Original Article

The Effect of N-Acetylcysteine as Adjuvant Therapy of Hypoxemia in COVID-19 Patients, Assessed by Interleukin-6 Level and PaO_2/FiO_2 Ratio

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Abstract

Background: Interleukin 6 (IL-6) is a cytokine that plays an essential role in lung damage and mortality. Arterial-to-inspired oxygen (PaO_2/FiO_2) ratio, also known as the Horowitz index, is a measure of hypoxemia in respiratory failure. N-Acetylcysteine (NAC) might be helpful in managing coronavirus disease 2019 (COVID-19) patients by decreasing the cytokine storm, which will lead to a decrease in disease severity. This study aims to analyze the effect of NAC as adjuvant therapy on IL-6 level and PaO_2/FiO_2 ratio in COVID-19 patients.

Methods: This is a quasi-experimental, non-equivalent control group designed study of confirmed COVID-19 patients moderate to critical in Saiful Anwar Hospital Malang. Seventy-five patients received NAC intravenously 5000mg/72 hours as adjuvant therapy for seven days, and 16 subjects in the control group. IL-6 level and PaO_2/FiO_2 ratio were measured on day one and day 8 in both groups from blood samples. Wilcoxon, Mann-Whitney U Test, and Pearson correlation were conducted for statistical analysis.

Results: The decrease in IL-6 level on days 1 to 8 in the NAC group is significantly lower (94.49±253.51) than in the control group (P=0.002). The increase in PaO_2/FiO_2 ratio from day 1 to day 8 in the NAC group is significantly improving (126.94±76.05), the same as the control group (P<0.001). There is a weak correlation between IL-6 level and PaO_2/FiO_2 ratio after administration of NAC (r=0.154, P=0.186).

Conclusion: There is a significant decrease in IL-6 level after administration of NAC. NAC has no significant effect on hypoxemia in COVID-19 patients.

Keywords: IL-6, N-acetylcysteine, PaO_2/FiO_2 ratio

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INTRODUCTION

On December 31, 2019, China reported a mysterious case of pneumonia of unknown origin. Samples isolates were studied, and the results showed the presence of coronavirus infection, a new type of beta-coronavirus named 2019 novel Coronavirus (2019-nCoV). On February 11, 2020, World Health Organization named the new virus Severa Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), and the disease was named Coronavirus disease 2019 (COVID-19).[1–3]

The total global confirmed cases of COVID-19 as of March 10, 2021, were 117,424,768 cases, with 2,608,231 deaths (CFR 2.2%) in 222 infected countries and 187 local transmission countries. The list of countries affected by COVID-19 is growing every day. In Indonesia alone, until March 10, 2021, there were 1,398,578 confirmed cases and 37,932 deaths due to COVID-19.[4]

In COVID-19 patients, IL-6 levels increase sharply, and this cytokine plays a role in the induction of B lymphocyte differentiation and antibody production, as well as the proliferation and differentiation of T lymphocytes. Cytokine storms in COVID-19 can increase vascular permeability. Fluid and blood cell movement in the alveoli will thus result in acute respiratory distress syndrome (ARDS), which can lead to death. The PaO_2/FiO_2 ratio assesses hypoxemia at different FiO_2 levels. The average ratio varies from 300 to 500 mmHg. Shock and organ

failures—such as the kidneys, heart, lungs, and liver, severely damaged due to a cytokine storm—are caused by an increase in inflammatory cytokines, including IL-6, IL-1β, TNF-, IL-8, IL -2, IL-17, G-CSF, GMCSF, CXCL10, CCL2, CCL3.[5,6]

IL-6 (Interleukin-6) is a pleiotropic cytokine with a wide range of biological activity, which affects immune regulation, hematopoiesis, inflammation, and oncogenesis. IL-6 levels increase rapidly in severe and critical COVID-19 patients. Coomes' systematic review and meta-analysis showed that IL-6 levels were significantly elevated in COVID-19 patients and were associated with poor clinical outcomes.[7–9]

There is currently no definitive therapy for COVID-19. The primary strategy for treating COVID-19 is symptomatic and supportive care by monitoring vital signs, maintaining oxygen saturation, and treating complications (e.g., secondary infections and organ failure).[10] At present, there is yet a specific antiviral targeting the virus.[11]

N-acetylcysteine (NAC) is an antidote drug against paracetamol poisoning, first discovered in 1960. It has been widely used since the 1970s as a phlegm-thinning drug. N-acetylcysteine is also a precursor of Glutathione — an excellent antioxidant and free radical scavenger in the body. Therefore, NAC has as potential as COVID-19 therapy through several possible mechanisms viz; increasing Glutathione, increasing T-cell response, and modulating inflammation. In an in-vitro study by Guo et al., NAC was proven to reduce IL-6 levels. Another study by Suter et al. proved that administration of NAC could improve the PaO_2/FiO_2 ratio in ARDS patients.[12–14]

Based on the theory and several supporting journals, the author was interested in the effect of giving NAC as adjuvant therapy on IL-6 levels and the PaO_2/FiO_2 ratio in COVID-19 patients at RSSA.

METHODS

This quantitative research was conducted using a quasi-experimental non-equivalent control group design. The study was conducted from June 2020 to July 2021 on confirmed COVID-19 subjects treated in the INCOVIT room of Dr. Saiful Anwar Regional General Hospital. The inclusion criteria in this study were patients who were: confirmed to have COVID-19 based on PCR, within moderate to critical stages, aged more than 14 years old, and were willing to participate in the study by signing an informed consent form. Subjects who were not treated or died before being treated in the INCOVIT room of dr. Saiful Anwar's hospital and were pregnant, asymptomatic, or confirmed with a mild case of COVID-19 were not included in this study.

The minimum number of samples is 16 for each dependent variable. The number of samples in the non-NAC group was less due to the difficulty of the study in obtaining the number of populations that agreed not to be given NAC because, as the guidelines developed, NAC has been included in the clinical practice guideline.

Samples were obtained by consecutive sampling that met the inclusion and exclusion criteria. Standard therapy provided according to clinical practice guidelines of dr. Saiful Anwar Hospital Malang refers to the Indonesia's local guideline and the decree of the minister of health, which develops dynamically in the research process: 1) Chloroquine phosphate, 500 mg oral/12 hours (days 1-3) followed by 250 mg/12 hours (days 4-10) OR Hydroxychloroquine dose 400 mg oral/ 24 hours (for five days); 2) Azithromycin 500 mg oral/ 24 hours (for five days) or levofloxacin 750 mg intravenous/24 hours (5 days); 3) Hydrocortisone 100 mg intravenous/24 hours (first three days); 4) Treatment of comorbidities and existing complications. Ninety-one subjects were measured for the PaO_2/FiO_2 ratio and the level of IL-6 on the 0th and eighth days after receiving NAC 5000mg/72 hours.

Data processing and analysis are carried out using IBM SPSS software version 16.0. Differences in IL-6 on D1 and D8 were analyzed by paired T-test or Wilcoxon. Comparison of IL-6 levels at D1 and D8 between the group given NAC and the group not was analyzed by independent T-test or Mann Whitney. The PaO_2/FiO_2 ratio comparison after given NAC between D1 and D8 was analyzed using paired T-test

or Wilcoxon. The comparison of PaO_2/FiO_2 at D1 and D8 between the group given NAC and the group was not analyzed using the independent T-test or Mann Whitney. The relationship between changes in IL-6 levels and the PaO_2/FiO_2 ratio was analyzed using the Pearson correlation test.

RESULTS

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.

There was a total of 91 subjects —16 in the group not given with NAC and 75 in the group given with NAC. The demographic characteristics of the two groups can be seen in Table 1. The median age of patients in the NAC group was 53.12 years, and this was not much different from the median in the group without NAC, which was 52.31 (*P*>0.05) years. Males dominated both groups — 58.7% in the NAC group and 75% in the non-NAC group, with *P*=0.225, and thus, it could be concluded that both groups had no significant difference.

The highest proportion of disease severity in the NAC group was moderate (58.7%), while in the non-NAC group was severe (68.8%). However, with *P*=0.526, the degree of severity between the NAC group and the group without NAC was not significantly different. 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%).

The test results obtained *P*=0.062, thus, indicating that there was no significant difference in the complaints of shortness of breath between both groups. As much as 80% of the subjects in the NAC group showed a symptom of cough, while in non-NAC, 100% of the subjects did. The test results obtained *P*=0.052, indicating that the cough symptom does not significantly differ between both groups. As much as 70.6% of the subjects in the NAC group showed a symptom of fever, and in non-NAC, all the subjects did (100%).

Table 1. Demographic Profile of Research Subjects

Variable and Category	NAC group (n=75)	non-NAC group (n=16)	*P*
Age (mean±SD), normally distributed	52.31±11.52	53.12±11.18	0.795
Age [median (min-max)]	56,00 (24–76)	52,50 (25–69)	
Sex			
Male	44 (58,7%)	12 (75%)	0.225
Female	31 (41,2%)	4 (25%)	
Severity			
Mild	33 (44,0%)	3 (18,8%)	
Severe	21 (28,0%)	11 (68,8%)	0.526
Critically ill	21 (28,0%)	2 (12,5%)	
Symptoms			
Shortness of breath	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
Indigestion	42 (56%)	10 (62,5%)	0.635
Smoking/History of smoking			
Yes	35 (46,67%)	10 (62,5%)	0.253
No	41 (54,67%)	6 (37,5%)	
Comorbid			
Yes	35 (46,67%)	7 (43,75%)	0.833
No	41 (54,67%)	9 (56,25%)	
Outcome			
Recovered	64 (85,3%)	15 (93,8%)	0.369
Dead	11 (14,7%)	1 (6,3%)	

The test results obtained *P*=0.013, thus, indicating that complaints of fever between both groups have a significant difference. As much as 20% of patients in the NAC experienced symptoms of anosmia/ageusia, and in the group, without NAC, there were 62.5% did. The test results obtained *P*=0.001, thus, indicating a significant difference between the symptoms of anosmia/ageusia in both groups. 56% of patients in the NAC group experienced indigestion, and in the group, without NAC there were 62.5% did. The test results obtained *P*=0.635, thus, indicating no significant difference between the complaints of indigestion in both groups.

There are 46.67% of the subjects in the NAC group had a smoking history. The test results obtained *P*=0.253, thus, indicating no significant difference in the smoking history between the group given with NAC and the group not.

The distribution of subjects with comorbidities in the two groups was similar and could be called balanced (46.67% in the NAC group and 43.75% in the non-NAC group). The test results obtained *P*=0.833, thus, indicating that the history of comorbidity between the NAC group and the group without NAC did not differ significantly. The most remarkable 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 test results obtained *P*=0.369, thus, indicating no significant difference between the outcome of both groups.

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

Variable	D1 Median (min-max)	D8 Median (min-max)	*P*
IL-6 with NAC (pg/ml)	121.62 (7.09–1058.32)	18.91 (1.12–970.15)	0.001
IL-6 without NAC (pg/ml)	41.33 (2.72–524.76)	34.68 (3.36–515.74)	0.408

As seen in Table 2, of 75 patients who were given adjuvant NAC therapy, the IL-6 levels on the first day had a median value of 121.62 pg/ml, and on the eighth day, the median value was 18.91 pg/ml. The p-value was 0.001, thus, indicating a significant difference in the levels of IL-6 in the group given adjuvant NAC therapy between D1 and D8, where on the eighth day after adjuvant NAC therapy, the levels of IL-6 had a median decrease of 102.71 pg/ml.

From the 16 patients in the group without NAC, the median value of IL-6 on the first day they had IL-6 was 41.33 pg/ml, and on the eighth day, the median value was 34.68 pg/ml. The Wilcoxon test resulted in *P*=0.408, thus, indicating no significant difference in the IL-6 levels between D1 and D8 in the group not given NAC. Although, on the eighth day after adjuvant NAC therapy, IL-6 decreased in value with a difference of 6.65 pg/ml in the median, the median value of IL-6 on D1 and D8 did not differ too much; thus, the test results showed no significant difference.

Table 3. Comparison of IL-6 levels on D1 and D8 between the group given NAC and the group not

Variable	Non-NAC group Median (min-max)	NAC group Median (min-max)	*P*
IL6 (pg/ml) on D1	41.33 (2.72–524.76)	121.62 (7.09–1058.32)	0.015
IL-6 (pg/ml) on D8	34.68 (3.36–515.74)	18.91 (1.12–970.15)	0.654

As seen in Table 3, the IL-6 levels on the first day of the 91 subjects showed *P*=0.015, thus, indicating a significant difference in IL-6 levels on the first day between NAC and non-NAC groups, with a median difference of IL-6 between the two groups on D1 of 80.28 pg/ml. This result concludes that the levels of IL-6 on the first day in the NAC group were higher than in the non-NAC.

As for testing IL-6 levels on day 8, of 91 samples, *P*=0.654 was obtained, thus, indicating no significant difference in IL-6 levels on the eighth day between the groups given NAC and the groups not. The median difference in IL-6 between the two groups on the first day was not too vast; thus, the difference was not statistically significant.

Table 4. Comparison of PaO2/FiO2 between D1 and D8

Variable	D1	D8	*P*
PaO_2/FiO_2 with NAC (mmHg)	182.18±78.28	309.12±68.42	<0.001
PaO_2/FiO_2 without NAC (mmHg)	171.46±67.89	296.26±74.97	<0.001

As seen in Table 4, from 75 samples of patients who were administered with NAC, the PF ratio on the first day had an average of 181.18 mmHg, and on the eighth day, the average ratio was 309.12 mmHg. The paired T-test resulted with *P*<0.001, thus, indicating a

significant difference in the PF ratio of the group given NAC between D1 and D8, where on the eighth day after adjuvant NAC therapy, the PF ratio increased by 136.94 mmHg.

Sixteen patients in the group without NAC had a PaO_2/FiO_2 ratio with an average value of 171.46 mmHg on the first day, and on day eight, the average value became 296.26 mmHg. The Wilcoxon test resulted with $P<0.001$, thus, indicating a significant difference in the PaO_2/FiO_2 NAC group between D1 and D8. For the eighth day after adjuvant therapy, the NAC PaO_2/FiO_2 increased in value with an average of 124.79 mmHg.

Table 5. Comparison of PF ratio between D1 and D8 between the group given NAC and the group not

Variable	Non-NAC group	NAC group	*P*
PF ratio (mmHg) on D1	171.46±67.89	182.18±78.28	0.613
PF ratio (mmHg) on D8	296.26±74.97	309.12±68.42	0.504

As seen in Table 5, the PF ratio on the first day of the 91 subjects had $P=0.613$, thus, indicating no significant difference in the PF ratio on the first day between the group given NAC and the group not. Although the median difference in the PF ratio between the two groups on the first day was 11.76 units, this difference is not too vast; thus, the test results showed no significant difference.

As for the PF ratio on day 8, of 91 subjects, $P=0.504$ was obtained, thus, indicating no significant difference in the PF ratio on the eighth day between the group given NAC and the group not. Although the median difference in the PF ratio between the two groups on the eighth day was 19.67 mmHg, this difference is not too vast; thus, the test results show no significant difference.

As for the relationship between changes in IL-6 levels and changes in the PF ratio of COVID-19 patients after administration of adjuvant NAC therapy, the correlation coefficient value is 0.154 with a significance value $P=0.186$, thus, indicating no positive and significant correlation between changes in IL-6 levels and changes in the PF ratio of COVID-19 patients after administration of adjuvant NAC therapy. In other words, the changes in IL-6 levels in COVID-19 patients in the group receiving adjuvant NAC therapy did not affect the changes in the PF ratio.

The effects of changes in IL-6 levels and changes in the PF ratio of COVID-19 patients after administration of adjuvant NAC therapy, in accordance to the results of this study, could be depicted in the form of a linearity graph as shown in Figure 1.

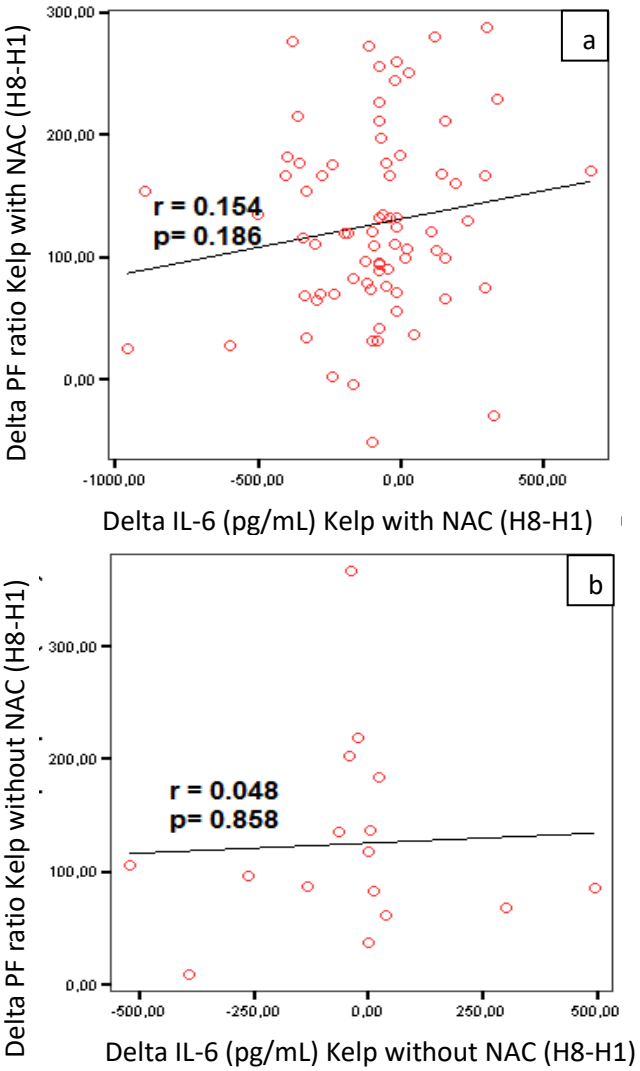

Figure 1. Linearity graph between changes in IL-6 levels and changes in the PF ratio of COVID-19 patients in a) the group with adjuvant NAC therapy; b) the group without adjuvant NAC therapy

Based on the linearity graph above, it can be seen that the regression line between changes in IL-6 levels and changes in the PF ratio of COVID-19 patients after administration of NAC adjuvant therapy points to the top right. These findings prove the linearity of IL-6 levels with changes in the PF ratio of COVID-19 patients after administration of adjuvant NAC therapy. However, because the test results

obtained insignificant results, it can be concluded that the high and low changes in IL-6 levels in COVID-19 patients in the group with NAC adjuvant therapy did not affect the high or low changes in the PF ratio.

These results prove the hypothesis—that the correlation test between changes in IL-6 levels (delta IL-6) and changes in the PF ratio (delta PF ratio) of COVID-19 patients in the group administered with adjuvant NAC therapy has a positive correlation. However, the correlation between the two variables is not significant.

The correlation test between changes in IL-6 levels (Delta IL-6) and changes in the PF ratio (Delta PF ratio) of COVID-19 patients in the group not administered with adjuvant NAC therapy resulted in a correlation coefficient value of 0.048 with a significance value *P*=0.858, thus, indicating no positive and significant correlation between changes in IL-6 levels and changes in the PF ratio of COVID-19 patients in the group without adjuvant NAC therapy. In other words, changes in IL-6 levels in COVID-19 patients not administered with adjuvant NAC therapy did not affect the level of change in the PF ratio.

The effect of changes in IL-6 levels and changes in the PF ratio of COVID-19 patients not administered with NAC therapy, based on the results of this study, is shown in the form of a linearity graph (Figure 1). The regression line between changes in IL-6 levels and changes in the PF ratio of COVID-19 patients in the group not given NAC adjuvant therapy tends to be horizontal but slightly points to the upper right. However, because the test result was not significant, it can be concluded that changes in IL-6 levels in COVID-19 patients in the group without adjuvant NAC therapy did not affect the level of change in the PF ratio.

These results prove the hypothesis — that the correlation test between changes in IL-6 levels (delta IL-6) and changes in the PF ratio (delta PF ratio) of COVID-19 patients in the group not administered with adjuvant NAC therapy has a positive correlation — to be true. However, the correlation between the two variables is not significant.

DISCUSSION

In the group administered with NAC, the mean age value was 53.12 years old, the youngest was 24 years, and the oldest was 76 years. This result does not differ much from the average age in the group not administered with NAC, which is 52.31 years old, where the youngest age was 25 years and the oldest was 69 years (*P*=0.795). Qiu et al. performed a systematic review and meta-analysis of 2,401 COVID-19 patients and found an average age of 69.9.[15] the result was comparable to the systematic review study and meta-analysis conducted by Baradaran et al., which showed that the average age was 51.[16]

Both groups were dominated by the male sex — 58.7% in the group NAC and 75% in the group not — with *P*=0.225, thus, indicating no significant difference in the gender of respondents of the two groups. Qiu et al., through a systematic review and meta-analysis, suggested that of the total 2,401 COVID-19 patients, 66.6% of the population was male. Females generally have more robust innate and adaptive immune responses than males, possibly due to X-chromosome and hormonal protection. For this reason, women are less likely to become infected with bacteria and viruses. On the other hand, males tend to have more bad habits than females (such as smoking, drinking, and more underlying illnesses), which may also be the reason why men infected with COVID-19 are more likely to die than women.[15]

The highest proportion of disease severity in the group given NAC was moderate (44.0%), while in the group not given NAC, the most significant proportion was severe (68.8%). They were not significantly different, with *P*=0.526. The degree of COVID-19 disease correlates with the course of the disease and levels of pro-inflammatory cytokines. The finding is evident from the study conducted by Han et al., where there is a significant difference in the levels of IL-6 and IL-10 in critically ill COVID-19 patients compared to severe, moderate, and mild COVID-19 patients.[17]

In the group of subjects administered with NAC, only 81.3% showed a symptom of shortness of breath,

while in the group not given NAC, all subjects showed said symptom (100%). This result had *P*=0.062, thus, indicating no significant difference in the symptom of shortness of breath between the two groups. 80% of the subjects administered with NAC had a cough, while all the subjects in the other group did (100%). This result had *P*=0.052, thus, indicating that the symptoms of cough between the group given NAC and the group were not significantly different.

As much as 70.6% of the subjects in the group given NAC had a fever, and in the group not given NAC, all subjects had a fever (100%). This result had *P*=0.013, thus, indicating a significant difference in fever symptoms between the two groups. 20% of the subjects in the NAC group experienced symptoms of anosmia/ageusia, and in the other group, 62.5% did. This result had *P*=0.001, thus, indicating a significant difference between the symptoms of anosmia/ageusia between the group given NAC and the group not. 56% of the subjects in the NAC group experienced digestive disorders, and in the other group, 62.5% did. This result had *P*=.635, thus, indicating that the symptoms of digestive disorders were not significantly different between the two groups.

For smoking history, there was 46.67% of the subjects in the group administered with NAC had a history of smoking; from the test results obtained *P*=0.253, thus, indicating that smoking history between the group given NAC and the group was not significantly different. A meta-analysis study with a total sample of 11,590 COVID-19 patients by Patanavanich showed a significant relationship between smoking and COVID-19 progression (OR=1.91; 95% confidence interval [CI]=1.42-2.59; *P*=0.001).[18] The same result was also proven by Reddy et al. through a meta-analysis study of 32,849 COVID-19 patients, of which there were 8,417 patients with a smoking history. Patients with a history of smoking have a significantly increased risk of COVID-19.[3]

The distribution of patients with comorbidities in the two groups is similar and could be considered balanced (46.67% in the group given NAC and 43.75% in the group not). The test results obtained *P*=0.833, thus indicating that the history of comorbidity between the NAC and non-NAC groups was not significantly different. Baradaran explained that the most common finding in confirmed COVID-19 patients was hypertension, which was found in 1/5 patients (21%). Comorbidities were also associated with a significantly increased risk of death, kidney disease (RR=4.90; 95% CI=3.04–7.88; *P*<0.00001), cerebrovascular disease (RR=4.78; 95% CI=3.39–6.76; *P*<0.00001), cardiovascular disease (RR=3.05; 95% CI=2.20–4.25; *P*<0.00001), respiratory disease (RR=2.74: 95% CI=2.04–3.67; *P*<0.00001), diabetes (RR=1.97; 95% CI=1.48–2.64; *P*<0.00001), hypertension (RR=1.95: 95% CI=1.58–2.40; *P*<0.00001), and cancer (RR=1.89; 95% CI=1.25-2.84; *P*=0.002), as suggested through a systematic review and meta-analysis conducted by Biswas et al..[19]

The most considerable outcome in both groups was the proportion of patients who recovered (85.3% in the group given NAC and 93.8% in the group not). This result had *P*=0.369, thus, indicating that the patient outcome between both groups was not significantly different.

Interleukin-6 (IL-6) is a cytokine that plays a central role in acute inflammation. IL-6 was first discovered by Weissenbach et al. in 1980. IL-6 is a multifunctional cytokine that plays a vital role in human metabolism, autoimmune cell differentiation, disease treatment, and others. This cytokine is tightly regulated, and the level is primarily low in healthy individuals. During infection, trauma, or other stress, IL-6 is expressed in much higher concentrations and has been implicated in the pathogenesis of several chronic disease conditions—including cardiovascular disease, atherosclerosis, and obesity.[20,21] In this study, 46.67% in the NAC group and 43.75% in the no-NAC group had comorbidities, where comorbidity could affect IL-6 levels.

N-acetylcysteine has been suggested as an adjunct therapy as standard treatment for SARS-CoV-2 infection. NAC is beneficial in its effect — increasing glutathione synthesis, enhancing immune function, and modulating the inflammatory response. Various studies have described the benefits and

effects of giving NAC on IL-6 levels, but more data are still needed for cases of COVID-19. The effect of NAC on IL-6 is through the inhibition of NFKB and ROS, as well as glutathione metabolism.[22]

IL-6 levels in the group administered with NAC decreased significantly with a median value of 102.71 pg/mL on day 8 (*P*=0.001), with an average decrease in IL-6 levels of 94.49 pg/mL (*P*=0.002). In the group not administered with NAC, the IL-6 levels also decreased with a median value of 6.65 pg/mL, and the value was lower on the eighth day compared to the first day (*P*=0.408), with an average decrease in IL-6 levels experienced by individuals of 37.5 pg/mL (*P*=0.539). This result, however, was not significant. These data indicate that using NAC as adjuvant therapy significantly reduces IL-6 levels — as IL-6 levels are associated with a poor prognosis in COVID-19 cases through various studies.

Saddadi et al. conducted a study to see the effectiveness of NAC on IL-6 and CRP levels in subjects undergoing hemodialysis. Twenty-four patients (nine males and fifteen females) on maintenance HD with an average age of 55.3 years were given oral NAC (600mg twice daily) for three months. Serum levels of biomedical parameters and IL-6 and hs-CRP were measured prior to and three months after initiation of treatment. There was a significant decrease in serum levels of CRP (22.4 vs. 5.2) and IL-6 (8.1 vs. 3.6).[23]

Paterson et al. conducted a study to see the effect of NAC on IL-6 and IL-8 levels in septic subjects. Twenty sepsis patients were divided into two groups of 10 people each. The first group was given a bolus of 150 mg/kg N-acetylcysteine in 100 mL 0.9% saline for 15 minutes, then 50 mg/kg in 100 mL 0.9% saline for 4 hours as a loading dose, and then a maintenance dose of 50 mg/kg in 200 mL of 0.9% normal saline over any 24 hours for a total of 72 hours. The second group was given a placebo. The result showed a decrease in IL-6 levels in both groups. However, the result was not significant.[24] This result was in contrast to our study, where there was a decrease in IL-6 levels in the group given NAC and without NAC on the eighth day, and the decrease of IL-6 levels in the group given NAC was significant.

Gosset et al. conducted a study to see NAC's effect on TNF-α, IL-6, and IL-8. There is a significant decrease in TNF-α, IL-6, and IL-8 in BAL fluid samples after administration of NAC for 48 hours. In our study, IL-6 levels decreased significantly after administration of adjuvant NAC therapy at a dose of 5gram/72 hours for eight days.

N-acetylcysteine has been shown to inhibit NF-κB and the replication of the human influenza virus (H5N1 strain, Vietnam/VN1203) in human lung epithelial cells. NAC also reduces the production of pro-inflammatory cytokines (IL-8, CXCL10, CCL5, and IL-6), thereby reducing monocyte chemotactic migration. It has been shown to have a protective effect against ARDS. N-acetylcysteine can also inhibit viral replication and the expression of pro-inflammatory molecules. This drug can inhibit pulmonary inflammation, myeloperoxidase (MPO) activity, neutrophil macrophages, IL-6, IL-1β, CXCL-10, and TNF-α.[25] It was proven in this study as there was a significant decrease in IL-6 levels after administration of adjuvant NAC therapy of 5 grams/72 hours for eight days.

There was a significant difference in the PF ratio of the group given NAC between D1 and D8; on the eighth day after administration of adjuvant NAC therapy, the PF ratio increased by an average value of 126.94 mmHg (*P*≤0.001). However, it turns out that the same result was also found in the group not given NAC, where there was a significant difference in PaO_2/FiO_2 between D1 and D8, and on the eighth day after administration of adjuvant NAC therapy, PaO_2/FiO_2 showed an increased average value of 124.79 mmHg (*P*≤0.001).

Moradi et al. conducted a study to see the effect of giving NAC on 27 ARDS patients treated in the ICU. The subjects were divided into two groups — 14 patients were administered with NAC, and 13 control patients were given a placebo. NAC was administered intravenously at a dose of 150 mg/kg on the first day, followed by 50 mg/kg/day for three days of administration. The ARDS criteria were following the American European Consensus Conference on ARDS. The study's results showed a significant increase in the PF ratio in the group of patients given

NAC compared to the group given placebo (*P*≤0.001).[21]

Similarly, in a previous study about the effect of administering NAC on ARDS patients admitted to the ICU, the subjects were divided into two groups; those who were given NAC intravenously at 40 mg/kg/day for three days (32 subjects) and those who were given a placebo (29 subjects). There was a significant increase in the PF ratio in patients given NAC, while in the placebo group, there was no significant increase in the PF ratio. In our study, there was no difference between the group given NAC and the group not; as in both groups, there was a significant improvement in PF ratio values on the eighth day.

The relationship between changes in IL-6 levels and changes in PF ratio of COVID-19 patients after administration of adjuvant NAC therapy shows correlation coefficient of 0.154 with *P*=0.186, thus, indicating a weak, positive, but not statistically significant correlation. In other words, the changes in IL-6 levels in COVID-19 patients receiving NAC adjuvant therapy had a weak influence on the change in PF ratio.

The correlation test between changes in IL-6 level (Delta IL-6) and changes in PF ratio (Delta PF ratio) of COVID-19 patients in the group without adjuvant NAC therapy showed correlation coefficient value of 0.048, with *P*=0.858, thus, indicating neither positive nor significant correlation. In other words, the change in IL-6 levels in COVID-19 patients not administered with adjuvant NAC therapy did not affect the change in PF ratio.

There are not many studies on the correlation between IL-6 and PF ratio. Hagau et al. studied the correlation between several cytokines — such as IL-6, IL-15, IL-8, and TNFα — and PF ratio in 31 patients infected with the severe H1N1 virus. The study showed a negative correlation between IL-6 levels and the PF ratio (r = -0.556, *P*=0.001).[26] In the study of Benucci et al., lung improvement estimated through the SpO_2/FiO_2 ratio was observed in 7 of 8 patients with COVID-19 pneumonia who received tocilizumab treatment. Clinical recovery is associated with increased lymphocyte counts, decreased levels of IL-6, and CRP.[27]

NAC decreased IL1β, IL18, IL6, and TNF-α in vitro. It inhibits downstream activity post TNF- α receptor activation, while NAC inhibits TNF- α and IL-6 gene expression under oxidative stress.[13] The effect of NAC on IL-6 is through the inhibition of NFkB and ROS, as well as glutathione metabolism. It is known that ROS plays an essential role in the pathogenesis of lung injury and that the alveolar epithelial lining of ARDS patients is deficient in Glutathione.[22] In this study, changes in IL-6 have a weak effect on changes in PF ratio; this is because IL-6 is not the only factor that affects PF ratio, as what could affect it is multifactorial.

LIMITATION

The control group was not comparative, with only 16 subjects due to obtaining more samples in the non-NAC population because it was difficult to get samples that agreed not to be given NAC, especially when NAC was included in the treatment guidelines.

CONCLUSION

There was a significant decrease in IL-6 levels in COVID-19 patients who were given NAC as adjuvant therapy compared to COVID-19 patients who did not receive adjuvant NAC therapy. There was a significant decrease in PaO_2/FiO_2 ratio in both COVID-19 patients who were given NAC as adjuvant therapy and COVID-19 patients who did not. There is no positive and significant correlation between decreased IL-6 levels and increased PaO_2/FiO_2 ratio in COVID-19 patients receiving adjuvant NAC therapy; neither is there in COVID-19 patients who did not receive adjuvant NAC therapy.

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CONFLICT OF INTEREST

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