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Original Research Article

Effect of N-acetylcysteine aerosol inhalation on serum levels of inflammatory factors and immune function in children with upper airway cough syndrome

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Abstract

Purpose: *To investigate the influence of N-acetylcysteine (NAC) aerosol inhalation on serum inflammatory factors and immune function in children with upper airway cough syndrome (UACS).*

Methods: *118 children with UACS who were on admission at Wenzhou Hospital of Integrated Traditional Chinese and Western Medicine, China between September 2021 and February 2023, were enrolled in the study. They were assigned to control and study cohorts, with 59 patients per group. Control group received routine treatment (3 mL of normal saline via nebulized inhalation 2 times/day), while the study group received 3 mL of NAC aerosol inhalation via nebulized inhalation 2 times/day. Both groups were treated for 2 weeks. Thereafter, the two groups were compared with respect to total treatment effectiveness, cough symptom and Lund-Kennedy ratings, levels of inflammatory factors (interleukin-13 (IL-13), interleukin-27 (IL-27), C-reactive protein (CPR)) and concentrations of immune function indices (CD_3^+, CD_4^+ and CD_8^+) before and after treatment, and adverse reactions.*

Results: *Treatment effectiveness (efficacy) was significantly higher ($p < 0.05$) in study cohort than in control cohort (89.83 vs 71.19 %). The 2 cohorts had significant reductions in scores on cough symptoms and Lund-Kennedy, with smaller values in study cohort. In both groups, the levels of IL-13 and CPR were decreased, while IL-27 level was increased. Levels of IL-13 and CPR in study group were significantly lower than those in control group, while IL-27 level was significantly higher than that in control group ($p < 0.05$).*

Conclusion: *N-acetylcysteine aerosol inhalation enhances curative effect, relieves clinical symptoms, reduces inflammation, and improves immunity in UACS children, and shows good degree of safety. However, its long-term efficacy needs to be studied.*

Keywords: *Upper airway cough syndrome, Children, N-acetylcysteine, Aerosol inhalation, Inflammatory factor, Immune function*

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INTRODUCTION

Upper airway cough syndrome (UACS) is caused by nasal diseases such as nasal polyps, rhinitis, chronic pharyngitis and sinusitis, and it often occurs in children. The main symptom of UACS is chronic cough which lasts for more than 4 weeks, with a serious impact on children's lives and daily learning**Error! Reference source not found.**. In treating UACS in clinical practice,

antibiotics and other drugs are used to alleviate the symptoms to a certain extent. However, long-term use of these drugs easily leads to drug resistance and poor treatment outcomes**Error! Reference source not found.**. Therefore, the treatment of UACS is an important study subject.

N-acetylcysteine (NAC) is an antioxidant. When administered through nebulized inhalation, NAC affects the nasal cavity and sinus mucosa, enhances mucus dissolution, and restores respiratory function. Studies have shown that the drug NAC produces good efficacy in treating chronic obstructive pulmonary disease (COPD), bronchitis, acute sinusitis, and other respiratory diseases**Error! Reference source not found.**. However, the effect of NAC on treatment of UACS has not been investigated. In this study, the efficacy of aerosol-inhaled NAC on UACS was investigated, to provide data for improving the treatment plan for children with UACS.

METHODS

Subject data

A total of 118 children with UACS who visited Wenzhou Hospital of Integrated Traditional Chinese and Western Medicine, Wenzhou, China, from September 2021 to February 2023, were selected for the study. They were randomly divided into control and study groups, with 59 children in each group. This study was approved by the ethics committee of Wenzhou Hospital of Integrated Traditional Chinese and Western Medicine (approval no. 2022110307310001930 13), and complied with the requirements of the Declaration of Helsinki of the World Medical Association [6]. All family members of the children were duly informed about the study, and they voluntarily signed informed consent.

Inclusion criteria

Children who met the diagnostic criteria of UACS**Error! Reference source not found.**; children aged 3 - 12 years, and those with cough duration > 4 weeks, were included in the study.

Exclusion criteria

Children with nose bleeding, innate immune deficiency and poor compliance, were excluded. Moreover, children on immune preparations and other drugs in the previous month; children with hepatic and renal insufficiency, and those who had blood diseases, neurological and endocrine diseases and chronic cough due to other diseases, were excluded. Children with other respiratory diseases and children who were allergic to drugs used in this study were also excluded.

Treatments

Basic treatment was used in the two groups. This included the use of anti-allergy, anti-infection, anti-cough, theophylline, ambroxol, oxygen therapy, and nebulized budesonide inhalation [7]. Children in the control group were given 3 mL of normal saline through aerosol inhalation, 2 times a day. Children in study group were given 3 mL of NAC solution (0.10g/mL, Zambon S.p.A., State Medical Permit no. H20150548) *via* atomized inhalation, 2 times/day. Both groups were treated for 2 weeks.

Evaluation of parameters/indices

Clinical treatment efficacy

Clinical treatment efficacy in children was evaluated before and after treatment**Error! Reference source not found.**. It was categorized as *cured* (absence of mucus in the throat, nasal symptoms and cough); *significantly effective* (presence of a small amount of mucus in the throat, but nasal symptoms no longer appeared, and cough was significantly relieved); *effective* (mucus level in the throat was reduced, and nasal symptoms and cough were alleviated), and *ineffective* (there was no change in the amount of mucus in the throat and nasal symptoms and coughing were not alleviated). Total effectiveness (T) was calculated using Eq 1.

T (%) = (N-Nu)/N)100 ……………….. (1)

where N is total number of patients, and Nu is total number of unresponsive patients.

Severity of cough

The severity of cough in patients was evaluated before and after treatment using the cough symptom score [9], with scale divided into daytime and night. Daytime: 0 points (i.e., no cough), 1 point (occasional cough), 2 points (repeated cough with slight influence from daily activities), and 3 points (repeated cough with obvious influence from daily activities); Night: 0 (no cough), 1 (occasional cough while asleep), 2 (cough which slightly interfered with night sleep), and 3 (cough which significantly interfered with night sleep).

Severity of symptoms

The severity of symptoms in children was evaluated before and after treatment, with Lund Kennedy score**Error! Reference source not found.** using nasal endoscopy *viz*: (a) polyps: 0 point (none), 1 point (not beyond the middle nasal passage), and 2 points (beyond the middle nasal passage); (b) rhinorrhea: 0 point (none), 1 point (thin and clear), and 2 points (purulent and, sticky); (c) edema: 0 point (none), 1 point (slight), and 2 points (obvious). Unilateral scores ranged from 0 to 6, while total scores ranged from 0 to 12. The higher the score, the more severe the symptoms.

Levels of inflammatory factors

The serum levels of interleukin-13 (IL-13), interleukin-27 (IL-27) and C-reactive protein (CPR) were assayed using ELISA after fasting venous blood was collected before and after treatment. The cell concentrations of $CD8^+$, $CD3^+$ and $CD4^+$ were measured with a flow cytometer (Beckman Coulter, Inc.).

Safety analysis

The occurrence of nausea, vomiting, diarrhea, headache, and rash were recorded in each group.

Statistical analysis

Statistical Package for Social Sciences (SPSS) 20.0 software was used for statistical analysis. Measured data are presented as mean ± standard deviation (SD), and a two-group comparison was done with *t*-test. Data from counting are presented in percentages (%) and were compared with chi-squared (χ^2) test. Values of $p < 0.05$ indicated the statistical significance of differences in data.

RESULTS

Basic biodata

Table 1 shows that the general data were comparable in the two cohorts.

Treatment effectiveness

Treatment efficacy was markedly higher in study cohort (89.83 %) than in control cohort (71.19 %; Table 2).

Cough symptom scores and Lund-Kennedy scores

Post-treatment scores on cough symptoms and Lund-Kennedy were significantly lower than the pre-treatment values, with significantly lower post-treatment values in study cohort ($p < 0.05$; Table 3).

Table 1: Comparison of general data between the two groups

Group	Sex		MA (years)	MDD (weeks)	CR	SDET	NS	AD	MDS
	M	*F*							
Control	36	23	6.45±1.20	5.52±1.18	27	9	7	4	12
Study	33	26	6.86±1.34	5.39±1.06	24	8	10	2	15
χ2/t	0.314		1.751	0.630	1.765				
P-value	0.575		0.083	0.530	0.779				

n = 59 per group. (MA: mean age; MDD: mean disease duration: CR: chronic rhinitis: SDET: second-degree enlargement of the tonsils: NS: naso-sinusitis: AD: adenoid hypertrophy: MDS: multiple diseases simultaneously)

Table 2: Treatment effectiveness in each cohort (n=59)

Group	Cured	Significantly effective	Improved	Ineffective	Total Effectiveness
Control	10 (16.95)	15 (25.42)	17 (28.81)	17 (28.81)	42 (71.19)
Study	23 (38.98)	19 (32.20)	11 (18.64)	6 (10.17)	53 (89.83)
χ^2					6.535
P-value					0.011

Table 3: Cough symptom scores and Lund-Kennedy scores in the two cohorts (n=59)

Group	Period	Cough symptom score		Lund-Kennedy score
		Daytime	*Night*	
Control	Pre-treatment	2.17±0.39	2.29±0.54	6.14±1.26
	Post-treatment	1.20±0.27*	1.43±0.32*	3.56±0.68*
Study	Pre-treatment	2.24±0.36	20±09.4.4	6.34±1.42
	Post-treatment	0.62±0.15*#	0.86±0.17*#	2.23±0.30*#

*$P < 0.05$, vs. pre-treatment (same cohort); #$p < 0.05$, vs. control post-treatment

Table 4: Comparison of inflammatory factor concentrations between the 2 cohorts (n=59)

Group	Period	IL-13 (ng/L)	IL-27 (ng/L)	CPR (mg/mL)
Control	Pre-treatment	46.12±5.58	7.81±1.04	74.84±9.75
	Post-treatment	33.34±4.70*	28.28±3.31*	46.08±7.32*
Study	Pre-treatment	45.65±5.22	7.29±1.16	72.46±9.30
	Post-treatment	28.80±4.39*#	32.15±3.64*#	31.62±5.18*#

*P < 0.05, pre-treatment comparison with same group; #p < 0.05, vs. control

Table 5: Levels of $CD3^+$, $CD4^+$ and $CD8^+$

Group	Period	CD_3^+	CD_4^+	CD_8^+
Control	Pre-treatment	48.56±7.24	31.34±5.47	36.61±4.18
	Post-treatment	53.74±7.56*	39.51±5.31*	30.55±5.20*
Study	Pre-treatment	48.82±7.32	32.47±5.51	37.54±4.84
	Post-treatment	62.26±8.45*#	42.83±6.64*#	27.96±4.14*#

*P < 0.05 vs same group before treatment; #p < 0.05 vs control post-treatment

Table 6: Incidents of adverse reactions (n=59)

Group	Nausea and Vomiting	Diarrhea	Headache	Erythrasma	Overall Incidence (%)
Control	2 (3.39)	0 (0.00)	2 (3.39)	1 (1.69)	5 (8.47)
Study	4 (6.78)	1 (1.69)	1 (1.69)	2 (3.39)	8 (13.56)
χ^2-value					0.778
P-value					0.378

Levels of inflammatory factors

Treatment reduced levels of IL-13 and CPR in both cohorts, with significantly lower IL-14 and CPR values in control cohort, while IL-27 concentration was significantly higher than pre-treatment value, with markedly higher value in study group (p < 0.05), as shown in Table 4.

Concentrations of immune function levels

In both groups, $CD3^+$ and $CD4^+$ were significantly raised after treatment, with higher $CD3^+$ and $CD4^+$ concentrations in study cohort, while post-treatment $CD8^+$ concentration was reduced, with lower levels in study cohort (p < 0.05; Table 5).

Safety

The indents of adverse reactions during treatment were comparable in the 2 cohorts (p > 0.05; Table 6).

DISCUSSION

Children have special anatomical and physiological structures. The sinus mucosa contains numerous lymphatic and mucosal vessels; the sinus opening is large, and the immune function is not optimal. These features make it easy for children to be infected with UACS after respiratory tract infection**Error! Reference source not found.**. The main cause of chronic cough is UACS, and the pathogenesis of UACS is controversial. However, three speculations have been made viz: (a) when the nasal mucosa is inflamed, most allergens become activated and stimulate the production of sensitized lymphocytes which act on the nasal cavity and lower respiratory tract, causing a high airway reactivity state, and when the stimulation is repeated, it produces a rhino-pulmonary reflex which causes cough**Error! Reference source not found.**; (b) inflammation impairs the function of the nasal mucosa, and when dry and cold air or air carrying particles is inhaled, it easily produces a stress response which provokes cough in the airway**Error! Reference source not found.**; and (c) mucosal secretions from the nose and sinuses flow into the pharynx or lower respiratory tract, thereby producing a cough reflex**Error! Reference source not found.**.

The conventional treatment for UACS is mainly symptomatic: it relieves nasal symptoms and foreign body sensation in the pharynx and reduces inflammation. However, due to its slow efficacy, children need to take medicines for a long time. However, some children find it difficult to adhere to the treatment course, a situation which may easily cause relapse of disease after discontinuing the drug**Error! Reference source not found.**. Therefore, there is a need to evolve more efficient treatment options for children with UACS.

N-acetylcysteine (NAC) is a novel mucolytic agent that is employed for treating respiratory diseases because of its various physiological functions**Error! Reference source not found.**.

This study has demonstrated that after treatment, total treatment effectiveness in study cohort (89.83 %) was superior to that in control cohort (71.19 %), while cough symptom score and Lund-Kennedy score in the treatment group were lower than those in the control group. These data indicate that treatment with NAC aerosol enhanced outcomes and effectively relieved cough and other clinical symptoms.

In the treatment of UACS, the mechanism of action of NAC may involve (a) expectorant effect: NAC has a special structure containing a large number of sulfhydryl groups which promote the disulfide bond cleavage of mucin in sputum, thereby reducing the viscosity of mucus. It also splits the DNA in sputum, makes sputum thinner, facilitates coughing out of sputum, prevents sputum accumulation, and reduces airway resistance [17]; (b) antioxidant effect: NAC interacts with hydroxyl radicals and other factors, thereby scavenging oxygen free radicals, and enhancing the production of reduced glutathione (GSH) which is an important antioxidant, thereby reducing airway damage through antioxidant action [18]; (c) bacteriostasis: NAC destroys pathogenic biofilms, inhibits the adhesion of pathogenic bacteria, and inhibits the growth of *Staphylococcus epidermidis* and other bacteria [19]; (d) anti-inflammation and immune regulation: NAC blocks the expressions of proteins involved in airway inflammation, and it protects the airway by activating immune response [20]; (e) NAC promotes the expression of nitric oxide, dilates pulmonary blood vessels, enhances pulmonary ventilation, improves oxygen supply to airway mucosa, and facilitates the repair of airway mucosa [21]; and (f) NAC stimulates the production of bronchial glandular serous fluid and enhances the activity of nasal mucosa cilia, thereby improving the function of nasal self-defense barrier [22]. Moreover, aerosol inhalation makes NAC work faster [23].

In this study, it was found that after treatment, study cohort had lower concentrations of IL-13 and CPR than those in control cohort, while IL-27 concentration was higher in control cohort, indicating that NAC aerosol inhalation inhibited inflammatory reactions, probably because NAC inhibits the growth of pathogenic bacteria, thereby reducing the extent of bacterial stimulation of the airway. Additionally, NAC reduced inflammatory reactions by blocking the expressions of nuclear transcription factors. In this study, it was also found that post-treatment concentrations of $CD3^+$ and $CD4^+$ were higher in study cohort, while $CD8^+$ concentration was higher in control cohort. These data confirm that NAC aerosol inhalation boosts immunity via the facilitation of recovery of immune function by regulating T cell levels. There were no significant differences in adverse outcomes between the two groups during treatment. This shows that NAC aerosol inhalation produces a good safety profile during treatment.

Limitations of this study

This study has some limitations: the selected sample size was small, and the observation time was insufficient. In subsequent studies, the sample size will be expanded and the observation time will be extended, to further investigate the effectiveness of nebulized NAC inhalation in the treatment of UACS children.

CONCLUSION

This study has demonstrated that NAC aerosol inhalation produces significant clinical efficacy in children with UACS: it significantly reduces cough and other clinical symptoms, reduces inflammatory response, and improves immune function, with a good safety profile. However, its long-term efficacy needs to be further studied.

DECLARATIONS

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Ethical approval

None provided.

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. Mengmeng

Zhong and Nan Zheng conceived and designed the study, and drafted the manuscript. Mengmeng Zhong, Nan Zheng and Hui Huang collected, analyzed and interpreted the experimental data. All authors revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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