The Effect of Nitric Oxide Supplement Intake on the Value of Hematological Examination of Health Workers Exposed to Covid-19

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Abstract

Objective: Examination of the diagnostic covid-19 gold standard with real time reverse polymerase chain reaction (RT-PCR) is still limited, so that the initial hematological examination (leukocytes, lymphocytes, neutrophils, platelets, hemoglobin, and neutrophil lymphocyte ratio (NLR) plays an important role in monitoring the course of covid-19 disease Macrophages release nitric oxide (NO) to kill parasites NO inhibits migration and adhesion of leucocytes to the endothelium. **Metode:** The research design used pre-experimental with one group pre-post test, the total sample was 80 health workers who were exposed to Covid-19, the independent variable was the provision of NO and the dependent variable was the result of a hematological examination (leukocytes, lymposites, platelets, neutrophils, hemoglobin and NLR). The intervention was given NO 500 mg mixed with warm water 250 cc, given 3 times a day for 5 days, data analysis used the T-test with a significance value of a<0.05. **Results:** There is a difference in the results of the pre and post-test hematology giving NO to the results of the examination of leucocytes a=0.001, lymphocytes a=0.000, platelets a=0.000, neutrophils a=0.000, hemoglobin a=0.031, and NLR a=0.000. **Conclusion:** Giving NO to health workers exposed to Covid-19 can improve the hematology and immune systems to fight the corona virus.

Keywords: *Nitric oxide, hematology, Covid-19*

Introduction

The body's reaction when infected with the Corona virus is to form the body's resistance to eradicate the virus [(1).] The immune system is unable to fight the Corona virus, severe Covid-19 symptoms can appear and there is a risk of complications [(2)] .During infection, the patient exhibits an uncontrolled immune response, which is caused by hyperactivation of macrophages and monocytes. This response results in a decrease in the total lymphocyte count [(3)]. This process will be detected by the immune system. After that, the immune system or immune system will react by sending white blood cells and forming antibodies to fight and kill the virus [(4)].

Macrophages are immune cells that are used to eat particles that enter the body and release nitric oxide. In its activity as cells that defend the body from parasites, macrophages release nitric oxide to kill these parasites.

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All parasites, including COVID-19 can be killed by using free radicals in the body, namely nitric oxide or NO. NO is released by macrophages in the body, macrophages are present in several organs such as the lungs and skin [5]. Nitric Oxide (NO) is a potent vasodilator for blood vessels, platelet inhibitor, is anti-inflammatory and anti-atherogenic, NO inhibits migration and adhesion of leucocytes to the endothelium [6]. NO is a signaling molecule that is widespread and plays an important role in every cell and organ function in the body. One of the factors that influence changes in hematological values is the role of Nitric oxide in the blood [7].

Nitric oxide is a molecule that is produced naturally by the body. NO production is essential for overall health as it allows blood, nutrients and oxygen to travel to every part of the body. Green leafy vegetables and some root vegetables (such as beets) have high concentrations of nitrates [8].

Table 1 Foods Containing NO

No	Foof	Content NO/100 g
1	Dark chocolate	1332 g
2	Beets	279 g
3	Melon	68 g
3	Pomegranate	1,3 g
4	Strawbery	9,4 g

Nitric oxide is a compound produced by many body cells. It relaxes vascular smooth muscle by binding to the heme portion of the cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of 3 ‘, 5’-monophosphate cyclic-guanosine, which in turn causes vasodilation. When it enters the body NO dilates the pulmonary blood vessels and, due to the efficient cleaning by hemoglobin, has minimal effect on blood vessels throughout the body [9].

Laboratory tests play an important role in handling COVID-19, starting from screening, diagnosis, monitoring therapy, determining prognosis, to surveillance [10]. A simple examination at an affordable cost, relatively fast processing time, and later results can be used for research purposes related to Covid-19 [11]. Apart from being an early detection, laboratory tests can be a means of monitoring the course of the COVID-19 disease. Several components of blood tests that are widely used as a monitoring tool and predictor of Covid-19 are leukocyte levels, lymphocyte levels, neutrophil levels, platelet levels, Hb, and neutrophil lymphocyte ratio (NLR) [12-14].

Method

The research design used pre-experimental with one group pre-post test [15]. The number of samples of 80 health workers who were exposed to Covid-19, the independent variable was the provision of NO and the dependent variable was the result of hematology tests (leukocytes, lymphocytes, platelets, neutrophils, hemoglobin and NLR). The group exposed to COVID-19 received food powder rich in NO from pomegranates, beets and dark chocolate contained in the powder, each patient received 1 sachet of powder every day for 5 days. The mass of 1 powder sachet is 500 mg NO which has been approved by BPOM RI MD 867013167071 and BPOM RI MD 867013219071 in 2020.

The groups exposed were taken from health workers who were in the tracing monitoring of the Kebonsari Health Center working area who carried out self-quarantine at home. Venous blood sampling was 5 cc and intervention was given NO 500 mg mixed with warm water 250 cc, given 3 times a day for 5 days, data analysis used the T-test with a significance value of $a<0.05$. The results of the leukocyte, lymphocyte, platelet, Hb and neutrophil counts were compared with the normal number of each variable [16]. Neutrophil and lymphocyte ratio or NLR was also compared with normal NLR [17].

Result

A. Characteristics of Respondents Data

The results of the study were data on the characteristics of respondents including gender, age, level of education, history of disease, BMI, and fasting blood sugar. This data is listed in the following table:

Table 2 Characteristics of respondents

VARIABEL		THE GROUP	
		N	%
Gender			
1. Men		10	12.5
2. Woman		70	87.5
Age			
1.	21-30 Year	31	38.8
2.	31-40 Year	29	36.3
3.	41-50 Year	18	22.5
4.	51-60 Year	2	2.5
Level of education			
1. Diploma		45	56.3
2. Bachelor		28	35.0
3. Post Graduate		7	8.8
Previous Disease History			
1. No		77	96.3
2. Yes		3	3.8
Body mass index (BMI)			
1. Low		13	16.3
2. Normal		39	48.8
3. Obesity		28	35.0
Fasting Blood Sugar			
1. Low		1	1.3
2. Normal		70	87.5
3. High		9	11.3

Based on table 1 data shows that the most respondents are gender 70 women (87.5%), age 21-30 years 31 (38.8%), education level 45 diplomas (56.3%), history of disease 77 (96.3 %), BMI was 39 normal (48.8%) and normal fasting blood sugar 70 (87.5%).

B. Hematology examination data

The results contained data on the number of leukocytes, lymphocytes, neutrophic platelets, Hb and neutrophil lymphocyte ratio NLR). The significance test is used to determine whether there is an effect of the independent variable, namely the provision of NO and the dependent variable is the result of a hematology examination. The test results are carried out with the criteria that if the P-value <significant alpha 5% or 0.05, then there is a significant effect of the independent variable on the dependent variable.

The results of the significance and model testing can be seen through figures and tables 2. Hypothesis testing on the effect of NO intake on blood hematological values includes NLR (p = 0.000), leucocytes (p = 0.001), hemogoblin (p = 0.031), lymphocytes (p = 0,000), neutrophils (p = 0,000), and platelets (p = 0,000), showed a significant effect on changes in blood hematological values after consuming NO intake. Thus it can be interpreted that giving NO to health workers who are exposed to Covid-19 is able to improve the hematology and immune systems to fight the corona virus.

Discussion

The role of NO intake is very important in increasing body immunity. NO plays a role in immunological processes, some of which are produced by tissue macrophage cells, due to the activation of various cytokines and endotoxin of pathogenic bacteria [18]. which is able to damage target cells or bacterial cells through its role as a cytotoxic material [19]. A study with a total sample of 41 people who were divided into 2 groups, namely the ICU patient group and the non-ICU patient group. In the ICU patient group, it was found that 54% had leukocytosis (leukocytes> 10 x 109 / L). Meanwhile, in the non-ICU group, it was found that 48% had normal leukocyte levels (4-10 x 109 / L) (20).

NO in the immune system is a nonspecific molecule and can cause damage to both host cells and pathogens [21]. Nitric oxide functions on a variety of cells, in the nonspecific immune system that occurs before the presence of a specific and complex infection involving lymphocytes [22]. NO production is increased during inflammation, so that if the production is not controlled, it will cause cell damage [23]. Another study showed that of the 40 COVID-19 patients, 13 were severe cases and showed a significant and sustained decrease in lymphocyte counts. The mean lymphocyte value in severe cases was 0.6 x 109 / L and in mild cases it was 1.1 x 109 / L [24].

The role of NO in regulating neutrophil migration has been investigated. Human neutrophil migration to interleukin (IL) -8 and NO NG-nitro-l-arginine methyl ester (L-NAME) synthase inhibitor significantly (P <0.001) increased IL-8-induced migration by up to 45% [25]. Antibodies to L-selectin or PSGL-1 had no effect on IL-8-induced migration but prevented increased migration to IL-8 induced by L-NAME. L-NAME produced neutrophil-derived microparticles that were significantly (P <0.01) larger than untreated neutrophils [26].

The accumulation and activation of inflammatory cells is essential for defense but can also lead to pathology. Neutrophils, for example, are essential for clearance of a wide range of pathogens but also cause injury and death to host tissue if their activity is misdirected or exaggerated [27]. In a study with 60 patients in Wuhan, the mean absolute neutrophil value was 2.8 x 109 / L. The percentage of COVID-19 patients with absolute neutrophil levels below the normal range (1.8-6.3 x 109 / L) was 11 patients (21%) while the percentage of COVID-19 patients with absolute neutrophil levels above the normal range was as much as 7 patients (13%) [28]. Another study in the same area, with a sample of 40 people, stated that absolute neutrophil levels were significantly higher in severe cases of COVID-19 than in mild cases. The magnitude of the increase in absolute neutrophil levels may indicate the intensity of the inflammatory response in COVID-19 patients [29].

Nitric oxide (NO), originally described as an endothelial-derived relaxation factor, is an important messenger molecule involved in many physiological and pathological processes [30]. Endothelium-derived NO causes vasorelaxation and also inhibits platelet adhesion and aggregation, thereby maintaining blood fluidity and preventing thrombosis [31]. An important alternative function of NO is in modulation of platelet production from megakaryocytes. Immune thrombocytopenic purpura (ITP) may be associated with decreased NO bioavailability contributing to thrombocytopenia

and other clinical characteristics associated with this condition [32]. At the same time, several treatment modalities for ITP may exert its beneficial effect by increasing NO levels and thereby increasing platelet production [33]. Several studies with mixed results have reported slightly lower platelet levels in mild cases of COVID-19 than in severe cases. The platelet level in mild cases was around 52.06-293.46 x 109 / L while the platelet level in severe cases was 129.98-393.78 x 109 / L (7). It was also stated that in mild cases of COVID-19, platelet levels decreased by about 5-17% [19].

NO, a multi-functional molecule, modulates the immune response mediated by the NLRP3 inflammasome [34]. Interestingly, our findings suggest that NO can be an intrinsic negative regulator of various diseases caused by abnormal inflammatory activation of NLRP3 [35]. Our observation that NLRP3 inflammation in humans is inhibited by NO further suggests the potential application of NO therapy in the treatment of inflammatory diseases. [36]. In this study, the group divided between acute and chronic COVID-19 infection, found that the NLR value in chronic infection patients (69 patients) was lower than in acute infection patients (24 patients). The mean NLR value of acute infection patients was 20.7 and chronic infection patients was 4.8. The results of this study indicate that the NLR value is related to the clinical outcome of COVID-19 patients and that the increase in the NLR value can be an independent prognostic parameter for patients with COVID-19 infection [37].

The rate at which hemoglobin reacts with nitric oxide (NO) is limited by how quickly NO can diffuse into the heme pouch [39]. The reaction is as fast as a ligand / protein reaction and the result, when the hemoglobin is oxygenated, is the formation of nitrates which is known as the oxygenation reaction. [40] Since nitrate, at the concentration produced by the oxygenation reaction, is biologically inert, the only role hemoglobin has ever thought to play a role in NO signaling is to block it [41]. Research has shown the importance of Hb compartmentalization in red blood cells in limiting the breakdown of NO by Hb. [42]. In addition, oxidation of Hb to form metHb reduces the ability of Hb to absorb or destroy NO [43]. The formation of N2O3 or S-nitrosthiols from the Hb / nitrite reaction may be the key to nitrite-mediated export of NO activity from red blood cells [44]. In a study with a sample of 41 people, divided into 2 groups, the mean hemoglobin level in mild cases of COVID-19 was 12.2 g / L, while the mean hemoglobin level in severe cases was 13.3 g / L. Another study, which sampled 67 patients, stated that the mean hemoglobin level in the ICU patient group was 13.2 g / L and that in the non-ICU patient group was 14.2 g / L (16,45).

Conclusion

The hematological profile of health workers exposed to COVID-19 can be an important supporting data to see the state of a person's immunity. Covid-19 patients with severe symptoms may experience a compromised immune response, which can lead to the development of hyperinflammation of the virus. Therefore, patients with severe COVID-19 symptoms should be examined for laboratory parameters for hyperinflammatory markers to improve mortality rates.

In its activity as cells that defend the body from parasites, macrophages release nitric oxide to kill these parasites. All parasites, including COVID-19 can be killed by using free radicals in the body, namely nitric oxide or NO. The provision of nitric oxide intake to health workers exposed to Covid-19 has an effect on changing the results of blood hematology examinations.

Conflict of Interest : Nil

Source of Fund : Universitas Airlangga

Ethical Clereance: The study was approved by ethical clereance certificate Number. 303/HRECC. FODM/VI/2020 from the institutional Health Research Etchical Clereance Commision, Airlangga University.

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