RESEARCH ARTICLE

Evaluation the efficacy and safety of N-acetylcysteine inhalation spray in controlling the symptoms of patients with COVID-19: An open-label randomized controlled clinical trial

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Abstract

The aim of this study was to evaluate the effect and safety of N-acetylcysteine (NAC) inhalation spray in the treatment of patients with coronavirus disease 2019 (COVID-19). This randomized controlled clinical trial study was conducted on patients with COVID-19. Eligible patients ($n = 250$) were randomly allocated into the intervention group (routine treatment + NAC inhaler spray one puff per 12 h, for 7 days) or the control group who received routine treatment alone. Clinical features, hemodynamic, hematological, biochemical parameters and patient outcomes were assessed and compared before and after treatment. The mortality rate was significantly higher in the control group than in the intervention group (39.2% vs. 3.2%, $p < 0.001$). Significant differences were found between the two groups (intervention and control, respectively) for white blood cell count (6.2 vs. 7.8, $p < 0.001$), hemoglobin (12.3 vs. 13.3, $p = 0.002$), C-reactive protein (CRP: 6 vs. 11.5, $p < 0.0001$) and aspartate aminotransferase (AST: 32 vs. 25.5, $p < 0.0001$). No differences were seen for hospital length of stay (11.98 ± 3.61 vs. 11.81 ± 3.52, $p = 0.814$) or the requirement for intensive care unit (ICU) admission (7.2% vs. 11.2%, $p = 0.274$). NAC was beneficial in reducing the mortality rate in patients with COVID-19 and inflammatory parameters, and a reduction in the development of severe respiratory failure; however, it did not affect the length of hospital stay or the need for ICU admission. Data on the effectiveness of NAC for Severe Acute Respiratory Syndrome Coronavirus-2 is limited and further research is required.

KEYWORDS

clinical trial, N-acetylcysteine, Severe Acute Respiratory Syndrome Coronavirus-2

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a highly infectious respiratory disease caused by a new coronavirus, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). COVID-19 disease emerged in Wuhan city, China in late 2019 and resulted in a global pandemic leading to more than 5.4 million deaths

worldwide.[1,2] The rapid transmission of this highly pathogenic virus has necessitated the urgent need for global development and immediate application of therapeutic approaches and preventive measures against COVID-19 disease.[3] Prevaccination, supportive care and oxygen therapy remain key management elements of COVID-19 though there is no effective treatment for acute COVID-19 disease.[4–6] The emergence of SARS-CoV-2 variants have

increased the transmissibility and severity of infection and may also reduce the level of immunity of current vaccines.[7–9] In addition, the unequal distribution of vaccines worldwide has made it difficult to curtail the pandemic,[10,11] hence the need to accelerate the discovery and development of safe and effective agents as adjunctive therapies.

The pathogenetic mechanism of COVID-19 is not fully understood[12]; however, upon reaching the lower respiratory tract, it appears that the virus spike protein binds to the angiotensin-converting enzyme 2 (ACE2), allowing it to enter the alveolar cells.[13] ACE2 is an enzyme that catalyzes the conversion of angiotensin II to angiotensin 1–7; therefore, elevated levels of serum angiotensin II in patients with COVID-19 indicate the inactivation of this enzyme.[14] The increased levels of angiotensin II after inactivation of ACE2 by SARS-CoV-2 leads to a redox imbalance in the alveolar epithelium cells, causing apoptosis, breakdown of the alveolar-capillary barrier resulting in impaired gas exchange and respiratory failure.[15–17] Evidence suggests that the excessive production of angiotensin II can be blocked by N-acetylcysteine (NAC), preventing its cleavage to angiotensin 1–7 by ACE2, which may reduce pulmonary disease severity.[18–20]

NAC has been used as a mucolytic in clinical practice for several decades to treat medical conditions including bronchitis, acute respiratory distress syndrome (ARDS), paracetamol intoxication, chemotherapy-related toxicity, doxorubicin cardiotoxicity, heavy metal intoxication, ischemia-reperfusion cardiac injury, human immunodeficiency virus infection or acquired immunodeficiency syndrome, and neuropsychiatric disorders.[21–24] NAC's antioxidant effect may attenuate the oxidative stress environment created by cytokine storm syndrome and the production of reactive oxygen species (ROS).[25] Given these properties, we hypothesized that NAC might be a potential adjuvant therapy against SARS-CoV-2. Therefore, we conducted this single-center, prospective, randomized, open-labeled, controlled clinical trial study to evaluate the therapeutic effect and safety of NAC inhalation spray in patients with COVID-19.

2 | MATERIALS AND METHODS

2.1 | Trial design

This single-center, prospective, randomized, open-labeled, controlled clinical trial was conducted to investigate the therapeutic efficacy of NAC inhalation spray in the clinical management of patients with COVID-19 admitted to Baqiyatallah hospital, Tehran, Iran, from May 2021 until August 2021. The protocol study was reviewed and approved by the Ethics Committees of Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1399.123), following the Declaration of Helsinki of the World Medical Association.[26] This study was registered at the Iranian Registry of Clinical Trials (IRCT20080901001165N55) dated 23-05-2020. Written informed consent was obtained from all patients or their legal representatives

if they could not provide consent. The study was conducted and reported following the recommendations of the Consolidated Standards of Reporting Trials (CONSORT) statement.[27]

2.2 | Study population

Symptomatic adult patients with a positive COVID-19 diagnosis were eligible for study participation if they met all the inclusion criteria. Inclusion criteria included (a) age of ≥18, (b) positive result on a reverse-transcriptase–polymerase-chain-reaction assay of a specimen collected from a nasopharyngeal swab that confirmed COVID-19 infection, (c) chest computed tomography (CT) results that confirmed COVID-19 infection and (d) less than 7 days since the onset of symptoms. The exclusion criteria included (a) any allergy or hypersensitivity to NAC, (b) signs of the imminent need for intubation or the need for intensive care unit (ICU) admission due to increased respiratory effort, decreased level of consciousness, and oxygen saturation (SpO_2) less than 90% with supplemental oxygen, (c) being a participant in another clinical trial at the same time, (d) pregnancy or breastfeeding and (e) unwilling to participate or unable to give informed consent.

2.3 | Randomization and intervention

Eligible patients were randomly assigned (1:1) to the NAC or the control groups. The Block randomization method was used to randomize the patients. The randomization code was generated by computer in permuted blocks of 6. Block randomization was performed using the sealed envelope technique and computer-generated random numbers by Random Allocation Software© (RAS; Informer Technologies, Inc.). All patients in the study groups received standard care regimens based on the latest version of the Ministry of Health of Iran protocols for COVID-19.[28] In addition to routine treatment, the intervention group received NAC spray (@sinadarou.co) one puff (200 μg per puff) every 12 h for 7 days.

2.4 | Data collection

Clinical data were collected on the specific case report forms for each patient that included: (a) demographics information: (age, gender, height, weight, and body mass index); (b) comorbidities: diabetes mellitus, hypertension, chronic kidney disease, malignancy, asthma and chronic obstructive pulmonary disease; (c) symptoms: fever, chills, dyspnea, chest pain, cough, sore throat, headache, myalgia, diarrhea, nausea, vomiting, olfactory, and generalized weakness; (d) vital signs or hemodynamic parameters: systolic blood pressure (SBP-mmHg), diastolic blood pressure (DBP-mmHg), heart rate (HR) beats per minute (BPM), respiratory rate (RR per minute) and oxygen saturation (SpO_2-%); (e) hematological data: white blood cell (WBC-cell/mm^3), neutrophil count (cells/μl), lymphocyte count (cell/mm^3),

platelet (Plt-cell/mm^3) and hemoglobin (Hb-g/dl); (f) biochemical data: erythrocyte sedimentation rate (ESR-mm/h), C-reactive protein (CRP-mg/L), creatinine (CR-mg/dl), urea (mg/dl), aspartate amino-transferase (AST-U/L) and alanine aminotransferase (ALT-U/L); (g) CT score based on chest CT scan (noted below); (h) National Early Warning Score (NEWS-noted below); (i) hospital length of stay (LOS-day); (j) need for ICU admission and (k) mortality. All symptoms, hemodynamic, hematological and biochemical parameters were collected for each patient before and after the intervention.

2.5 | Chest CT scan

All patients underwent chest CT examinations in a supine position before and after the intervention. CT scan examinations were performed with a 16-row detector CT scanner (general electric GE; optima) and reviewed by two radiologists with 12 and 10 years of experience in thoracic imaging. To quantify the extent of lung lesions, a thin-section CT involvement score was assigned based on all abnormal areas shown: each of the five lobes of the lung was assigned a visual score: score 0, no involvement; score 1, less than 5% involvement; score 2, 5%–25% involvement; score 3, 26%–49% involvement; score 4, 50%–75% involvement; and score 5, greater than 75% involvement. There was a score of 0–5 for each lobe, with a total possible score of 0–25.[29]

2.6 | Calculation of NEWS score

NEWS score included the following seven vital signs parameters: RR, oxygen saturation (SpO$_2$) that was measured by pulse oximetry, supplementary oxygen requirement, systolic arterial blood pressure (SBP), pulse rate (PR), body temperature (T), and AVPU (Alert, responds to Voice, responds to Pain, Unresponsive) score based on the Glasgow Coma Scale (GCS) (The AVPU score was derived from the GCS as follows: $A = 14$–15, $V = 9$–13, $P = 4$–8, $U = 3$).[30] Patients with a score between 0 and 4 were considered to have low risk, those with a score of 5 or 6 were considered to have medium risk, and those with a score ≥ 7 were considered to have a high risk.[30]

2.7 | Statistical analysis

The Shapiro–Wilk test was used to test data normality. Normal distributed data were reported as mean and standard deviation for continuous variables and percentage (%) for categorical character-istics. Non-normal distributed continuous data were reported as median and interquartile range. Comparison of demographic char-acteristics and clinical variables between two study groups were assessed using χ^2 or Fisher's exact tests for categorical variables and Mann–Whitney u test for non-normally distributed continual variables. To compare symptoms as categorical variables, we used the χ^2 test or Fisher exact test between two groups and the

McNemar test within groups pre and postintervention. Mann–Whitney u test and Wilcoxon Ranks test was used for comparing the non-normal distributed continuous variables (hemo-dynamic, hematological, biochemical, CT score, and NEWS score) between groups and within groups pre and postintervention. All statistical analysis was carried out using SPSS software (ver.21) (SPSS Inc.). In all analyses, p Values less than 0.05 were considered significant.

3 | RESULTS

3.1 | Trial population

From May 2021 to August 2021, 250 out of 260 patients with COVID-19 referred to Baqiyatallah Hospital in Tehran, Iran, met the inclusion criteria and gave informed written consent to participate in the study. Patients were randomly assigned through block random-ization 1:1 into the intervention group ($n = 125$) or the control group ($n = 125$). No patient (in either study group) was eliminated during the follow-up period, and 250 patients completed the study and were included in the analysis. Figure 1 shows the consolidated standards of reporting trials (CONSORT) flowchart of participants through each stage of a randomized trial.

3.2 | Demographic characteristics and clinical outcomes

A comparison of the demographic characteristics and clinical outcomes between the two groups is presented in Table 1. Two hundred and fifty patients with COVID-19, including 138 (55.2%) men and 112 (44.8%) women, were enrolled in this study. Patients in the intervention and control groups showed similar demographic characteristics and clinical data. There was no difference between the two groups in terms of gender ($p = 0.203$), smoking status ($p = 0.811$), comorbidities ($p = 0.899$), height (0.548), weight ($p = 0.112$), and BMI ($p = 0.052$). However, the mean age of patients in the intervention group was significantly higher than in the control groups (57.25 ± 15.80 vs. 52.77 ± 16.21, $p = 0.02$). Regarding the clinical outcomes, there was no difference between the two groups for hospital LOS ($p = 0.814$) and the need for ICU admission ($p = 0.274$); however, nearly 40% of patients in the control group died, which was significantly higher than the intervention group (39.2% vs. 3.2%, $p < 0.001$).

3.3 | Symptoms

A comparison of pre and postintervention symptoms between two study groups of study is shown in Table 2. Four common symptoms in both groups were cough (60.4%), myalgia (51.2%), dyspnea (50.4%), and fever (46.4%). There were no differences in the frequency of
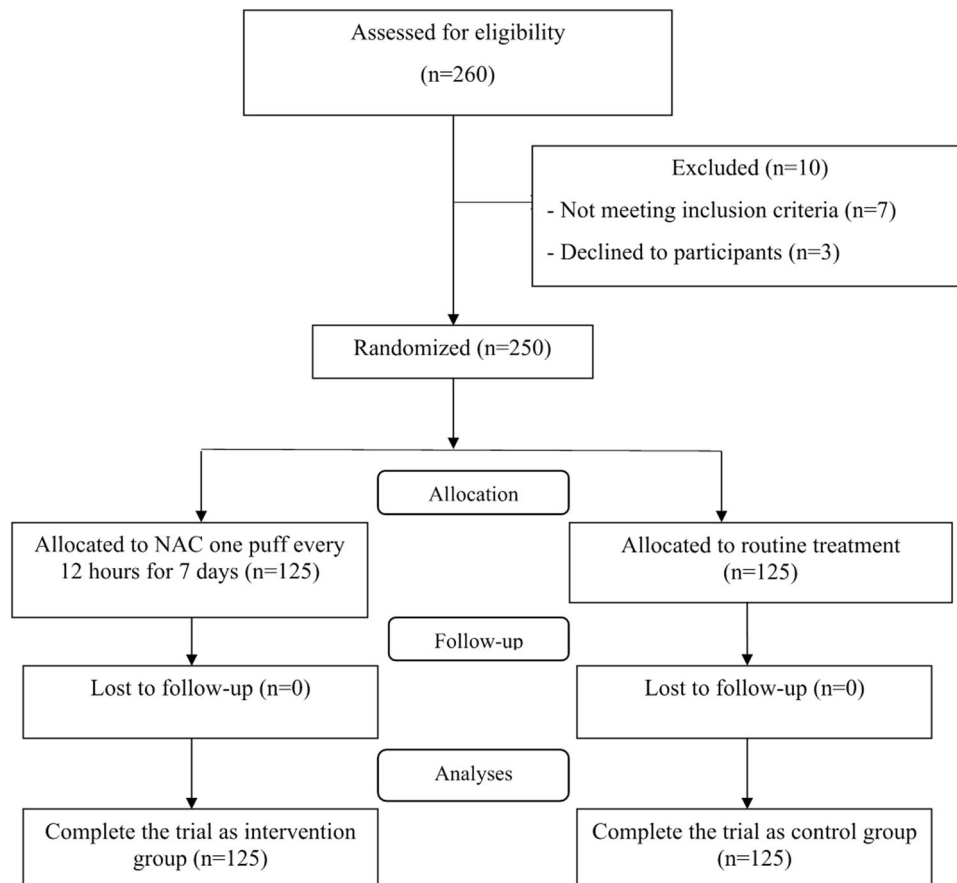
FIGURE 1 CONSORT flow diagram for this study. CONSORT, Consolidated Standards of Reporting Trials.

symptoms preintervention among patients in the NAC and control groups, indicating the homogeneity of the participants in the study ($p > 0.05$). In postintervention, dyspnea (7.2% vs. 19.2%, $p = 0.005$) and generalized weakness (3.2% vs. 9.6%, $p = 0.039$) were significantly reduced in the NAC group than the control group. In addition, all symptoms were significantly decreased within groups pre and postintervention ($p < 0.001$).

3.4 | Vital signs, hematological, and biochemical parameters findings

Comparison of vital signs and hematological parameters pre and postintervention between two groups of study are shown in Table 3. There were no differences between the two groups in terms of vital signs and hematological parameters, except (intervention vs. control) RR (18 vs. 17, $p = 0.036$), SpO_2 (93% vs. 92%, $p = 0.014$) and lymphocytes (6.8 vs. 11.0, $p < 0.001$). The results for postintervention (intervention vs. control) showed significant differences in the median of WBC (6.2 vs. 7.8, $p < 0.001$) and Hb (12.3 vs. 13.3, $p = 0.002$) between groups. Significant differences were seen for pre and postintervention for vital signs and hematological parameters ($p < 0.001$), except for DBP and WBC.

Comparison of biochemical parameters pre and postintervention between the two groups of study are presented in Table 4.

There were no differences between the two groups, except for urea (27 vs. 17, $p < 0.0001$) and ALT (31 vs. 37, $p = 0.009$), which were significantly higher and lower in the NAC group than in the control group, respectively. The results postintervention (intervention vs. control) showed significant differences in median CRP (6.0 vs. 11.5, $p < 0.0001$) and AST (33 vs. 25.5, $p < 0.0001$). Significant differences pre and postintervention were seen for biochemical parameters ($p < 0.001$), except ALT within the control group ($p = 0.315$).

3.5 | CT scores and NEWS scores

Comparison of CT score and NEWS score pre and postintervention between the intervention and control groups are shown in Table 5. There were no differences in the median CT and NEWS scores between NAC and control groups preintervention. The median score of CT was significantly lower in the intervention group than in the control group post-intervention (6 vs. 7, $p < 0.0001$); however, the median score of NEWS was not different between the two groups postintervention (2 vs. 1, $p = 0.184$). CT scores differed significantly between groups ($p < 0.0001$), and both CT and NEWS scores were significantly decreased with groups for both the intervention and control groups ($p < 0.0001$).

TABLE 1 Comparison of demographic characteristics and clinical data between two groups of study

Variables		Total participants (n = 250)	Intervention group (n = 125)	Control group (n = 125)	p Value
Gender	Male (%)	138 (55.2)	64 (51.2)	74 (59.2)	0.203[a]
	Female (%)	112 (44.8)	61 (48.8)	51 (40.8)	
Age (year)	Mean ± SD (Range)	55.01 ± 16.13 (18–90)	57.25 ± 15.80 (18–90)	52.77 ± 16.21 (24–89)	**0.022**[*,b]
	Median (IQR)	54 (41–67)	56 (45–71)	53 (39–65)	
Hight (cm)	Mean ± SD (Range)	169.76 ± 10.24 (124–194)	169.53 ± 10.62 (150–194)	169.99 ± 9.88 (124–191)	0.548[b]
	Median (IQR)	170 (161–178)	168 (160–179)	169.9 (162. 5–178)	
Weight (kg)	Mean ± SD (Range)	77.86 ± 17.08 (50–171)	76.72 ± 18.87 (50–171)	78.96 ± 15.08 (50–120)	0.112[b]
	Median (IQR)	76 (67–86)	75 (64–85)	78 (67.5–87.5)	
BMI	Mean ± SD (Range)	27.07 ± 5.79 (16.79–60.55)	26.77 ± 6.63 (16.79–60.55)	27.37 ± 4.81 (19.53–43.57)	0.052[b]
	Median (IQR)	26.19 (23.30–29.37)	25.05 (22.8–28.7)	26.66 (23.8–29.7)	
Smoking status	Yes (%)	19 (7.6)	10 (8)	9 (7.2)	0.811[a]
	No (%)	231 (92.4)	115 (92)	116 (92.8)	
Comorbidities	Yes (%)	111 (44.4)	56 (44.8)	55 (44)	0.899[a]
	No (%)	139 (55.6)	69 (55.2)	70 (56)	
Comorbidity	DM (%)	49 (19.6)	23 (18.4)	26 (20.8)	0.633[a]
types	HTN (%)	63 (25.2)	29 (23.2)	34 (27.2)	0.466[a]
	IHD (%)	26 (10.4)	14 (11.2)	12 (9.6)	0.679[a]
	CKD (%)	20 (8.1)	8 (6.4)	12 (9.6)	0.351[a]
	Cancer (%)	6 (2.3)	5 (4)	1 (0.8)	0.213[c]
	Asthma (%)	8 (3.2)	6 (4.8)	2 (1.6)	0.281[c]
	COPD (%)	4 (1.6)	3 (2.4)	1 (0.8)	0.622[c]
Hospital LOS	Mean ± SD (Range)	11.89 ± 3.56 (7–37)	11.98 ± 3.61 (8–31)	11.81 ± 3.52 (7–37)	0.814[b]
	Median (IQR)	11 (10–13)	11 (10–13)	12 (10–13)	
ICU needed	Yes (%)	23 (9.2)	9 (7.2)	14 (11.2)	0.274[a]
	No (%)	227 (90.8)	116 (92.8)	111 (88.8)	
Mortality	Yes (%)	53 (21.2)	4 (3.2)	49 (39.2)	**<0.001**[*,a]
	No (%)	197 (78.8)	121 (96.8)	76 (60.8)	

Note: Bold values are statistically significant p values.

Abbreviations: CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; IHD, ischemic heart disease; HTN, hypertension.

[a]p Value based on χ^2 test;

[b]p Value based on Mann–Whitney u test;

[c]p Value based on Fisher exact test;

*p < 0.05 considered as significant.

4 | DISCUSSION

In this single-center, prospective, randomized, controlled trial, and 250 patients with COVID-19 infection were randomly divided into two equal groups. Patients who received standard of care were compared with patients who additionally received a single puff (200 μg/puff) of inhalation spray of NAC every 12 h for 7 days. Symptoms in both study groups were significantly improved on the seventh day compared to the first day of hospitalization. Treatment with NAC led to

significantly lower frequencies of dyspnea and weakness compared to the control group. There were significant differences between the two groups in terms of WBC, Hb, CRP and AST postintervention. Furthermore, both CT score and NEWS score were significantly reduced within both groups; however, NAC treatment significantly reduced the CT score compared to the control group. The mortality rate was significantly lower in the intervention group than in the control group despite the increased age of the subjects randomized to the intervention arm. No differences were observed in vital signs, the requirement for

TABLE 2 Comparison of symptoms on pre- and post-intervention between two groups of study

Symptoms		Intervention group (n = 125)	Control group (n = 125)	p Value[a]
Fever	Before (yes, %)	63 (50.4)	53 (42.4)	0.205
	After (yes, %)	4 (3.2)	1 (0.8)	0.175
	p Value[b]	**<0.001***	**<0.001***	
Chills	Before (yes, %)	58 (46.4)	56 (44.8)	0.800
	After (yes, %)	0	0	
	p Value[b]	**<0.001***	**<0.001***	
Cough	Before (yes, %)	73 (58.4)	78 (62.4)	0.518
	After (yes, %)	12 (9.6)	18 (14.4)	0.243
	p Value[b]	**<0.001***	**<0.001***	
Dyspnea	Before (yes, %)	60 (48.4)	66 (53.2)	0.446
	After (yes, %)	9 (7.2)	24 (19.2)	**0.005***
	p Value[b]	**<0.001***	**<0.001***	
Chest pain	Before (yes, %)	33 (26.4)	33 (26.4)	0.970
	After (yes, %)	8 (6.4)	5 (4)	0.393
	p Value[b]	**<0.001***	**<0.001***	
Sore throat	Before (yes, %)	25 (20)	27 (21.6)	0.755
	After (yes, %)	0	0	
	p Value[b]	**<0.001***	**<0.001***	
Headache	Before (yes, %)	30 (24)	38 (30.4)	0.256
	After (yes, %)	0	0	
	p Value[b]	**<0.001***	**<0.001***	
Myalgia	Before (yes, %)	58 (46.4)	70 (56)	0.129
	After (yes, %)	5 (4)	5 (4)	0.999
	p Value[b]	**<0.001***	**<0.001***	
Diarrhea	Before (yes, %)	19 (15.2)	28 (22.4)	0.145
	After (yes, %)	0	0	
	p Value[b]	**<0.001***	**<0.001***	
Nausea	Before (yes, %)	40 (32)	35 (28)	0.490
	After (yes, %)	0	0	
	p Value[b]	**<0.001***	**<0.001***	
Vomiting	Before (yes, %)	10 (8)	14 (11.2)	0.390
	After (yes, %)	0	0	
	p Value[b]	**<0.001***	**<0.001***	
Olfactory	Before (yes, %)	34 (27.2)	24 (19.2)	0.057
	After (yes, %)	11 (8.8)	10 (8)	0.668
	p Value[b]	**<0.001***	**<0.001***	
Weakness	Before (yes, %)	43 (34.4)	54 (43.2)	0.153
	After (yes, %)	4 (3.2)	12 (9.6)	**0.039***
	p Value[b]	**<0.001***	**<0.001***	

Note: Bold values are statistically significant p values.

[a]p Value based on χ^2 test or Fisher exact test;

[b]p Value based on McNemar test;

*$p < 0.05$ considered as significant.

TABLE 3 Comparison of vital signs and hematological parameters on pre and postintervention between two groups of study

Parameters		Intervention group (n = 125)	Control group (n = 125)	p Value[a]
SBP (mmHg)	Before	130 (110–135)	130 (120–139.5)	0.194
	After	125 (120–130)	125 (120–130)	0.332
	p Value[b]	**0.043***	**0.009***	
DBP (mmHg)	Before	80 (73–89)	80 (75–90)	0.533
	After	80 (75–85)	80 (75–85)	0.816
	p Value[b]	0.582	0.223	
HR (BPM)	Before	96 (87–103)	95 (87–100)	0.402
	After	83 (78–90)	83 (78–91)	0.427
	p Value[b]	**<0.0001***	**<0.001***	
RR (RPM)	Before	18 (16–19)	17 (16–18)	**0.036***
	After	14 (14–15)	15 (14–16)	0.091
	p Value[b]	**<0.0001***	**<0.0001***	
SpO2 (%)	Before	93 (93–96)	92 (89–94)	**0.014***
	After	94 (93–96)	94 (93–95)	0.581
	p Value[b]	**<0.0001***	**<0.0001***	
WBC	Before	6.97 (5.08–9.505)	6.86 (5.20–9.35)	0.981
(×10³ cell/mm³)	After	6.2 (5.06–8.050)	7.80 (5.89–10.17)	**<0.001***
	p Value[b]	0.070	0.088	
Neutrophils	Before	82 (75.05–87.95)	78.2 (71.2–86)	**0.011***
(cells/μL)	After	69 (58–78)	67.9 (59–78)	0.851
	p Value[b]	**<0.0001***	**<0.0001***	
Lymphocyte	Before	6.8 (4.15–10.75)	11 (6.800–15.1)	**<0.001***
(×10³ cell/mm³)	After	24.4 (17.0–32.7)	23 (13.85–33.0)	0.205
	p Value[b]	**<0.0001***	**<0.0001***	
Hb (g/dl)	Before	13.5 (11.6–15)	14 (12.5–15.4)	0.078
	After	12.3 (10.35–13.9)	13.3 (11.35–14.6)	**0.002***
	p Value[b]	**<0.0001***	**<0.0001***	
Platelet	Before	195 (150.5–251.5)	200 (154.5–240.5)	0.955
(×10³ cell/mm³)	After	260 (205.5–317.5)	251 (195–327.5)	0.764
	p Value[b]	**<0.0001***	**<0.0001***	

Note: Bold values are statistically significant p values.

Abbreviations: DBP, diastolic blood pressure; Hb, hemoglobin; HR, heart rate; RR, respiratory rate; SBP, systolic blood pressure; SpO$_2$,Saturated pressure of oxygen; WBC, white blood cell.

[a]p Value based on Mann–Whitney u test;

[b]p Value based on Wilcoxon Ranks test;

*$p < 0.05$ considered as significant.

ICU admission and hospital LOS between the intervention and control groups. No adverse drug reaction occurred in any of the study subjects treated with NAC; thus, NAC can be considered a safe adjunct treatment in coronavirus disease.

To date, few clinical studies have examined the therapeutic effect of NAC on COVID-19 disease.[31] However, the rationale for using NAC in the prevention or adjunct treatment of COVID-19 has been largely inferred from previous research on other viruses, such as influenza and respiratory syncytial virus.[32–34] SARS-CoV-2, by binding to the ACE2 receptors in the host's body, can infect the cells of many structures related to the respiratory, cardiac, neurological, hematological, renal and gastrointestinal systems.

TABLE 4 Comparison of biochemical parameters on pre and postintervention between two groups of study

Biochemical parameters		Intervention group (n = 125)	Control group (n = 125)	p Value[a]
ESR (mm/h)	Before	32 (13–50.5)	29 (14.5–54)	0.878
	After	12 (7–19)	13 (9–29)	0.057
	p Value[b]	**<0.0001***	**<0.0001***	
CRP (mg/L)	Before	19.7 (10.8–41.65)	18.10 (6.55–62.70)	0.423
	After	6 (2–11.80)	11.5 (6.55–25.1)	**<0.0001***
	p Value[b]	**<0.0001***	**<0.0001***	
CR (mg/dl)	Before	1.1 (0.930–1.33)	1.1 (1–1.3)	0.600
	After	1 (0.875–1.23)	1.01 (0.90–1.200)	0.546
	p Value[b]	**<0.003***	**0.003***	
Urea (mg/dl)	Before	27 (22–39)	17 (13–31)	**<0.0001***
	After	19 (14–31)	19 (13–34)	0.764
	p Value[b]	**<0.0001***	0.1780	
AST (U/L)	Before	33 (27–43)	32 (20–41.75)	0.087
	After	32 (26–51)	25.5 (19–41.75)	**<0.0001***
	p Value[b]	**0.039***	**0.031***	
ALT (U/L)	Before	31 (24–45)	37 (28–51.5)	**0.009***
	After	37 (28.5–51.5)	37 (23–53)	0.385
	p Value[b]	**0.001***	0.315	

Note: Bold values are statistically significant p values.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CR, creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

[a]p Value based on Mann–Whitney u test;

[b]p Value based on Wilcoxon Ranks test;

*p < 0.05 considered as significant.

TABLE 5 Comparison of CT score and NEWS score on pre- and post-intervention between two groups of study

Biochemical parameters		Intervention group (n = 125)	Control group (n = 125)	p Value[a]
CT score	Before	9 (7–13)	10 (7–14)	0.375
	After	6 (4–8)	7 (5–12)	**<0.0001***
	p Value[b]	**<0.0001***	**<0.0001***	
NEWS score	Before	6 (3–7)	5 (3–6)	0.148
	After	2 (1–3)	1 (1–2)	0.184
	p Value[b]	**<0.0001***	**<0.0001***	

Note: Bold values are statistically significant p values.

[a]p Value based on Mann–Whitney u test;

[b]p Value based on Wilcoxon Ranks test;

*p < 0.05 considered as significant.

COVID-19 induces a severe cytokine storm syndrome in pulmonary structures, leading to oxidative stress, inflammation and alveolar damage.[35,36] A high ratio of ROS to glutathione (GSH) appears to reflect the severity of symptoms and prolonged hospitalization of COVID-19 patients.[37] NAC, which acts as a precursor of GSH inside cells, has been used in many conditions to restore or protect against GSH depletion and has a wide safety margin. Promising results have been reported in the ability of NAC to synthesize glutathione, improve the T lymphocyte proliferative response and modulate the inflammatory pathway. Glutathione precursors such as NAC have been suggested as a potential therapeutic approach to block nuclear factor kappa-light-chain-enhancer of activated B cells activation and address cytokine storm syndrome and respiratory distress in patients with COVID-19 pneumonia.[38–41] During the period of our study, little information was available on the administration of NAC. It was derived mainly from scattered case reports of intravenous or oral

administration of NAC, and no information was available on its inhalation. Until now, inhalation administration of NAC has generally been associated with mucolytic activity, in contrast to oral administration of NAC, which is mainly associated with antioxidant activity. This molecule, as well as other thiol derivatives, acts primarily on the lower respiratory tract to loosen mucus, as the main target of the drug is mucin. However, some studies have shown that inhaled NAC is also effective on oxidative stress and that patients with higher oxidative stress may be good responders to inhaled NAC therapy, as the glutathione replenished by inhaled NAC can reverse the oxidant-antioxidant imbalance.[37,42]

In accord with the current study, a multi-center, prospective cohort study by Assimakopoulos et al.[43] in 2021 was conducted on hospitalized patients with moderate or severe COVID-19: patients who received standard of care were compared with patients who additionally received oral NAC (600 mg) for 14 days. The results showed that oral NAC administration (1200 mg/day) in patients with COVID-19 pneumonia reduces the risk for mechanical ventilation (MV) and 14-day and 28-day mortality. In addition, NAC improved the PO_2/FiO_2 ratio over time and decreased WBC, CRP, D-dimer and lactic acid dehydrogenase (LDH) levels. A randomized study by Gaynitdinova et al.[44] in 2021 evaluated NAC efficacy in the treatment of moderate COVID-19-associated pneumonia in 22 patients who received standard therapy (hydroxychloroquine, azithromycin, enoxaparin, dexamethasone, and tocilizumab) compared with 24 patients who received NAC (1200–1500 mg/day) intravenously. A significant increase in SpO_2 and ventilatory function and a more rapid reduction in the degree of lung damage occurred in the patients receiving NAC compared to the control group. The rate of CRP reduction and the decrease of hospitalization duration in the NAC group was significantly higher compared to the standard treatment group. Moreover, a single-center, randomized, double-blind, and placebo-controlled trial by Altay et al.[45] in 2021 evaluated a combined metabolic cofactor supplementation (CMCS) consisting of L-serine, NAC, nicotinamide riboside, and L-carnitine tartrate in 309 adult patients with laboratory-confirmed COVID-19 infection, reported reductions in recovery time and liver enzymes associated with hepatic function in CMCS compared to placebo.

Contrary to these positive findings, in a pilot randomized clinical trial, 92 patients with mild-moderate COVID-19 associated ARDS were treated with standard-of-care treatment and either placebo (n = 45) or intravenous NAC 40 mg/kg/day for three consecutive days besides standard-of-care treatment (n = 47). No differences were observed in rates of 28-day mortality, ICU and hospital stay and invasive MV use between the intervention and control groups.[46] In addition, a double-blind, placebo-controlled, randomized, single-center trial conducted at the Emergency Department of a hospital in Brazil, where 135 patients diagnosed with severe COVID-19 were assigned 1:1 to either 21 000 mg (approximately 300 mg/kg) of intervenous NAC or placebo.[47] This study found no difference in the progression to severe respiratory failure requiring invasive or noninvasive MV, hospital LOS, admission to ICU and mortality.[47] These conflicting results may be related to differences in the study population (moderate or severe COVID-19 patients), research environment (ICU or general wards), long-term or short-term treatment with NAC, or different doses of NAC that were used. Therefore, well-designed prospective clinical trials are essential to confirm that NAC can be considered adjunctive therapy with the standard COVID-19-based protocol.

To the best of our knowledge, this is the first study to use the NAC inhaler spray. The main strength of our study was that it was randomized and well-designed, with accurate follow-up and observation of all subjects. All patients were admitted under the same conditions and received standard treatment protocols based on WHO and Ministry of Health of Iran guidelines for COVID-19 alongside NAC for the intervention group. However, the results of the current study should be interpreted in light of the study's limitations. A drug synergism effect with other components of the standardized protocol-driven care cannot be ruled out and therapeutic dose monitoring was not performed in patients. Due to the unique circumstances of the pandemic, repeating, and conducting some important laboratory indices were not possible for all patients.

5 | CONCLUSION

The present study provides evidence that the use of 400 μg/day of NAC inhaler spray for 7 days in patients with COVID-19 pneumonia prevents the development of severe respiratory failure. Despite admitted COVID-19 patients treated with NAC being older and therefore at greater risk of serious COVID-19 disease, the results showed that NAC administered by inhaled spray was associated with better survival when added to standard treatment in hospitalized patients with COVID 19 pneumonia. However, NAC therapy had no effect on hospital LOS and the requirement for ICU admission. These findings need to be confirmed by future prospective clinical trials; however, until then, considering the excellent safety profile and low cost of NAC, its use as adjunct therapy in COVID-19 should be considered.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data collected and analyzed during the current study are available from the authors on a reasonable request.

ETHICS STATEMENT

The protocol study was reviewed and approved by the Ethics Committees of Baqiyatallah University of Medical Sciences (IR.BM-SU. REC.1399.123), in accordance with the Declaration of Helsinki of the World Medical Association. This study Registered at Iranian Registry of Clinical Trials (IRCT20080901001165N55) dated 23-05-2020. Written informed consent was obtained from all patients or their legal representatives if they were unable to provide consent. In addition, the study was conducted and reported in accordance with the recommendations of the Consolidated Standards of Reporting Trials (CONSORT) statement.

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