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The Effects of N-Acetylcysteine as Adjuvant Therapy To Reduce TNF-A Level And Increase SPO_2/FIO_2 Ratio In Improving Hypoxemia In COVID-19 Patients

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ABSTRACT

Tumor Necrosis Factor Alpha (TNF-α) is a pro-inflammatory cytokine that plays a crucial role in COVID-19 disease progression. N-acetylcysteine (NAC) works throughout several GSH-mediated mechanisms and is known to eliminate oxidative stress in acute respiratory distress syndrome (ARDS) in COVID-19. This study aims to analyze the e ect of the N-Acetylcysteine as Adjuvant Therapy to reduce TNF-α levels and Increase SpO_2/FiO_2 ratio in Improving hypoxemia in COVID-19 Patients. This is a quasi-experimental, non-equivalent control group design study. There were 91 subjects selected using non-random sampling, which consisted of 75 patients in the NAC group and 16 patients in the control group. The TNF-α level was measured using the ELISA method, and SpO_2/FiO_2 ratio was calculated on day 1 (on admission) and day eight after NAC 5000mg/ 72 hours was given. Statistical analysis was conducted using Wilcoxon and Mann-Whitney U Test. There is a significant decrease in TNF-α level in the treatment group (median 1.49±5.22) (p=0.016) compared with the control group (median 1.64±1.99) (p=0.005). The Median SpO_2/FiO_2 ratio on day 1 is 163.70±69.64 in the control group and 121.49±40.41 in the treatment group (p=0.005). The Median SpO_2/FiO_2 ratio on day 8 is 249.69±132.26 in the control group and 151.29±59.18 in the treatment group (p=0.001). There is a positive correlation between serum TNF-α level and SpO_2/FiO_2 ratio after administration of adjuvant therapy NAC (r=0.240, p=0.038). There is a positive correlation and significant decrease of serum TNF-α and SpO_2/FiO_2 ratio after adjuvant NAC therapy, which improves hypoxemia in COVID-19 patients.

Keywords: *N-acetylcysteine, TNF-α, SpO_2/FiO_2 ratio*

ABSTRAK

Tumor Necrosis Factor Alpha (TNF-α) merupakan sitokin proinflamasi yang berperan penting dalam perkembangan penyakit COVID-19. N-acetylcysteine (NAC) bekerja melalui beberapa mekanisme yang dimediasi GSH dan diketahui menghilangkan stres oksidatif pada acute respiratory distress syndrome (ARDS) pada COVID-19. Penelitian ini bertujuan untuk menganalisis pengaruh NAC sebagai terapi Adjuvant untuk menurunkan kadar TNF-α dan Meningkatkan rasio SpO_2/FiO_2 dalam Memperbaiki Hipoksemia pada Pasien COVID-19. Penelitian ini merupakan quasi-experimental, non-equivalent control group designed study. Subyek yang dipilih sebanyak 91 orang dengan non random sampling, yang terdiri dari 75 pasien pada kelompok NAC dan 16 pasien pada kelompok kontrol. Kadar TNF-α diukur menggunakan metode ELISA dan Rasio SpO2/FiO2 diukur pada hari ke-1 (saat masuk) dan hari ke-8 setelah pemberian NAC 5000mg/72 jam. Analisis statistik dilakukan dengan menggunakan Wilcoxon dan Mann-Whitney U Test. Terdapat penurunan kadar TNF-α yang signifikan pada kelompok perlakuan (median 1,49±5,22) (p=0,016) dibandingkan dengan kelompok kontrol (median 1,64±1,99) (p=0,005). Median Rasio SpO_2/FiO_2 pada hari 1 adalah 163,70±69,64 pada kelompok kontrol dan 121,49±40,41 pada kelompok perlakuan (p=0,005). Median rasio SpO_2/FiO_2 pada hari ke-8 adalah 249,69±132,26

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pada kelompok kontrol dan 151,29±59,18 pada kelompok perlakuan (p=0,001). Terdapat hubungan positif antara kadar TNF-α serum dengan rasio SpO_2/FiO^2 setelah pemberian terapi adjuvant NAC (r=0,240, p=0,038). Terdapat hubungan positif dan penurunan signifikan kadar TNF-α dan peningkatan rasio SpO_2/FiO_2 setelah terapi adjuvant NAC dalam memperbaiki hipoksemia pasien COVID-19.

Kata kunci: *N-Acetylcysteine, TNF-α, Rasio SpO_2/FiO_2*

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INTRODUCTION

In December 2019, there was an outbreak of coronavirus infection in Wuhan, China. On Feb 11, 2020, the World Health Organization (WHO) named the virus as "severe acute respiratory syndrome-coronavirus-2" (SARS-CoV-2), and the disease caused by this virus was referred to as "coronavirus disease-2019" (COVID-19). The ongoing COVID-19 pandemic has been hurting human health and the health care system, people's lives, and the global economy.[1]

As of Jul 28, 2020, there were 16,341,920 confirmed cases worldwide and 650,805 deaths due to COVID-19, while in Indonesia, as of Jul 28, 2020, there were 102,051 confirmed cases and 4,910 tolls due to COVID-19.[2] The high mortality rate is thought to be due to a "cytokine storm"—also known as cytokine storm syndrome. Cron and Behrens defined the cytokine storm as the activation of a cytokine-producing cascade resulting from dysregulation of the host immune response to di erent triggers. These triggers can be in the form of infection, malignancy, rheumatic disorders, etc.[3] This cytokine storm is reported to be capable of causing epithelial and endothelial cell apoptosis, vascular leakage, and ultimately acute respiratory distress syndrome and death.[4]

The host immune response to SARS-CoV-2 causes an exaggerated inflammatory reaction. Several studies analyzed the cytokine profile of COVID-19 patients and reported that cytokine storms are directly associated with lung injury, multiple organ failure, and poor prognosis of COVID-19 patients. Three pro-inflammatory cytokines are essential in innate immune response, namely Interleukin (IL)-1, IL-6, and Tumor necrosis factor (TNF)-α. The cytokine storm in COVID-19 came after a sudden increase in circulating pro-inflammatory cytokines, including elevated levels of IL-1, IL-6, and TNF-α.[5] The increasing cytokines cause an influx of various immune cells, including macrophages, neutrophils, and T cells, from the circulation to the site of infection. This reaction has a destructive e ect on human tissues caused by the process of endothelial cell destabilization, vascular barrier damage, capillary damage, diffuse alveolar damage, multiple organ failure, and ultimately death.[6]

Tumor necrosis factor-α (TNF-α) is one of the overproduced pro-inflammatory cytokines. Tumor necrosis factor-α has been associated with a poor prognosis in patients with Severe Acute Respiratory Syndrome (SARS).[7] Increase of cytokines could be seen in various inflammatory conditions, including cytokine release syndrome (CRS). TNF-α serum levels elevate in COVID-19 patients, especially those with severe symptoms.[8]

Inflammation and oxidative stress are closely related. Cell exposure to either the hydroxyl radical (-OH) or superoxide radical anion (O_2 -) causes the release of pro-inflammatory cytokines. Liposaccharides (LPS) induces intracellular accumulation of reactive oxygen species (ROS) and further increase the release of IL-1β, IL-6, and TNF-α. In addition, the nuclear factor kappa B (NF-kB) pathway is also involved in the regulation of pro-inflammatory cytokines, where this process is further amplified in Glutathione (GSH) depletion.[9]

N-acetylcysteine (NAC) works through various mechanisms by GSH within the cells. NAC has been long known as a ROS-eliminating agent, especially hypochlorous acid (HOCL) and –OH. NAC inhibition against ROS that produces Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase can prevent hypertension and various pathological conditions associated with inflammation, i.e., atherosclerosis.[10] NAC has also been shown to have a protective effect against ARDS. ROS plays an essential role in the pathogenesis of lung injury, and the amount of GSH in the alveolar epithelial lining is deficient in ARDS patients. N-acetylcysteine can inhibit viral replication and the expression of pro-inflammatory molecules as well. N-acetylcysteine (NAC) can inhibit pulmonary inflammation, myeloperoxidase (MPO) activity, neutrophils, macrophages, IL-6, IL-1β, CXCL-10, and TNF-α. [9]

SpO_2/FiO_2 ratio (S/F ratio) was widely used recently as an alternative to PaO_2/FiO_2 (P/F ratio) to assess hypoxemia in acute respiratory failure; It is preferred due to its simplicity and non-invasiveness. In addition, it has served as a useful prognostic marker for hypoxemic respiratory failure, especially in the setting of COVID-19.

Based on the mentioned theory above and suggestions from several supporting journals, the author was interested in examining the e ect of NAC adjuvant therapy to inhibit the manifestation of the SARS-CoV2 virus through several pathways—one of which is negative regulation of NF-kB—thereby reducing the secretion of pro-inflammatory cytokines such as TNF-α.

MATERIALS AND METHODS

The study was conducted using a quasi-experimental non-equivalent control group design. The time frame was between June 2020 - July 2021. The study was conducted on patients with confirmed COVID-19 treated in the Instalasi Covid Terpadu (INCOVIT) room of RSUD dr. Saiful Anwar Malang. The subjects in this study were approved to have COVID-19 by PCR, of more than 14 years of age, and were willing to participate in the study and signed an informed consent form. Subjects who died before being treated in the INCOVIT room of dr. Saiful Anwar Hospital, where pregnant and were asymptomatic or confirmed with mild cases of COVID-19, were not included in this study.

The minimum number of samples from each dependent variable is 17. Samples were obtained by consecutive sampling. Ninety-one subjects who met the inclusion and exclusion criteria were measured for their SpO_2/FiO_2 ratio and TNF-α levels on day-0 and day-8 after receiving NAC therapy. The administration of NAC was per systemic route. We gave a bottle of 5-gram NAC via syringe pump to be given continuously over 72 hours, according to the antioxidant dose of NAC found in the literature (1,200 – 1,800 mg/day). NAC infusions are available in 5 gr bottles in our setting. The safety profile of NAC is very high as we know that a known potentially lethal dose of NAC may reach up to 100 mg/kg/ day. The antioxidant dose we use in our study is relatively safe with almost no potential side e ects, with gastrointestinal side e ects being the most frequent possible side effect found in the literature**.** Samples are also divided into two groups, the control group (treated with Standard of Care (SoC) treatments including antivirals, azithromycin, vitamin C, vitamin D, and vitamin E) and the treatment group (SoC with NAC asadjuvant therapy).

Data processing and analysis are carried out using IBM SPSS software version 16.0. The subjects were divided into the groups subjected with NAC as adjuvant therapy and without. Di erences in TNF-α on day-1 and eight were analyzed by paired T-test or Wilcoxon. The comparison of TNF-α levels at day-1 and day-8 between the groups was analyzed by independent T-test or Mann Whitney. The comparison of SpO_2/FiO_2 in the group given with NAC between day-1 and day-8 was analyzed using paired T-test or Wilcoxon. The comparison of SpO_2/FiO_2 on day-1 and eight between the groups was analyzed using the independent T-test or Mann Whitney. The relationship between changes in TNF-α levels and differences in the SpO_2/FiO_2 ratio of COVID-19 patients was analyzed using the Pearson correlation test.

RESULT AND DISCUSSION

This study was carried out from June 2020 to July 2021 in the INCOVIT room of dr. Saiful Anwar hospital, Malang. Ninety-one subjects who met the inclusion and exclusion criteria and were willing to participate in the study by signing an informed consent were found (see in Table 1).

Table 1. Demographic Profile of Research Subjects

Variable and category	NAC group (n=75)	NAC group (n=16)	P value
Age (mean ± SD, median (min-max)	52.31±11.52 56,00 (24 -76)	53.12±11.18 52,50 (25 – 69)	0.795
Sex			0.225
- Male	44 (58,7%)	12 (75%)	
- Female	31 (41,2%)	4 (25%)	
Severity			0.526
- Moderate	33 (44,0%)	3 (18,8%)	
- Severe	21 (28,0%)	11 (68,8%)	
- Critically ill	21 (28,0%)	2 (12,5%)	
Complaints			
- SOB	61 (81,3%)	16 (100%)	0.062
- Cough	60 (80%)	16 (100%)	0.052
- Fever	53 (70,6%)	16 (100%)	0.013
- Anosmia/ Ageusia	15 (20%)	10 (62,5%)	0.001
- GI disturbance	42 (56%)	10 (62,5%)	0.635
Smoker			0.253
- Yes	35 (46,67%)	10 (62,5%)	
- No	41 (54,67%)	6 (37,5%)	
Comorbidities			0.833
- Yes	35 (46,67%)	7 (43,75%)	
- No	41 (54,67%)	9 (56,25%)	
Outcome			0.369
- Recovery	64 (85,3%)	15 (93,8%)	
- Death	11 (14,7%)	1 (6,3%)	

There are 91 subjects—16 in the group not given with NAC and 75 in the group shared with. The demographic characteristics of the two groups can be seen in Table 1. The median age of patients in the NAC group was 56.00 years; this was not much di erent from the median without NAC, which was 52.50 years. Both groups were dominated by males—58.7% in the NAC group and 75% in the non-NAC group. The highest proportion of disease severity in the NAC group was moderate (58.7%), while in the non-NAC group, it was severe (68.8%). Only 81.3% of patients in the NAC group experienced shortness of breath, while those in the non-NAC group all experienced shortness of breath (100%). 46.67% of subjects in the NAC group had a history of smoking, while 62.5% in the non-NAC group had. We also noted the comorbidities on our samples, including hypertension, diabetes mellitus, asthma, chronic obstructive pulmonary disease (COPD), and cerebrovascular diseases. The distribution of patients with comorbidities in the two groups was similar and balanced (46.67% in the NAC group and 43.75% in the non-NAC group). The most significant outcome in both groups was the proportion of patients who recovered (85.3% in the NAC group and 93.8% in the non-NAC group). The NAC group's treatment length was 13.56 days, similar to the group without NAC, which was 13.87 days.

Table 2. Comparison of TNF-α levels in D1 and D8

Variable	D1	D8	p-value
TNF-α with NAC (pg/ml)	Median: 5.196 (0.97 – 16.29)	Median: 3.346 (0.26 – 17.37)	0.041
TNF-α without NAC (pg/ml)	Mean: 5.13±0.79	Mean: 6.78±2.39	0.005

From Table 2, it can be seen that of 75 subjects in the NAC group, the median value of TNF-α was 5.196 pg/ml on the first day and 3,346 pg/ml on the 8th. The Wilcoxon test resulted in a p-value of 0.041 ($p<0.05$), thus, it can be concluded that there was a significant di erence in TNF-α of the NAC group between D1 and D8, in which on D8, after administration of NAC as adjuvant therapy, TNF-α showed a median decrease of 1.85 pg/ml.

Of the 16 subjects in the group without NAC, the average of TNF-α on the first day was 5.13 pg/ml, and on the 8th day, the standard became 6.78 pg/ml. The Wilcoxon test resulted in a p-value of 0.005 ($p<0.05$), thus, indicating a significant difference of TNF-α between D1 and D8 in the non-NAC group, in which on D8, after administration of NAC as adjuvant therapy, TNF-α increased by 1.64 pg/ml.

Table 3. Comparison of TNF-α levels on D1 and D8 between the group with NAC and the group without

Variable	Non-NAC group	NAC group	P-value
TNF-α (pg/ml) on day-1 Median (min-max)	5.185 (3.65-6.40)	5.196 (0.97 – 16.29)	0.934
TNF-α (pg/ml) on day-8 Median (min-max)	5.986 (4.61-14.6)	3.346 (0.26 – 17.37)	< 0.001

Table 3 showed that, on the first day, the TNF-α levels of 91 subjects had a p-value of 0.934 (p>0.05), thus, indicating no significant di erence between the group with NAC and the group without. Furthermore, there was only a slight di erence in the TNF-α levels between the two groups on the first day (0.011 pg/ml), thus, making the difference not statistically significant.

As for the TNF-α levels on the 8th day, of 91 subjects, the p-value was 0.000 (p <0.05), thus, indicating a significant difference between the group with NAC and the group without. The difference in TNF-α levels between the two groups on day-8 was 2.64 pg/ml, thus, indicating that the TNF-α levels in the group with NAC were lower than TNF-α levels in the group without.

Table 4. Comparison of SpO2/FiO2 levels between D1 and D8

Variable	D1	D8	P-value
SpO2/FiO with NAC (unit)	Median : 108.89 (43.33 – 272.22)	Median : 138 (70.56-342.86)	<0.001
SpO2/FiO2 without NAC (unit)	Mean : 163.70±69.64	Mean : 249.69±132.26	0.001

As can be seen in Table 4, of 75 subjects in the NAC group, on the fi rst day, the median value of SpO_2/FiO_2 was 108.89 units, and on the eighth day, the median value of SpO_2/FiO_2 became 138.0 units. The Wilcoxon test obtained a p-value of 0.000 (p<0.05), thus, indicating a signifi cant di erence in the SpO_2/FiO_2 of the group with NAC between D1 and D8, in which, on D8, after adjuvant therapy, SpO_2/FiO_2 increased by 29.11 units, see Table 4.

Of the 16 subjects in the non-NAC group, on the first day, the average SpO_2/FiO_2 value was 163.70 units, and on the 8th day, the average SpO2/ FiO2 value became 249.69 units. The Wilcoxon test obtained a p-value of 0.001 (p<0.05), thus, indicating a significant di erence in the SpO_2/FiO_2 of the group without NAC between D1 and D8, in which on H8, after adjuvant therapy, SpO_2/FiO_2 increased by 85.99 units.

Table 5. Comparison of SpO_2/FiO_2 value between D1 and D8 between the group with NAC and the group without

Variable	non-NAC group	NAC group	P-value
SpO2/FiO2 (unit) on D1 median (min-max)	138 (70,56-342,86)	108,89 (43,33-272,22)	0.005
SpO2/FiO2 (unit) on D8 median (min-max)	193,75 (108,11-461,43)	141 (96,5-350)	0.001

The SpO_2/FiO_2 on the first day of 91 subjects showed a p-value of 0.005 (p<0.05), thus, indicating a significant di erence in SpO2/FiO2 on the first day between the group with NAC and the group without, with a di erence in SpO2/FiO2 between the two groups valuing -29.11 units, thus, indicating that SpO_2/FiO_2 on the first day in the group with NAC was lower than SpO_2/FiO_2 in the group without, see Table 5.

As for the SpO_2/FiO_2 test on the eighth day, of 91 subjects, a p-value of 0.001 (p<0.05) was obtained, thus, indicating a significant di erence in SpO_2/FiO_2 on day-8 between the group with NAC and the group without, with a di erence of SpO_2/FiO_2 between the two groups valuing -98.40 units, thus, indicating that SpO_2/FiO_2 on day-8 in the group with NAC was lower than SpO_2/FiO_2 in the group without.

Table 6. Pearson correlation test between the changes of TNF-α (pg/mL) and the changes of SpO_2/FiO_2 ratio (D8-D1)

	Delta Ratio SpO2/ FiO2 NAC group	Delta Ratio SpO2/FiO2 non-NAC group
Delta TNF-α (pg/mL) NAC group (H8-H1)	r = 0,240 p = 0,038 n = 75	r = 0,292 p = 0,272 n = 16

The Pearson correlation test for the relationship between changes in TNF-α levels and SpO_2/FiO_2 ratio of COVID-19 patients after administration of NAC as adjuvant therapy resulted in the correlation coefficient value of 0.240 with a significance value (p) of 0.038 ($p<0.05$), thus, indicating a positive and significant correlation between changes in TNF-α levels and changes in the SpO_2/FiO_2 ratio. A positive coe cient value could be interpreted as the higher the change in TNF-α levels in COVID-19 patients after administration of adjuvant therapy, the higher the SpO_2/FiO_2 ratio will be. On the other hand, the lower the change in TNF-α levels in COVID-19 patients after adjuvant treatment, the lower the SpO_2/FiO_2 ratio, see Table 6.

The results of this test prove that the hypothesis of the correlation test between changes in TNF-α levels and di erences in the SpO_2/FiO_2 ratio of COVID-19 patients after administration of adjuvant NAC therapy is true. There is a positive correlation between changes in TNF-α levels and changes in the SpO_2/FiO_2 ratio of COVID-19 patients after administration of adjuvant NAC therapy.

The relationship between changes in TNF-α levels and SpO_2/FiO_2 ratio of COVID-19 patients in the group without adjuvant NAC therapy shows a correlation coe cient value of -0.292 with a significance value (p) of 0.272 ($p>0.05$), thus, indicating neither positive nor significant correlation between changes in TNF-α levels and di erences in the SpO_2/FiO_2 ratio of COVID-19 patients in the group without adjuvant therapy. In other words, the level of change in TNF-α levels in COVID-19 patients in the group without adjuvant NAC therapy did not a ect the level of change in the SpO_2/FiO_2 ratio.

The results of this test prove that the hypothesis of the correlation test between changes in TNF-α levels and di erences in the SpO_2/FiO_2 ratio of COVID-19 patients in the group without adjuvant NAC therapy that there is a positive correlation between changes in TNF-α levels and changes in the SpO_2/FiO_2 ratio of COVID-19 patients who did not receive adjuvant NAC therapy is not valid, because the correlation coe cient was negative and not significant.

The characteristics of the groups with NAC and those without did not di er by much in age. The median age in the group with NAC was 56.00 years, while in the group without was 52.50 years. NAC works as an antioxidant and anti-inflammatory.[11] The occurrence of inflammation and oxidation increases with age[12], as age may be one of many factors a ecting clinical outcomes in COVID-19 patients. However, in this study, the median age did not di er much. Thus, the baseline for the two groups was the same.

Both groups in this study were dominated by the male gender—58.7% in the NAC group and 75% in the non-NAC group. This result should be considered as it can lead to confusion in data analysis. Gender is one of the predictors of COVID-19 outcome, where a male is associated with a worse prognosis compared to a female.[13]

There were some di erences in the clinical profile between the two groups in this study. First, the highest proportion of disease severity in the NAC group was moderate (58.7%), while in the non-NAC group, the highest proportion was severe (68.8%). Second, only 81.3% of the subjects in the NAC group experienced shortness of breath, while in the group without, all subjects experienced symptoms of shortness of breath (100%). Third, the most significant outcome in both groups was the proportion of patients who recovered (85.3% in the NAC group and 93.8% in the non-NAC group). Di erences in these clinical conditions can be confounding factors, and thus, need to be considered in interpreting the results.

The distribution of subjects with comorbidities was similar in the two groups (46.67% in the NAC group and 43.75% in the non-NAC group). In addition, the length of treatment for the NAC group was 13.56 days, comparable to the group without NAC, which was 13.87 days. These two indicators do not di er much in the two groups; thus, comorbid factors and length of treatment are not considered things that can confound the results.

TNF-α in the group with NAC showed a reduction of 1.49 pg/ml and a statistically significant di erence. TNF-α in the NAC group was lower in D8 than D1 ($p=0.041 <0.05$). In the group without NAC, TNF-α increased on the eight

day compared to the first day (p=0.005 <0.05). This is by the hypothesis that NAC administration as an adjuvant to COVID-19 therapy can reduce TNF-α.

TNF-α is one of the pro-inflammatory cytokines that worsens the outcome of COVID-19 infection. TNF-α is produced by cells in the immune system, such as macrophages, dendritic cells, and T cells, during inflammation.[14] In severe COVID-19 infection, TNF-α is produced in excess, causing an increase in the systemic inflammatory response that results in tissue damage. One of the functions of TNF-α is to increase the recruitment of phagocytic cells and increase the autolysis reaction of cells.[15] TNF-α is associated with acute lung injury and predicts prognosis.[5]

Oxidative stress and inflammation are closely related to each other. Cell exposure to either the hydroxyl radical (-OH) or superoxide radical anion (O2-) causes the release of pro-inflammatory cytokines. Liposaccharides (LPS) induces intracellular accumulation of reactive oxygen species (ROS) and further increase the release of IL-1β, IL-6, and TNF-α. In addition, the nuclear factor kappa B (NF-kB) pathway is also involved in the regulation of pro-inflammatory cytokines, where this process is further amplified under the conditions of Glutathione (GSH) depletion.[9]

N-acetylcysteine has recently been suggested as adjuvant therapy in standard treatment for SARS-CoV-2 infection. NAC benefits by increasing glutathione synthesis, enhancing immune function, and modulating the inflammatory response. There are two therapeutic mechanisms of action of NAC: 1) Mucolytic e ect caused by the free sulfhydryl groups, which reduces disulfide bonds in the mucus glycoprotein matrix, thereby reducing mucus viscosity; 2) Antioxidant effect caused by direct interaction with free radicals, as well as indirect e ect as cysteine precursor required for glutathione biosynthesis, and replenishment of thiol pool which is central to redox regulation and control.[16]

N-acetylcysteine (NAC) acts through various mechanisms mediated intracellularly by GSH. NAC has long been known as a ROS-eliminating agent, especially hypochlorous acid (HOCL) and –OH. NAC inhibition against ROS that produces the oxidation of Nicotinamide adenine dinucleotide phosphate (NADPH) can prevent hypertension and various pathological conditions associated with inflammation, such as atherosclerosis.[10] In addition, NAC has also been proven for its protective e ect against ARDS, as ROS plays an essential role in the pathogenesis of lung injury, and the alveolar epithelial lining of ARDS patients is deficient in GSH. N-acetylcysteine can also inhibit viral replication and the expression of pro-inflammatory molecules. N-acetylcysteine (NAC) has been proven to inhibit pulmonary inflammation, myeloperoxidase (MPO) activity, neutrophil macrophages, IL-6, IL-1β, CXCL-10, and TNF-α.[9]

Previous studies found that NAC administration significantly decreased CRP and NEWS2 scale levels. The duration of hospitalization was also considerably shorter in the NAC group. However, all other clinical outcomes—transfer to ICU, need for non-invasive or invasive mechanical ventilation, and 28-day mortality—do not di er between the two groups.[17]

Several studies have also examined the e cacy of NAC in hospitalized patients with COVID-19. In respirator-dependent patients, intravenous administration of NAC results in clinical improvement and reduction of CRP and Ferritin.[18] Another study by Alamdari et al. had shown that administration of NAC in combination with high doses of methylene blue and vitamin C as a last resort therapy resulted in a significant recovery and clinical response in four out of five critically ill patients with COVID-19.[19] At the same time, de Alencar et al. suggested that the administration of high doses of NAC does not a ect the evolution of severe COVID-19.[20]

In both groups, the SpO_2/FiO_2 ratio increased significantly on the eighth day compared to the first day. However, when comparing the increase of SpO_2/FiO_2 ratio between the two groups, it was found that the group without adjuvant therapy had a higher increase in the SpO_2/FiO_2 ratio. This finding was by another study, which found that the administration of NAC could significantly increase the SpO_2FiO_2 ratio.[17]

Most of the subjects in the non-NAC group had severe degrees of illness. However, the outcome in the group without NAC was also better, in which 93.8% recovered, while in the NAC group, only 85.3%. This causes the increase in the SpO_2/FiO_2 ratio on the 8th day in the group without NAC to be higher than the group with NAC.

A commensurate baseline is needed to obtain unbiased results, where the clinical conditions are similar for the groups given NAC and the groups not. Therefore, further research using a larger sample size or a placebo-controlled experimental study (randomized controlled trial) is necessary.

Changes in TNF-α levels and alterations in SpO_2/FiO_2 ratio of COVID-19 patients in the group without adjuvant NAC therapy showed a positive correlation. On the other hand, in the group that did not receive adjuvant NAC therapy, the correlation coe cient was negative and not significant.

This finding is by the previous studies, in which it was found that the administration of NAC can increase the SpO_2/FiO_2 ratio.[16] The conclusions of this study prove that there is the involvement of TNF-α in the mechanism of action of NAC. NAC interferes with the NLRP3 inflammasome pathway by suppressing the expression of NLRP3 and caspase-1 activation mRNA. NAC decreased IL1β, IL18, IL6 and TNF-α in vitro. NAC inhibits downstream activity post-TNF-α receptor activation, and under oxidative stress, NAC inhibits TNF-α and IL-6 gene expression.[11]

CONCLUSION

There was a significant decrease in TNF-α levels after NAC administration as adjuvants. In addition, there was also a substantial increase in the SpO_2FiO_2 ratio after administration of adjuvant NAC therapy in patients with COVID-19. However, there was no change in SpO_2/FiO_2 in the subjects given adjuvant NAC therapy and not. This study also showed a significant positive correlation between changes in TNF-α levels and di erences in the SpO_2/FiO_2 ratio in COVID-19 patients after administration of adjuvant NAC therapy. On the other hand, there was no positive correlation between changes in TNF-αlevels and diferences in the SpO_2/FiO_2 ratio in COVID-19 patients who did not receive adjuvant NAC therapy.

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CONFLIC OF INTEREST

The authors declare that they have no conflict of interest.

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