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Administration of N-Acetylcystein (NAC) as Adjunctive Therapy for COVID-19 Patients by Improving Oxidative Stress And Inflammation Through Assessment of LDH, CRP, D-Dimer, And Ferritin

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ABSTRACT

INTRODUCTION

Lactate dehydrogenase (LDH), C-Reactive Protein (CRP), D-dimer and feritin are acute phase proteins which levels may rise in the presence of an inflammatory process of COVID-19. N-Acetylcysteine (NAC) is an antioxidant which has also been widely considered as adjunctive therapy in COVID-19. We aim to analyze the effect of NAC administration in improving oxidative stress and inflammation in COVID-19 through assessment of LDH, CRP, D-dimer, and feritin levels. **METHOD** A quasi-experimental study with pre-post design. LDH, CRP, D-dimer, and feritin levels were measured in admission and day 8 of administration of 5000 mg/72 hours of NAC. **RESULT** There is a significant decrease in CRP, LDH, D-dimer and Ferritin levels between admission and day 8 of NAC therapy. CRP levels were reduced from 185.31 ± 181.30 to 97.60 ± 161.86 ($p<0.001$), LDH levels were reduced from 4.65 ± 4.13 to 1.87 ± 0.96 ($p<0.001$), D-dimer levels were reduced from 185.31 ± 181.30 to 97.60 ± 161.86 ($p<0.001$) and Ferritin levels were reduced from 4.65 ± 4.13 to 1.87 ± 0.96 ($p<0.001$).

DISCUSSION

There is a significant decrease of LDH, CRP, D-dimer, and feritin levels from day-1 (admission) to day-8 of NAC administration in accordance with existing theories. This implies the ability of NAC to reduce oxidative stress and inflammation in COVID-19 patients. **CONCLUSION** There was a decrease in CRP, LDH, D-dimer and Ferritin after administration of NAC in COVID-19 patients and a positive correlation between severity and mortality towards acute phase protein in COVID-19 patients.

Keywords: COVID-19, N-Acetylcysteine, Acute phase protein, Inflammatory markers

INTRODUCTION

On December 31, 2019, China reported a mysterious case of pneumonia of unknown cause. In 3 days, the number of patients with these cases amounted to 44 patients and continues to grow until now there are thousands of cases. Samples of isolates from patients were studied with the results showing the presence of a coronavirus infection, a new type of betacoronavirus, named 2019 novel Coronavirus (2019-nCoV). On February 11, 2020, the World Health Organization named the new virus Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the name of the disease as Coronavirus disease 2019 (COVID-19). Until now, this virus is still spreading and researches are still ongoing.[1]

The lungs are the preferred target of Covid-19, where the lungs are also one of the most oxygenated organs of the body. Multiple lung diseases contribute to the increased production of the characteristic reactive oxygen species (ROS) from oxidative stress conditions. Oxidative stress is an important factor that causes metabolic and physiological changes and various diseases in the body. Covid-19 infection triggers an inflammatory reaction that releases the proinflammatory cytokines characteristic of acute lung damage. Good association between pro-inflammatory elements and reactive oxygen species (ROS) in different lung diseases including Coronavirus infection is associated with inflammation and oxidative stress.[2]

N-acetylcysteine has recently been suggested as an adjunctive therapy to the standard care for SARS-CoV-2 infection considering the favorable risk and benefit ratio and its effects on synthesizing glutathione, improving immune function, and modulating inflammatory response; also reduce D-dimer levels which concomitant with high levels of fibrin degradation products and low antithrombin activity render COVID-19 patients to be at risk of hypercoagulability and thrombotic complications.[3]Another study show on Day 10, NAC therapy led to significant reduction in D-dimer levels compared to the controls (0.8 (0.6; 0.9) vs 0.6 (0.2; 0.7), p=0.07).[4]As an anti-inflammatory compound, NAC can reduce levels of tumor necrosis factor-alpha (TNF-α) and interleukins (IL-6 and IL-1β) by suppressing the activity of nuclear factor kappa B (NF-κB).[5]

METHOD

This is a quasi-experimental study with pre-post design to assess the effects of NAC administration as adjunctive therapy in COVID-19 confirmed patients. This study was conducted with ethical approval No 400/149/K.3/302/2020 by Saiful Anwar General Hospital Ethics Committee. Study population is confirmed COVID-19 patients by RT-PCR nasopharyngeal swab result admitted in our hospital. Inclusion criteria includes confirmed COVID-19 patients admitted both in non-intensive and intensive wards and exclusion criteria are those who passed away before RT PCR swab results can be obtained (probable cases), pregnant woman, and asymptomatic cases. The study was commenced in Saiful Anwar general Hospital, Malang Regency, East Java, Indonesia between May 2020 to July 2021. Samples were checked for full laboratory workup including inflammatory markers (LDH, Ferritin, CRP and D-dimer) in admission and in day 8 after administration of 5000 mg/72 hours of NAC. The data was collected and analyzed using appropriate statistical analysis.

RESULT

We screened 74 patients in the analyses which fulfilled the included criteria. Men had a higher number for infection with COVID-19 than women (44 (59.5%) vs 30 (40.5%)). With a higher number for severe and critically ill of COVID-19's severity (40 (54%) vs 34 (46%)). The analyses also showed that patients admit to hospital with many complaint, such as fever (53 (71.6) dyspnea (61 (82.4%)), cough (60 (81.1%)), and GIT disturbances (42 (56.8%)). Recent smoker or active smoker predominantly admit to hospital with 43 (58.1%) vs 31 (41%). Only 33 (44.59%) have a comorbid on this analyses which higher number of recovery outcome (64 (86.5%)) of all subject compared with death outcome (10 (13.5%)).

Table 1. Patients' Demographic

Variable and Cathegory	Frequency	Percentage
Age	53.67 yo ± 10.81 (min. 24 yo with max. 75 yo)	N sample = 74 (100%)
Gender - Male - Female	44 30	59.5% 40.5%
Severity - Moderate - Severe - Critically Ill	34 32 8	45.9% 43.2% 10.8%
Admission's Complaint - Fever - Shortness of Breath - Cough - GIT disturbances	53 61 60 42	71.6% 82.4% 81.1% 56.8%
Recent Smoker or History of Smoking - Yes - No	43 31	58.1% 41.9%
Comorbidities - Yes - No	33 41	44.59% 55.40%
Corticosteroid Used - Yes - No	40 34	54.1% 45.9%
Outcome - Recovery - Death	64 10	86.5% 13.5%

NAC treatment results in a increase in the concentration of CRP, LDH, D-dimer and Ferritin, markedly from day 1 to day 8. CRP concentrations were reduced at day 8 after NAC treatment (185.31 ± 181.30 to 97.60 ± 161.86, p<0.001). LDH concentrations also were reduced by NAC treatment on days 1–8 (4.65 ± 4.13 to 1.87 ± 0.96, p<0.001). And also D-dimer concentrations which decrease after 8th day of NAC treatment (185.31 ± 181.30 to 97.60 ± 161.86). Similar to the CRP, LDH, and D-dimer, NAC treatment result in a decrease in the Ferritin concentrations on days 1–8 (4.65 ± 4.13 to 1.87 ± 0.96, p<0.001).

Table 2. Pre- and Post-NAC Results of Test Markers

Parameter	Pre (Mean ± SD)	Post (Mean ± SD)	P value
CRP	8.17 ± 8.16	3.73 ± 6.86	< 0.001
LDH	736.48 ± 256.89	606.59 ± 252.33	< 0.001
D-dimer	3.24 ± 8.37	2.07 ± 5.29	0.044
Ferritin	1053.01 ± 818.20	788.85 + 716.96	< 0.001

DISCUSSION

There are several biomarkers that has been widely used in COVID-19 to evaluate progression and disease severity. LDH is an enzyme involved in energy production and is found in almost all cells in the body. Tests that measure the concentration of LDH in the blood are usually used to quantify cell damage and indicate viral infection or lung damage, such as pneumonia caused by SARS-CoV-2. Serum LDH is a rapidly, affordable, and widely available measurement that can predict the risk of death in COVID-19 patients.[6] LDH and CRP may be associated with respiratory function (PaO2/FiO2) and be predictors of respiratory failure in CoVID-19 patients. LDH and CRP should be considered as useful tests for early diagnosis of patients requiring closer observation and more aggressive supportive therapy to avoid a poor prognosis.[7]

C-reactive protein (CRP) is a non-specific acute phase reactant elevated in infection or inflammation. CRP levels can be used for early diagnosis of pneumonia, and patients with severe pneumonia had high CRP levels. It is an important index for the diagnosis and assessment of severe pulmonary infectious diseases.[8,9] CRP can be used to assist with differentiation between viral and bacterial infections, for example, influenza produces a mean CRP level of 25.65 mg/L [95% confidence interval (CI) 18.88 to 32.41 mg/L] versus bacterial pneumonia which produces a mean CRP level of 135.96 mg/L (95% CI 99.38 to 172.54 mg/L). Elevated levels of serum C-reactive protein (CRP) have been observed in patients with COVID-19 and used to assist with triage, diagnostics, and prognostication. Higher levels of CRP have been used as an indicator of COVID-19 disease severity and the magnitude of the acute inflammatory response. The use of CRP as a biomarker in COVID-19 may present a quick and accessible tool in clinical management, trigger longer periods of enhanced observation, provide information around likely disease progression, and assist with early therapeutic, ventilation, and palliative care discussions.[1,9,10]

Serum ferritin is an iron storage protein that is commonly used to assess iron levels, but it is also a well-known inflammatory marker. Inflammation and a variety of disorders can cause large increases in serum ferritin levels. Connelly et al. tested serum ferritin levels in patients at risk for and with ARDS as

early as 1997, and discovered that serum ferritin was a predictor of ARDS. Patients with hyperferritinemia (500 ug/L) were more likely to develop bilateral lung infiltration and a more severe illness course, according to our findings. Because serum ferritin levels are linked to the severity of systemic and pulmonary inflammation, it's plausible to assume that hyperferritinemia is linked to disease severity in COVID-19 patients.[11]

Although the processes underlying the link between hyperferritinemia and disease severity in COVID-19 patients are unknown, there are a few theories to consider: 1) Interleukin-I (IL-l), a proinflammatory cytokine, and tumor cells TNF-alpha and IL-6 have been shown to stimulate ferritin synthesis.[12] As a result, we hypothesized that SARS-CoV-2-induced production of proinflammatory cytokines (such as IL-6 and TNF- alpha), which are known to be high in COVID-19, would boost ferritin synthesis early in the inflammation process.2) Inflammation-induced cellular damage can lead to intracellular ferritin leakage, which raises serum ferritin levels.[13] 3) In acidosis, the microvascular environment and increased production of reactive oxygen species (ROS) may liberate iron from ferritin, and it is this unliganded iron that can participate in Haber-Weiss and Fenton reactions, generating hydroxyl radicals, further cellular damage[13], and worsening tissue inflammation, resulting in a vicious cycle of inflammation. Similarly, one study discovered that chaperone-mediated ferritinis involved in the assembly of Middle East Respiratory Syndrome (MERS) coronavirus nanoparticles.[14] However, more research is needed to confirm the relevance of serum ferritin levels in COVID-19 etiology.

It was observed that the combination of corticosteroids and intravenous N-acetylcysteine resulted in a significant reduction in inflammatory markers (C-reactive protein and ferritin). A group of nine COVID-19 patients were given intravenous N-acetylcysteine and showed a significant reduction in inflammatory markers (C-reactive protein and ferritin). During IV N-acetylcysteine treatment, the median C-reactive protein level was 55 mg/dL, which was significantly lower than before administration (143 mg/dL) or after N-acetylcysteine termination (69 mg/dL). COVID-19-associated cytokine storm was found to be mitigated by N-acetylcysteine, which also elicited progressive clinical improvement and facilitated hospital discharge readiness.[15] Similar results are also observed and there's ongoing clinical trial that studies the effect of NAC to levels of ferritin in COVID-19.[16]

Based on the table above, it can be seen that from 74 samples of patients with NAC, on the first day they had an average CRP of 8.17 mg/L, and on the 8th day the average CRP was 3.73 mg/L. Based on the results of the statistical test, a p-value of 0.001 ($p<0.05$) was obtained, so it could be concluded that there was a significant difference in the CRP value after administration of NAC between H1 and H8, where on H8 after administration of adjuvant NAC therapy, CRP decreased by 4.44mg/ L.

Previous studies found that NAC administration significantly decreased CRP and NEWS2 scale scores compared to the control group. The duration of hospitalization was also significantly 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) did not differ between groups.[4] Several studies have also examined the efficacy of NAC in hospitalized patients with COVID-19. In respirator dependent patients, intravenous NAC causes clinical improvement and reduces CRP and ferritin.[15]

There is a significant decrease of ferritin level between the administration of NAC therapy and day 8 (1053.01 ± 818.20 and $788.85 + 716.96$, respectively) ($p<0.001$). The significant reduction of ferritin suggests that NAC therapy could also reduce the risk of cytokine storm. This result is consistent with the result of study by Ibrahim et al.[15] Conversely, this also suggests that NAC therapy could reduce

disease severity and ARDS, since high level of ferritin is linked with both higher disease severity and the severity of ARDS.

In our study, it was found that MDA levels in covid 19 patients increased (3000.70 ± 2017,98), this is in line with the study of Mehri et al, where MDA as a marker of oxidative stress increased significantly in Covid-19 patients.[17] Elevated levels of MDA in COVID-19 patients compared to the control group showed an overproduction of free radicals that destroy membrane lipids with the formation of MDA as a by-product.[18] After administration of NAC, MDA decreased significantly after administration of NAC for 7 days ($p<0.001$), this is in accordance with the study of Cazzola et al which stated that NAC significantly reduced the pro-oxidant response caused by LPS. by reducing the level of peroxidase activity by 30% and levels of H2O2, malondialdehyde (MDA), and nitric oxide (NO).[19]

From the results of our study, we found an increase in LDH levels in covid 19 patients (736.48 ± 256.89). This is in line with the research which stated that there was an increase in the average LDH of 245 U/L in covid 19 patientsand that the LDH increased higher in COVID-19 patients.[20] There is an association between increased LDH values and poor outcomes in patients with COVID-19. In particular, there was a >6-fold increase in severity and a >16-fold increase in the probability of death in patients with high LDH.[21]

In our study there was a significant decrease in LDH levels between H1 and H8 with NAC administration where on H1 (before administration of NAC adjuvant therapy, LDH levels were 736.48 ± 256.89 and H8 after NAC administration, LDH levels decreased by an average of 606.59 ± 252.33 ($p < 0.001$) This is in line with research by Assimakopoulos et al which stated that NAC increased the PO2/FiO2 ratio and decreased leukocytes, CRP, D-dimer and LDH.[22]

There is a significant decrease of ferritin level between the administration of NAC therapy and day 8 (1053.01 ± 818.20 and 788.85 + 716.96, respectively) ($p<0.001$). The significant reduction of ferritin suggests that NAC therapy could also reduce the risk of cytokine storm. This result is consistent with the result of study by Ibrahim et al.[15] Conversely, this also suggests that NAC therapy could reduce disease severity and ARDS, since high level of ferritin is linked with both higher disease severity and the severity of ARDS.

DECLARATION OF CONFLICTING INTEREST

The authors declare no conflicts of interest in preparing this article

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CONCLUSION

There was a decrease in levels of acute protein phase such as CRP, LDH, D-dimer and Ferritin after administration of N-acetylcsteine in Covid-19 patients. There is also a positive correlation between severity and mortality towards acute phase protein in Covid-19 patients.

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