Short Communication

The role of N-acetylcysteine in decreasing neutrophil-lymphocyte ratio in COVID-19 patients: A double-blind, randomized controlled trial

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Abstract

N-acetylcysteine has antioxidant and anti-inflammatory activities that could potentially improve the clinical outcomes of coronavirus disease 2019 (COVID-19) patients. N-acetylcysteine potentially inhibits NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome and results in control oxidative stress and cytokine release in COVID-19 patients. The aim of this study was to assess the effect of N-acetylcysteine in reducing the neutrophil-lymphocyte ratio (NLR) in COVID-19 patients. A randomized controlled clinical trial was conducted among severe and moderate COVID-19 patients. The treatment group received oral 1200 mg daily of N-acetylcysteine (three times a day) and the standard care for COVID-19, while the control group received standard care for COVID-19 and a placebo. The NLR was determined on the first day of admission and after the seventh day of treatment. A paired Student t-test was used to compare the NLR before and after treatment while independent Student t-test was used to compare the NLR between treatment and control groups. A total of 40 severe and moderate COVID-19 were enrolled, 20 people in each group, with a mean age was 44.68±13.24 years old. The mean NLR on the first day was 9.44 in the treatment group and 8.84 in the control group. After the seventh day, the mean NLR was 4.27 and 11.54 in the treatment group and control group, respectively. The mean changes of NLR (the pre-treatment compared to post-treatment) in the treatment and control group were reduced 4.05 and increased 3.34, respectively. The NLR in treatment group significantly decreased compared to the control group (p<0.001). In conclusion, N-acetylcysteine 1200 mg daily could reduce the NLR in severe and moderate COVID-19 patients.

Keywords: COVID-19, N-acetylcysteine, neutrophil-lymphocyte ratio, COVID-19 treatment, clinical trial

Introduction

Coronavirus disease 2019 (COVID-19), a respiratory infectious disease caused by the new emerging coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused significant health problems [1-4]. COVID-19 still became a health problem in many nations globally [5]. The main concern of COVID-19 is inflammation and cases with evidence of cytokine storm tend to have a high mortality rate [6]. Studies suggested a correlation between cytokine storms and the deterioration of COVID-19 patients [2,6]. Many antiviral agents and immunotherapy are being developed as potential therapies against COVID-19 [7]. However, until

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now, no definitive treatment for COVID-19 since the development of effective treatment for a new disease is a long and complex process [8-10].

Two viroporins—protein E and ORF3a—are present in SARS-CoV-2 [11]. The E protein participates in a number of signaling processes that ultimately lead to inflammation during infection. Additionally, it plays a part in the syntenin protein-protein interaction that activates the p38 MAPK and the inflammatory NF-kB pathway. The E protein causes lysosomal breakdown and ion redistribution, triggering the NLRP3 (NOD, LRR-, and pyrin domain-containing 3) inflammasome [11]. The NLRP3 inflammasome is a critical component of the innate immune system that mediates caspase-1 activation and the secretion of proinflammatory cytokines such as interleukin 1 (IL-1) [12]. Increased production of IL-1, IL-6, and tumor necrosis factor-α (TNF-α) is caused by the activation of NLRP3, and this cytokine storm causes inflammation and cellular damage in COVID-19 patients [11]. Since NLRP3 inflammasome plays a critical role in COVID-19 pathogenesis, it is a possible therapeutic target for the treatment of COVID-19 [11].

Neutrophil-lymphocyte ratio (NLR) is a marker of inflammatory conditions [13], and it has been proposed as an independent prognostic indicator for patients with COVID-19 [14]. Moreover, NLR was recently considered a marker for decision-making processes concerning the admission/recovery of patients with COVID-19 [15]. Several studies found that high NLR was associated with the severity of patients with COVID-19 [15-18]. NLR is also a marker for oxidative stress [19].

N-acetylcysteine (NAC) is a mucolytic drug that is well tolerated and usually used to treat coughing. NAC has been proposed as prevention and therapy for various respiratory system diseases, mainly caused by oxidative stress, one of which is COVID-19 [20]. NAC works by inducing mucus secretion that adheres to the mucosa, increases glutathione S-transferase activity, triggers detoxification, and works directly as a scavenger of free radicals [21]. NAC is a potent antioxidant with a potential therapeutic effect in diseases characterized by increased formation of free oxidant radicals [15]. It also could work as an anti-inflammatory agent by limiting the production of pro-inflammatory cytokines in the early stages of immune responses [16]. An in-vitro study using SARS-CoV-2 showed that NAC could inhibit the activation of NLRP3 inflammasome [20]. Unfortunately, the evidence from clinical trials regarding the effect of NAC as an antioxidant and an anti-NLRP3 inflammasome in respiratory tract infections remain limited [22]. The aim of this study was to assess the effect of NAC in improving NLR in severe and moderate COVID-19 patients.

Methods

Study design and registration

This was a double-blind, randomized controlled clinical trial to assess the improvement of NLR after NAC treatment in patients with COVID-19. Moderate and severe COVID-19 patients were recruited and treated with oral NAC or with placebo. The NLR was determined before and after seven days of treatment. This clinical trial was registered in www.clinicaltrial.gov (number NCT05658549).

The patients, sample size, and sampling

Hospitalized COVID-19 patients with moderate and severe severity at Universitas Sebelas Maret Hospital Kartasura, Indonesia, were recruited through simple random sampling. The inclusion criteria were: (1) a moderate or severe grade of COVID-19 based on WHO classification [23]; (2) aged between 18 and 60 years old; and (3) willing to entice the clinical trial. Patients who had comorbidities such as diabetes mellitus, heart failure, chronic kidney disease, or liver disease, and those who died during the treatment period were excluded.

Randomization and blinding

The sample size was determined using the sample size formula by Dahlan [24] for paired numeric analytical research type, with a 5% type I error and 20% type II error. The minimal sample size was 18 patients for each arm. The number was round up to 20 subjects for each group. With simple random sampling approach, the patients were divided into control and treatment groups.

The treatment group received NAC, while the control group received a placebo. NAC and placebo tablets were similar in size and shape, making they were difficult to be distinguished. Patients and investigators did not know whether to get a placebo or NAC. The allocation of treatment and placebo was conducted behind a closed system. The data were opened at the end of the study.

Interventions

The patients within treatment group received standard care of COVID-19 with oral NAC 1200 mg (400 mg three times a day). The control group received COVID-19 standard care and a placebo.

Outcome

The outcome of this study was the NLR of COVID-19 patients. The components of NLR (neutrophile and lymphocyte) were measured using XP-300 Hematology Analyzer (Sysmex, Singapore). The NLR was measured twice for each patient, at the time of admission and after the seventh day of the treatment with NAC. NLR was determined as neutrophil counts (per microliter) divided by the lymphocyte counts per microliter.

Statistical analysis

A paired Student t-test was used to compare the NLR before and after treatment while independent Student t-test was used to compare the NLR between treatment and control groups. The $p<0.05$ was considerate statistically significant. Data analysis using SPSS v25 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the patients

A total of 40 severe and moderate COVID-19 were enrolled and completed clinical trial and the detailed flow diagram of each step from recruitment till analysis is presented in **Figure 1**. Baseline demographic and clinical characteristics of the patients are presented in **Table 1**. Out of total patients, 22 men (55%) were men, twelve in the treatment group and ten in the control group. The mean age for both groups was 44.68±13.24 years; 42.30±14.88 years in control group and 47.05±11.25 years for treatment group. The mean length of stay for both groups was 11±45 day; 11±5 day in control group and 11±4 day for treatment group. The mean NLR before treatment was 8.84±3.45 in control group and 9.44±6.42 for treatment group. The mean NLR after treatment was 11.54±4.30 and 4.27±3.66 for control and treatment group, respectively. The change of NLR was increased 3.34 in control group and reduced 4.05 for the treatment group.

Table 1. Baseline demographic and clinical characteristics of the COVID-19 patients included in the study

Patient characteristics	Group		*p*-value
	Treatment (n=20) Mean±SD	Control (n=20) Mean±SD	
Sex			0.502 [a]
Female	9 (50%)	9 (50%)	
Male	12 (55%)	10 (45%)	
Age (year)	47.05±11.25	42.30±14.88	0.509 [b]
Length of stay (day)	11±4	11±5	0.375 [b]
NLR pre-treatment	9.44±6.42	8.84 ±3.45	0.140 [b]
NLR pos-treatment	4.27±3.66	11.54±4.30	0.019 [b]
Changes of NLR	4.05±6.24	-3.34±3.48	<0.001 [b]

SD: standard deviation
[a] Analyzed using Chi-squared test
[b] Analyzed using independent Student t-test

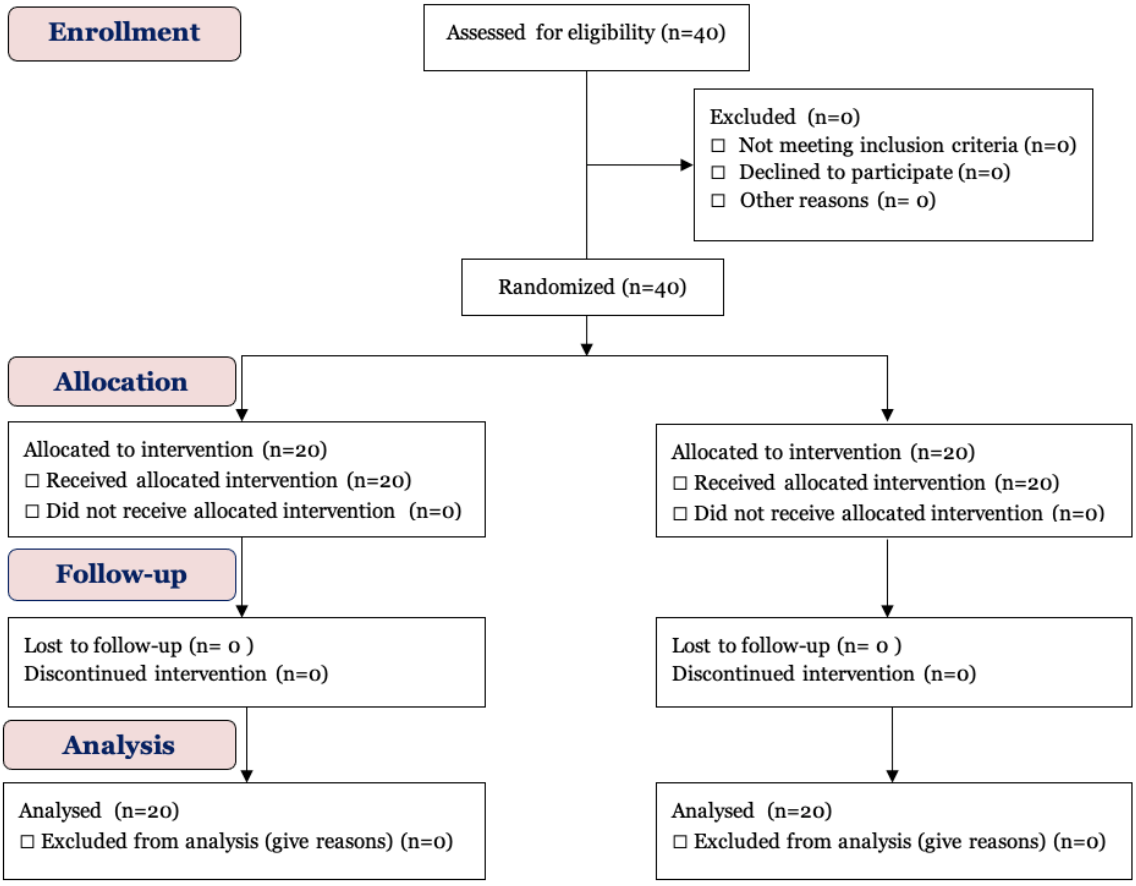

Figure 1. Consort flow diagram of the clinical trial.

Comparation of NLR pre- and post-treatment in treatment and control groups

To assess the changes in NLR before and after seven days of treatment of COVID-19 patients within control or treatment group, a paired Student t-test was employed. Our data indicated that the NLR was siginifacntly different between pre- and post-tretment within control and treatment groups (p<0.001). The control group's mean delta NLR level is negative, indicating a worsening inflammatory condition in control group patients (**Figure 2**). Meanwhile, the mean delta NLR level of the treatment group NAC 1200mg/day treatment group was positive indicating the decreasing NLR level and an improvement of the inflammatory condition in treatment group patients.

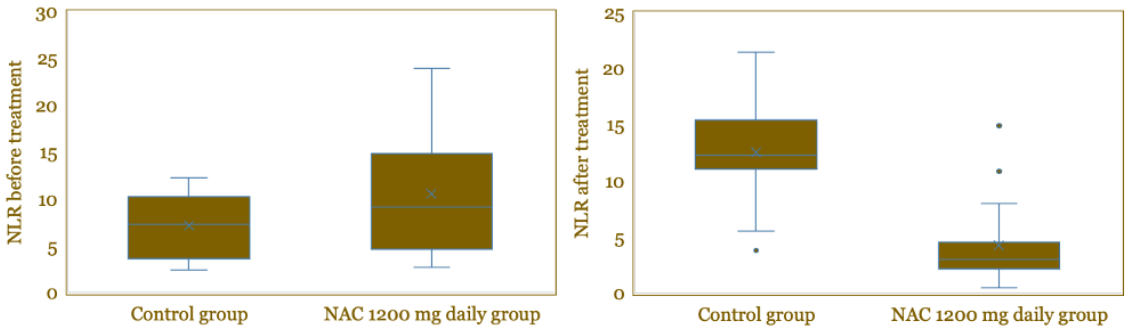

Figure 2. Comparison of neutrophil-lymphocyte ratio before and after treatment between the control and treatment groups.

Changes in neutrophil-lymphocyte ratio between treatment and control groups

The average value of delta NLR (pre-treatment compared post-treatment) of control group was -3.34±5.68 while the delta NLR in treatment group was 4.05±7.07 indicating the NLR increased and reduced in control and treatment group, respectively (**Figure 3**). An independent Student t-

test was used to compare for the delta NLR between both groups. Our data indicated a significant different of delta NLR between the control and treatment groups (p<0.001).

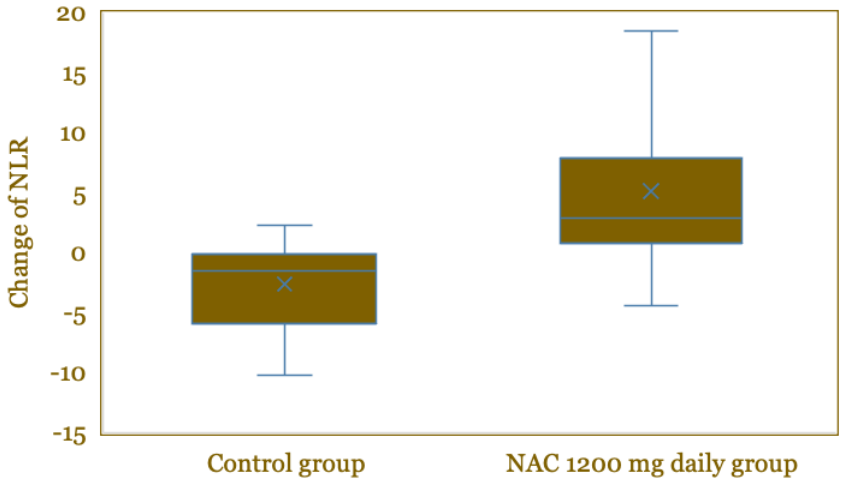

Figure 3. The changes of neutrophil-lymphocyte ratio (NLR) (pre-treatment compared to post-treatment) in treatment and control groups. The data indicating that the NLR worsen and improved in control and treatment group, respectively.

Discussion

Inflammation is a part of the body's defense mechanism against infection [25]. Although inflammation plays a role in the healing process, uncontrolled or excessive inflammation conditions that aren't treated can potentially lead to more severe symptoms and even death. Some inflammatory diseases, especially those caused by infection, require immediate medical treatment [26]. Management of anti-inflammatory and antioxidant agents has been proven to relieve inflammatory conditions [27-29].

The NLR seems much more terrific in patients with severe or life-threatening illnesses than in individuals with less severe diseases [16]. NLR is a dependable indicator for predicting COVID-19 illness severity. Several mechanisms regarding neutrophils and lymphocytes' resistance to Sars-Cov-2 virus infection have been postulated. Neutrophils stimulate the immune system and generate reactive oxygen species, which may trigger cell DNA damage and eliminate viruses that are subsequently targeted by antibodies. Moreover, neutrophils produce the production of many cytokines and effector molecules. On the other hand, although viral infection predominantly induces a lymphocyte response, systemic inflammation with exceptionally high Interleukin 6 paradoxically reduces the number of lymphocytes and the consequent cellular immunity. These two elements enhance NLR. Consequently, a greater NLR forecasts the intensity of inflammation [30].

The mechanism of NAC is anti-inflammatory by suppressing the release of IL-6, IL-10, IL-1β, and TNF-α. Through the NF-κB inhibitory pathway, NAC can inhibit the production of pro-inflammatory cytokines in COVID-19 patients. So NAC decreases the cytokine storm in COVID-19 patients. Meanwhile, primary antioxidants of NAC work to reduce the intensity of intracellular oxidative stress in COVID-19 [23]. The condition of lymphopenia and the decrease in glutathione concentration caused by COVID-19 was also considered to be controlled by administering a high dose of 1200 mg oral NAC, which works by blocking the apoptotic signal of T lymphocytes, which can then increase adaptive immunity. Administration of 1200 mg oral NAC has been shown to reduce ROS production by neutrophils that cause oxidative stress conditions without interfering with the function of neutrophils in phagocytosing SARS-CoV-2 itself. NAC's ability to effectively deal with oxidative stress prevents other cytokine storms, pulmonary edema, and acute respiratory distress syndrome. The results of reports from several case reports, adding NAC to COVID-19 patients can improve the clinical outcomes of these patients [21].

This study suggested that NAC's anti-inflammation and antioxidant reduced NLR in COVID-19 patients. This could be due to NLRP3 inflammasome inhibition. The inflammasome protein

increases in COVID-19 patients because the SARS-CoV-2 activates the TLR2 pathway. A previous in vitro study [20] found that NAC can inhibit inflammasome by suppressing Nf-κB and NLRP3.

This study has some limitations. The sample size was relatively small in this preliminary clinical trial. The NAC's anti-inflammation and antioxidant activities were only assessed using NLR. Some confounding factors such as ethnicity, vaccination number, and viral load were also not controlled in this clinical trial.

Conclusion

N-Acetylcysteine can reduce the NLR level of COVID-19 patients. NAC acts as an anti-inflammation by reviewing the significant decrease in NLR level compared to the control group. According to the study that has been conducted, the results of the treatment of NAC 1200mg/day (three times a day) orally can be used as anti-inflammatory and antioxidant agents to treat hyperinflammatory conditions and oxidative stress in COVID-19 patients.

Ethics approval

The Health Research Ethics Committee of Dr. Moewardi Hospital Surakarta has approved this research by approval Number: 149/II/HREC/2021. All participants provided informed consent before the study started.

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Conflict of interest

The authors declare that they have no competing interests.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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