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# Regulating effect of N-acetyl cysteine adjuvant therapy on airway inflammation, remodeling and so on in patients with stable COPD

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ABSTRACT

**Objective:** To analyze the regulating effect of N-acetyl cysteine adjuvant therapy on airway inflammation, remodeling and so on in patients with stable COPD. **Methods:** A total of 108 cases of COPD patients who were treated in our hospital were included for study and were in stable phase after detection. According to different treatment methods, they were divided into control group 58 cases who received routine treatment and observation group 50 cases who received additional N-acetyl cysteine adjuvant therapy. Differences in levels of serum inflammation-related factors, airway remodeling indicators, Keap1-Nrf2-ARE signaling pathway, oxidation-antioxidation levels, etc were compared between two groups after treatment. **Results:** Serum MIP-1 α, sTREM-1, IL-13, IL-8 and IP-10 values of observation group after treatment were lower than those of control group; serum TGF-β$_1$ and Ang-Ⅱ values of observation group after treatment were lower than those of control group, Ang-Ⅰ value was higher than that of control group and lesion bronchia T, WA and WA% values were lower than those of control group; Keap1, Nrf2 and ARE values in serum and induced sputum of observation group were higher than those of control group; serum MDA and LPO values of observation group were lower than those of control group while SOD, GSH-Px and T-AOX values were higher than those of control group. **Conclusion:** N-acetyl cysteine adjuvant therapy for patients with stable COPD optimizes airway inflammation, remodeling and so on, and is of positive significance in controlling long-term disease, improving outcome and so on.

## 1. Introduction

Chronic obstructive pulmonary disease (COPD) has higher clinical incidence, and is one of the main diseases endangering the respiratory function and quality of life of middle-aged and elderly patients. There is a certain degree of inflammation and oxidative stress in stable COPD patients, COPD can be acute when inflammation expands or the body's constitution is weakened, so such patients should still receive positive intervention[1,2]. N-acetyl cysteine (NAC) is oxygen free radical scavenger containing sulphur, which can not only interfere with the generation of free radicals, but can also regulate cell metabolism. NAC has anti-cytotoxic effect and protects tissues from endogenous and exogenous injury, and at the same time, anti-inflammatory and anti-airway remodeling and other effects of NAC are with great pertinence and effectiveness in the treatment of COPD[3]. In the research, the regulating effect of N-acetyl cysteine adjuvant therapy on airway inflammation, remodeling and so on in patients with stable COPD was mainly analyzed, hereby reported as follows.

## 2. Case information and treatment methods

### 2.1 Case information

A total of 108 cases of COPD patients who were treated in our hospital were included for study and were all in stable phase after

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detection. Treatment scheme and test results of stable phase for all included patients were retrospectively analyzed. Inclusion criteria: 1) $FEV_1/FVC<70\%$, 30% $FEV_1<50\%$; 2) the time in stable phase was more than 4 weeks; 3) patients who didn't smoke or quit smoking for more than 2 years; 4) patients and families signed informed consent forms. Exclusion criteria: 1) the patients with bronchial asthma or allergic rhinitis; 2) patients with acute respiratory tract infection; 3) those with other chronic respiratory diseases; 4) those with obviously abnormal liver and kidney function.

According to different treatment methods, patients were divided into control group 58 cases who received routine treatment and observation group 50 cases who received additional N-acetyl cysteine adjuvant therapy. Control group included 30 male cases and 28 female cases, they were 38-72 years old, the average was (61.28±8.53) years, the course of disease was 3-12 years and the average was (6.28±0.85) years; observation group included 27 male cases and 23 female cases, they were 39-73 years old, the average was (60.76±8.53) years, the course of disease was 4-11 years and the average was (6.76±0.91) years. Differences in baseline information were not significant between two groups, $P>0.05$ and they were comparable.

### 2.2 Treatment methods

Included subjects received 4-week washout period and then stopped using all anticholinergic drugs and corticosteroids, and when patients were with abnormal respiratory disorder, short-acting $\beta_2$ agonist could be temporarily used. After finishing the washout period, all patients entered into research-based treatment process, control group received regular phlegm-eliminating, asthma-relieving and other symptomatic treatment. Observation group received additional N-acetyl cysteine adjuvant therapy, which was specifically as follows: oral administration of N-acetyl cysteine 600 mg, once a day. Species and methods of other drug treatment were the same as those of control group.

### 2.3 Observation indicators

Peripheral venous blood was drawn from patients after treatment and centrifuged to collected supernatant and cryopreserved in - 80 ℃ refrigerator for test. All included patients received high resolution CT (HRCT) examination after treatment, and 1cm above and 1 cm below tracheal carina, right pulmonary veins, right lateral diaphragm and other places in airway were measured. All patients in the research inhaled aerosolized hypertonic (%) saline 7 mL after treatment, patients' positive expectoration was encouraged after inhalation, nasal cavity was cleaned up and then sputum was collected. When the sputum coughed up reached the required amount, the induction was stopped, and the total inhalation time was controlled within 20-30 min. After centrifugation, induced sputum supernatant was collected to analyze the content of target molecules in it.

Inflammation-related factors: macrophage inflammatory protein-1 α (MIP-1 α), soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), interleukin-13 (IL-13), interleukin-8 (IL-8) and interferon-G-induced protein (IP-10). Airway remodeling indicators: TGF-$\beta_1$, Ang-Ⅰ and Ang-Ⅱ; airway wall thickness (T), airway wall area (WA) and percentage of airway wall area to the total cross-sectional area of the airway (WA%). Protein expression of Keap1-Nrf2-ARE signaling pathway-related factors: sample Kelch-like epichlorohydrin-associated protein-1 (Keap1), nuclear transcription factor erythron-2p45 (NF-E2) and antioxidant response element (ARE). Oxidation/antioxidation levels: malondialdehyde (MDA), lipid peroxide (LPO), superoxide dismutase (SOD), glutathione peroxidase (gsh-px) and total antioxidant capacity (T-AOX).

### 2.4 Statistical methods

Data obtained in the research was analyzed by SPSS 23.0 software, measurement data was in terms of Mean ± SD, comparison between two groups was by t test, and $P<0.05$ was set as the standard of statistical significance in differences.

## 3. Results

### 3.1 Serum inflammation-related factors

COPD patients are complicated with local airway and systemic inflammatory state, and both excessive inflammatory factors and

**Table 1.**
Comparison of serum inflammation-related factor levels between two groups after treatment.

| Groups | MIP-1 α (pg/mL) | sTREM-1 (ng/mL) | IL-13 (pg/mL) | IL-8 (pg/mL) | IP-10 (μg/L) |
|---|---|---|---|---|---|
| Observation | 19.82±1.77 | 38.72±3.09 | 63.27±5.88 | 4.51±0.43 | 198.27±13.29 |
| Control | 37.95±3.41 | 61.35±5.76 | 75.88±7.12 | 6.72±0.58 | 321.66±29.85 |
| *t* | 7.384 | 8.923 | 7.173 | 6.384 | 12.384 |
| *P* | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

the resulting inflammatory cascade reaction can lead to acute onset and progress of COPD. For stable COPD patients, detecting serum inflammation-related factor levels is an effective way to evaluate patients' disease condition, predict disease trend and evaluate treatment effect. Detection of the values of serum MIP-1α, sTREM-1, IL-13 and other indicators in patients after treatment showed that serum MIP-1α, sTREM-1, IL-13, IL-8 and IP-10 values of observation group after treatment were lower than those of control group ($P$<0.05), shown in Table 1.

### 3.2 Airway remodeling indicators

Airway remodeling is one of the most typical changes in COPD patients, and an important cause of irreversible progression of patients' condition. Airway remodeling can be divided into microscopic and macroscopic indicators, levels of some factors in serum can indirectly reflect the degree of body's airway remodeling, and direct HRCT examination of lesion bronchia can directly reflect the degree of airway remodeling. In the research, serum and airway indicators were detected, and results showed that serum TGF-$\beta_1$ and Ang-Ⅱ values of observation group after treatment were lower than those of control group, Ang-Ⅰ value was higher than that of control group ($P$<0.05) and lesion bronchia T, WA and WA% values were lower than those of control group ($P$<0.05), shown in Table 2.

### 3.3 Keap1-Nrf2-ARE signaling pathway

Keap1-Nrf2-ARE signaling pathway is involved in the occurrence and development of COPD, and the expression levels of its downstream Keap1, Nrf2, ARE and other factors can directly decide the activity of the pathway and the trend of COPD. After different treatment, serum and induced sputum Keap1-Nrf2-ARE signaling pathway activity of all included patients was detected, and results showed that protein expression levels of Keap1, Nrf2 and ARE in serum and induced sputum of observation group were higher than those of control group ($P$<0.05), shown in Table 3.

### 3.4 Oxidation/antioxidation levels

Oxidation/antioxidation imbalance is one of the important links causing aggravated COPD, and both increased oxygen free radicals and reduced antioxidant capacity will cause aggravated COPD lung tissue injury and exacerbated disease progression. Detection of serum oxidation and antioxidation-related factor levels of two groups after treatment showed that serum MDA and LPO values of observation group were lower than those of control group while SOD, GSH-Px and T-AOX values were higher than those of control group ($P$<0.05), shown in Table 4.

## 4. Discussion

N-acetyl cysteine (NAC) is widely applied clinical antioxidant that has potent anti-inflammation, elastase-inhibiting and other effects at the same time, and its effect in in COPD patients is recognized.

**Table 2.**
Comparison of airway-remodeling-related indicator values between two groups after treatment.

| Groups | Serum indicators (ng/L) | | | Lesion bronchia-related indicators | | |
|---|---|---|---|---|---|---|
| | TGF-$\beta_1$ | Ang-Ⅰ | Ang-Ⅱ | T (mm) | WA ($mm^2$) | WA% (%) |
| Observation | 473.28±34.17 | 43.28±4.71 | 36.27±4.11 | 1.13±0.12 | 10.78±1.21 | 68.36±6.12 |
| Control | 583.66±59.26 | 21.29±2.07 | 57.05±5.38 | 1.37±0.14 | 12.15±1.34 | 73.21±7.03 |
| $t$ | 11.832 | 7.394 | 8.293 | 5.209 | 6.281 | 8.384 |
| $P$ | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

**Table 3.**
Keap1-Nrf2-ARE signaling pathway-related factor levels in serum and induced sputum of two groups after treatment.

| Groups | Serum | | | Induced sputum | | |
|---|---|---|---|---|---|---|
| | Keap1 | Nrf2 | ARE | Keap1 | Nrf2 | ARE |
| Observation | 129.37±14.58 | 131.47±12.05 | 123.27±11.28 | 132.84±11.28 | 150.28±14.37 | 143.26±15.95 |
| Control | 85.39±8.11 | 92.37±9.56 | 75.46±6.88 | 76.59±8.54 | 91.27±8.65 | 89.75±8.43 |
| $t$ | 9.283 | 8.293 | 11.482 | 8.293 | 9.172 | 10.039 |
| $P$ | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

**Table 4.**
Comparison of serum oxidation/antioxidation levels between two groups after treatment.

| Groups | MDA (μmol/L) | LPO (μmol/L) | SOD (nu/mL) | GSH-Px (U) | T-AOX (U/mL) |
|---|---|---|---|---|---|
| Observation | 3.17±0.29 | 4.36±0.37 | 81.29±7.88 | 162.36±14.38 | 11.18±1.43 |
| Control | 7.32±0.66 | 6.11±0.59 | 70.34±7.45 | 127.55±13.07 | 7.05±0.68 |
| $t$ | 6.293 | 5.395 | 8.273 | 9.263 | 6.172 |
| $P$ | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

COPD is disease characterized by limited airflow, and both airway inflammation and oxidation/antioxidation imbalance are of important significance in the occurrence and development of disease[4]. For stable COPD patients, in addition to eliminating phlegm and long-acting inhalation of corticosteroids to maintain condition, the control of inflammation, oxidative stress, etc., should be strengthened to prevent acute inflammation-caused COPD attack and aggravation. NAC has a variety of clinical effects at the same time, is an ideal drug for stable COPD and was added to the treatment of observation group in the research, and the change of airway inflammation, airway remodeling changes, etc. brought by different treatment was mainly elaborated.

Macrophage inflammatory protein-1 α (MIP-1 α) belongs to acidic protein, is secreted by mononuclear macrophages, and as chemokine, can effectively affect the local inflammatory cell infiltration and enhance endothelial activity. Study has shown that MIP-1 α level in circulating blood of COPD patients is significantly higher than that of healthy controls, indicating that MIP-1 α is involved in the inflammatory response in patients with COPD. Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) is selectively expressed in neutrophils, and can activate neutrophils, precipitate inflammatory response, etc[5]. Interleukin-13 (IL-13) is secreted by activated Th2 cells, is in first place in airway inflammation-related factors, can separately mediate asthma, COPD, etc, and is associated with both airway inflammatory response and immune regulation. Studies have confirmed that IL-13 can induce bronchial smooth muscle to secrete VEGF while stimulate transforming growth factors to produce TGF-β$_1$. Interleukin-8 (IL-8) can activate neutrophils, which releases the neutrophil elastase and cause airway damage[6,7]. Interferon-G-induced protein 10 (IP-10) can induce mononuclear macrophage migration and activation, adjust cytokine network and accelerate related factors recruitment in inflammatory area. Research has confirmed that the TP-10 plays an important role in the COPD acute onset. The research results showed that serum MIP-1 α, sTREM-1, IL-13, IL-8 and IP-10 values of observation group were lower after treatment, indicating that N-acetyl cysteine treatment effect was significant on inhibiting the inflammatory response in patients with COPD.

TGF-β$_1$ is currently one of the most important cytokines that cause fibrosis, is produced by macrophages, platelets, etc, can regulate cell growth and extracellular matrix deposition, and promotes pulmonary fibrosis in many links[8]. Angiotensin Ⅰ (Ang-Ⅰ) is a potent cytokine that promotes blood vessel growth, and under the stimulation of local inflammation, Ang-Ⅰ can activate the serine/threonine protein kinase, raise level of apoptosis-inhibiting factors and stabilize cell vitality. Studies have shown that giving Ang-Ⅰ to COPD model rats can reduce neutrophil adhesion and significantly reduce local inflammation, and plays a positive role in restraining local airway inflammation and airway remodeling[9,10]. Angiotensin Ⅱ (Ang-Ⅱ) activates AGTR1 to increase fibroblast MAPK activity and DNA formation, further raise the TGF-β$_1$ expression, form positive feedback effect and accelerate the airway and surrounding tissue fibrosis and compliance weakening. High-resolution CT (HRCT) is the most direct way of judging the degree of airway remodeling in patients with COPD, airway wall thickness (T), airway wall area (WA) and the percentage of airway wall area to the total cross-sectional area of the airway (WA%) can all represent airway remodeling in lesion bronchia of patients, and the larger the above values, the more serious the airway remodeling[11]. Blood gas ELISA and HRCT inspection in the research showed that serum TGF-β$_1$, Ang-Ⅱ, T, WA and WA% values of observation group were lower while Ang-Ⅰ value was higher, it indicated that after N-acetyl cysteine auxiliary treatment, airway remodeling in patients with COPD was improved and the process of airway remodeling was restrained, and it showed that N-acetyl cysteine has a positive role in improving patients' local lesion airway remodeling.

There are many researches on the pathogenesis of COPD, and they currently focus on chronic inflammation, oxidative stress, protease/antiprotease imbalance and other aspects, of which oxidative stress is the focus of research in recent years. Oxidative stress can lead to oxidant and antioxidant expression imbalance and cause oxidative damage and even direct necrosis of histocytes. Study has confirmed there is systemic and local oxidation and antioxidation imbalance in both acute and stable COPD patients[12]. Keap1-Nrf2-ARE signaling pathway is a newly discovered protective transduction pathway that resists external oxidation and chemical stimulation, and high expression of Keap1-Nrf2-ARE signaling pathway in patients with COPD may be an effective symbol of strong self-defensive ability and stable condition in the body. There is mainly the expression of Kelch-like epichlorohydrin-associated protein-1 (Keap1), nuclear transcription factor erythron-2p45 (Nrf2), antioxidant response element (ARE), etc in Keap1 Nrf2-ARE signaling pathway downstream. Nrf2 belongs to the family of transcription factor CNC, and its deletion or activation disorder can lead to increased sensitivity of cells to stressor. Keap1 is the key factor that regulates Nrf2, it controls Nrf2 in cytoplasm under the normal state, the two are dissociated and enter into the nucleus in cases of stimulation, Nrf2 and Maf protein form dimers, and the dimmer is combined with ARE and leads to two-phase detoxifying enzyme gene transcription[13]. ARE is the specific DNA-promoter sequences, and signal molecules are mediated by Keap1 and Nrf2 and conducted into the intranuclear ARE. In the research, the detection of Keap1-

Nrf2-ARE signaling pathway activity in serum and induced sputum showed that Keap1, Nrf2 and ARE expression levels of observation group were higher after treatment, indicating that N-acetyl cysteine treatment and intervention could significantly increase anti-inflammatory and antioxidant properties in the COPD patients, and had positive significance in optimizing the disease.

Given the decisive significance of oxidation/antioxidant imbalance in the development of COPD, the levels of oxidation and anti-oxidation-related factors in serum of patients were further detected to intuitively measure oxidation/antioxidation status in the body[14]. In acute exacerbation period of COPD, malondialdehyde (MDA) and lipid peroxide (LPO) levels increase significantly, it indicates that there is lipid peroxidation damage in the body, and the process can produce a large number of oxygen free radicals and further consume superoxide dismutase (SOD), glutathione peroxidase (gsh-px) and other antioxidants, and lead to broken oxidation and antioxidant balance and further aggravated disease. Total antioxidant capacity (T-AOX) represents the body's overall antioxidant effect, and when there are many oxygen free radicals in the body and antioxidants are severely consumed, T-AOX value reduces[15]. In the research, after observation group received N-acetyl cysteine auxiliary treatment, serum MDA and LPO values were lower while SOD, GSH-Px and T-AOX values were higher, and it was because that NAC sulfydryl, as $H^+$ donor, directly played an antioxidant role, improved intracellular GSH biosynthesis, etc.

To sum up, it is concluded as follows: N-acetyl cysteine adjuvant therapy for patients with stable COPD optimizes airway inflammation, remodeling and so on, and is worth popularization and application in clinical practice in the future.

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