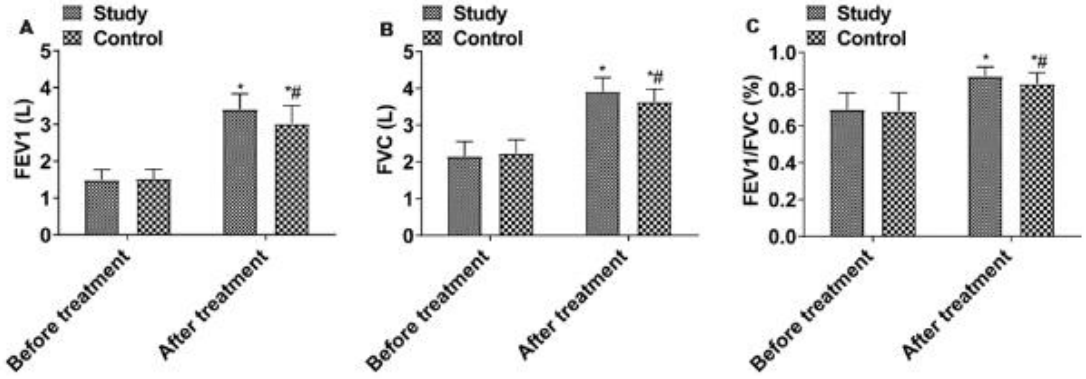

Figure 1: Lung function parameters. A: Study group showed significantly higher FEV1 compared to control group. B: Study group showed significantly higher FVC compared to control group. C: Study group showed significantly higher FEV1/FVC compared to control group. *$P < 0.05$ vs before treatment, $^{\#}p < 0.05$ vs study group

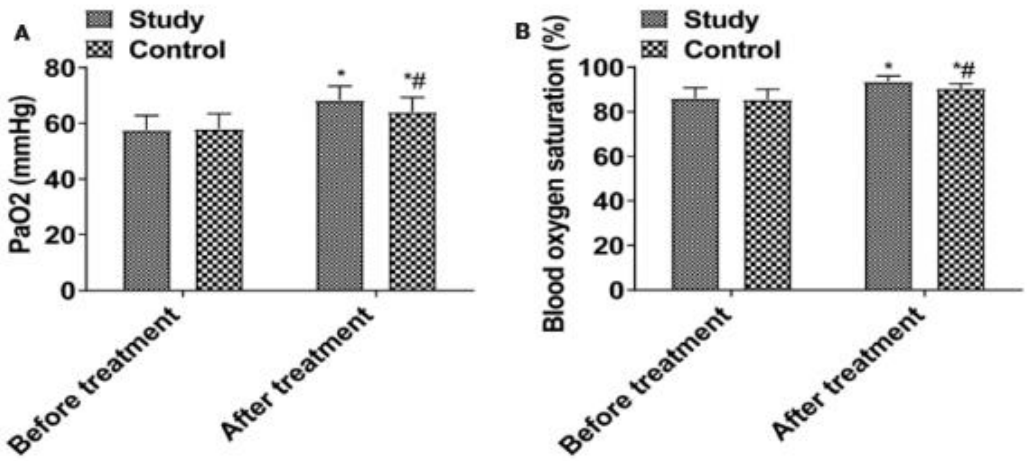

Figure 2: Elevated PaO_2 and oxygen saturation. A: Partial pressure of oxygen (PaO_2) was significantly higher in study group after treatment compared to control group. B: Blood oxygen saturation was significantly higher in study group compared to control group. *$P < 0.05$ vs before treatment, $^{\#}p < 0.05$ vs study group

Serum levels of lung fibrosis markers

There was no significant difference in lung fibrosis indices before treatment in both groups ($p > 0.05$). However, levels of PDGF (Figure 3 A), TGF-β (Figure 3 B), and VCAM-1 (Figure 3 C) significantly decreased in study group compared to control group ($p < 0.05$).

Serum inflammatory and angiogenic factors

There was no significant difference in TNF-α, IL-6 levels and VEGF levels between the two groups before treatment ($p > 0.05$). However, TNF-α (Figure 4 A), IL-6 (Figure 4 B), and VEGF (Figure 4 C) after treatment were significantly lower in study group compared to control group ($p < 0.05$).

Oxidative stress indices

There was no significant difference in oxidative stress indices between the two groups before treatment ($p > 0.05$). However, levels of SOD (Figure 5 A) and GSH (Figure 5 B) were significantly higher and MDA (Figure 5 C) was significantly lower in study group compared to control group after treatment ($p < 0.05$).

Incidence of adverse drug reactions

During the treatment period, neither group experienced serious adverse reactions.

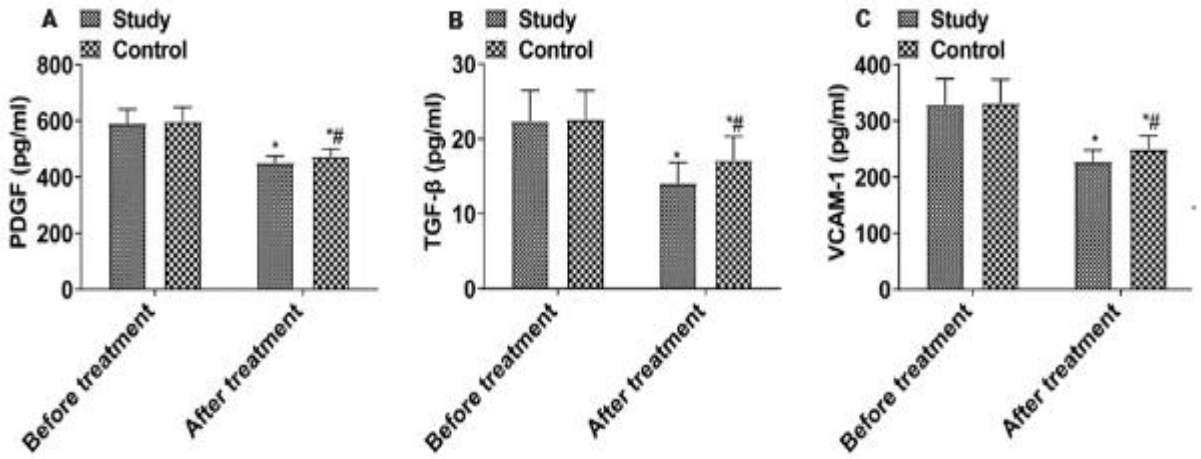

Figure 3: Serum levels of lung fibrosis markers. A: Platelet derived growth factor (PDGF) significantly decreased after treatment in study group compared to control group. B: Transforming growth factor (TGF-β) significantly decreased after treatment in study group compared to control group. Vascular cell adhesion molecule – 1 (VCAM) significantly decreased after treatment in study group compared to control group. *$P < 0.05$ vs before treatment, $^{\#}p < 0.05$ vs study group

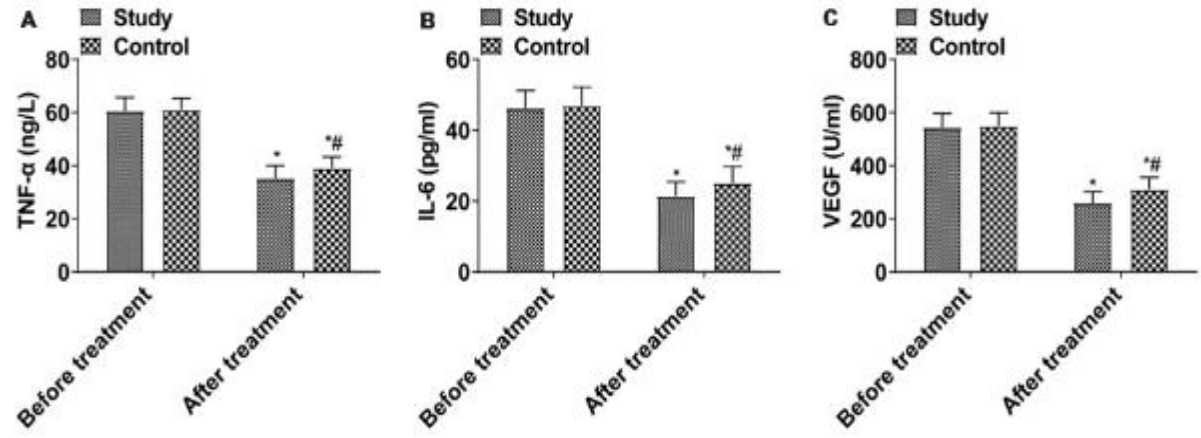

Figure 4: Inflammatory and angiogenic indices**.** A: Tumor necrosis factor (TNF-α) significantly decreased after treatment in study group compared to control group. B: Interleukin-6 (IL-6) significantly decreased after treatment in study group compared to control group. C: Vascular endothelial growth factor (VEGF) significantly decreased after treatment in study group compared to control group. *$P < 0.05$ vs before treatment, $^{\#}p < 0.05$ vs study group

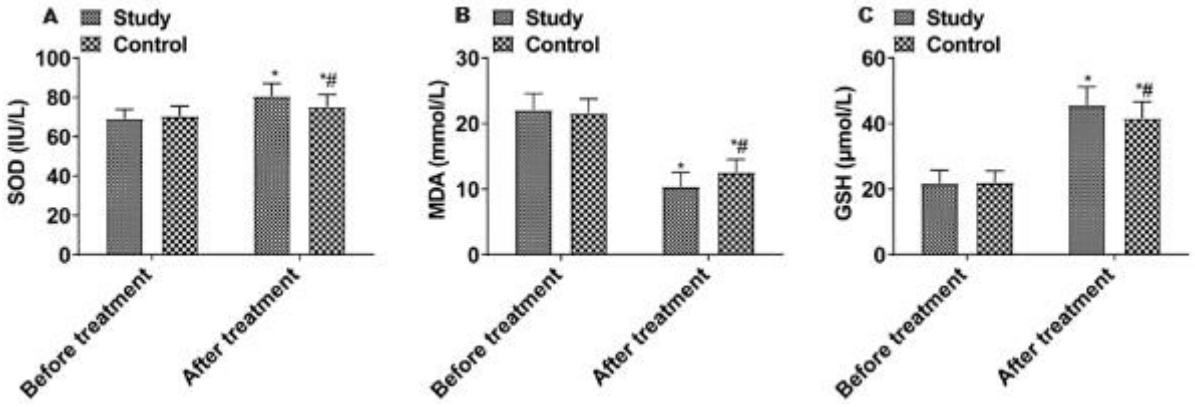

Figure 5: Oxidative stress indices**.** A: Superoxide dismutase (SOD) significantly increased after treatment in study group compared to control group. B: Malondialdehyde (MDA) significantly decreased after treatment in study group compared to control group. C: Glutathione (GSH) significantly increased after treatment in study group compared to control group. *$P < 0.05$ vs before treatment, $^{\#}p < 0.05$ vs study group after treatment

DISCUSSION

Pulmonary interstitial fibrosis (PIF) is a common pathological change in COPD. The main feature of this disease is airflow restriction. Pulmonary interstitial fibrosis (PIF) may occur in the middle and late stages of COPD, and its progression may lead to respiratory failure or hypoxemia [6]. In clinical treatment of patients with COPD-PIF, targeted measures should be taken based on the treatment of the primary disease, so as to reverse PIF as much as possible, and effectively control disease development. Currently, low-flow oxygen inhalation and glucocorticoids are the main treatment methods for COPD-PIF. However, studies have found that clinical efficacy of this treatment regimen is still poor, and as such combination therapy is advised [7,8].

Clinical data showed the involvement of oxidative stress in COPD [9]. N-acetylcysteine is a sulfur-based compound containing cysteine. N-acetylcysteine, as an expectorant, has antioxidant and anti-fibrosis role in high oral doses, but its clinical efficacy is still not clear. Therefore, this study investigated the efficacy of N-acetylcysteine in COPD-PIF. The results revealed that effective rate, FEV1, FVC, FEV1/FVC, PaO_2, and blood oxygen saturation were significantly greater in study group compared to control group. This suggests that N-acetylcysteine enhances clinical effectiveness, improves lung function, and reduces hypoxia. This may be because N-acetylcysteine inhibits VCAM-1-mediated vascular endothelial adhesion, blocks endogenous and exogenous coagulation system activated by injured endothelial cells, and inhibits PIF caused by pulmonary inflammatory response. Also, it corrects protease/antiprotease system disorders and improves lung ventilation function by improving airway elasticity [10,11]. This study showed that N-acetylcysteine reduced levels of serum markers of PIF (PDGF, TGF-β and VCAM-1), suggesting that it may reduce the degree of pulmonary fibrosis. This is primarily due to the inhibitory effects of N-acetylcysteine on TGF-β expression, resulting in suppression of collagen synthesis, fibroblast proliferation, and interstitial fibrosis progression. Also, N-acetylcysteine blocks the formation of the TGF-β-induced PDGFq receptor and reduces the bioactivity of PDGF in pulmonary fibrosis [12].

N-acetyl cysteine, a sulfhydryl compound with a cysteine structure, undergoes conversion to cysteine by removal of the acetyl group. This compound serves as an antioxidant and stabilizes cell membrane structure by reducing oxidant generation and increasing antioxidant levels [13]. It has been reported that N-acetylcysteine improves oxidative stress response in COPD [14]. This study demonstrated that administration of N-acetylcysteine resulted in increased levels of SOD and GSH, as well as decreased levels of MDA, suggesting that N-acetylcysteine treatment may mitigate oxidative stress. This effect is likely due to the ability of the decomposition product of N-acetylcysteine to reduce glutathione, and to scavenge oxygen free radicals effectively, thereby reducing lung damage caused by oxidative stress. Also, N-acetylcysteine exhibits scavenging properties against hydroxyl free radicals, hypochlorous acid, hydrogen peroxide, and other oxidizing agents, potentially inhibiting progression of pulmonary fibrosis [15].

Epithelial-mesenchymal transformation is related to PIF in interstitial pneumonia, and TGF-β1 activates tyrosine kinase receptors on alveolar epithelial cells via the PI3K/Akt pathway. Many

protein tyrosine kinases are associated with the onset and progression of fibrosis, with the highest expressions being PDGF and VEGF. Vascular endothelial growth factor (VEGF) is induced by TGF-β1 activation which in turn releases signaling molecules as a key step in promoting fibroblast differentiation [16].

Studies have also demonstrated that serum levels of TGF-β1, IL-6 and VEGF reflect the severity of PIF to a certain extent [17,18]. This current study revealed that N-acetylcysteine significantly reduced levels of TNF-α, IL-6, and VEGF, indicating that this treatment method reduces levels of inflammatory factors and VEGF. This may be because N-acetylcysteine inhibits oxidative stress response, and oxygen free radicals that damage the endoplasmic reticulum, thereby mediating activation of Smad and Src signal transduction pathways. Aerosolized N-acetylcysteine effectively reduces the concentration of oxygen free radicals in alveolar cells, and thus interferes with the signal transduction process of epithelial cell-mesenchymal transformation in interstitial pneumonia which in turn reduces VEGF levels [19,20]. Furthermore, this study revealed no significant difference in adverse reactions in both groups indicating a high safety margin.

Limitations of this study

There are some limitations of this study. This study included a small sample size and did not further investigate the downstream regulatory mechanism of N-acetylcysteine in COPD-PIF.

CONCLUSION

N-acetylcysteine combination regimen improves clinical efficacy, lung function, and blood gas parameters, lowers markers of lung fibrosis, reduces inflammation, and mitigates oxidative stress with no serious adverse effects. A larger sample size as well as multicenter, randomized trials are required to validate these findings and to investigate the downstream regulatory mechanism of N-acetylcysteine in COPD-PIF.

DECLARATIONS

Acknowledgements

This study was approved by Study on the Mechanism of Schisandrin B Regulating Autophagy and Epithelial Interstitial Transformation in Preventing Pulmonary Fibrosis (no. WZ19Q12).

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None provided.

Ethical approval

The Ethics Committee of Wuhan Fourth Hospital, China approved this study (201906WH031).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Jie Yang and Yuanping Cai conceived and designed the study, and drafted the manuscript. Qiaofa Lu and Bo Zhang collected, analyzed and interpreted the experimental data. All authors revised the manuscript for important intellectual content. All authors read and approved the final draft of the manuscript for publication.

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