# Comparison of vitamin d deficiency with neutrophil lymphocyte ratio and crp levels in covid-19 patients

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## ABSTRACT

**Objective:** Objective: We aimed to evaluate the association of neutrophil-lymphocyte ratio (NLR) and C reactive protein (CRP), ferritin, sedimentation levels with vitamin D concentrations in Covid-19 patients and to investigate  their effect of  levels on Covid-19 patients.

**Materials and Methods:** Forty-six patients aged 18-85 were included in our study. Our study is a retrospective study. Patients were divided into 2 groups with the first group consisting of those with vitamin D level 20 and below and 2nd group consisting of those with vitamin D level above 20. After Vitamin D, CRP, neutrophil, and lymphocyte values were retrospectively investigated with the hospital database, vitamin D levels were compared to CRP and neutrophil/lymphocyte ratio.

**Results:** A positive correlation between CRP and SED, CRP and ferritin, NLR, and ferritin was observed in Covid-19 positive patients with Vitamin D level > 20. A positive correlation between NLR and CRP was observed in Covid-19 positive patients with Vitamin D level ⩽ 20.

**Conclusion:** In patients with Vitamin D levels >20, there was a significant correlation between vitamin D and NLR and a moderate correlation between Vitamin D and CRP. In patients with Vitamin D levels <20, there was a positive correlation of Vitamin D with CRP and NLR.

**Keywords:** Covid-19, Vitamin D, Neutrophil Lymphocyte Ratio, CRP, SARS-CoV-2

## INTRODUCTION

The world's first cases of a highly contagious, deadly new type of coronavirus infection were identified in December 2019. There have been applications to the hospital with cases of pneumonia with serious complications. The cause of which is unknown, and it was later understood that this is a new type of coronavirus infection (**1**).  The World Health Organization reported 11,125,245 cases and 528,204 deaths worldwide as of July 6, 2020 (**2**).

Coronavirus was first described by Tyrell and Bynoe in 1966. They are enveloped positive single chain RNA viruses. Although it has 7 subgroups that can infect people, beta coronavirus makes the deadliest, most serious disease. SARS-COV-2 is one of these beta coronavirus (**3**).

Coronaviruses have been found to be responsible for 2 diseases affecting the entire world in recent years; Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (**4**). The genetic sequence of the new type of coronavirus is similar to the genetic sequence of SARS co-V at 80% and MERS at 50% (**5**).

Vitamin D is produced on the skin from 7-dehydrocholesterol by the effect of UVB rays. It converts to 25(OH)D in the liver and then 1.25(OH)2D metabolites in the kidney (**6**). Vitamin D has a protective effect on many viral infections. It reduces viral replication. By reducing proinflammatory cytokines, it shows an anti-inflammatory effect. A study has shown that vitamin D reduces the risk of colds and viral infections in cold patients, both by its effect on the immune system and by its physical effect on tight junctions (**7, 8**).

Neutrophil lymphocyte ratio and CRP are our routine laboratory tests. They are easily accessible, inexpensive, routine biochemical parameters. CRP is an acute-phase protein synthesized from the liver. In cases of acute infection and inflammation, the amount in the blood increases (**9**). Neutrophil lymphocyte ratio is also a marker that reflects inflammation.

We aimed to evaluate the relationship of neutrophil-lymphocyte ratio and CRP level with vitamin d concentrations, evaluate the relationship of Vitamin D deficiency with the disease. We wanted to investigate the deficiency of vitamin d and the relationship with the disease.

## MATERIAL and METHODS

This study was approved by the Committee of Tokat Gaziosmanpaşa University, Education and Research Hospital with Decision No. 20-KAEK-218 in the scientific meeting which was held on 13.08.2020. Patients diagnosed with Covid-19 in our hospital were taken to the study. Forty-six patients aged 18-85 were included. These patients were retrospectively examined. Patients admitted with cough, fever, or upper respiratory tract complaints and followed up with Covid-19 diagnosis according to PCR and tomography results were included in the study. Examinations of patients were obtained through the hospital database. After Vitamin D values, also CRP, neutrophil, and lymphocyte values were retrospectively obtained from the hospital database; Vitamin D levels and CRP and neutrophil and lymphocyte values were compared. Being under 18 and over 85 are the criteria for being excluded from the study.

Neutrophil and lymphocyte values were studied with Sysmex XN 1000 hemogram device in our hospital. A laser optical method is used.

CRP is studied with Roche Cobas 8000 device by the Turbidimetric method. Vitamin D is studied by the chemiluminescence method with Siemens Advia Centaur XP hormone device.

**Statistics:** GraphPad Prism version 7.00 was used for statistical analysis (GraphPad Software, La Jolla California USA). After calculating quantitative data, the existence of the relationship between variables was investigated by applying the Spearman correlation test. Correlation coefficient (r) was evaluated as weak from 0.00 to 0.24, medium from 0.25 to 0.49, strong from 0.50 to 0.74, very strong from 0.75 to 1.00. The parameters measured in Covid-19 positive male and female patients were compared with the non-parametric test (Mann-Whitney test).

Results were obtained at a 95% confidence interval and a statistical significance level of p <0.05.

## RESULTS

There was a positive strong correlation between n/L and CRP, and a moderate positive correlation between CRP and age, n/L and age, sed and age, sed and CRP, sed and n/L, fer and n/l. A weak positive correlation between fer and age, fer and CRP, fer, and sed was noted.

* The correlation coefficient (r) shows that the relationship between parameters is statistically significant (p<0.05).

There was a very strong positive correlation between CRP and sed, CRP and fer, n/l and fer, and a strong positive correlation between age and n/l, age and sed, CRP and n/l, n/l and sed, sed and fer, age and CRP, and moderately positive correlation between age and fer.

* Correlation coefficient (r) shows that the relationship between parameters is statistically significant (p<0.05).

**Table 1.** Independent t-test results between biochemical results of Covid-19 patients with Vitamin D levels ⩽20 and >20

| Parameters | Vit d ⩽ 20 (n=33) (13.06 ± 1.10) | Vit d > 20 (n=13) (27.12 ± 1.76) | p value* |
|---|---|---|---|
| **Age** | 52.61 ± 2,415 | 44.62 ± 6.19 | 0.3612 |
| **Crp** | 23.36 ± 6.49 | 28.96 ± 14.20 | 0.9185 |
| **n/l** | 2.69 ± 0.42 | 2.64 ± 0.62 | 0.7770 |
| **Sed** | 34.42 ± 4.67 | 27.08 ± 5.0 | 0.5344 |
| **Fer** | 126.3 ± 19.59 | 151.6 ± 47.49 | 0.9089 |

* Those with a p-value of less than 0.05 were considered statistically significant.

**Table 2.** Inter-parameter correlation analysis in Covid-19 positive patients with Vitamin D level ⩽20

| Parameters | | age | CRP | n/l | sed | fer |
|---|---|---|---|---|---|---|
| **age** | r | 1 | 0.421* | 0.313 | 0.367* | 0.246 |
| **CRP** | r | 0.421* | 1 | 0.650* | 0.495* | 0.124 |
| **n/l** | r | 0.313 | 0.650* | 1 | 0.334 | 0.252 |
| **sed** | r | 0.367* | 0.495* | 0.334 | 1 | 0.090 |
| **fer** | r | 0.246 | 0.124 | 0.252 | 0.090 | 1 |

Correlation coefficient (r) was evaluated as weak from 0.00 to 0.24, medium from 0.25 to 0.49, strong from 0.50 to 0.74, and very strong from 0.75 to 1.00.

**Table 3.** Correlation analysis between parameters in Covid-19 positive patients with Vitamin D level > 20

| Parameters | | age | CRP | n/l | sed | fer |
|---|---|---|---|---|---|---|
| age | r | 1 | 0.392 | 0.535 | 0.609* | 0.417 |
| CRP | r | 0.392 | 1 | 0.694 | 0.862* | 0.801* |
| n/l | r | 0.535 | 0.694* | 1 | 0.538 | 0.774* |
| sed | r | 0.609* | 0.862* | 0.538 | 1 | 0.704* |
| fer | r | 0.417 | 0.801* | 0.774* | 0.704* | 1 |

Correlation coefficient (r) was evaluated as weak from 0.00 to 0.24, medium from 0.25 to 0.49, strong from 0.50 to 0.74, very strong from 0.75 to 1.00.

## DISCUSSION

Our study is a retrospective study with 46 patients. Patients with vitamin D >20 had a strong correlation between vitamin D and NLR, and a moderate correlation between vitamin D and CRP. In other words, the increased vitamin D level of the patients did not show itself in the form of a decrease in inflammation and a decrease in the parameters indicative of this. It was increasing in correlation with Vitamin D.

Patients with Vitamin D <20 also had a positive correlation between Vitamin D and CRP and NLR. This means that in patients with low Vitamin D levels, the infection is expected to be more severe and reflected in the form of high NLR and CRP, but the opposite happens, and NLR and CRP also fall.

NLR and CRP are important markers of increased inflammation and sepsis. Vitamin D reduces the apoptosis of alveolar cells, increases surfactant synthesis, and has been shown in many studies to be protective in respiratory infections with its anti-inflammatory role (10). Many studies conducted so far showed that vitamin D deficiency increases the risk of Covid-19, and its supply is protective (11).

In Indonesia, Raharus P and friends (12) studied with 780-person and noted that low vitamin D levels were associated with increased mortality in Covid-19 patients.

But in some recent studies, they have not found an association between Vitamin D levels and Covid-19 severity, as in our study. Hastie C.E and friends (13) could not show an association between Vitamin D concentration and Covid-19 in a large-scale study involving 502,604 participants aged 37-73 through UK Biobank. We did not see an increase in CRP and NLR as expected in people with vitamin D below 20 who were expected to have more serious infections. On the contrary, we observed a decline in them with a positive correlation. Sabetta J.R and friends (14) conducted a large-scale study to examine the relationship between Covid-19 risk and serum vitamin D and found no significant relationship.

Despite this, Mardani R (15) noted that low serum vitamin D levels increased hospitalization rates in Covid-19 and they observed higher neutrophil-lymphocyte ratio and vitamin d levels in Covid-19 patients compared to the control group. They found a statistically significant difference.

In a study with 348 598 UKBiobank participants, Hastie C.E and friends did not reach any findings indicating a relationship between the risk of infection and vitamin D level (16).

## CONCLUSION

Our study was retrospective, so we did not know about the socioeconomic levels of patients, their living conditions, whether they had a sedentary lifestyle, or their ultraviolet exposure. While evaluating the deficiency of vitamin D, it should be considered the contribution of this situation to occurrence of the covid-19 disease or as a result of covid-19 disease. For the purpose of clarifying the subject, we believe that studies with a larger number of groups will be useful. In addition, the fact that we did not show a direct relationship in our study, it does not mean that patients are deprived of vitamin D supplements. Vitamin D support is required and should be done as needed.

## REFERENCES

1.  Lu H.Drug treatment options for the 2019-new coronavirus. BioScience Trends. 2020; 14(1):69-71.DOI: 10.5582/bst.2020.01020

2.  World Health Organization. Coronavirus disease 2019 (COVID-19): situation report, vol. 167 https://apps.who.int/iris/bitstream/handle/10665/333146/nCoVsitrep05Jul2020-eng.pdf?sequence=1&isAllowed=y Available 22.01.2021

3.  Zhou P, Yang XL, Wang XG, Zhang L, Zhang W, Si HR et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579(7798): 270–273.Published online 2020 Feb 3. doi: 10.1038/s41586-020-2012-7

4.  Drosten C, Günther S. , Preiser W, Werf S., Brodt H., Becke. s Identification of a novel coronavirus in patients with severe acute respiratory syndrome N Engl J Med 2003 May 15;348(20):1967-76. doi: 10.1056/NEJMoa030747.

5.  Ren L.L., Wang Y.M., Wu Z.Q  Wu, Zhi-Qiang4; Xiang,et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. Chinese Medical Journal: May 5, 2020 - Volume 133 - Issue 9 - p 1015-1024doi: 10.1097/CM9.0000000000000722

6.  Holick, M.F. Vitamin D deficiency N. N Engl J Med 2007; 357:266-281 DOI: 10.1056/NEJMra070553

7. Rondanelli, M.; Miccono, A.; Lamburghini, S. Avanzato I , Riva A 2, Allegrini Pet al. Self-Care for Common Colds: The Pivotal Role of Vitamin D, Vitamin C, Zinc, and Echinacea in Three Main Immune Interactive Clusters (Physical Barriers, Innate and Adaptive Immunity) Involved during an Episode of Common Colds-Practical Advice on Dosages and on the Time to Take These Nutrients/Botanicals in order to Prevent or Treat Common Colds Evid Based Complement Alternat Med 2018 Apr 29;2018:5813095.doi: 10.1155/2018/5813095. eCollection 2018.

8. Schwalfenberg, G.K A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency. Mol Nutr Food Res2011 Jan;55(1):96-108. doi: 10.1002/mnfr.201000174.

9. Marnell L. Mold C, Du Clos TW. C-reactive protein: ligands, receptors and role in inflammation. Clinical Immunology Volume 117, Issue 2, November 2005, Pages 104-111 https://doi.org/10.1016/j.clim.2005.08.004

10. Cannell JJ, Vieth R, Umhau JC, et al. Vitamin D attenuates lung injury via stimulating epithelial repair, reducing epithelial cell apoptosis and inhibits TGF-β induced epithelial to mesenchymal transition . Epidemiol Infect.134( 2006),pp.1129-40.

11. Glicio EJ. Vitamin D level of mild and severe elderly cases of COVID-19: a preliminary report

12. Raharusun P., Priambada S., Budiarti C.,. Agung E, Budi C et al.Patterns of COVID-19 mortality and vitamin D: an Indonesian study. SSRN J. (2020), pp1-14

13. Hastie C.E, Mackay D.F, Ho .F, Morales C.A.C Katikireddi V. S. Niedzwiedz C.L. et al. Vitamin D concentrations and COVID-19 infection in UK Biobank. Diabetes & Metabolic Syndrome: Clinical Research & Reviews.14 (2020) pp 561-565 https://doi.org/10.1016/j.dsx.2020.04.050

14. Sabetta J.R. Depetrillo P,Cipriani.R.J. Smardin J, Burns L.A, Landry ML et al. Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults. PLoS One 2010 Jun 14;5(6):e11088. doi: 10.1371/journal.pone.0011088.

15. Mardani R, Alamdary A, Nasab M.S, Gholami R. Ahmadi N. Gholami A. et al. Association of vitamin D with the modulation of the disease severity in COVID-19 Virus Research Volume 289, November 2020, 198148 https://doi.org/10.1016/j.virusres.2020.198148

16. Hastie. C, Mackay D.F, Ho F, Carlos A., Celis-Morales C.A., Katikireddi V.S., Claire L. Niedzwiedz C.L. et al. Vitamin D concentrations and COVID-19 infection in UK Biobank. Diabetes Metab Syndr. 2020 Jul-Aug; 14(4): 561–565.doi: 10.1016/j.dsx.2020.04.050