# ANALYSIS BETWEEN VITAMIN D-25OH LEVELS AND CT VALUES OF SARS – COV-2 INFECTION

**Dewi Inderiati*, Citra Amaniah Anhar, Sulis Ernawati**
Poltekkes Kemenkes Jakarta III, Indonesia
**dregina.biomedic@gmail.com*

***ABSTRACT***

*Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) infection is an infection that increased risk of severity can be influenced by levels of vitamin D-25 OH. This condition can be influenced by the amount of virus (viral load), which can be seen from the Cycle threshold (Ct) value results . This research aims to analyze between vitamin D-25 OH levels and CT values of SARS-COV2 infection. This type of research is a cross-sectional study. The research data came from 85 medical records patients infected with SARS-CoV2 during January - December 2021 using the consecutive sampling technique in Prodia Laboratory Clinical. The majority of subjects were women, as many as 55 people (67%), with the age category of elderly old (46-65 years) as many as 34 people (41%), experienced insufficiency (10-39 ng/mL) of 43 people (51%) with positive Ct values of SARS-CoV-2 RNA (30-37) of 43 people (52%). Vitamin D levels and Ct values in patients with confirmed SARS-CoV-2 infection have a correction with a p-value of 0.000 and a correlation coefficient of 0.393. There was significant relationship between vitamin D -25 OHlevels and Ct values in patients with confirmed SARS-CoV2 infection*

***Keywords****: CT Values; Vitamin D-25OH; SARS-COV2 Infection*

## INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) infection is an infection that causes Corona Virus Disease (COVID-19), which spreads quickly and has been declared a pandemic that has occurred worldwide (WHO, 2020). SARS-CoV2 infection can cause mild to severe symptoms, depending on the individual's resistance to the virus. Someone with a good immune system may not experience any symptoms, but they are still at risk of transmitting the virus. When the virus enters a person's body with weak immunity, it incubates for 5–6 days, and then symptoms appear (Kemenkes, 2020). Common symptoms that often occur and are reported are fever, cough, and shortness of breath. Meanwhile, the severe symptoms that occur are pneumonia, acute respiratory syndrome, kidney failure, and even death (Shihab, 2020).

Severe symptoms increase the severity of the SARS-CoV-2 virus infection compared to mild symptoms. The increased risk of severity can be influenced by levels of vitamin D-25 OH (Mubina & Wahyuni, 2021). Vitamin D reduces the cytokine storm caused by the virus infection through interferon-gamma and tumor necrosis factor-alpha. Vitamin D can also interact with the protein angiotensin-converting enzyme 2 (ACE-2), a receptor for the SARS-CoV-2 virus (Haq et al., 2021). When the SARS-CoV-2 virus enters the body of someone who is experiencing decreased or deficient levels of vitamin D-25 OH, it can accelerate the formation of inflammatory proteins in the body by stimulating the renin-angiotensin system to be overactive and lead to decreased lung function. SARS-CoV-2 infection with vitamin D deficiency can cause severe clinical symptoms requiring intensive care in the hospital (Meltzer et al., 2020).

There are two types of diagnostic tests for SARS-CoV-2 infection: viral antigen detection tests and antibody tests for the body's immune reaction to infection. The Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method for testing viral RNA is the Gold Standard for examination (WHO, 2020). The examination targets at least two viral gene targets, including the E, RdRP, N, and S genes in SARS-CoV-2, and the interpretation of the results is according to what is listed on the insert kit. The confirmation method for SARS-CoV-2 infection is based on detecting specific viral sequences through nucleic acid amplification tests (NAAT), such as real-time Reverse-Transcription Polymerase Chain Reaction (rRT-PCR) (Chakraborty et al., 2020). RT-PCR examination on nasopharyngeal and oropharyngeal swabs has high specificity and sensitivity, which depend on several things; viral load, method of isolation or RNA extraction used, and time for taking the swab depending on the phase of disease that the patient experienced. The results of the real-time PCR examination were declared positive when an accumulation of fluorescent signals was obtained (PDS PatKlin, 2020). The cycle threshold value (Ct) is the number of cycles required for the fluorescent signal to pass through the threshold. The Ct value is inversely proportional to the amount of target nucleic acid in the sample, meaning that the lower the Ct value, the greater the amount of nucleic acid detected in the sample. A low CT value generally indicates a high concentration of viral genetic material associated with an increased risk of infection. In contrast, a high CT value usually indicates a low concentration of viral genetic material related to low disease risk (Coleman et al., 2022). The CT value in the early stages of infection is generally below 20–30 and tends to increase afterwards, reflecting a decrease in viral RNA due to the role of the immune system (Manurung & Sukohar, 2021).

The potential role of vitamin D-25 OH in determining the severity and mortality of SARS-CoV2 infection makes this helpful research a contribution to society. Therefore, the author is interested in raising this issue as material for the research "Analysis Between Vitamin D-25 OH Levels and CT Values of SARS-CoV2 Infection.

## METHOD

This research received ethical approval from the ethics committee of Budhi Asih Hospital with number 266/KEP-ETIK/VI.2022 on June 7, 2022. This research is a descriptive-analytical study with a cross-sectional approach using secondary data from the results of vitamin D- 25 OH and CT value of SARS-CoV-2 RNA in patients infected with SARS-CoV-2. On examination of vitamin D-25 OH levels, the results will be classified into four categories, namely deficiency (<10 mg/mL), insufficiency (10-300 ng/mL), and sufficiency (31-100 ng/mL). Meanwhile, for the Ct value of SARS-CoV2 RNA, the results will be classified into three categories, namely strong positive (<29), positive (30-37), and weak positive (38-40). The samples for this study were patients who had confirmed infection with the SARS-CoV-2 virus through antigen testing using a PCR tool. The sample size used was 82 people with a consecutive sampling technique, which means that the sample was taken by determining the subjects who met the inclusion criteria within a certain time. The inclusion criteria were patients confirmed to have SARS-CoV-2 infection and who performed vitamin D-25 OH and Ct values for SARS-CoV-2 RNA at the Prodia Bekasi clinical laboratory from January to December 2021. This research data processing and analysis techniques were univariate and bivariate analysis. Univariate analysis was presented in tabular form to examine patient characteristics (gender and age) and calculate the average, minimum, maximum, and standard deviation of vitamin D levels and Ct values of SARS-CoV-2 RNA. Bivariate analysis was carried out by testing using the spearman test to see the correlation between vitamin D-25 OH and the Ct value of SARS-CoV-2 RNA

## RESULTS AND DISCUSSION

1. Patient Characteristics

   The data used in this research is secondary data, with a sample used in 82 people based on inclusion criteria. The overall description of the variables studied is as follows:

Tabel 1. Characteristics of patients with confirmed SARS-CoV-2 infection

| Characteristics | Total | |
|---|---|---|
| | n | % |
| Gender | | |
| Man | 27 | 33 |
| Woman | 55 | 67 |
| Age | | |
| Children (5-11 years) | 1 | 1 |
| Teenager (17 – 25 years) | 6 | 7 |
| Mature (26 – 45 years) | 27 | 33 |
| Elderly old (46 – 65 years) | 34 | 41 |
| Senior old (> 65 years) | 14 | 18 |

Based on Table 1 above, the patient characteristics in the gender group obtained the number of males - 27 people (33%) more significant and while in women, there were 55 people. Based on Table 1 above, the patient characteristics in the gender group obtained the number of males - men are more prominent with 27 people (33%), and women are 55 people (67%). In the age group, it is classified into five groups, namely the children group (5-11 years), as many as 1 (1%), teenagers (17-25 years), as many as six people (7%), mature (26-45 years) as many as 27 people ( 33%), elderly (46-65 years) as many as 34 people (41%), and senior old (> 65 years) as many as 14 people (18%).

2. Vitamin D-25 OH Levels

The vitamin D levels in the patient's serum are divided into 3, namely deficiency, insufficiency, and sufficiency. An overview of the frequency distribution of vitamin D levels in the serum of patients with confirmed SARS-CoV-2 infection at the Prodia Clinical Laboratory can be seen in Table 2.

Table 2. Frequency Distribution of Vitamin D-25 OH Levels

| Variable | Total | |
|---|---|---|
| | N | % |
| Interpretation of result of Vitamin D-25 OH levels | | |
| Deficiency (<10 ng/mL) | 3 | 4 |
| Insufficiency (10 – 30 ng/mL) | 43 | 51 |
| Sufficiency (31 – 100 ng/mL) | 37 | 45 |
| Variable | Mean ± SD (ng/mL) | Median (min – max ) |
| Vitamin D-25 OH levels | 29,7 ± 19,1 | 27,8 (4 – 65,7) |

Based on Table 2, it can be seen that the levels of vitamin D-25 OH in patients with a deficiency category were three people (3.7%), insufficiency was 42 people (51.2%), and deficiency 37 people (45.1%) on vitamin C. D-25OH. The average level of vitamin D-25 OH was 29.7 ng/mL, with the lowest value being 4,0 ng/mL and the highest value 65.7 ng/mL. Several factors, including gender and age, influence serum vitamin D-25OH levels in patients. An overview of the frequency of factors that can affect vitamin D-25OH in patients can be seen in Table 3.

Table 3. Characteristics of Vitamin D-25 OH Levels from of patients with confirmed SARS-CoV2 infection

| Variable | Deficiency | | Insufficiency | | Sufficiency | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| Gender | | | | | | |
| Man | 0 | 0 | 17 | 63 | 10 | 37 |
| Woman | 3 | 5 | 25 | 46 | 27 | 49 |
| Ages | | | | | | |
| Children (5-11 years) | 0 | 0 | 0 | 0 | 1 | 100 |
| Teenager (17 – 25 years) | 0 | 0 | 2 | 33 | 4 | 67 |
| Mature (26 – 45 years) | 1 | 4 | 16 | 59 | 10 | 27 |
| Elderly old (46 – 65 years) | 2 | 6 | 13 | 38 | 19 | 56 |
| Senior old (> 65 years) | 0 | 0 | 11 | 79 | 3 | 21 |

Table 3 shows that based on gender characteristics, the highest prevalence of vitamin D insufficiency was in male patients at 17% (63%). Meanwhile, based on age characteristics, the results showed the most insufficiency in the mature group (26-45 years), with 16 people (59%).

Vitamin D-25 OH levels in research subjects had an average of 29.6 ng/ml included in the category of insufficiency of vitamin D (based on table 3). In the categorial age group, two issues (33%) had insufficient vitamin D levels, and in adults, as many as 16 people (59.3%). This situation can be influenced by several factors, including lack of exposure to sunlight (UVB) and low intake of vitamin D. Less exposure to sunlight is caused by a lack of outdoor activity or working indoors for a long time, a lifestyle that tends to avoid sunlight, the use of clothing materials that are difficult to absorb sunlight or the habit of wearing long clothes, the use of body protection such as hats, umbrellas, sunscreen/sunblock. In addition, with the low intake of foods containing lots of vitamin D, such as fatty fish, milk, and fortified foods, there is a tendency to reduce high-fat foodstuffs, ultimately resulting in vitamin D deficiency (Rimahardika et al., 2017). In the elderly old group, the number of

research subjects who had insufficient levels of vitamin D (insufficiency) was 13 people (38%), and in the senior old group, there were 11 people (79%). Reduced mobility, which caused less exposure to sunlight, reduced production of 1, 25 hydroxyvitamins from the kidneys, and decreased intake of food sources of vitamin D, are risk factors for vitamin D deficiency in the elderly old (Mohan et al., 2020).

3. Ct Values of SARS-CoV-2 RNA

The Ct value category is divided into three groups. It was strong positive, positive, and weak positive. The classification of these categories is based on the results of the Ct values listed in the results of the RT PCR examination. An overview of the frequency distribution of Ct values in patients with confirmed COVID-19 at the Prodia Clinical Laboratory can be seen in Table 4

Table 4. Frequency Distribution of Ct values of SARS-CoV-2 RNA

| Variable | Total | |
|---|---|---|
| | n | % |
| Interpretation of result of Ct values of SARS-CoV-2 RNA | | |
| High Positive (<29) | 39 | 48 |
| Positive (30-37) | 43 | 52 |
| Weak positive (38-40) | 0 | 0 |
| Variable | Mean ± SD | Median (min – max ) |
| Ct values of SARS-CoV-2 RNA) | 30,1 ± 4,9 | 31,1 (13,9 – 36,9) |

Based on Table 4 above, it can be seen that there were 39 patients (47.6%) who showed a high positive Ct value, 43 people (52.4%) were positive, and there were no patients who had a weakly positive Ct value. From the description of these results, it can be seen that more patients have a positive Ct value compared to respondents who have a high positive Ct value. The average CT value of SARS-CoV-2 RNA was 30,1 with the lowest value being 13.9 and the highest being 36.9. Several factors, including gender and age, influence the CT value in patients. An overview of the frequency of factors that can affect Ct values in patients can be seen in Table 5.

Tabel 5. Characteristics of Ct Values of SARS-CoV-2 RNA from of patients with confirmed SARS-CoV-2 infection

| Variable | High Positive | | Positive | |
|---|---|---|---|---|
| | n | % | n | % |
| Gender | | | | |
| Man | 12 | 44 | 15 | 56 |
| Woman | 27 | 49 | 28 | 51 |
| Age | | | | |
| Children (5-11 years) | 0 | 0 | 1 | 100 |
| Teenager (17 – 25 years) | 2 | 33 | 4 | 67 |
| Mature (26 – 45 years) | 13 | 48 | 14 | 52 |
| Elderly old (46 – 65 years) | 20 | 59 | 14 | 41 |
| Senior old (> 65 years) | 4 | 29 | 10 | 71 |

Based on Table 5, the frequency of Ct values based on gender, female patients showed a strong positive result in 27 people (49%) compared to 12 male patients (44%). Based on the age factor, the most patients who showed strong positive results were the elderly old group (46-65 years), totaling 20 people (59%).

Ct values of SARS-CoV-2 RNA stretcher real-time PCR in this study had an average of 30.1, included in the category of high positive. This result indicates a moderate amount of nucleic acid in the sample of the research subject. In this research (based on table 4), the frequency of positive Ct values was more for men, namely 55.6%, while for women, it was 50.9% of the total number of each sex. This result is because men are more sensitive to SAR-CoV-2 due to the habit of men leaving the house more due to working conditions and being more in society than women. Besides that, men tend to pay less attention to social distancing issues and are more active smokers (Bullard et al., 2020). From the age factor, the most infected with SARS-CoV2 were the elderly, totaling 34 people or 41.5%, with the most Ct values being strongly positive, namely 58.8%, which means that there was a lot of RNA in the sample of the study subjects. It can be caused by degeneration of the body so that the immune system's competence decreases. This degeneration manifests in two forms, namely a decrease in the number of cells (neutrophil cells, T lymphocyte cells, and dendritic cells), a reduction of the number of receptors involved in the immune response (TLR receptors and cell surface receptors for monocytes and macrophages), and a decrease in the ability to differentiate (lymphocyte cells). B). Making it difficult for elderly old group to fight various kinds of bacteria or viruses that cause disease, including SARS-CoV2 infection. Another factor that causes more elderly old group to be infected is the presence of co-morbidities that cause a weak body condition(Singh et al., 2020).

4. Correlation of vitamin D-25 OH levels with Ct values from patients with confirmed SARS-CoV2 infection

a. Data normality test for vitamin D-25 OH and Ct values of SARS-CoV-2 RNA

Before the correlation test was carried out, the data normality test was first performed using the Kolmogorov-Smirnov test. The results of the data normality test can be seen in Table 6.

Table 6. Normality of data on Vitamin D-25 OH levels and Ct value of SARS-CoV-2 RNA (Kolmogrov-Spinov Test with α=0,05)

| Variable | Kolmogrov-Sminov | |
|---|---|---|
| | n | Sig |
| Vitamin D-25 OH Levels | 82 | 0,015 |
| Ct values of SARS-CoV-2 RNA | 82 | 0,000 |

Based on the data in Table 6, it can be seen that the results of the data normality test analysis with a 95% confidence level, the variable vitamin D levels obtained a p-value of 0.015 and the variable Ct value p-value of 0.000. The results of the p-value of these two variables <0.05 mean that the data is not normally distributed

b. Correlation Test of Vitamin D-25 OH levels with Ct Values of SARS-CoV-2 RNA

Because the results of the two data were not normally distributed, the correlation test was continued using the Spearman correlation test. The results of the correlation test can be seen in Table 7.

Table 7. Correlation of vitamin D-25 OH levels and Ct values (Spearman Test with α=0,05)

| Variable | p-value | Correlation coeffecient |
|---|---|---|
| Vitamin D-25 OH Levels - Ct values of SARS-CoV-2 RNA | 0,000 | 0,393 |

Based on the data in Table 7, the p-value for the two variables above is 0.000 at the 95% confidence level. It means that the p-value < 0.05 so that Ha is accepted, and it can be concluded that there is a significant relationship between vitamin D levels and Ct values in patients with confirmed SARS-CoV2 infection with a correlation coefficient of 0.393.

Vitamin D is immunomodulating in the body's response to viral infections that attack the respiratory system. The lungs are the organs most affected by infection with the SARS-CoV-2 virus. Vitamin D receptors (VDR) and CYP27B1 are present in the immune system, bronchial and lung epithelial cells. They are upregulated after ligating specific receptors by extracellular pathogens, implicating vitamin D in innate immunity. The natural immune system is the first defense against pathogenic organisms such as viruses. 1,25(OH)2D enhances this defense by inducing endogenous antimicrobial peptides (AMP) such as cathelicidin in monocytes, neutrophils, and epithelial cells, which will kill pathogenic organisms such as bacteria, viruses, and fungi, which will then initiate an adaptive immune response. In a state of vitamin D deficiency, the immune response will be disrupted because less 25-OHD is available for synthesizing 1,25(OH)2D, which causes impaired innate immune function (Brenner, 2021).

Vitamin D also plays a role in regulating anti-inflammatory cytokines, thereby enabling a reduction in mortality and severity of COVID-19 infection by inducing regulatory T cells and Treg cells by 1,25(OH) 2D. Vitamin D can also enhance antiviral immunity by inducing cathelicidins and defensins, which can block the entry of viruses into cells and suppress viral replication. Vitamin D deficiency in individuals with risk factors for SARS-CoV2 infection, such as men, and elderly old people, can lead to a higher risk of disease severity (Mohan et al., 2020). This condition can be influenced by the amount of virus (viral load) entering the body, which can be seen from the Cycle threshold (Ct) value results. A low Ct value indicates a high concentration of viral genetic material (high viral load), which is usually associated with a risk of infectivity. In this research, the average research subject had a moderate amount of nucleic acid and insufficient levels of vitamin D, so it can be concluded that the lower the vitamin D-25 OH level in a person's body, the lower the Ct value; this means that the individual's body is deficient in vitamin D-25OH can be found a large enough amount of virus that can affect the severity of the disease and increase mortality (Grant et al., 2020).

## CONCLUSION

Based on data analysis and discussion of the research, it can be concluded that There was significant relationship between vitamin D-25 OH levels and Ct values in patients with confirmed SARS-CoV2 infection. It is necessary to check the levels of vitamin D-25 OH in individuals with risk factors for SARS-CoV-2 infection such as men, elderly old people, and someone with a comorbid disease


## ACKNOWLEDGEMENT

We would like to express our appreciation to everyone who contributed significantly to the completion of this research. We would like to express our gratitude to Poltekkes Kemenkes Jakarta III and Dumai Hospital for granting permission to do this research and use their facilities


## REFERENCES


Brenner, H. (2021). Vitamin D supplementation to prevent COVID-19 infections and deaths—accumulating evidence from epidemiological and intervention studies calls for immediate action. *Nutrients*, *13*(2), 411.

Bullard, J., Dust, K., Funk, D., Strong, J. E., Alexander, D., Garnett, L., Boodman, C., Bello, A., Hedley, A., & Schiffman, Z. (2020). Predicting infectious severe acute respiratory syndrome coronavirus 2 from diagnostic samples. *Clinical Infectious Diseases*, *71*(10), 2663–2666.

Chakraborty, C., Sharma, A. R., Sharma, G., Bhattacharya, M., & Lee, S. S. (2020). SARS-CoV-2 causing pneumonia-associated respiratory disorder (COVID-19): diagnostic and proposed therapeutic options. *Eur Rev Med Pharmacol Sci*, *24*(7), 4016–4026.

Coleman, K. K., Tay, D. J. W., Tan, K. Sen, Ong, S. W. X., Than, T. S., Koh, M. H., Chin, Y. Q., Nasir, H., Mak, T. M., & Chu, J. J. H. (2022). Viral load of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in respiratory aerosols emitted by patients with coronavirus disease 2019 (COVID-19) while breathing, talking, and singing. *Clinical Infectious Diseases*, *74*(10), 1722–1728.

Grant, W. B., Lahore, H., McDonnell, S. L., Baggerly, C. A., French, C. B., Aliano, J. L., & Bhattoa, H. P. (2020). Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths. *Nutrients*, *12*(4), 988.

Haq, A. D., Nugraha, A. P., Wibisana, I. K. G. A., Anggy, F., Damayanti, F., Syifa, R. R. D. M., Widhiani, N. P. V., & Warnaini, C. (2021). Faktor–Faktor Terkait Tingkat Keparahan Infeksi Coronavirus Disease 2019 (COVID-19): Sebuah Kajian Literatur. *JIMKI: Jurnal Ilmiah Mahasiswa Kedokteran Indonesia*, *9*(1), 48–55.

Kemenkes, R. I. (2020). *Pedoman pencegahan dan pengendalian coronavirus disease (covid-19)*.

Manurung, J. J., & Sukohar, A. (2021). Hubungan Antara CT Value pada Test RT-PCR Terhadap Parameter Klinis Pasien COVID-19. *Medical Profession Journal of Lampung*, *11*(1), 119–124.

Meltzer, D. O., Best, T. J., Zhang, H., Vokes, T., Arora, V., & Solway, J. (2020). Association of vitamin D status and other clinical characteristics with COVID-19 test results. *JAMA Network Open*, *3*(9), e2019722–e2019722.

Mohan, M., Cherian, J. J., & Sharma, A. (2020). Exploring links between vitamin D deficiency and COVID-19. *PLoS Pathogens*, *16*(9), e1008874.

Mubina, J. F., & Wahyuni, A. (2021). Pengaruh Vitamin D terhadap Keparahan dan Mortalitas COVID-19. *Medical Profession Journal of Lampung*, *11*(1), 183–189.

PDS PatKlin. (2020). Panduan Tatalaksana Pemeriksaan Tes Cepat Molekuler (TCM) dan Polymerase Chain Reaction (PCR) SARS-COV-2. In *JPDS PatKlin: akarta Selatan*.

Rimahardika, R., Subagio, H. W., & Wijayanti, H. S. (2017). Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal of Nutrition College*, *6*(4), 333–342.

Shihab, N. (2020). *Covid-19: Kupasan Ringkas yang Perlu Anda Ketahui*. Lentera Hati Group.

Singh, N. P., Kumar, R., Singh, A., Takhelchangbam, N. D., & Chauhan, M. (2020). COVID-19 Outbreak: Reviewing Various Factors Affecting its Fate. *Int J Med Sci Curr Res*, *3*(3), 184–193.

WHO. (2020). *COVID-19 diagnostic testing in the context of international travel: scientific brief, 16 December 2020*. World Health Organization.