

Correlation Between Serum Vitamin D Levels and Neutrophil Lymphocyte Ratio (NLR) with Mortality of COVID-19 Patients at H Adam Malik General Hospital Medan

Anandita Putri¹, Zuhrial Zubir², Ananda Wibawanta Ginting³

^{1,2,3}Pulmonology Allergy Immunology Division, Department of Internal Medicine, Faculty of Medicine, H. Adam Malik General Hospital - University of Sumatera Utara, Medan, Indonesia

Corresponding Author: Anandita Putri

DOI: <https://doi.org/10.52403/ijrr.20230362>

ABSTRACT

Background: Coronavirus Diseases 2019 (COVID 19) is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Vitamin D deficiency shows 6 times more risk of being infected with severe degree of COVID 19 and 15 times more risk of dying. An increase in the neutrophil lymphocyte ratio (NLR) was significantly related to the severity of the disease. The aim of this study was to determine the relationship between serum vitamin D levels and neutrophil lymphocyte ratio with mortality of COVID 19 patients at H Adam Malik General Hospital Medan.

Method: This was an observational study with cross-sectional design with retrospective data collection from medical record sources. This research was conducted from January 1 to November 31 2022 at H Adam Malik General Hospital in Medan. The sample for this study were all COVID 19 patients from January 1 until November 31 2022 at H Adam Malik General Hospital Medan who met the inclusion and exclusion criteria. Serum vitamin D levels, neutrophils, lymphocytes, neutrophil lymphocyte ratio, mortality and comorbidities were recorded and data analysis was carried out.

Results: The number of samples that met the inclusion criteria in this study were 125 people, the group that died was 60 people while the group that alive was 65 people. There was a significant difference in vitamin D levels between living and deceased COVID 19 sufferers ($p < 0.001$) and there was a significant

difference in NLR between living and deceased COVID 19 sufferers ($p = 0.046$).

Conclusion: There is an association between serum vitamin D levels and neutrophil lymphocyte ratio with the mortality of COVID 19 patients at H Adam Malik General Hospital Medan.

Keywords: COVID-19, vitamin D, NLR, comorbids

INTRODUCTION

In December 2019, several cases of pneumonia occurred in Wuhan, Hubei Province, China due to a new type of beta variant coronavirus. The disease is named by the World Health Organization (WHO) as Coronavirus Diseases 2019 (COVID-19). The Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses named the organism that causes the disease as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2).^[1] As of September 21, 2021, there are 204 countries infected with COVID-19 with 226 million positive confirmed cases worldwide and 4.6 million of them have died.^[2]

Studies show that SARS-CoV-2 can recognize angiotensin converting enzyme-2 (ACE2) in humans and other animals such as cats, civet cats and bats. In the lungs, ACE2 protein is present in alveolar epithelial cells. ACE2 protein is a zinc-

metallopeptidase which acts as an antagonist to ACE. These two enzymes balance the renin-angiotensin system (RAS). Renin is the initial hormone in the cascade process of the RAS system, where vitamin D has a role between these systems. ACE converts angiotensin I to angiotensin II as a vasoconstrictor and vitamin D has an angiotensin II blocking effect on the renin-angiotensin system which is very relevant to the course of COVID 19 disease.^[3]

Vitamin D can increase the immune system to fight COVID 19 and reduce the severity, especially in patients with comorbidities. According to the study in Germany, it was found that vitamin D deficiency was 6 times more at risk of being infected with severe degree of COVID-19 and 15 times more at risk of dying.^[4] Study of Baktash, et al. in 2020 reported that vitamin D deficiency in the elderly can lead to worsening of COVID-19 and severe morbidity outcomes.^[5] A multicenter study by Alipio et al, in 2020 in countries in Southeast Asia showed that there was a significant difference in average vitamin D levels between mild, severe and critical COVID 19 degrees, and vitamin D status showed a significant relationship to clinical manifestations.^[6]

Neutrophilia and lymphocytopenia are physiological responses of the natural immune system to systemic inflammation. The occurrence of neutrophilia, lymphocytopenia and increased NLR in COVID19 patients is affected by the expression of ACE2 which is a receptor for the COVID 19 disease virus, while lymphocytopenia occurs due to infection and loss of immune cells. This causes the risk of bacterial infection resulting in an increase in the neutrophil count.^[7] In addition, because the cytokine storm plays an important role in pathogenesis, cytokines including IL-10, IL-6 and tumor necrosis factor- α can trigger neutrophil activation and proliferation as well as lymphocyte apoptosis and lymphatic tissue destruction. Therefore, this whole process results in an increase in NLR.^[8]

A recent study by Jimeno in 2021 assessed NLR in predicting moderate-severe acute respiratory distress syndrome in patients with COVID 19 infection. The study reported that NLR in acute respiratory distress syndrome was significantly higher and a good predictor of moderate acute respiratory distress syndrome -heavy. Other studies have also shown a significantly higher NLR in patients who are critically ill compared to those with a milder degree of disease.^[8] Uncontrolled inflammation has an important role in COVID-19 disease and the NLR ratio and platelet lymphocyte ratio (RPL) have been recognized as inflammatory factors in lung regions such as lung cancer and obstructive lung disease.^[9] Sisca's study in 2022 showed that 10,000 IU of vitamin D3 supplementation had a better effect than 1,000 IU on the clinical outcome of moderate COVID-19 patients in improving length of stay, length of conversion, clinical manifestations, functional capacity and inflammatory markers of NLR.^[10] In conclusion, there is not enough evidence to know the relationship between vitamin D and NLR levels with COVID 19 mortality. In addition, efforts to prevent disease in reducing the risk of infection, worsening and severity need further review.

MATERIALS & METHODS

This research was an observational study with unpaired numerical comparative analytic type of two groups with a cross-sectional design with retrospective data collection from medical record sources. This research was conducted from January 1 to November 31 2022 in the isolation inpatient unit at H Adam Malik General Hospital in Medan. The sample for this study were all COVID 19 patients from January 1 to November 31 2022 at H Adam Malik General Hospital Medan who met the inclusion and exclusion criteria. The inclusion criteria in this study were age \geq 18 years, male and female gender, diagnosed with COVID 19 based on RT-PCR examination, and patients alive when

discharged and died during treatment. The exclusion criteria were patients returning home at their own request, dying outside H Adam Malik Hospital, or incomplete data. Subjects in this study were selected using consecutive sampling. The selected subjects recorded their name, medical record number, age, sex, serum vitamin D levels, neutrophils, lymphocytes, neutrophil lymphocyte ratio, mortality and comorbidities. After all the data has been collected, data tabulation is performed to further analyze the data

STATISTICAL ANALYSIS

The data obtained in this study will be presented descriptively using tables. All numerical data in this study will be assessed for distribution using the Kolmogorov Smirnov test. If the data is normally distributed, the relationship between serum vitamin D levels and neutrophil lymphocyte

ratio with the mortality of COVID 19 patients will be analyzed using an independent sample t-test. However, if the data is not normally distributed, it will be tested using the alternative Mann Whitney U test with a 95% confidence level using a computerized system.

RESULT

The number of samples that met the inclusion criteria in this study were 125 people, the group that died was 60 people while the group that lived was more, namely 65 people. The mean age of those who died was 56 years while the mean age of those who were alive was 48 years. Gender Males in the group that died were 34 people while in the group that lived were 33 people. Gender The female group who died was 26 people while the group who lived was 32 people.

Table 1. Frequency Distribution of Research Subjects

Variable	Die N=60	Live N=65	p value
Age (years), Mean ±SD	56.18±14.76	48.83±14.01	
Gender, n (%)			0.509
Man	34 (56.7)	33 (50.8)	
Woman	26 (43.3)	32 (49.2)	
DM, n (%)			0.006
Yes	23 (38.3)	41 (63.1)	
No	37 (61.7)	24 (36.9)	
Hypertension, n (%)			0.873
Yes	35 (58.3)	37 (56.9)	
No	25 (41.7)	28 (43.1)	
Strokes, n (%)			0.932
Yes	30 (50)	32 (49.2)	
No	30 (50)	33 (50.8)	
Cardiovascular, n (%)			0.278
Yes	25 (41.7)	21 (32.3)	
No	35 (58.3)	44 (67.7)	
CKD, n (%)			0.283
Yes	22 (36.7)	18 (27.7)	
No	38 (63.3)	47 (72.3)	
SLE, n (%)			1,000
Yes	1(1,7)	2(3,1)	
No	59 (98.3)	63 (96.9)	
Blood cancer			1,000
Yes	1(1,7)	2(3,1)	
No	59 (98.3)	63 (96.9)	
Lung tumor, n (%)			1,000
Yes	0 (0)	1 (1.5)	
No	60 (100)	64 (98.5)	
Degrees, n (%)			<0.001
Moderate	31 (51.7)	60 (92.3)	
Severe	29 (48.3)	5 (7.7)	

Based on table 1, there was significant relationship between mortality in COVID-19 and DM (p=0.006) and the degree of

COVID-9 (p<0.001). There was no significant relationship between mortality in COVID 19 and hypertension (p=0.932),

stroke (p=0.932), cardiovascular comorbidities (p=0.278), CKD (p=0.283), and SLE (p=1.000).

There was no significant relationship between blood cancer and mortality in COVID 19 (p=1,000). In the death group, 1 person had comorbid blood cancer, while 59 people did not have blood cancer. In the living group, 2 people had blood cancer, while 63 people did not have blood cancer.

There was no significant relationship between lung tumors and mortality in COVID 19 (p=1,000). There were no comorbid lung tumors in the group that died. In the living group, 1 person had a lung tumor, while 64 people did not have a lung tumor.

Table 2. Relationship between Vitamin D Levels and COVID-19 Mortality

Vitamin D (ng/L)	Median	Min-Max	p value
Died, n=60	12,7	6,7-18,5	<0,001
Alive, n= 65	29,8	12,6-39,8	

Based on table 2, it was found that the mean vitamin D level of the dead group was 12.7 ng/L while the surviving group had more than 2 times the vitamin D level, namely 29.8. Data was statistically analyzed with Mann-Whitney test, the result shows significant difference in vitamin D levels between living and deceased COVID 19 sufferers (p<0.05).

Table 3. Relationship between NLR and COVID-19 Mortality

NLR	Median	Min-Max	p value
Died, n=60	9,12	0,17-63,3	0,046
Alive, n= 65	5,19	0,60-68,7	

Table 3 above shows that the NLR of the dead group was 9.12 while the surviving group had a lower average level of 5.19. Data was statistically analyzed with Mann-Whitney test, the result shows there is a significant difference in NLR between COVID 19 sufferers who live and die (p<0.05).

DISCUSSION

In this study, there was a significant relationship between DM and mortality in COVID 19. In the death group, 23 people had comorbid DM, while 37 people did not

have DM. In the living group, 41 people had DM, while 24 people did not have DM. This is in accordance with previous research conducted by Hunafa in 2020 with the results of a high percentage of mortality in patients with confirmed COVID 19 accompanied by DM, especially higher in the group of patients with uncontrolled DM, namely 11% with a risk ratio value of 2.56.^[11] In another study, it was found that people with type 2 DM had a significantly higher risk of getting COVID 19 compared to people who did not have diabetes.^[12]

There was no significant relationship between hypertension and mortality in COVID 19 in this study. In the death group, 35 people had comorbid hypertension, while 25 people did not have hypertension. In the living group, 37 people had hypertension, while 28 people did not have hypertension. In another study, similar results were obtained by Trisanto in 2022, namely that there was a relationship between a history of hypertension and diabetes mellitus and the mortality of COVID 19 patients at Ummi Bogor Hospital.^[13]

There was no significant relationship between stroke and mortality in COVID 19 in this study. In the death group, 30 people had comorbid strokes, while 30 people did not have strokes. In the living group, 32 people had a stroke, while 32 people did not have a stroke. A different study was found by Utomo 2022 on the relationship between dyslipidemia and the occurrence of stroke in patients with COVID 19.^[14]

There was no significant relationship between cardiovascular comorbidities and mortality in COVID 19 in this study. In the death group, 25 people had cardiovascular comorbidities, while 35 people did not have cardiovascular disease. In the living group, 21 people had CHD, while 44 people did not have cardiovascular disease. Another different study stated that patients with cardiovascular comorbidities are at higher risk of experiencing more severe manifestations if infected with COVID 19 and are associated with higher mortality. Although dominated by respiratory clinical

manifestations, COVID 19 can also cause severe cardiovascular disorders. ACE2 acts as a receptor for SARS-CoV-2. It is suspected that patients with cardiovascular disease may have more severe clinical manifestations due to the higher expression of ACE2 in this population. Cardiovascular complications in COVID 19 can include myocardial injury, myocarditis, acute myocardial infarction, acute heart failure, thromboembolism and arrhythmias. In patients with cardiovascular comorbidities, optimization of conservative medical therapy needs to be prioritized. Emergency intervention measures may be considered in selected cases with hemodynamic instability.^[15]

There was no significant relationship between CKD and mortality in COVID19 in this study. In the death group, 22 people had comorbid CKD, while 38 people did not have CKD. In the living group 18 people had CKD while 47 people did not have CKD. Drew found something different in 2021, namely from the results of the study it was concluded that a history of hypertension and chronic kidney failure increases the risk of mortality in positive COVID-19 patients in East Jakarta.^[16]

In this study, there was no significant relationship between SLE and mortality in COVID 19. In the death group, there was 1 person who had comorbid SLE while 59 people did not have SLE. In the living group 2 people had SLE while 63 people did not have SLE. A different study was found by Suryaputra in 2022 that there was a significant relationship between comorbidities and mortality, 1% of the 132 samples were patients with comorbid SLE.^[17]

The high number of deaths in COVID-19 patients is associated with co-morbidities such as hypertension, diabetes mellitus, cardiovascular disease, chronic obstructive pulmonary disease, kidney disease, cancer and liver disease. The pathophysiological mechanisms of comorbidities in exacerbating symptoms and increasing the risk of mortality in COVID 19 patients are

very complex and diverse. In patients with hypertension, chronic obstructive pulmonary disease, and kidney disease, there is an increase in the expression of ACE-2 receptors causing them to be very susceptible to infection with COVID 19. Decreased and weakened immune systems play an important role in worsening symptoms of COVID 19 in patients with cancer, liver disease and cardiovascular disease. In diabetic patients, impaired T-cell function and increased interleukin-6 (IL-6) levels play a role in increasing the degree of COVID-19 disease.^[18]

In this study, there were significant differences in vitamin D levels between living and deceased COVID 19 sufferers. The same thing was obtained by Fathan in 2021, namely that there is a correlation between vitamin D and a reduction in the severity and mortality of COVID 19.^[19] Vadir found a different study in 2020 that there was no correlation between vitamin D levels and COVID 19 cases and death rates.^[5]

The NLR in the dead group was 9.12 while the living group had a lower average level of 5.19. There is a significant difference in NLR between COVID 19 sufferers who live and die. Kannan's 2020 study stated that NLR in patients with severe degrees was higher than in mild patients. Apart from playing a good role as a biomarker in predicting severity, NLR can also be a predictor of mortality in COVID-19 patients. 19 Similar results were found by Aidil in 2022 that NLR had significant association with mortality of COVID 19 patients.^[20]

CONCLUSION

There was an association between vitamin D levels, NLR, DM comorbidities and the mortality of COVID 19 patients.

Declaration by Authors

Ethical Approval: Approved

Acknowledgement: None

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Mardani R, Alamdary A, Mousavi Nasab SD, Gholami R, Ahmadi N, Gholami A. Association of vitamin D with the modulation of the disease severity in COVID-19. *Virus Res* [Internet]. 2020 Nov;289:198148. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0168170220310558>
2. World Health Organization. Clinical management Clinical management Living guidance COVID-19. 2021B. 2021;(January):16–44.
3. Zapata I. Vitamin d and inflammation-potential implications for severity of COVID-19. *Ir Med J*. 2020;113(6):1–3.
4. Radujkovic A, Hippchen T, Tiwari-Heckler S, Dreher S, Boxberger M, Merle U. Vitamin D Deficiency and Outcome of COVID-19 Patients. *Nutrients* [Internet]. 2020 Sep 10;12(9):2757. Available from: <https://www.mdpi.com/20726643/12/9/2757>
5. Baktash V, Hosack T, Patel N, Shah S, Kandiah P, Van den Abbeele K, et al. Vitamin D status and outcomes for hospitalised older patients with COVID-19
6. Pal R, Banerjee M, Bhadada SK, Shetty AJ, Singh B, Vyas A. Vitamin D supplementation and clinical outcomes in COVID-19: a systematic review and meta-analysis. *J Endocrinol Invest* [Internet]. 2022 Jan 24;45(1):53–68. Available from: <https://link.springer.com/10.1007/s40618-021-01614-4>
7. Fouad SH, Allam MF, Taha SI, Okba AA, Hosny A, Moneer M, et al. Comparison of hemoglobin level and neutrophil to lymphocyte ratio as prognostic markers in patients with COVID-19. *J Int Med Res* [Internet]. 2021 Jul 11;49(7):030006052110301. Available from: <http://journals.sagepub.com/doi/10.1177/03000605211030124>
8. Prozan L, Shusterman E, Ablin J, Mitelpunkt A, Weiss-Meilik A, Adler A, et al. Prognostic value of neutrophil-to-lymphocyte ratio in COVID-19 compared with Influenza and respiratory syncytial virus infection. *Sci Rep* [Internet]. 2021 Nov 2;11(1):21519. Available from: <https://www.nature.com/articles/s41598-02100927-x>
9. Jimeno S, Ventura PS, Castellano JM, García-Adasme SI, Miranda M, Touza P, et al. Prognostic implications of neutrophil-lymphocyte ratio in COVID-19. *Eur J Clin Invest* [Internet]. 2021 Jan 25;51(1). Available from: 59 <https://onlinelibrary.wiley.com/doi/10.1111/eci.13404>
10. Oliy SA. Uji Klinis Randomisasi Pengaruh Suplementasi Vitamin D Terhadap Luaran Klinis dan Neutrophil-to-Lymphocyte Ratio (NLR) Pasien COVID-19 Derajat Sedang. Universitas Hasanudin; 2022.
11. Hunafa AF, Harahap SP, Yulianti R, Nugraha Y. Hubungan Diabetes Melitus dengan Kejadian Mortalitas pada Pasien Terkonfirmasi Covid-19 Tahun 2020 : Systematic Review The Correlation between Diabetes Mellitus and Mortality of Patient with Confirmed COVID-19: Systematic Review. *eJKI* [Internet]. 2021;9(1):67–73. Available from: <https://ejki.fk.ui.ac.id/index.php/journal/article/view/20/11>
12. Kevin. Analisa COVID 19 pada pasien Diabetes Melitus. Universitas Hasanudin Makasar; 2021.
13. Trisanto D, Djannatun T, Arifandi F. Korelasi antara Riwayat Hipertensi dan Diabetes Melitus dengan Mortalitas Pasien Covid-19 di Rumah Sakit Ummi Bogor dan Tinjauannya Menurut Pandangan Islam. *Jr Med J* [Internet]. 2022;1(2):174–80. Available from: <https://academicjournal.yarsi.ac.id/index.php/jmj/article/view/2882>
14. Utomo TY. Hubungan faktor risiko terhadap kejadian stroke pada penderita corona virus diseases-2019. hubungan faktor risiko terhadap kejadian stroke pada penderita corona virus Dis. 2022;2(8.5.2017):2002.
15. Willim HA, Ketaren I, Supit AI. Dampak Coronavirus Disease 2019 terhadap Sistem Kardiovaskular. *e-Clinic*. 2020;8(2):237–45.
16. Drew C, Adisasmita AC. Gejala dan komorbid yang memengaruhi mortalitas pasien positif COVID-19 di Jakarta Timur, Maret-September 2020. *Tarumanagara Med J*. 2021;3(1):42–51.
17. Suryaputra GP, Apriningsih H, Wardani MM. Hubungan Komorbid dengan

- Mortalitas dan Lama Rawat Inap pada Pasien COVID-19 di Rumah Sakit UNS Surakarta. *Plex Med J.* 2022;1(1):32–41.
18. Nartha KPPP. Penyakit Komorbid Sebagai Faktor Risiko Mortalitas Pasien Covid-19. *Unram Med J.* 2022;11(3):1079–84.
19. Fathan Mubina J, Wahyuni A. Pengaruh Vitamin D terhadap Keparahan dan Mortalitas COVID-19 Effect of vitamin D for COVID-19 severity and mortality. 64 2021;11(April):183.
20. Aidil M, Ramadhan A, Sawitri E, Wahyunie S. Artikel Penelitian Association of Neutrophil-Lymphocyte Ratio (NLR) and

D-Dimer Levels with COVID-19 Mortality. 2022;5(2):167–73.

How to cite this article: Anandita Putri, Zuhrial Zubir, Ananda Wibawanta Ginting. Correlation between serum vitamin D levels and neutrophil lymphocyte ratio (NLR) with mortality of COVID-19 patients at H Adam Malik General Hospital Medan. *International Journal of Research and Review.* 2023; 10(3): 560-566. DOI: <https://doi.org/10.52403/ijrr.20230362>
