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Original Article

Impact of N-Acetylcysteine on Modulating Inflammation of Hospitalized Patients with COVID-19 Infections: A Prospective Randomized Trial

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ABSTRACT

N- acetylcysteine (NAC) is a widely used safe mucolytic, that demonstrated positive impacts on various respiratory diseases via its anti-inflammatory and antioxidant effects. The study aimed to evaluate the potential benefit of adding high-dose oral N- acetylcysteine in hospitalized moderate-severity COVID-19 patients. A prospective, single-center, randomized clinical trial on 60 hospitalized moderate COVID-19 patients who were randomly assigned to the NAC group (30); received NAC daily at 1800 mg added to the institutional protocol, or non-NAC group (30); received only the institutional protocol. *Outcomes.* The primary outcome was the change in plasma TNF-α, IL-6, and glutathione peroxidase levels. Secondary outcomes were the length of hospital stay, need for oxygen support, duration of oxygenation, and mortality rate between the two study groups. At the study end, a significant decline in TNF-α levels ($p< 0.001$) and a significant increase in glutathione peroxidase in the NAC-treated group ($p= 0.001$) were evident. Groups were comparable in IL-6 levels ($p= 0.810$). The duration of oxygen support significantly decreased in the NAC group ($p= 0.005$). On the contrary, hospital stay length and oxygen support need was not affected by the addition of NAC (p- values, 0.45, 0.42, respectively). The mortality rate was comparable in both groups. In conclusion, the Addition of 1800 mg of NAC to the institutional treatment protocol for moderate COVID-19 patients has led to a decline in the levels of plasma TNF-α and increased glutathione peroxidase levels. Moreover, the duration required for oxygen support decreased in patients needing supplemental oxygenation.

Keywords: *NAC; TNF-α; IL-6; glutathione peroxidase; oxygen support; Covid-19.*

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1. Introduction

Toward the end of 2019, many pneumonia cases appeared in Wuhan (Hubei, China) of unknown origin **[1]**. These cases were later identified through deep sequencing analysis of the patient's respiratory samples to be caused by a novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) **[2]**. The disease caused by this virus has been named COVID-19 (Coronavirus disease 2019) by the World Health Organization (WHO) **[3]**. By March 2020, the WHO announced the disease as a pandemic **[4]**. Afterward, the disease rapidly spread around the globe leading to the death of thousands of people which necessitated the urge

of developing effective treatment strategies **[5]**.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seems to be an evolution from its two ancestors the severe acute respiratory syndrome coronavirus (SARS-Cov) and the Middle East respiratory syndrome coronavirus (MERS-Cov) leading to SARS and MERS pandemic in the 2002 and 2012 respectively **[1, 6]**. SARS-Cov2 has the higher transmission rate among the mentioned coronaviruses **[7]**. The symptoms of COVID-19 range widely, from asymptomatic, mild, self-limiting upper respiratory tract infection symptoms to severe and sometimes lethal symptoms **[8, 9]**. Critically ill patients, in particular, are prone to develop sepsis, multiple organ dysfunction, or even respiratory failure mandating the need for mechanical ventilation and admission to the Intensive Care Unit (ICU) **[10, 11]**.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is spread mainly through droplet transmission from an infected individual **[6]**. Once the virus enters the human body, it targets the upper respiratory tract via the nasal epithelia, or directly attacks the lower respiratory tract infecting the bronchial and alveolar epithelial cells **[12]**. SARS-CoV-2 penetrates the host cell via Angiotensin-converting enzyme 2 (ACE 2) receptor leading to its downregulation **[13, 14]**. This downregulation results in the accumulation of Angiotensin II (AngII) which binds to the angiotensin type I receptor (AT1R) initiating inflammation and lung tissue fibrosis **[15-18]**. The AT1 receptor stimulation activates a pathologic inflammatory reaction through the nuclear factor kappa B (NF-ƙB) pathway **[15, 19, 20]**. This pathway leads to the augmentation in interleukin-6 (IL-6), tumor necrosis factor alfa (TNFα), and interleukin-1 (IL-1) release **[21]**. The blood level of IL-6 and TNF α are observed to be higher in patients with COVID-19 and positively correlates with the disease severity and the occurrence of a cytokine storm **[18, 22]**.

Moreover, Ang II utilizes the mitochondrial Reactive oxygen species (ROS) for intracellular signaling under normal physiological conditions **[23]**. However, increased Ang II level causes excess mitochondrial ROS generation leading to overt oxidative stress, cell apoptosis, or necrosis **[24]**. Oxidative stress and inflammation are strongly correlative as cell exposure to ROS induces the release of proinflammatory cytokines such as IL-2, IL-6, and TNF α resulting in further cell damage **[25-27]**. Furthermore, inflammation causes the overproduction of ROS, resulting in significant oxidative stress at the site of inflammation **[28, 29]**.

N-acetylcysteine (NAC) is considered a well-tolerated, inexpensive, and safe medication that has been used all across the world in a variety of medical conditions for several decades **[30]**. The drug has been used as a mucolytic agent and for paracetamol intoxication **[31]**. NAC has antioxidant properties by increasing the level of glutathione in the cells as well as scavenging the ROS **[32]**. The consumption of ROS leads to the inhibition of Ang II action as they appear to be important mediators of its action **[33]**. Additionally, NAC has anti-inflammatory characteristics through its ability to decrease the proinflammatory cytokines release in the early stage of the immune system activation **[28, 34]**. The antioxidant and anti-inflammatory properties of NAC make it a cost-effective and promising adjuvant treatment that might decrease the progression of COVID-19.

Previous trials have evaluated the impact of NAC in COVID-19 patients with severe infections and those who developed Acute Respiratory Distress syndrome **[35, 36]**. Hence, this study aimed to assess the effect of NAC in moderate COVID-19 patients and to evaluate its

impact on the progression of the disease.

2. PATIENTS AND METHODS

2.1. Study design and patients:

The present study was a randomized, controlled study. Patients were recruited from El-Asema Hospital, Cairo, Egypt, from the period of March 2021 to April 2022. The most predominant strain in the study was the B.1.617.2 mutant strain, called the delta strain which was prevalent in Egypt in the middle of 2021. Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis of nasopharyngeal swabs was used to confirm COVID-19 patients. COVID-19 pneumonia was confirmed by the presence of bilateral ground-glass opacities on a chest Computed tomography (CT) scan.

Patients with COVID-19 were transferred to the isolation ward and were assessed for eligibility. Patients were included if presented with moderate COVID-19 infection as classified by the management protocol of the Egyptian Ministry of Health and Population **[37]**. Patients were excluded if they were; < 18 years, pregnant or lactating, allergic to NAC, critically ill, or mechanically ventilated.

According to the management protocol of the Egyptian Ministry of Health and Population, infection with COVID-19 was classified into mild, moderate, or severe infection. Mild COVID-19 refers to respiratory symptoms without signs of pneumonia or hypoxia, while moderate cases have pneumonia findings on radiology along with symptoms. Severe cases exhibit one of the following clinical signs of pneumonia: respiratory rate > 30 breaths/min; SaO_2 < 92 at room air, PaO_2/FiO_2 ratio < 300 **[37]**.

To our knowledge, there are no previous trials assessing the impact of NAC in moderate COVID-19 patients. Thus, the sample size was determined utilizing the guide of a previous study assessing the effect of NAC in non-COVID-19 pneumonia patients to identify the change in the TNF-α level between the two studied groups of 3.25 with a pooled standard deviation of 3.9 **[29]**. According to these findings, a minimum sample size of 24 patients in the test group and 24 patients in the control group was enough at a power of 0.8 with a Type I error of 0.05. The sample was increased by 25% to compensate for the probable loss to follow-up to 30 cases in each group.

2.2. Intervention

Eligible patients were randomly allocated using a simple randomization procedure with a (1:1) allocation ratio, to one of the following two groups. Group 1(non- NAC group); 30 moderate COVID-19 patients receiving standard care or Group 2 (NAC group); 30 Patients receiving the standard care, in addition to NAC at a dose of 1800 mg in 3 divided doses **[38, 39]**. Patients received treatment for a maximum of 2 weeks or until one of the following: Discharge from the hospital, intolerance to the medication, or death. The study drug, Acetylcysteine® 600 mg sachets was purchased from the South Egypt Drug Industries Company (SEDICO), Egypt.

All patients with COVID-19 who participated in the study were managed following the treatment protocol issued by the institution. The protocol included antibiotics (azithromycin, linezolid, ceftazidime, levofloxacin), corticosteroids (dexamethasone 8 mg once daily), and remdisivir (200 mg IV administrated on day 1 followed by 100 mg IV every day since day 2 for 5 days) as well as supplementary vitamins and minerals; as Vitamin C (1000 mg p.o once daily), Zinc (50 mg p.o once daily), Vit D3 (42000 IU every week) **[37, 40]**.

2.3. Data and Sample Collection

Baseline Evaluation: All patients were

evaluated for the following: history taking, clinical assessment, and laboratory assessment. The laboratory evaluation consisted of Complete blood count (CBC), renal function tests, C-reactive protein (CRP), serum ferritin, and D-Dimer test along with plasma TNF-α, IL-6 and oxidative stress marker (glutathione peroxidase). Follow-up: Both arms were assessed every day for the following: signs of infection resolution versus deterioration, incidence, and severity of adverse effects.

Venous blood samples were collected from all the participants in EDTA tubes and then centrifuged 1500g for 10 min. Plasma was separated and kept at –80 °C till analysis. Plasma IL-6 and TNF- α were assessed by ELISA technique, where plasma glutathione peroxidase level was detected through colorimetric assay.

2.4. Primary and Secondary Outcomes

The primary outcome was the change in plasma TNF-α, IL-6, and glutathione peroxidase levels at the end of the study in the NAC and non-NAC groups. The secondary outcomes included the length of hospital stay, the need for oxygen support, the duration of oxygenation, and the mortality rate between the two study groups.

2.5. Safety Profile

The addition of high-dose NAC to the standard therapy was evaluated in terms of efficacy as well as safety. The reported adverse effects of high-dose NAC in the previous studies were gastrointestinal symptoms such as mild nausea, vomiting diarrhea, and flatulence **[41, 42]**. These anticipated adverse events were closely monitored throughout the study.

2.6. Ethical Consideration

Before the initiation of this trial, approval was attained from the Scientific Research Ethics Committee of the Faculty of Pharmacy, Ain Shams University, and was recorded at clinical trial.gov (NCT04792021). Written informed consent was provided to all participants or their surrogates.

2.7. Statistical Analysis

Statistical analysis was carried out through IBM SPSS® Statistics version 26 (IBM® Corp., Armonk, NY, USA). The expression of numerical data was performed using the mean and standard deviation or median and interquartile range as appropriate. Frequency and percentage are used to express qualitative data. The relation between qualitative variables was examined via Pearson's Chi-square test or Fisher's exact test. The comparison of quantitative data between the two groups was performed using either the Student t-test for normally distributed data or the Mann-Whitney test (non-parametric t-test) for not normally distributed data. To compare two consecutive measures of numerical variables, the Wilcoxon-signed ranks test (non-parametric paired t-test) was used. To examine the correlation between numerical variables, the Spearman-rho method was applied. All tests were two-tailed. A p-value < 0.05 was considered significant.

3. Results

3.1. Demographics and baseline characteristics

During the trial period, 152 patients were assessed for eligibility; only 60 patients met the eligibility criteria and were included in the study. All the study's participants received the institutional protocol for COVID-19 and the NAC group received additional NAC. **Fig. 1,** displays the consort flow diagram. Just one patient chose not to continue taking NAC due to its bitter aftertaste. No patients were excluded from the analysis. Patients' demographics and baseline characteristics are presented in **Table 1**. Participants' age, vital signs, laboratory findings, and presenting symptoms were comparable between the 2 groups. Around 73.3% of the

inpatients in the NAC group had comorbidities vs 76.7 in the non-NAC group; hypertension was the most predominant comorbidity in the study's participants with no significant difference between the 2 study groups (**Fig. 2 & 3)**. The baseline plasma level of the inflammatory cytokines (TNF-α, IL-6), as well as the glutathione peroxidase plasma level, did not statistically differ in both groups.

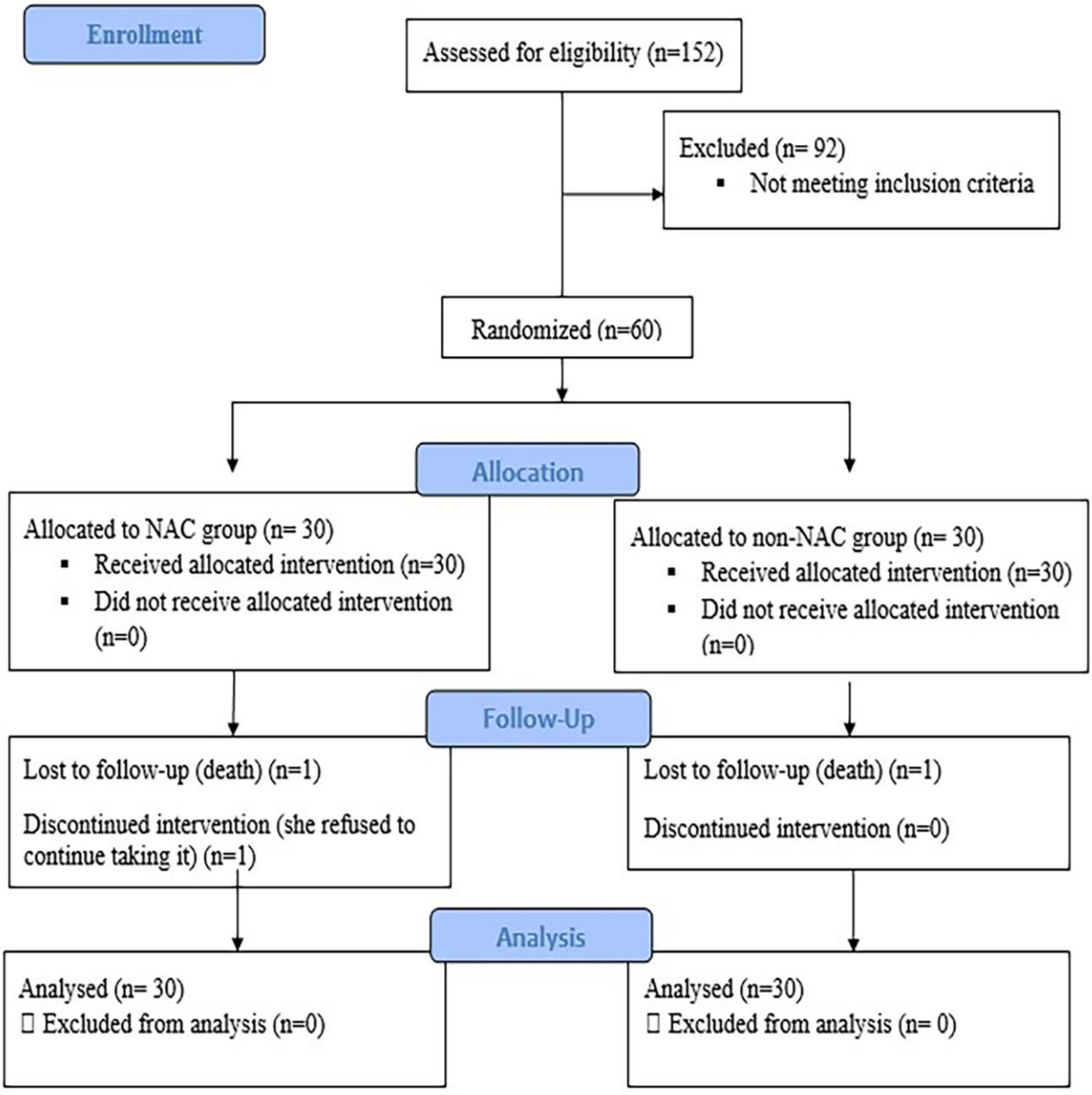

Fig. 1. Consort Flow Diagram

Table 1. Baseline Demographics, laboratory, and clinical data in both groups

Parameter	NAC group (30)	Non-NAC group (30)	p- Value
Age (years); Mean ±SD	56.3±8.4	59.5±9.5	0.17 #
Sex; n (%)			
Male	17 (56.7)	15 (50)	0.605@
Female	13 (43.3)	15 (50)	
Comorbidities; n (%)			
HTN	20 (66.7)	19 (63.3)	0.787 @
D.M	9 (30)	7 (23.3)	0.559@
CVD	3 (10)	2 (6.7)	1ψ
Asthma	1 (3.3)	2 (6.7)	x
Others	0	1	x
No comorbidities for each patient; n (%)			
0 comorbidities	8 (26.7)	7 (23.3)	
1 comorbidity	11 (36.7)	17 (56.7)	0.203 @
2 comorbidities	11 (36.7)	5 (16.7)	
3 comorbidities	0	1 (3.3)	
Presenting Symptoms; n (%)			
Fever	19 (63.3)	17 (56.7)	0.598@
Cough	26 (86.7)	24 (80)	0.488@
Dyspnea	20 (66.7)	17 (56.7)	0.426@
Diarrhea	2 (6.7)	2 (6.7)	1ψ
Headache	5 (16.7)	3 (10)	0.706ψ
No of symptoms; n (%)			
Only 1 symptom	4 (13.3)	7 (23.3)	
2 symptoms	10 (33.3)	13 (43.3)	0.360@
3	15 (50)	10 (33.3)	
4	1 (3.3)	0	
Days to hospital admission	6.7±2.43	7.5±2.72	0.2 #
Vital Signs; Mean± SD			
HR (bpm)	84.1 ±4	83.2±3.8	0.373#
RR (bpm)	20.6±2.5	20.9±3	0.680#
SBP (mm Hg)	129.4±13.4	132.5±11.8	0.346#
DBP (mmHg)	78.3±6.2	80.5±6.7	0.2#
SaO2 (%)	93.9±1.2	93.8±1.2	0.588#
Kidney function; Mean± SD			
SCR (mg/dL)	0.65±0.9	0.70±0.14	0.091#
Urea (mg/dL)	30.9±7.8	30.1±6.7	0.647#
Laboratory parameters; Median (IQR)			
Ferritin(µg/L)	338 (247.5-457.5)	352.5 (249-481)	0.971$
D-Dimer (g/L)	0.32(0.24-0.52)	0.34 (0.23-0.6)	0.912$
LDH (U/L)	439 (274.3-588.5)	444 (311-550.5)	0.955$
Platelets (*10^3/µL)	182.5 (159.75-196.5)	187.5 (159- 219.5)	0.294$
CRP (mg/L)	28.5 (14.75-86.25)	21 (13.75 – 77.50)	0.539$
TLC (*10^3/µL)	7 (5.50-11)	8.5 (6.40 – 11.35)	0.301$
IL-6 (pg/ml)	40.82 (34.42-47.4)	41.60 (33.88-51.82)	0.525$
TNF-α (pg/ml)	32.8 (15.8-48)	35.2 (16.70-48)	0.935$
GPx (mU/mL)	43.12 (38.8-50.58)	40.9 (36.69-48)	0.233$

CRP; C-reactive protein, CVD; cardiovascular disease, D.M; Type 2 diabetes mellitus, DBP; diastolic blood pressure, GPx; glutathione peroxidase, HR; heart rate, HTN; hypertension, IL-6; interleukin-6, LDH; lactate dehydrogenase, n; number of patients, RR; respiratory rate, SaO2; oxygen saturation, SBP; systolic blood pressure, Scr; serum creatinine, IQR; interquartile range, TLC; Total Leucocyte Count, TNF-α; tumor necrosis factor alfa. Statistical test; @ Chi-square, ψ Fisher's exact, $ Mann-Whitney, # T-test. X, no p-value due to a small number of cases within subgroups. * $P < 0.05$ is considered significant

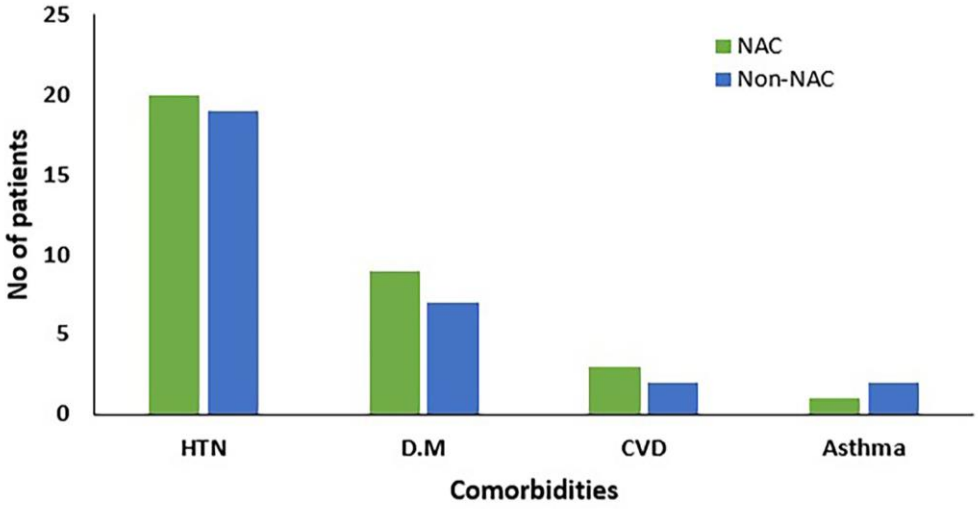

Fig. 2. Comorbidities in both study groups

CVD: cardiovascular diseases; D.M: diabetes mellitus; HTN: hypertension. NAC group: Moderate hospitalized COVID-19 patients who received 600 mg NAC three times daily in addition to the institutional protocol. Non-NAC group: Moderate hospitalized COVID-19 patients who received only medications according to the institutional protocol.

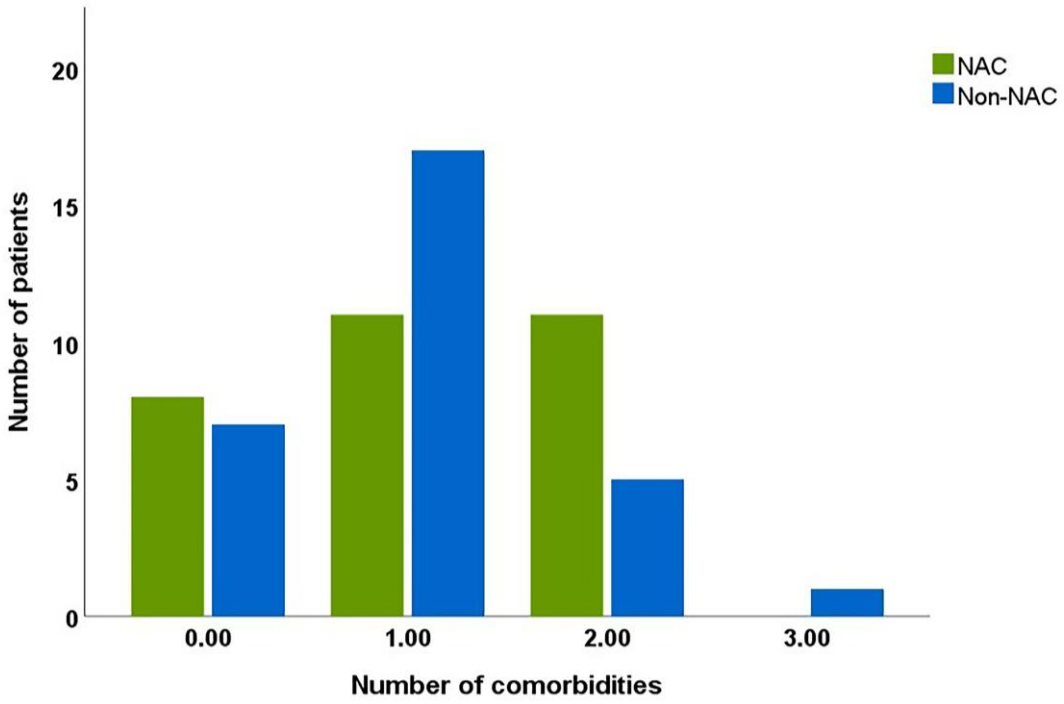

Fig. 3. No of Comorbidities in non-NAC and NAC group

NAC group: Moderate hospitalized COVID-19 patients who received 600 mg NAC three times daily in addition to the institutional protocol. Non-NAC group: Moderate hospitalized COVID-19 patients who received only medications according to the institutional protocol. Statistical test: Chi-square test, p= 0.203 (non-significant).

3.2. Comparison of inflammatory cytokines and oxidative stress markers

At the end of the study, the level of TNF-α in plasma was significantly decreased in NAC versus non-NAC group (p<0.001; **Fig. 4**). A decline was observed in the plasma IL-6 level in the NAC group and non-NAC group compared (p=0.81; **Fig. 5**). The plasma level of glutathione peroxidase was markedly elevated in the NAC group (p= 0.001; **Fig. 6**). CRP levels were lower in the NAC group while the TLC level increased to normal levels, yet, both changes did not reach significance (p; 0.474, 0.050 respectively). The results are displayed in **Table 2.** Using Spearman rho correlation at the end of the research revealed a fair negative correlation between the change in TNF-α levels and the change in the glutathione peroxidase level.

Table 2. Inflammatory and oxidative stress markers at the end of the study in both groups

Parameters; Median (IQR)	On admission	End of study	changes	p-value change between groups $	p-value overtime within group ¥
IL-6 (pg/mL)					
NAC	40.82 (34.42-47.4)	12.6 (9.25-16.96)	-27.2 (-36.45-(-19.37))	0.810	NAC, <0.001
Non-NAC	41.60 (33.88-51.82)	12.5 (8.84-21.51)	-29.9 (-40.7-(-16.03))		
					Non-NAC, <0.001
TNF-α (pg/mL)					
NAC	32.8 (15.8-48)	18.4 (6.30-37.95)	-12 (-13.30-(-10))	*<0.001	NAC, < 0.001
Non-NAC	35.2 (16.70-48)	29.30 (13.45-45)	-5 (-6-(-3.45))		
					Non- NAC, 0.001
GPx (mU/mL)					
NAC	43.12 (38.8-50.58)	126.20(118.9-136.2)	84.2 (70.02- 91.86)	*0.001	NAC, <0.001
Non-NAC	40.9 (36.69-48)	108.9 (90.35-122.7)	66.2 (49.41-77.95)		
					Non-NAC, <0.001
CRP (mg/L)					
NAC	28.5(14.75-86.25)	7 (5-10.5)	-25(-79-(-9.5))	0.474	NAC, <0.001
Non-NAC	21 (13.75-77.50)	6.5 (5-9.5)	-14 (-65-(-9))		
					Non-NAC, <0.001
TLC(*10^3/µL)					
NAC	7 (5.50-11.03)	10.9 (9.7-11.6)	3 (0.35-4.75)	0.050	NAC, <0.001
Non-NAC	8.5 (6.4-11.35)	10.30 (9.5-11.25)	1.1 (-0.6-3.55)		
					Non-NAC, 0.012

CRP; C-reactive protein, GPx; glutathione peroxidase, IL-6; interleukin-6, IQR; interquartile range, TLC; Total Leucocyte Count, TNF-α; tumor necrosis factor alfa. Statistical test; $ Mann-Whitney, ¥ Wilcoxon signed ranks. * P < 0.05 is considered significant.

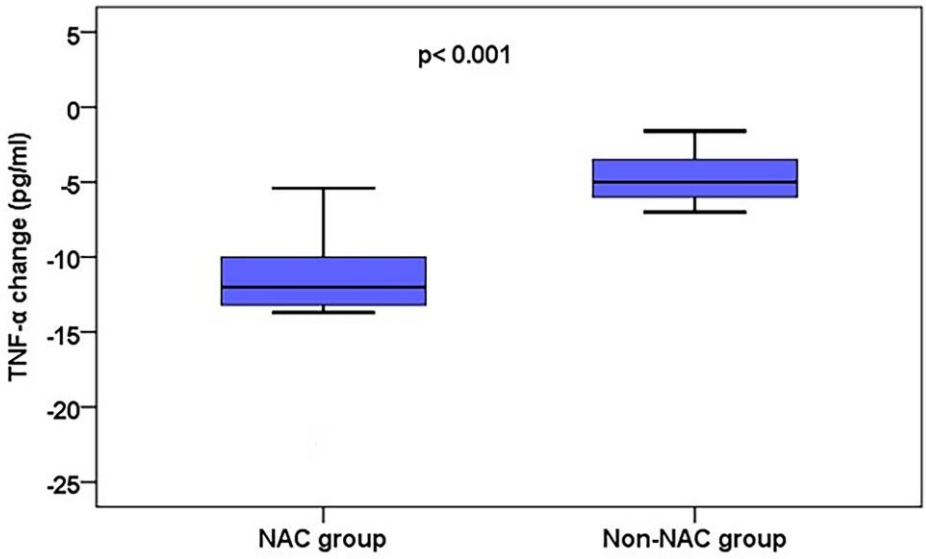

Fig. 4. Change in plasma TNF-α level in non-NAC and NAC groups from day 0 to the end of the study

NAC group: Moderate hospitalized COVID-19 patients who received 600 mg NAC three times daily in addition to the institutional protocol. Non-NAC group: Moderate hospitalized COVID-19 patients who received only medications according to the institutional protocol. Statistical test: Mann-Whitney test, p<0.001 (significant)

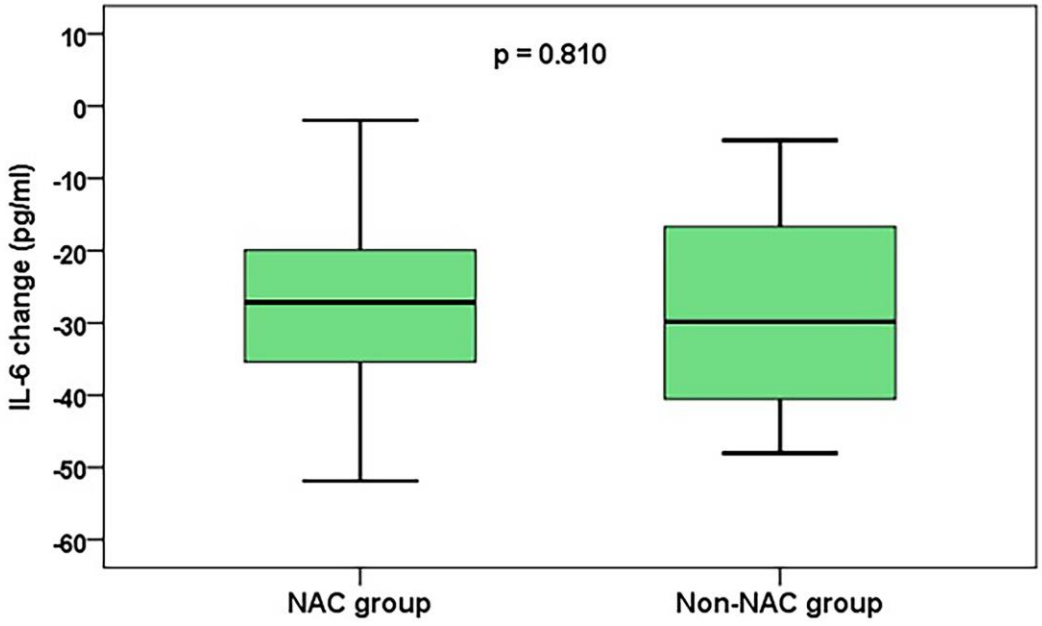

Fig. 5. Change in plasma IL-6 level in non-NAC and NAC groups from day 0 to the end of the study

NAC group: Moderate hospitalized COVID-19 patients who received 600 mg NAC three times daily in addition to the institutional protocol. Non-NAC group: Moderate hospitalized COVID-19 patients who received only medications according to the institutional protocol. Statistical test: Mann-Whitney test, p= 0.810 (non-significant).

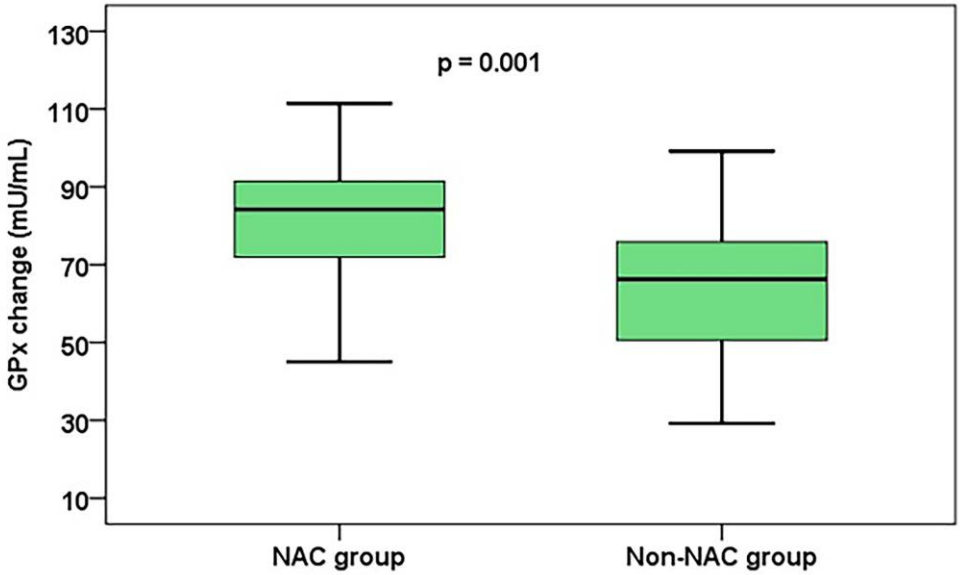

Fig. 6. Change in plasma GPx level in NAC and Non-NAC groups from day 0 to the end of the study

NAC group: Moderate hospitalized COVID-19 patients who received 600 mg NAC three times daily in addition to the institutional protocol. On-NAC group: Moderate hospitalized COVID-19 patients who received only medications according to the institutional protocol. Statistical test: Mann-Whitney test, p= 0.001 (significant).

3.3. Clinical outcomes

Hospital stay length was shorter in NAC receiving group, where the median time of stay in the NAC group was 7 days while that of the non-NAC group was 8 days. This difference did not reach significance (p=0.45). The same results were found regarding the number of patients who required oxygen support. Seventeen out of 30 patients in the NAC treatment group (56.7%) and 20 out of 30 patients in the test group (66.7%) needed oxygen support (nasal cannula) (p=0.426). Yet, adding NAC significantly decreased the duration of oxygen support to be 4 days (3-6.5) compared to 6 days (5-8) in the control group (p=0.005; **Fig. 7**). Mortality was comparable in the two groups, one patient died from each group. Results are presented in **Table3**

Table 3. Clinical Outcomes in both groups

	NAC group (30)	**Non-NAC (30)**	**p-value**
Length of hospital stay (days); Median (IQR)	7 (6-9)	8 (6.5-9.5)	0.45$
Need for oxygen support; n (%)	17 (56.7)	20 (66.7)	0.426@
Oxygen support duration (days); Median (IQR)	4 (3-6.5)	6 (5-8)	*0.005$
Number of deaths	1	1	-

IQR; interquartile range, n; number of patients. Statistical test; @ Chi-square, $ Mann-Whitney. *P < 0.05 is considered significant

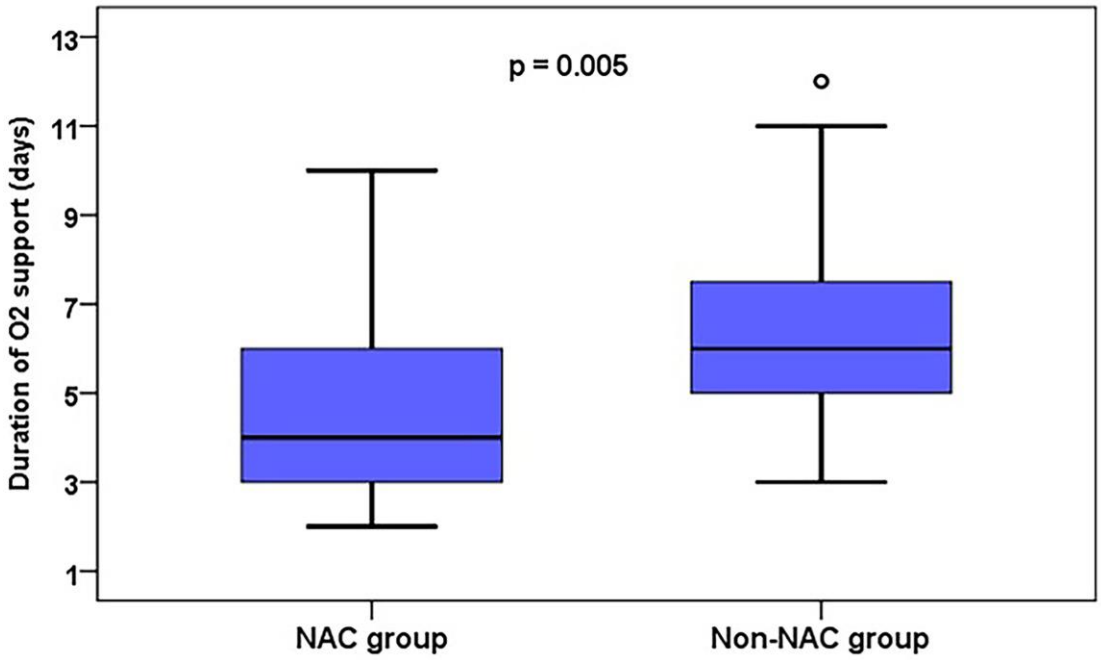

Fig. 7. Duration of oxygen therapy in the NAC group vs non-NAC group

NAC group: Moderate hospitalized COVID-19 patients who received 600 mg NAC three times daily in addition to the institutional protocol. Non-NAC group: Moderate hospitalized COVID-19 patients who received only medications according to the institutional protocol. Statistical test: Mann-Whitney test, p=0.005 (significant).

3.4. Safety and adverse events

N-acetylcysteine appears to be very well tolerated. There were no documented severe adverse events during the trial that mandated discontinuation, only one patient refused to keep taking the NAC sachets for their unpleasant taste.

4. Discussion

To the best of our knowledge, this is the first prospective randomized controlled trial that investigated the effectiveness and safety of high-dose oral NAC in hospitalized moderate COVID-19 patients. The findings of this trial revealed that adding oral NAC to standard treatment caused a decrease in the TNF-α plasma levels as well as an increase in the glutathione peroxidase plasma level. In addition, the NAC administration reduced the duration of oxygen support for those who were in need.

Coronaviruses are associated with alterations in ROS-producing and scavenging pathways, which cause inflammation and tissue damage **[43]**. COVID-19 occurrence, progression, and severity are heavily influenced by ROS overproduction and antioxidant system deficiency **[44]**. ROS and free radicals are formed in the bodies of infected persons with serious COVID-19 infections, usually accompanied by a cytokine storm characterized by increased concentrations of cytokines that promote inflammation, such as interleukin (IL)-6, IL-1, and tumor necrosis factor- (TNF-α) [45].

NAC is a molecule with a pleiotropic effect; it has a mucolytic, anti-oxidant, and anti-inflammatory effect. It exerts a dual anti-oxidant effect by directly eliminating ROS or indirectly by being a cysteine donor essential for glutathione synthesis **[46]**. NAC exerts its anti-inflammatory property by inhibiting nuclear factor kappa B (NF-ƙB) activation leading to regulation in the cytokine synthesis **[46]**.

Furthermore, NAC has an indirect anti-viral activity as it has been shown that RNA viruses, coronavirus included, need the NF-ƙB pathway to be active to help them replicate within the host cells **[47]**. Prior, in vitro research has demonstrated that NAC was associated with prevention in the proliferation of other influenza A and B, as well as other respiratory human pulmonary epithelial cells infected with syncytial virus **[48]**. In light of these findings, this trial was done to test the efficacy of NAC as a promising drug that may prevent the progression of moderate COVID-19 patients to a severe state.

Previous studies have investigated the role of NAC in different disease states, where oxidative stress and inflammation are parts of its etiology. However, these studies had conflicting results. In the current trial, the administration of 1800 mg of oral NAC significantly decreased TNF-α level. This result was following the Zhang et al trial that revealed a significant decline of TNF- α levels in patients with community-acquired pneumonia with the administration of oral NAC **[29]**. Furthermore, a meta-analysis, for controlled clinical trials testing the effect of NAC on inflammatory and oxidative stress markers has revealed that in patients older than 40 years old and NAC doses above 1-1.5 g per day, NAC significantly decreased the levels of TNF-α **[49]**. The same meta-analysis declared that upon performing sensitivity analysis, NAC decreased the level of IL-6 significantly. The latter finding was not replicated in our study where NAC administration failed to significantly decrease the levels of IL-6. This could be explained by the administration of dexamethasone as a standard treatment to the patients which might have hindered the effect of NAC on IL-6 levels. Also, higher NAC doses could have contributed to a detectable significant change. In other words, NAC has no additive effect in lowering IL-6 compared to the standard regimen. The same finding was replicated regarding the level of CRP where the administration of 1800 mg of NAC did not cause a significant decrease in the level of CRP. Although an RCT has assessed the effect of 1200 mg of NAC in acute exacerbation of chronic obstructive pulmonary disease (COPD) and a significant reduction in CRP was evident **[50]**. The contradiction in the result might be also explained by the hindrance of the COVID-19 standard therapy to the effect of NAC on CRP. Besides, this result matches the one concluded by a meta-analysis that has found no effect of NAC on the level of CRP [49]**.**

A study showed that the level of Glutathione peroxidase enzyme was reduced in COVID-19 patients in comparison with controls **[51]**. The current study demonstrated that NAC administration significantly increased glutathione peroxidase levels. A similar result was shown in a trial that showed the effectiveness of NAC in increasing the level of glutathione peroxidase in people with low glutathione levels **[52]**.

Concerning the clinical outcome, our study revealed that the administration of NAC resulted in a significant decrease in the duration of oxygenation in patients requiring oxygen support. Similarly, a cross-sectional trial evaluating NAC effects in moderate to severe COVID-19 patients documented similar results **[53]**. This result could be interpreted by the fact that NAC has mucolytic properties. It lessens the mucus' viscosity by rupturing the disulfide bonds in the high molecular weight glycoproteins that are present in the mucus which in turn decreases the resistance of the airway, dyspnea, and the work of breathing **[54]**. On the other hand, the current study failed to show a difference with NAC administration in decreasing neither the duration of hospital stay nor the number of patients who required oxygen support. Moreover, the number of deaths was comparable in the 2 study groups.

An observational retrospective cohort study assessing the effect of administration of 600 mg

of NAC every 8 hours in 2071 patients diagnosed with COVID-19 withdrew a conclusion that a high dose of NAC was accompanied by significantly lower mortality rates compared to using standard therapy alone **[55]**. The same study found no effect on the mean duration of hospital stay similar to the findings of the current study.

Previously, the twice daily oral administration of 600 mg NAC for 6 months has been found to decrease the rate of recurrence and the severity of influenza episodes in elderly people **[56]**. Consequently, it has been suggested that the administration of 600 mg twice daily of NAC decreases the risk of being infected with COVID-19 in high-risk populations during the pandemic **[57]**. Furthermore, the administration of the same dose in intubated ICU patients was proven to reduce the likelihood of acquiring pneumonia associated with mechanical ventilation and decreased the length of ICU stay as well as hospital stay in those patients **[58]**. Yet, another randomized clinical trial assessing the effect of daily intravenous administration of 40 mg/kg of NAC for 3 days in patients with mild to moderate COVID-19-associated acute respiratory distress syndrome (ADRS), showed an insignificant difference in the duration of hospital stay as well as the need for mechanical ventilation **[59]**. The lack of significant benefit with high dose NAC in this cohort of patients is understandable due to the complexity of ARDS in addition to the variability of administration timing concerning the ARDS severity.

It can hence be concluded from the previously mentioned studies that the earlier administration of NAC at the beginning of symptoms might potentially lead to improvement in symptoms' severity and probably faster recovery. Thus, the timing of the drug administration could have a pivotal role in affecting the progression of the disease and preventing the disease's complications.

Regarding the safety of NAC, it shows to have a benign adverse effect profile that does not vary significantly from placebo in most clinical trials **[41]**. A dose of 1800 mg/day was administrated for 5 weeks in patients with polycystic ovary disease and no adverse event was detected **[60]**. This finding goes in alignment with our study. Besides, NAC was found to be safe and well tolerated in studies of high doses (up to 3000 mg/day) in respiratory diseases with specific reports on safety. In general, the safety profile at high and standard doses is comparable **[61]**.

The current study bears the limitations of being single-centered, having a relatively small sample size, and the possibility of drug synergism with other medications provided following the institutional standard protocol cannot be ruled out. It is hence recommended to replicate the findings of the current study by a multi-center analysis that will also increase the power of the analysis. Moreover, higher doses beyond those tested should be examined for their potential benefit on clinical outcomes and other inflammatory responses.

Conclusion

In summary, this study demonstrates that the addition of 1800 mg of oral NAC to the standard COVID-19 treatment exhibited an increase in the plasma glutathione peroxidase level as well as a decline in the plasma TNF-α level. Moreover, it decreased the duration of oxygen support required. Thus, the study suggests considering the addition of oral NAC in moderate hospitalized COVID-19 patients as it has the potential to minimize inflammatory and oxidative damage in those patients.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Scientific Research Ethics Committee, Faculty of Pharmacy, Ain Shams University (No. 128). Written informed consents were signed and collected from all the study participants.

Consent to publish

All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

The data generated or analyzed during this study are included in the main manuscript file.

Competing interests

The authors have no competing interests.

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