Colchicine reduces the degree of inflammation in COVID-19 patients

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Abstract. COVID-19 increases vulnerability for populations living in regions significantly impacted by the adverse effects of climate change. There is currently no definitive treatment for COVID-19. Colchicine is a drug that can reduce the severity of COVID-19 by inhibiting the NLRP3 inflammasome. This study aims to determine the effect of colchicine administration on the high-sensitivity C-reactive protein (HsCRP) and Neutrophil to Lymphocyte Ratio (NLR) levels in COVID-19 patients. This study was conducted at the UNS Hospital in February-March 2021. The inclusion criteria were moderate-grade COVID-19 patients. HsCRP and NLR examinations were carried out before and after giving the treatment. The treatment group received 2x0.5 mg colchicine for 7 days and standard therapy, while the control group received placebo and standard therapy. Statistical test using paired t-test and independent t-test. P is significant if p is less than 0.05. The study subjects were 40 patients, with 20 patients in the control group and 20 in the treatment group. There was a decrease in NLR and HsCRP levels in the treatment group before therapy (NLR = 7.89 ± 3.45; HsCRP = 5.41 ± 3.24 mg/dL) compared to after therapy (NLR = 3.59 ± 2.25; HsCRP = 1.41 ± 1.13 mg/dL) with p = 0.001. Colchicine reduces the degree of NLR and HsCRP in COVID-19 patients.

1. Introduction
COVID-19 (coronavirus disease 2019) has been circulating in Indonesia for over a year. COVID-19 is a public health issue that affects people of all ages and backgrounds. COVID-19 has had a massive effect, and evidence indicates it will take more than a decade for the planet to recover. In terms of health, socially, and financially [1]. On January 8, 2021, there were 818,484 cases of COVID-19 in Indonesia, with 120,928 active patients and 23,947 deaths. In Central Java alone, there are 90,670 COVID-19 cases (11.1 percent of the total national cases), with 25,558 active cases and 3,725 deaths. Central Java has the fourth highest number of confirmed COVID-19 cases in Indonesia, but the death toll is second only to East Java. COVID-19 is a severe problem in Central Java, with a high mortality rate, as shown by this condition [2].

Converging evidence would suggest that there are potential links between climate change, emissions, and epidemics/pandemics, such as the recent SARS-CoV-2 (evere acute respiratory syndrome-related coronavirus 2) outbreak. COVID-19 and climate change are the two most serious public health challenges at the moment, with each exacerbating the risks of the other. The COVID-19 pandemic clearly reveals our ecosystem's fragility and vulnerability, as well as our inability to defend ourselves from pollutants. It once again highlights our failings and indifference to disasters caused by climate change, pollution, or the effects of human actions outside of natural ecosystems, all of which are increasing the risk of virus transmission from animals to humans. Pollution and climate change are now
well recognized as having negative effects on a wide range of physiological processes and organs in individuals of all ages, including perinatal problems, respiratory and cardiovascular problems, cancer, allergies, and neurological and mental diseases [3, 4].

Acute respiratory distress syndrome (ARDS) in COVID-19 patients is caused by the activation of inflammatory signaling pathways and cytokine storms [5–8]. The innate immune system is dysregulated when pro-inflammatory cytokines and chemokines are secreted in excess. Cytokine storms attract a large number of inflammatory cells to lung tissue, causing immunological damage. Aside from immune system dysregulation, COVID-19 patients’ deaths are linked to renin-angiotensin system (RAS) dysfunction caused by ACE2 downregulation. Both methods are linked to cytokine storms, which enhance vascular hyperpermeability, edema, and hypercoagulation, as well as multiorgan damage [5].

Strong inflammatory response leads to a weakened adaptive immune response, resulting in an immune response imbalance. As a result, biomarkers that reflect inflammatory and immunological state might be used to predict COVID-19 patients’ prognosis [9–12]. The neutrophil to lymphocyte ratio (NLR) is an inflammatory response indicator that has been explored as a useful predictor of outcome in patients with viral pneumonia [13]. High sensitivity C-Reactive Protein (hs-CRP) is a very sensitive systemic marker of the acute phase response to inflammation, infection, and tissue damage that may be used to detect inflammation. COVID-19 severity and Hs-CRP had a favorable relationship [14].

Colchicine is a drug that is routinely used in the treatment of arthritis gout. Colchicine will suppress the NLPR3 inflammasome so that it will reduce the production of pro-inflammatory cytokines like IL-1B. The introduction of PRRs (Pattern Recognition Receptors) is one of the pathogenesis of SARS-CoV-2 once the virus enters the body. It will then activate the NLRP3 inflammasome and Caspase-1 activation leads to the activation of interleukin 1B, leading in cytokine storms. The mechanism of colchicine in preventing cytokine storms causes colchicine to become one of the target therapies in COVID-19 [5]. This study aims to determine the effect of colchicine administration on HsCRP and Neutrophil to Lymphocyte Ratio (NLR) levels in COVID-19 patients.

2. Material and methods

2.1. Study design
This study used a pretest-posttest control group design in an experimental randomized controlled trial. The treatment group received 2x0.5 mg colchicine for 7 days and standard therapy, while the control group received placebo and standard therapy. HsCRP and NLR examinations were carried out before and after giving the treatment.

2.2. Population and study setting
The population of this study was patients at the UNS Hospital during the period February-May 2021 with the following criteria:
Inclusion criteria:
   a. Ages 18-64 years
   b. Positive COVID-19 swab with moderate to severe symptoms
   c. Willing to take part in research by signing research concentration informants.
Exclusion criteria:
   a. Creatinine above 1.5 mg / dL.
   b. Pregnant for women
The sampling technique used in this study was the Randomized Controlled Trial. Subjects who met the criteria were taken for each group with the division of the groups randomly.

2.3. Variables and data collection
NLR (Neutrophil to Lymphocyte Ratio) is a comparison of the number of neutrophils divided by lymphocytes. Hs-CRP is an acute phase reactant whose levels are increased in the presence of infection.
The examination of neutrophils and lymphocytes used a hemocytometer, while the hs-CRP test used the ELISA examination in the UNS Hospital laboratory.

2.4. Data analysis
The statistical test before and after treatment was carried out at the end of the study with paired t-test. The independent T-test was employed for the difference test between variables, and P was significant if p 0.05.

2.5. Ethical clearance
The ethical committee of the Universitas Sebelas Maret Faculty of Medicine approved this study.

3. Results

![Graph showing the comparison of NLR and HsCRP levels before and after treatment with Colchicine and Control Group.](image)

**Figure 1.** Colchicine reduces the degree of NLR and HsCRP in COVID-19 patients.

The graph (Figure 1) shows that Colchicine reduces the degree of NLR and HsCRP in COVID-19 patients. The study subjects were 40 patients, with 20 patients in the control group and 20 in the treatment group. There was a decrease in NLR and HsCRP levels in the treatment group before therapy (NLR = 7.89 ± 3.45; HsCRP = 5.41 ± 3.24 mg/dL) compared to after therapy (NLR = 3.59 ± 2.25; HsCRP = 1.41 ± 1.13 mg/dL) with p = 0.001.

4. Discussion
The usage of colchicine reduces NLR and HsCRP in COVID-19 patients, according to this study. The virus enters the host, binds to the host cell receptor, and replicates. This is the pathophysiology of SARS-CoV-2 infection. The ACE2 receptor and a membrane protein expressed in the lungs, kidneys, heart, liver, testes, and intestines allow the virus to reach specific cells. By endocytosis, the pathogen's spike protein (S-protein) attaches to the ACE2 receptor with the aid of TMPRSS2 (transmembrane protease serine 2) cellular protease and other clathrin proteins [7, 8]. During a viral assault, the immune system successfully removes the virus from the body, but the body eventually collapses. This immune system dysfunction eventually leads to a cytokine storm, a COVID-19 hyperinflammatory stage [9]. Excessive production of pro-inflammatory cytokines (IL-1/Interleukin-1, IL-2R, IL-6, interferon; IFN-/interferon-, tumor necrosis factor-/TNF-), chemokines (CCL-2/ C-motive chemokine ligands-2, CCL-3, CCL-10), and other inflammatory substances leads to uncontrolled systemic inflammation. TNF-, IL-1, and IL-6 levels are all high. Cytokine storms worsen infection and can lead to ARDS or multi-organ failure, which
can lead to death [11]. COVID-19 has been linked to a variety of problems, including cardiovascular, neurological, thrombotic, hematological, and rhabdomyolysis, according to studies. In the pathophysiology of SARS-CoV-2, the angiotensin-renin system is also important. ACE2 regulates systemic hypotension, hypokalemia, and lung injury, and greater activation of the angiotensin II receptor 1 (AT1R) promotes damage and illness [12]. (COVID-19 is a disease for which scientists are still searching for a particular vaccination or cure [8].

The innate immune system is made up of components that respond fast to infection and is not unique to infectious pathogens. Inflammation is triggered by chemical substances produced by wounded cells, and it serves as a physical barrier to disease transmission and supports tissue repair following pathogen clearance. Acute inflammation is triggered by cells that are already present in all tissues. Neutrophils, macrophages, dendritic cells, histiocytes, Kupfer cells, and natural killer (NK) cells are primary effector cells that rapidly migrate to the site of infection or tissue injury, resulting in infection resolution and tissue repair [5].

Pathogens Associated Molecular Patterns (PAMPs) expressed by pathogenic organisms and Danger Associated Molecular Patterns (DAMPs), namely molecules from necrotic cells, are recognized by PRRs (Pattern Recognition Receptors), which are immune system cell receptors that play a role in recognizing pathogens such as bacteria and viruses [5]. When PRR introduces microbial compounds, it activates a number of signaling pathways that control the form, severity, and duration of the inflammatory response.

Inflammasomes are proteins that cause inflammation. NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3) is required for immunological defense against viral infections, but it has also been associated to the pathophysiology of inflammatory illnesses such as cytokine storm. The NLRP3 inflammasome has also been identified as a key mediator of DAMPs and PAMPs-independent virus-induced inflammation. Viroporins may impair membrane permeability and alter ion homeostasis in the cellular compartment, which might lead to the production of IL-18 and IL-1b [5].

The sensor protein (PRR) of the NLRP3 inflammasome oligomerizes to produce a pro-caspase-1 activation platform in response to DAMPs or PAMPs. Caspase-1 is activated by NLRP3-induced autocatalytic activation, and it now cleaves pro-interleukin-1b (pro-IL-1b) and pro-interleukin-18 (pro-IL-18) cytokines into mature, physiologically active forms [5]. In the case of COVID-19, research have found greater levels of IL-18 and IL-1b, implying enhanced NLRP3 inflammasome activation. Protein E and protein 3a, which operate as ion conducting pores in the planar lipid bilayer and are essential for optimum SARS-CoV-2 replication and pathogenicity, are two viroporins found in Coronavirus. By stimulating the inflammasome, they can potentially cause Cytokine Storm [5]. Inflammatory mediators are crucial in the pathophysiology of ARDS, which is the primary cause of mortality in SARS-CoV2 patients. ARDS is caused by a number of cytokines, including IL-6, IL-8, and IL-1b. Furthermore, ARDS develops as a result of unregulated epithelial cell growth and disturbed tissue deformation in later stages, resulting to lung fibrosis and mortality [5].

Colchicine is an alkaloid derived from the Colchicum genus (October crocus) that has been shown to reduce a number of inflammatory processes. TNF-α receptor expression in macrophages is reduced by colchicine, as are the cytokines IL-1b, IFNγ, IL-18, and IL-6 [5]. Colchicine inhibits NLRP3 inflammation, however the mechanism is uncertain. This method might be linked to the interruption of mitochondrial transport to the endoplasmic reticulum, which is dependent on microtubules. To activate the inflammasome, NLRP3 must be assembled in the endoplasmic reticulum with its adaptor [5].

According to studies, individuals infected with COVID-19 may have lung consolidation because to the proliferation of fibroblasts in the alveolar air gaps [7]. As a microtubule destabilizing drug, colchicine has an antifibrotic action. Myofibroblast differentiation is important for wound healing and fibrosis etiology. Colchicine suppressed myofibroblast development in human lung fibroblasts in an in vitro investigation employing dependent Rho/serum response factor (SRF) [5]. The limitations of this study are that the sample size is still too small, the research is still in one educational center only, and the viral load or genotype of the virus was not examined.
5. Conclusion
This study shows that colchicine supplementation in COVID-19 patients will decrease the degree NLR and HsCRP. Further research needs to be done to evaluate this study further so that a new drug for COVID-19 drugs can be obtained for the patient. The use of Colchicine in overcoming inflammation in COVID-19 patients will indirectly reduce the severity and degree of death in COVID-19 patients so that it plays an essential role in this pandemic.

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