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THE EFFECTS OF NITRIC OXIDE SUPPLEMENT INTAKE ON NEUTROPHILS, LYMPHOCYTES, AND NLR OF HEALTH WORKERS EXPOSED TO COVID-19 IN INDONESIA

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ABSTRACT

Objective: The COVID-19 pandemic has hit around 139 countries in the world. This virus can reduce immunity in humans and failure to control it can result in death. The purpose of this study was to determine the effect of NO supplementation on neutrophil, lymphocyte, and NLR values. **Method:** 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 (lymphocytes, neutrophils, and NLR). The intervention was given NO 500 mg mixed with warm water 250 ccs, given 3 times a day for 5 days, data analysis used the T-test with a significance value of $\alpha < 0.05$. **Results:** There is a difference in the results of the pre and post-test hematology giving NO to the results of the examination of lymphocytes $\alpha = 0.000$, neutrophils $\alpha = 0.000$, and NLR $\alpha = 0.000$. **Conclusion:** Giving NO to health workers exposed to COVID-19 can normalize the neutrophil, lymphocyte, and NLR values and the immune system to fight the coronavirus.

INTRODUCTION

All viruses both of animal and human origin can reduce immunity in the human body. Coronavirus originating from bats is now a world pandemic. Its ability to reduce immunity in the human body and become a ferocious virus has killed thousands of people on earth. All parasites, including the Coronavirus, can be killed by using Nitric Oxide or NO (1). NO is released by macrophages in the body. This is found in several organs such as the lungs and skin (2–6). Currently, in China, inhaled nitric oxide has been investigated as COVID-19 therapy, and currently, clinical trials are being carried out both as a potential treatment for patients and as a precautionary measure for healthcare providers with research sponsored by Massachusetts General Hospital and Hong Kong University of China Kong (7). Naturally, the human body has a macrophage that can kill parasites in our body (Figure 1) and produce Th1 and IFN- γ which will again become macrophages.

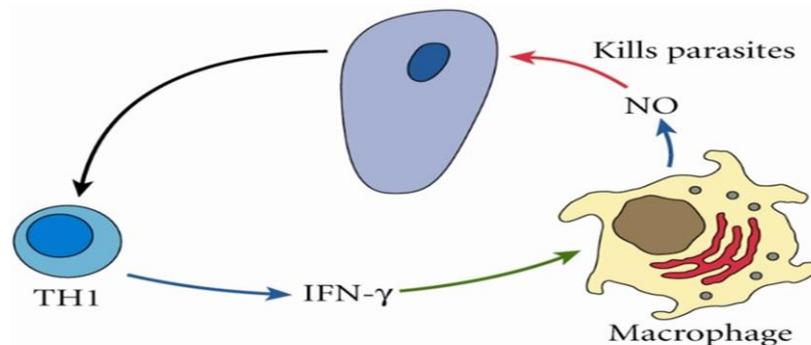


Figure 1. Macrophage kills a parasite (8)

NLR is an indicator to determine the effectiveness of treatment for healing disease. This is used as a new marker in predicting the failure of medical treatment of a disease in a patient (9). If the patient in medical care has a low NLR, the higher the effectiveness of the treatment for healing the disease (9–12). It can also be used as a marker of the effectiveness of treatment for healing COVID-19 patients. In controlling COVID-19, modulation of NO through NO-rich foods to enhance the patient's immunity as well as to kill COVID-19 is not available (13). NO-rich foods such as dark chocolate, beets, and pomegranates are effective for boosting the patient's immunity and killing the virus. Research to be carried out is to use foods rich in NO or nitric oxide to kill COVID-19. An indicator of the effect of NO rich foods on COVID-19 is the normality of NLR.

Sources of Nitrate can be obtained from vegetables, especially celery, lettuce, beetroot, and spinach. Other types of food, including fruits, pure chocolate, garlic, and red wine, also contain polyphenols and other compounds that can increase the production of nitric oxide in our bodies (14). With regard to its effect on viral infections, nitric oxide helps the immune system by acting as an antiviral and provides many of the much-needed protective effects during the onset of the viral infection (15). Referring to the phenomenon that occurred, researchers paid special attention to health workers who were exposed to Covid-19 for their health problems, especially to increase immunity.

METHOD

The research design used a pre-experimental with one group pre-post test (16). The number of samples of 80 health workers who were exposed to COVID-19, the independent variable presents NO and the variable in charge of checking the results of examinations for neutrophils, lymphocytes, and NLR. The group exposed to COVID-19 received NO supplements from pomegranates, beets, and dark chocolate contained in the powder, each patient received 1 sachet powder for every day for 5 days. The mass of 1 sachet powder is 500 mg NO which has been approved by BPOM RI MD 867013167071 and BPOM RI MD 867013219071 in 2020.

The exposed groups were taken from health workers who were in the tracing monitoring of the Kebonsari Community Health Center working area who carried out self-quarantine at home. The sampling of venous blood was 5 cc, the research instrument used was a 3 ml syringe/vacutainer, turnover, K2EDTA tube, microscope counter with 10 times ocular magnification and 100 times objectively with the help of immersion oil, and the intervention was given NO 500 mg supplement 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 $\alpha < 0.05$. The results of the neutrophil, lymphocyte, and NLR count were compared with the normal number of each variable. The result of lymphocyte and neutrophil count compared with normal lymphocyte count in children is 9 ml, normal lymphocytes in adults 3 ml, normal neutrophils 1.5-8 μ l. Neutrophil and lymphocyte ratio or NLR also compared to normal NLR is less than 6 (17). Neutrophil and lymphocyte ratio or NLR was also compared with normal NLR (18).

RESULTS

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 the disease, BMI, and fasting blood sugar. This data is listed in the following table:

Table 2 Characteristics of respondents

Variable		
	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. Neutrophil, lymphocyte and NLR examination values

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

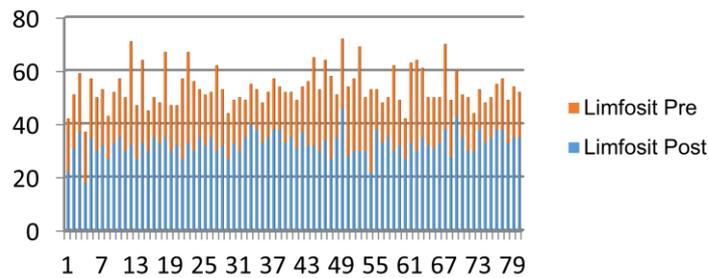


Figure 2. Value of Pre and Post Lymphocytes

Figure 2. The value of pre and post lymphocyte examination after NO intervention. The results of the lymphocyte analysis showed the data for the group pre mean (9,98250), CI lower (-13,415) and upper (-9,993), standard deviation (6.405), and post mean (8.19375), lower CI (-13.416) and upper (-9.991), the standard deviation (4.359). Lymphocyte P-value $\alpha=0.000$.

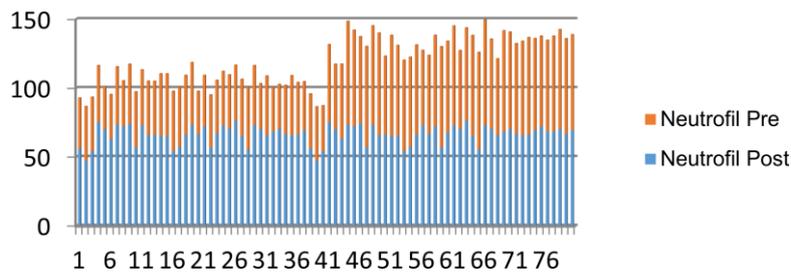


Figure 3. Value of Pre and Post Neutrophils

Figure 3. The value of pre and post neutrophil examination after NO intervention. The results of the analysis on neutrophil showed the data for the group pre mean (52,736), CI lower (-17.2840) and upper (-10.0485), standard deviation (14.9361), and post mean (66.402), lower CI (-17.2963) and upper (-10.0362), the standard deviation (1.3591). Neutrophil P value $\alpha=0.000$

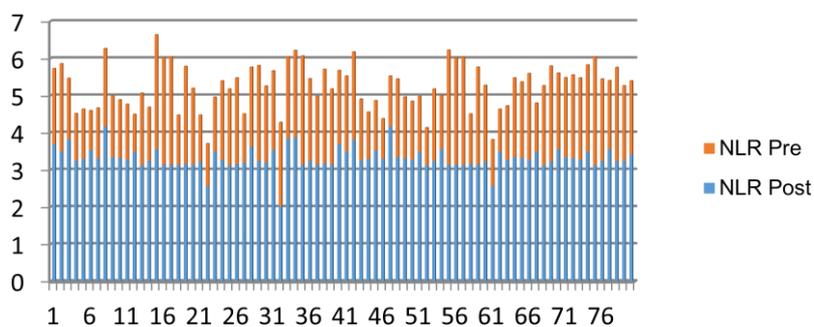


Figure 4. Value of Pre and post NLR

Figure 4. The value of pre and post NLR examination after the intervention giving NO. The results of the analysis on the NLR show the data for the pre mean (1.9497), lower CI (-1.51243) and upper (-1.23757), standard deviation (0.54637), and post mean (3.3247), lower CI (-1.51274) and upper (-1.23726), the standard deviation (0.29798). P-value NLR $\alpha=0.000$.

DISCUSSION

The relationship between NO supplements and the respondent group on lymphocyte levels in the blood.

Based on the results of the study, there is an influence between the intake of NO-rich foods on the normality of lymphocyte levels in the blood of the respondent group. Lymphocytes are a source of immunoglobulins that are important in the body's cellular immune response. Lymphocytes have a role to fight infections caused by viruses or bacteria. Absolute lymphopenia occurs in severe cases (19). When the lymphocyte counts decreases, the body's ability to fight infection will be impaired. A common laboratory abnormality in COVID-19 patients is a decrease in the absolute lymphocyte count (20). Another study showed that of the 40 COVID-19 patients, 13 were severe cases and showed a significant and sustained decrease in lymphocyte count. The mean lymphocyte value in severe cases was $0.6 \times 10^9 / L$ and in mild cases, it was $1.1 \times 10^9 / L$ (21).

NO that enters the body from food is released by macrophages when a virus enters the body. Macrophages will work together with T lymphocytes to kill Listeria bacteria (22). Macrophages will phagocyte Listeria bacteria through the phagocytosis process and T lymphocytes will produce IFN- γ , TNF- α cytokines, and stimulate NK (Natural Killer) cells (23). The benefits of NO on the body has been widely studied, one of the most important is its role in the body's immune system. NO cooperate with the lysosomes of macrophages to kill pathogens such as bacteria, fungi and viruses. Nitric oxide help to protect the body from bacteria that enter through the digestive tract. Normal flora that live in the mouth and throat convert nitrate to nitrite in the diet will be changed to NO when exposed to stomach acid. NO this will kill nearly all pathogens ingested with food (24). Gunawijaya stated that two cells play a role in the synthesis of NO by macrophage cells, namely macrophage cells and T lymphocytes. The c-NOS enzyme triggers the production of NO in small amounts which only causes temporary vasodilation, while i-NOS triggers large amounts of production and can cause vasodilation that is severe and prolonged (25). An increase in the immune response can be seen in addition to an increase in phagocytic activity, it can also be seen from an increase in NO levels. Based on this explanation, it can be clearly seen that there is a relationship between the intake of NO-rich foods with lymphocyte levels in the blood. This relationship suggests that NO intake affects the normality of lymphocytes in the blood (26).

The relationship between NO supplements and the respondent group on neutrophil levels in the blood.

The results showed that there was an effect of NO-rich foods on the normality of neutrophil levels in the blood of the respondent group. The results stated that the NO produced by eNOS (Endothelial Nitric Oxide Synthase) has a protective effect, namely decreasing platelet aggregation, preventing leukocyte adhesion and increasing vasodilation of arteries and blood flow, and regulating contractility (27). It can convert abnormal neutrophils into normal neutrophils. Nitric Oxide is a potent vasodilator for anti-inflammatory blood vessels (28). If therapy using NO is done properly and given according to recommended doses, then the symptoms of COVID-19 such as pneumonia can be prevented. This is because increasing the consumption of foods rich in NO can increase the normality of neutrophils in the blood. Neutrophils are the first leukocytes to reach the area of inflammation and initiate defense. host against pathogens. Neutrophil activation also acts to fight infection effectively, along with monocytes and macrophages via phagocytosis and microorganisms or through the release of inflammatory components such as oxygen radicals, proteases, or peroxidases (16).

Neutrophils are the body's first defense mechanism when damaged body tissue or foreign objects enter the body. Neutrophils serve as the body's line of defense against foreign substances, especially against bacteria. It is phagocytic and can enter infected tissue. Neutrophils in the blood that circulate for about 10 hours can live for 1-4 days while in the extravascular tissue. Neutrophils are the largest type of leukocyte cells, which are about 50-70% among other leukocyte cells (29). Neutrophil cell function is closely related to antibody activation and the complement system. The interaction of this system with neutrophils increases the ability of these cells to phagocytosis and break down various particles. Neutrophils are capable of releasing enzymes into their own cytoplasm to destroy ingested material or phagocytosis and neutrophils can also release myeloperoxidase enzymes into the surrounding environment. The release of NO can also reduce the aggregation and adhesion of platelets and leukocytes and inhibit Vascular Cell Adhesion Molecule-1 (VCAM-1) (31). NO consumption also affects neutrophil normality (30).

The relationship between NO supplements and the respondent group on NLR levels in the blood.

Based on the results of the study, there was an effect between the provision of NO-rich foods on the normality of the blood NLR levels of the respondent group. This statement is in line with previous studies which stated that intake of NO supplements affected the normality of lymphocytes and neutrophils which linearly affected the normality of NLR levels (32-33). The normality of NLR depends on the normality of lymphocytes and neutrophils (34). NO supplement (food) can increase the normality of lymphocytes and neutrophils, causing normality in NLR. The neutrophil/lymphocyte ratio (NLR) has been used as a marker of systemic inflammation (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, in the group divided between acute and chronic COVID-19 infection, the NLR value in chronic infection patients (69 patients) was lower

than in acute infection patients (23 patients). The mean NLR value of acute infection patients was 20.7 and chronic infection patients were 4.8. The results of this study indicate that the NLR value is associated with the clinical outcome of COVID-19 patients and that an increase in the NLR value can be an independent prognostic parameter for patients with COVID-19 infection (37). The addition of these foods not only increases NO concentration but also maintains NO in the body (38). Excess NO in the body can form nitroso compounds such as nitrosamine which has an effect on cancer. Beets help prevent the formation of nitroso thereby avoiding cancer (19). In a reasonable amount of NO compounds also controlling an important physiological role in the cell signaling processes and inflammation, however in excessive amounts of NO have a characters as reactive oxidative, genotoxic and destruktif for human cells (40).

In addition to providing NO with pomegranates, beets, and dark chocolate ingredients, it is also recommended that other ingredients be able to increase the amino acid L-arginine which produces nitric oxide in the body. L-Arginine is one of the substances that regulate Nitric Oxide (NO) synthesis, antibody production and B cell development, T cell receptor expression which causes L-Arginine to be important in the innate immune system and adaptive immune system (39). A diet high in nitrate and antioxidant-rich vegetables or the use of supplements, such as L-arginine, which is found in seafood, watermelon juice, nuts, seeds, algae, meat, protein concentrates, and soy protein isolation (40). L-Arginine is needed in various physiological processes of the body including modulation of the immune system and has a role in increasing the immune system (41). The intervention of NO supplement intake for health workers exposed to Covid did not show signs and symptoms of certain side effects. Side effects do not necessarily occur in every NO supplement intake, but if there are excessive side effects, stop consuming NO intake and must be treated immediately by the medical.

CONCLUSION

The hematological profile of the neutrophil, lymphocyte, and NLR values of health workers who are exposed to COVID-19 can be important for supporting data to see the state of a person's immunity. In their activity as cells that protect the body from parasites, macrophages release nitric oxide to kill these parasites. All parasites, including COVID-19, can be killed using free radicals in the body, namely nitric oxide or NO. Giving nitric oxide intake to health workers who are exposed to COVID-19 has an impact on changes in the results of hematological examinations, the value of neutrophils, lymphocytes, and blood NLR.

Ethical Clearance

The study was approved by the ethical clearance certificate Number. 303/HRECC.FODM/VI/2020 from the institutional Health Research Ethical Clearance Commission, Airlangga University.

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Conflict of Interest

The authors have no financial conflicts of interest to declare

REFERENCES

1. Keyaerts E, Vijgen L, Chen L, Maes P, Hedenstierna G, Van Ranst M. Inhibition of SARS-coronavirus infection in vitro by S-nitroso-N-acetylpenicillamine, a nitric oxide donor compound. *Int J Infect Dis.* 2004;8(4):223–6.
2. Bakker I, van der Voordt TJM, de Boon J, Vink P. Red or blue meeting rooms: Does it matter?: The impact of colour on perceived productivity, social cohesion and wellbeing. *Facilities.* 2013;31(1):68–83.
3. Annisa DF, Ifdil I. Konsep Kecemasan (Anxiety) pada Lanjut Usia (Lansia). *Konselor.* 2016;5(2):93.
4. Abdelfattah I. Treatment of highly polluted industrial wastewater utilizing clean and low cost technologies: Review article. *J Environ Account Manag.* 2018;6(2):167–84.
5. Sikap, perilaku dan faktor sosial pemilik hewan rentan Rabies dalam upaya pengendalian Rabies : suatu studi sosiologis di Jakarta Selatan. :71152.2015
6. Prado CM, Martins MA, Tibério IFLC. Nitric Oxide in Asthma Physiopathology. *ISRN Allergy.* 2011;2011(January 2014):1–13.
7. Hale C. FDA opens up Bellerophon’s gas therapy for COVID-19, stock rockets [Internet]. *Fierce Biotech.* 2020
8. Protozoan and Helminth Parasites [Internet]. *Pathogen Profile Dictionary.* 2020
9. Alay I, Kaya C, Karaca I, Eren E, Hosgoren M, Aslanova F, et al. The effectiveness of neutrophil to lymphocyte ratio in prediction of medical treatment failure for tubo-ovarian abscess. *J Obstet Gynaecol Res.* 2019;45(6):1183–9.
10. Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recomm Reports.* 2015;
11. Akkurt MÖ, Yalçın SE, Akkurt İ, Tatar B, Yavuz A, Yalçın Y, et al. The evaluation of risk factors for failed response to conservative treatment in tubo-ovarian abscesses. *J Turkish Ger Gynecol Assoc.* 2015;
12. Yildirim M, Turkyilmaz E, Avsar AF. Preoperative neutrophil-to-lymphocyte ratio has a better predictive capacity in diagnosing tubo-ovarian abscess. *Gynecol Obstet Invest.* 2015;
13. A. C, A. B, L. ZDV, A. T, F. L, M. G, et al. Neutrophil-to-lymphocyte ratio and clinical outcome in COVID-19: a report from the Italian front line. *Int J Antimicrob Agents.* 2020;(15892):106017.
14. Angelina dkk., Asupan, H., Nitric, S., Dengan, O., & Darah, T. (2019). Program Studi Pendidikan Dokter , Fakultas Kedokteran

- Universitas Udayana. Vol. 8 No. 7 (Juli) 2019
15. Health and Wellness Alerts. The Best Way to Maintain Heart-Healthy Nitric Oxide Levels. Published: 2018-10-16.
 16. Tualeka AR. Metodologi Penelitian Kesehatan dan Keselamatan Kerja. 1st ed. Surabaya: Airlangga University Press; 2019. 89 p.
 17. Sudiartha IPG, Wiargitha IK, Gde T, Mahadewa B. Perbedaan nilai Neutrophil Lymphocyte Ratio (NLR) terhadap pemeriksaan kultur darah dalam mendiagnosis sepsis pada pasien peritonitis di RSUP Sanglah , Bali , Indonesia. 2020;11(1):165–71.
 18. Tiara D, Tiho M, Mewo YM. Gambaran kadar limfosit pada pekerja bangunan. J e-Biomedik. 2016
 19. Liu, Xuan. Shen, Yong. et al. Prognostic Significance of Neutrophil to Lymphocyte Ratio in Patients with Sepsis: A Prospective Observational Study. Research article: Hindawi. 2016
 20. Fu L, Wang B, Yuan T, Chen X, Ao Y, Fitzpatrick T, et al. Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: A systematic review and meta-analysis. Journal of Infection. 2020; 80:65665
 21. Liu, Jingyuan. Liu, Yao. Dkk. Neutrophil to Lymphocyte Ratio Predicts Severe Illness Patients with 2019 Novel Coronavirus in the Early Stage. Journal: medRxiv. 2020
 22. Sulistiani RP, Rahayuningsih HM. Pengaruh Ekstrak Lompong Mentah (*Coloscasia esculenta* L Schoot) terhadap Aktivitas Fagositosis dan Kadar NO (Nitrit Oksida) Mencit BALB/C Sebelum dan Sesudah Terinfeksi *Listeria Monocytogenes*. J Nutr Coll. 2015;4(2):409–15.
 23. Sobko T, Marcus C, Govoni M, Kamiya S. Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. Nitric Oxide - Biol Chem [Internet]. 2010;22(2):136–40.
 24. Farmasi, A., Yogyakarta, I., & Pusat, Y. I. Gambaran Kadar Nitric Oxide (NO) Pada Masyarakat Yogyakarta. Jurnal Kefarmasian Akrafindo. 2015; 19–24.
 25. Gunawijaya E, BNP A. Peran Nitrogen Oksida pada Infeksi. Sari Pediatr. 2000;2(2):113–9.
 26. Cd R, Double CD, Cells NT, Kim EY, Juvet SC, Zhang L. Suppression and Regulation of Immune Responses. 2011;677:85–98.
 27. Syachrumayah M, Suciati, Suhartono E, Setiawan B. Aktivitas Scavenging Nitrit Oksida oleh Hemoglobin dan Tekanan Darah pada Preeklampsia. Mutiara Med J Kedokt dan Kesehat. 2006;6(1):1–8.
 28. Secco DD, Paron JA, De Oliveira SHP, Ferreira SH, Silva JS, Cunha FDQ. Neutrophil migration in inflammation: Nitric oxide inhibits rolling, adhesion and induces apoptosis. Nitric Oxide - Biol Chem. 2003;9(3):153–64.
 29. Bradley A. Maron, M.D. and Nazzareno Galiè MD. Pulmonary Arterial Hypertension Diagnosis, Treatment, and Clinical Management in the Contemporary Era Bradley. 2017;1(9):1056–65.
 30. De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA, et al. Nitric oxide decreases cytokine-induced endothelial activation: Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. J Clin Invest. 1995;96(1):60–8.

31. Wallace JL. Nitric oxide as a regulator of inflammatory processes. *Mem Inst Oswaldo Cruz.* 2005;100(SUPPL. 1):5–9.
32. Nakamura-Utsunomiya A, Tsumura M, Okada S, Kawaguchi H, Kobayashi M. Downregulation of endothelial nitric oxide synthase (eNOS) and endothelin-1 (ET-1) in a co-culture system with human stimulated X-linked CGD neutrophils. 2020;15(4):1–19.
33. Dubey M, Nagarkoti S, Awasthi D, Singh AK, Chandra T, Kumaravelu J, et al. Nitric oxide-mediated apoptosis of neutrophils through caspase-8 and caspase-3-dependent mechanism. *Cell Death Dis.* 2016;7(9):1–12.
32. Jhuang YH, Kao TW, Peng TC, Chen WL, Li YW, Chang PK, et al. Neutrophil to lymphocyte ratio as predictor for incident hypertension: a 9-year cohort study in Taiwan. *Hypertens Res.* 2019;42(8):1209–14.
33. Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. *J Infect.* 2020.
34. Nakahira K, Haspel JA, Rathinam VA, dkk. Protein autophagy mengaturreponimunbawaandenganmenghambatpelepasan DNA mitokondria yang dimediasiolehinflamasi NALP3. *Nat Immunol.* 2011; 12 : 222–230.
35. Yang AP, Liu JP, Tao WQ, Li HM. The Diagnostic and Predictive Role of NLR, d-NLR and PLR in COVID-19 Patients. *International Immunopharmacology.* 2020; 84:1-7.
36. Shepherd AI, Wilkerson DP, Dobson L, Kelly J, Winyard PG, Jones AM, et al. The effect of dietary nitrate supplementation on the oxygen cost of cycling, walking performance and resting blood pressure in individuals with chronic obstructive pulmonary disease: A double blind placebo controlled, randomised control trial. *Nitric Oxide - Biol Chem.* 2015;48:31–7.
37. McDonagh STJ, Wylie LJ, Webster JMA, Vanhatalo A, Jones AM. Influence of dietary nitrate food forms on nitrate metabolism and blood pressure in healthy normotensive adults. *Nitric Oxide - Biol Chem.* 2018;72:66–74.
38. Acharya, A. Das,I., Chandhok, D., Saha, T. Redox regulation in cancer:A double-edged sword with therapeutic potential, *Oxid Med Cell Longev,* 2010: 3(1):23-34.
39. Lewis,B., Langkamp-Henken,B. Arginine enhances In vivo immune responses in young, adult and aged mice. *J Nutr.* 2000; 130(7):1827-30.
40. Wu,G., Bazer,F.W., Davis,T.A. Arginine metabolism and nutrition in growth, health and disease. *Amino acids.* 2009; 37(1):153-168.
41. Guo,X., Oldham,M.J., Kleinman,M.T., Phalen,R.F., Kassab,G.S. 2006. Effect of cigarette smoking on nitric oxide, structural, and mechanical properties of mouse arteries. *American Journal of Physiology -Heart and Circulatory Physiology* 2000; 291(5): 2354-2361.