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CLINICAL STUDY

Is there a relationship between vitamin D levels, inflammatory parameters, and clinical severity of COVID-19 infection?

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ABSTRACT

OBJECTIVES: This study is aimed to determine the relationship between 25-OH vitamin D levels, inflammatory parameters of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), c-reactive protein (CRP) levels and the disease severity of COVID-19 infection.

BACKGROUND: Inflammation plays a key role in the pathogenesis of COVID-19 while identifying the clinical course and prognosis. The effect of vitamin D deficiency on contribution to inflammation in COVID-19 is unclear.

METHODS: Based on the classification of the clinical course of COVID-19, the patients were divided into three groups, i.e., with mild (Group 1), moderate (Group 2) and severe/critical cases (Group 3). The 25-OH vitamin D values were defined as deficient, insufficient or normal.

RESULTS: There were no statistically significant differences in the distribution rates of 25-OH vitamin D levels ($p>0.05$) between the groups. Inflammatory parameters in Group 3 were statistically significantly higher as compared to Groups1 and 2 ($p<0.05$). Multivariate logistic regression analysis revealed that NLR was an independent predictor of disease severity.

CONCLUSION: There is no relationship between the severity of COVID-19 infection and 25-OH vitamin D deficiency. Inflammatory parameters are associated with the disease severity, while NLR is an independent predictor of severe COVID-19. There was no correlation between 25-OH vitamin D and inflammatory markers *(Tab. 4, Fig. 1, Ref. 38).* Text in PDF *www.elis.sk*

KEY WORDS: COVID-19 pandemic, severity of illness, inflammation, vitamin D.

Introduction

In December 2019, cases of pneumonia with clinical findings such as fever, cough, and dyspnea were reported in Wuhan, China. Genome analysis from respiratory samples revealed that this was caused by a new type of beta coronavirus infection manifesting as a severe acute respiratory syndrome. On January 30, 2020, the World Health Organization named this syndrome a coronavirus disease of 2019 (COVID-19) (1).

It is known that vitamin D deficiency increases the susceptibility to respiratory virus infections and severity of infections. Vitamin D reduces the incidence of microbial infections and mortality by means of three mechanisms: it prevents the disruption of tight, *adherens*, and gap junctions with physical barrier mechanisms, increases cellular immunity, and modulates adaptive immunity (2).

Therefore, Vitamin D can suppress cytokine production (3). Inflammatory storms play a key role in the pathogenesis of COVID-19 while identifying the clinical course and prognosis COVID-19 (4).

C-reactive protein (CRP) is a response to inflammation to prevent damage in the tissues and may be related to the severity of COVID-19 (3, 5). The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are also indirectly related to the patient's inflammatory status. It has been stated in the literature that NLR and PLR can be used in the diagnosis and prognosis of inflammatory diseases (6, 7).

Qun S et al (4) suggested that lymphocytopenia, which is common in COVID-19, may be associated with disease severity and mortality.

Vitamin D receptors are present in many organs and tissues such as immune and cardiovascular systems, particularly in the heart, lungs, kidneys, liver, as well as in nervous and intestinal systems, bones, and parathyroid gland (8, 9). Vitamin D deficiency has been associated with many diseases such as oncological disorders, cardiovascular diseases, and immune and inflammatory disorders (10). It has been reported that the risk factors for mortality in COVID-19 include hypertension, advanced age, male sex, obesity and coagulation associated with COVID-19 (11). Thrombocytopenia is

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common in COVID-19 and may be associated with disease severity (12). Therefore, vitamin D deficiency may also be associated with COVID-19 severity and mortality. Additionally, vitamin D may prevent multiorgan damage (13, 14).

Hence, many authors have argued that vitamin D can be used as a preventive and therapeutic treatment for COVID-19. To date, several studies investigated the relationship of the severity of COVID-19 infection with 25-OH vitamin D levels or other laboratory values (13), but there is a lack of those investigating the association between 25-OH vitamin D and inflammatory parameters of NLR and PLR in COVID-19 infection. The aim of this study was to determine the relationship between 25-OH vitamin D levels, inflammatory laboratory parameters and disease severity of COVID-19 infection.

Materials and methods

Patient enrolment methods and study parameters

In this retrospective study conducted at two tertiary care hospitals in Turkey, patients diagnosed with COVID-19 were retrospectively reviewed between April 1, 2020 and October 1, 2020. The study included 300 PCR test-positive patients whose 25-OH vitamin D levels were measured in the past 6 months. Patients with COVID-19 whose 25-OH vitamin D levels were not measured 6 months before the COVID-19 diagnosis were excluded from the study. Based on the classification of the clinical course of the disease, the participants were separated into three groups (15), namely group with *mild* cases (Group 1); participants showing mild clinical symptoms without pneumonia, group with *moderate* cases (Group 2)*;* participants with fever, other respiratory symptoms, and pneumonia findings based on radiological imaging, and group with *severe* or *critical* cases (Group 3)*;* participants with at least one of the symptoms as follows: hypoxia (≤93 % oxygen saturation), respiratory distress (RR > 30 times per minute), partial pressure of arterial blood oxygen (paO_2)/ fraction of inspired oxygen (FiO_2) ≤300 mmHg, chest imaging showing significant lung damage development within 24 to 48 hours, respiratory failure requiring mechanical ventilation, signs of septic shock with multiple organ failure requiring intensive care unit admission. Cases with severe or critical category of symptoms were placed in Group 3. The 25-OH vitamin D values below 20 ng/ml were defined as deficiency, values of 21–29 ng/ml were defined as insufficiency, and values of 30 ng/ml and above were defined as normal (10). All serum 25-OHvitamin D measurements were done by the chemiluminescent microparticle immunoassay method, ARCHITECT system brand kits were used with the Abbott i2000SR immunological analyzer. SARS-CoV-2 infection was confirmed in all 300 patients by positive real-time reverse-transcriptase PCR (RT-PCR) testing for SARS-CoV-2 nucleic acid on nasopharyngeal swabs (vNAT 2019-nCoV assay, Sariyer, Istanbul, Turkey). Patients' age, gender, comorbid diseases (hypertension, diabetes mellitus, chronic cardiovascular disease, chronic pulmonary disease, rheumatic disease, malignancy, chronic kidney disease, chronic cerebrovascular disease, endocrine diseases, immunosuppression), and laboratory values (25-OH vitamin D, white blood cell count, neutrophil count, neutrophil ratio, lymphocyte count, lymphocyte ratio, platelet count, hemoglobin count, CRP, NLR and PLR) were recorded. Demographic, comorbidity-related, and laboratory data of the patients included in the study were retrieved from electronic health records. This study was approved by the Ethics Committee of University Health Sciences Fatih Sultan Mehmet Training and Research Hospital (approval number: 2020/44).

This trial was registered at www.clinicaltrials.gov (Clinical Trial Number: NCT04945577).

Statistical analysis

In the evaluation of the results of the study, IBM SPSS Statistics 22 (IBM Corporation, NY, USA) software was used. In the evaluation of the study data, the normal distribution of the parameters was evaluated using the Shapiro-Wilk test. In the evaluation of the study data, descriptive statistical methods (mean, standard

Tab.1. Demographic characteristics, laboratory values. inflammatory parameters and serum 25-OH vitamin D levels in patients.

	Min–Max	Mean ± SD
Age	16–97	52.64±17.6
Vitamin D status(ng/ml)	0–68	17.97±11 (17.2)
	n	%
Sex		
Male	96	32
Female	204	68
Vitamin D status(ng/ml)		
Normal	33	11
Insufficient	77	25.7
Deficient	190	63.3
Death		
No	293	97.7
Yes	7	2.3
Comorbidity		
No	98	32.7
Yes	202	67.3
Hypertension	113	37.7
Diabetes mellitus	63	21
Cardiovasculer disease	34	11.3
Pulmonary disease	30	10
Rheumatic disease	26	8.7
Malignancy	9	3
Kidney disease	6	2
Cerebrovasculer disease	30	10
Immunsupression	2	0.7
Endocrine disease	24	8
White blood cell count (x10³/μL)	1.9–26.8	6.98±3.27 (6.2)
Neutrophil count (x10³/μL)	1–25.5	4.56±3.03 (3.8)
Neutrophil ratio (%)	6.5–95.2	62.34±13.22 (60.8)
Lymphocyte count (x10³/μL)	0.1–4.7	1.81±0.81 (1.8)
Lymphocyte ratio (%)	1.7–63.8	27.93±11.22 (29.3)
Neutrophil to lymphocyte ratio	0.34–46	3.62±5.07 (2.1)
Platelet to lymphocyte ratio	34.06–1120	165.12±126.54 (136.5)
Hb (g/dL)	6.2–17.10	12.85±1.85 (12.9)
Platelet count (x10³/μL))	9–643	240.84±83.74 (226)
C-reactive protein levels (mg/dL)	0.05–328	12.91±37.8 (2.9)

SD: standard deviation (p<0.05 statistically significant)

Tab. 2. Comparison of age, sex distribution, incidance rate of comobidities, laboratory values, inflammatory parameters and 25-OH vitamin D levels between groups.

Variables	Group1 Mean ± SD/Number (%) n = 130	Group2 Mean ± SD/Number (%) n = 132	Group3 Mean ± SD/Number (%) n = 38	Total Mean ± SD/Number (%) n = 300	p
Age					
<65	118 (90.8%)	92 (69.7%)	11 (28.9%)	221 (73.7%)	0.000*
≥ 65	12 (9.2%)	40 (30.3%)	27 (71.1%)	79 (26.3%)	
Sex					
Male	31 (23.8%)	48 (36.4%)	17 (44.7%)	96 (32%)	0.019*
Female	99 (76.2%)	84 (63.6%)	21 (55.3%)	204 (68%)	
Vitamin D status					
Normal	15 (11.5%)	14 (10.6%)	4 (10.5%)	33 (11%)	
Insufficient	36 (27.7%)	36 (27.3%)	5 (13.2%)	77 (25.7%)	0.420
Deficient	79 (60.8%)	82 (62.1%)	29 (76.3%)	190 (63.3%)	
Death					0.000*
No	130 (100%)	132 (100%)	31 (81.6%)	293 (97.7%)	
Yes	0 (0%)	0 (0%)	7 (18.4%)	7 (2.3%)	
Comorbidity					0.000*
No	55 (42.3%)	40 (30.3%)	3 (7.9%)	98 (32.7%)	
Yes	75 (57.7%)	92 (69.7%)	35 (92.1%)	202 (67.3%)	
Hypertension	36 (27.9%)	56 (42.4%)	21 (55.3%)	113 (37.8%)	0.003*
Diabetes mellitus	23 (17.7%)	30 (22.7%)	10 (26.3%)	63 (21%)	0.419
Cardiovasculer disease	6 (4.6%)	16 (12.1%)	12 (31.6%)	34 (11.3%)	0.000*
Pulmonary disease	12 (9.2%)	11 (8.3%)	7 (18.4%)	30 (10%)	0.175
Rheumatic disease	13 (10%)	11 (8.3%)	2 (5.3%)	26 (8.7%)	0.648
Malignancy	0 (0%)	4 (3%)	5 (13.2%)	9 (3%)	0.000*
Kidney disease	2 (1.5%)	4 (3%)	0 (0%)	6 (2%)	0.615
Cerebrovasculer disease	9 (6.9%)	8 (6.1%)	13 (34.2%)	30 (10%)	0.000*
Immunsupression	1 (0.8%)	1 (0.8%)	0 (0%)	2 (0.7%)	1.000
Endocrine disease	13 (10%)	10 (7.6%)	1 (2.6%)	24 (8%)	0.328
White blood cell count (x10^3/μL)	7.23±3.19 (6.4)	6.31±2.26 (5.9)	8.48±5.36 (6.8)	6.98±3.27 (6.2)	0.038*
Neutrophil count (x10^3/μL)	4.58±2.88 (3.9)	3.95±1.85 (3.7)	6.6±5.24 (5)	4.56±3.03 (3.8)	0.033*
Neutrophil ratio (%)	60.53±11.23 (58.8)	60.81±12.56 (59.8)	73.84±16.05 (73.5)	62.34±13.22 (60.8)	0.000*
Lymphocyte count (x10^3/μL)	1.99±0.78 (1.9)	1.78±0.76 (1.8)	1.28±0.87 (1)	1.81±0.81 (1.8)	0.000*
Lymphocyte ratio (%)	29.57±10.11 (30.4)	29.08±10.63 (29.9)	18.3±12.32 (17.6)	27.93±11.22 (29.3)	0.000*
Neutrophil to lymphocyte ratio	2.74±2.77 (1.9)	2.81±2.46 (2)	9.41±10.96 (4.3)	3.62±5.07 (2.1)	0.000*
Platelet to lymphocyte ratio	142.63±60.51 (133)	157.99±98.77(132.6)	266.82±263.66(165.6)	165.12±126.54(136.5)	0.046*
Hb (g/dL)	13.19±1.62 (13.1)	12.88±1.75 (12.9)	11.59±2.4 (11.8)	12.85±1.85 (12.9)	0.000*
Platelet count (x10^3/μL)	252.92±68.54 (253)	234.93±87.48 (218.5)	220.08±110.07 (205.5)	240.84±83.74 (226)	0.011*
C-reactive protein levels (mg/dL)	7.79±34.33 (0.9)	13.41±36.54 (2.9)	28.66±48.6 (10.4)	12.91±37.8 (2.9)	0.000*

SD: standard deviation (p<0.05 statistically significant)

deviation, frequency) were used, and in comparison of the quantitative data, one-way ANOVA test was used to compare parameters with normal distribution between the groups and Tukey HDS test to identify the group that caused the difference. Kruskal-Wallis test was used in intergroup comparisons of parameters without normal distribution and Dunn's test to identify the group that caused the difference. Student t-test was used for comparing parameters with normal distribution between two groups, and Mann-Whitney U test was used for comparing parameters without normal distribution between two groups. Chi-square test, Fisher's exact test, Fisher-Freeman-Halton test, and Yate´s correction for continuity were used to compare qualitative data. The multivariate logistic regression analysis was used for determining independent associations with disease severity. The receiver operating characteristic (ROC) analysis was performed for optimal cut-off values to predict the disease prognosis. Significance was evaluated at the level of $p<0.05$.

Results

Patient demographics characteristics and baseline laboratory values

In this study, 300 patients aged 52.64±17.60 years had their 25-OH vitamin D measurement result during the period of 6 months before their admission to the hospital. Of the 300 patients diagnosed with COVID-19 infection included in the study, 130 (43.3 %) were mild, 132 (44 %) were moderate, and 38 (12.7 %) were severe/critical cases. The most common comorbidities were hypertension (37.7 %), diabetes mellitus (21 %) and heart diseases (11.3 %). Table 1 presents baseline laboratory values of study.

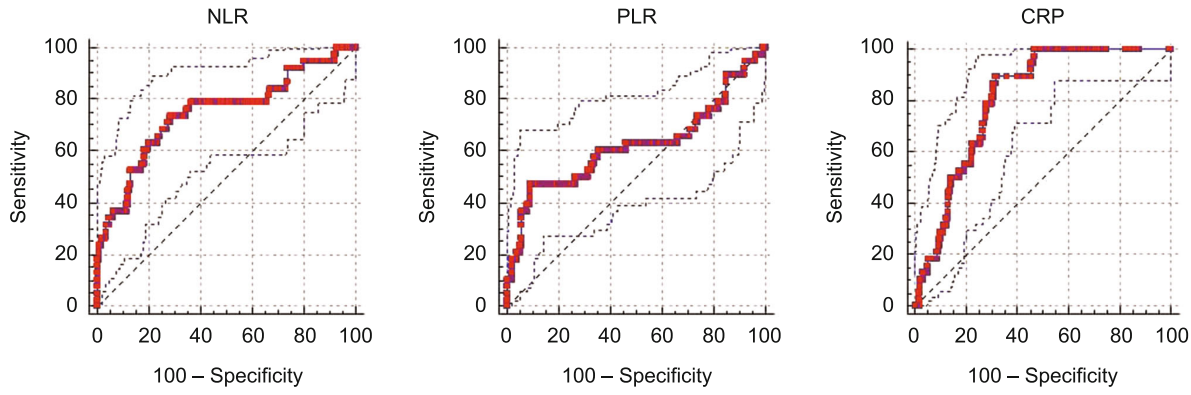

Fig. 1. Receiver operating characteristic curves (ROC) of NLR, (PLR) and C-reactive protein (CRP) for differentiating severe critical group from mild and moderate groups.

Clinical features and laboratory findings of COVID-19 patients stratified by illness severity

The mean age, incidence rate of mortality and incidence rate of comorbidities in Group 3 were higher as compared to those in Groups 1 and 2 ($p<0.05$). The mean age and incidence rate of comorbidities in Group 2 were statistically significantly higher compared to those in Group 1 ($p<0.05$). The female gender ratio in Group 1 was statistically significantly higher compared to those in Groups 2 and 3 ($p<0.05$). The incidence rate of HT in Group 1 was statistically significantly lower compared to those in Groups 2 and 3 ($p<0.05$). The incidence rate of cardiac diseases in Group 3 was statistically significantly higher compared to those in Groups 1 and 2 ($p<0.05$). The incidence rate of cardiac diseases in Group 2 was statistically significantly higher compared to that in Group 1 ($p<0.05$). The incidence rates of oncological and neurological diseases in Group 3 were statistically significantly higher compared to those in Groups 1 and 2 ($p<0.05$). Based on paired comparisons to determine the difference, white blood cell values of Group 1 were statistically significantly higher in comparison to Group 2 ($p<0.05$). Neutrophil count in Group 1 was statistically significantly lower as compared to Group 3 ($p<0.05$). Lymphocyte count and percentage in Group 3 were statistically significantly lower as compared to Groups 1 and 2 ($p<0.05$). Neutrophil percentage values in Group 3 were statistically significantly higher as compared to Groups 1 and 2 ($p<0.05$). Hemoglobin values in Group 3 were lower as compared to Groups 1 and 2 ($p<0.05$). Platelet count in Group 1 was statistically significantly higher as compared to Groups 2 and 3 ($p<0.05$). PLR and NLR in group 3 were statistically significantly higher as compared to Groups 1 and 2 ($p<0.05$). CRP values in Group 3 were statistically significantly higher compared to those in Groups 1 and 2 ($p<0.05$). CRP values in Group 1 were statistically significantly lower as compared to Group 2 ($p<0.05$). Table 2 summarizes the comorbid disease rates and laboratory values between groups.

25-OH vitamin D levels and vitamin D status

The mean 25-OH vitamin D level was 17.97±11 ng/mL. The numbers of patients with vitamin D insufficiency and deficiency were 77 (25.7 %) and 190 (63.3 %), respectively, while 11 % of the patients had a normal level of serum 25-OH vitamin D. No statistically significant differences in distribution rates of 25-OH vitamin D levels were found between the groups ($p > 0.05$). Vitamin D insufficiency and deficiency were present respectively in 27.7 % and 60.8 % of the patients with mild COVID-19. Vitamin D insufficiency and deficiency were present respectively in 27.3 % and 62.1 % of the patients with moderate COVID-19. Vitamin D insufficiency and deficiency were present respectively in 13.2 % and 76.3 % of the patients with severe/critical COVID-19 (Tab. 2).

Correlation between 25-OH vitamin D and inflammatory markers

There were no correlations with age ($r=0.006$; $p=0.920$), white blood cell count ($r=–0.023$; $p=0.686$), neutrophil count ($r=–0.005$; $p=0.930$), neutrophil ratio ($r=–0.001$; $p=0.993$), lymphocyte count ($r=–0.039$; $p=0.499$), lymphocyte ratio ($r=$

Tab. 3. Logistic regression analysis showing independent predictors of severe/critical COVID19 patients.

	OR	%95Cl	p
Age	8.734	3.557–21.446	0.000*
Malignancy	5.303	1.087–25.879	0.039*
Cerebrovasculer disease	8.170	2.893–23.073	0.000*
Neutrophil to lymphocyte ratio	1.182	1.090–1.281	0.000*

Logistic regression (forward LR), Cl – confidence interval, OR – odds ratio ($p<0.05$ statistically significant)

Tab. 4. ROC analysis for severe-critical COVID 19 patients.

	AUC (85% CI)	Cut-off	Sensitivity %	Specificity %	p
NLR	0.748 (0.695–0.796)	>2.8	73.7	71.8	0.001*
PLR	0.620 (0.563–0.675)	>236.1	47.4	91.2	0.048*
CRP	0.807 (0.758–0.850)	>3.13	89.5	68.3	0.001*

AUC – area under ROC curve; CRP – C-reactive protein; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio ($p < 0.05$ statistically significant)

–0.015; p=0.802), hemoglobin count (r=0.113; p=0.051), platelet count (r=0.025; p=0.669), CRP level (r=0.104; p=0.071), NLR (r=–0.002; p=0.975), and PLR (r=0.066; p=0.252).

Disease severity and its determinants

Multivariate logistic regression analysis revealed that only increased age, cerebrovascular disease, malignancy and NLR were significant independent predictors of disease severity in COVID-19 patients (Tab. 3).

ROC curve analysis

The optimal cut-off values calculated by the ROC analysis, and ROC curves are presented in Figure 1. The areas under the curve (AUC) of CRP, NLR, and PLR were found to be 0.807 (p=0.001), 0.748 (p=0.001), and 0.620 (p=0.048), respectively. Table 4 presents the optimal cut-off values for all the parameters.

Discussion

Our study results suggest that there is no relationship between the severity of COVID-19 infection and 25-OH vitamin D deficiency. Vitamin D insufficiency (25.7 %) and deficiency (63.3 %) were common in patients with COVID-19. A proportion of 11 % of the COVID-19 patients had a normal level of serum 25-OH vitamin D in our study. The logistic regression analysis showed that the 25-OH vitamin D level was not associated with disease severity and revealed that only age, cerebrovascular disease, malignancy, and NL ratio were significant independent predictors of disease severity. According to ROC analysis CRP, NLR, and PLR might be considered as effective prognostic tools. However, no correlation was found between 25-OH vitamin D, CRP, NLR and PLR.

The pathogenesis of COVID-19 involves the activation of innate and adaptive immune responses (4). Coronavirus enters the cell via angiotensin converting enzyme 2 (ACE2) receptors (16, 17). ACE2 degrades angiotensin 2 to angiotensin 1–7, angiotensin 1 to angiotensin 1–9, and shows anti-inflammatory, antioxidative, and vasodilator effects (16, 18). COVID-19 leads to a decrease in ACE2 activity and increase in angiotensin 2 and causes acute lung injury. Increased proinflammatory cytokines and chemokines lead to a cytokine storm, which affects the clinical course and prognosis of the disease by causing multiple systemic and respiratory symptoms (16, 19).

Vitamin D has anti-inflammatory, antioxidative, and immunomodulatory properties as well as important effects on the musculoskeletal system (20, 21). Vitamin D reduces the incidence of microbial infections and mortality by regulating innate and adaptive immunity (2). Vitamin D simultaneously increases the innate immune response, suppresses cytokine production, decreases pathogen load, reduces the overactivation of adaptive immunity, and suppresses Th1-mediated inflammatory cytokines and chemokines, thereby creating an adequate response to the pathogen load (16, 18). Dysregulation of the adaptive and innate immune response due to vitamin D deficiency contributes to the cytokine storm (21). Vitamin D can limit the inflammatory cytokine storm that may lead to ARDS and acute lung injury (16).

Calcitriol, the active form of vitamin D ($1,25(OH)_2D_3$), is an important modulator of both innate and adaptive forms of immunity (22) and is protective against respiratory viral infections (17, 18, 20). Extrarenal conversion of 25-OH vitamin D to calcitriol, takes place in the bronchial epithelium and immune cells (20). Calcitriol prevents acute lung injury and progression to ARDS by modulating the renin-angiotensin-aldosterone system and ACE2 (17, 20, 23). ACE2-receptor-expressing type 2 pneumocytes are one type of the cells that COVID-19 targets. Loss of function in type 2 pneumocytes causes impaired surfactant synthesis and an increase in surface tension in the respiratory tract. Calcitriol decreases pneumocyte apoptosis and increases surfactant synthesis (17, 20).

Considering the safety, cost effectiveness, and ease of availability of vitamin D, it is important to determine its efficacy in the management of coronavirus infection (20, 24). Several studies on vitamin D and disease severity reported that vitamin D is associated with disease severity and mortality (16, 25–27). On the other hand, Hastie et al (28) found no relationship between 25-OH vitamin D levels and coronavirus infection. In their study, baseline measurements including 25-OH vitamin D levels were obtained a decade ago. Hastie et al (28) emphasized that they could have reached this conclusion due to potential confounders such as ethnicity. Similarly, Raisi-Estabragh et al (29) found no relationship between 25-OH vitamin D levels and COVID-19 positivity. Although both COVID-19-negative and COVID-19-positive patients were found to have low vitamin D levels, Raisi-Estabragh et al (29) stated that calcitriol was actually responsible for immune functions and that the results of the study could be controversial as they evaluated 25-OH vitamin D levels. They also emphasized the potential confounders such as ethnicity and body mass index (29). Similarly in the present study, we found no relationship between vitamin D level and disease severity, however, the vitamin D levels were low in all study groups. This may be attributed to the following: relatively small number of patients, the fact that vitamin D levels were not measured right before the COVID-19 diagnosis, and presence of confounders such as age, gender, obesity, prescribed drugs and comorbidities. However, considering the functions of active vitamin D, calcitriol, we believe that more objective data can be obtained in future studies by directly evaluating the serum level of calcitriol instead of measuring 25-OH vitamin D levels. Additionally, $1,25(OH)_2D_3$ synthesized in extrarenal tissues is of utmost importance in terms of immunomodulation in local tissues. The level of vitamin D does not solely depend on the 25-OH vitamin D level; it is also necessary to measure $1,25(OH)_2D_3$ levels (17, 30). Mok et al (24) stated that calcitriol could be used as a prophylactic adjunct against a potential COVID-19 infection.

Inflammatory parameters have a prognostic value in systemic inflammatory diseases (31). CRP is an easily measured inflammatory marker (32). Recently, NLR and PLR have also been used as inflammatory markers in different diseases, including infections (33). Vitamin D, another inflammatory marker, has skeletal and extra-skeletal functions (8, 34). Vitamin D deficiency is associated with inflammation-related diseases such as those affecting

the cardiovascular, renal, and autoimmune systems, infections, anemia, depression, cognitive dysfunction, and cancer (33, 34). Studies on the relationship between vitamin D and inflammatory markers have provided conflicting results (31–35). Although we have stated the anti-inflammatory properties of vitamin D in our study, some studies have reported that inflammation leads to decreased vitamin D levels (34). However, it is difficult to establish the causal relationship between any disease and vitamin D, as insufficient daylight or nutritional deficiencies can also cause vitamin D deficiency (10, 36). In order to elucidate this issue, randomized controlled studies with a large number of patients are warranted in the future. In literature, there are studies emphasizing that CRP is associated with vitamin D deficiency in coronavirus infection (3, 19), and this is attributed to the relationship among vitamin D deficiency, inflammation, and cytokine storm in patients with COVID-19 (3). Unlike the findings of previous studies, the results in this study did not show a correlation between vitamin D levels and inflammatory markers.

Leukocytosis, neutrophilia, thrombocytopenia, and lymphopenia are observed in COVID-19, particularly in patients with severe disease progressing to ARDS (7). COVID-19 leads to a decrease in platelet production as well as to an increase in thrombocyte consumption due to lung damage (37, 38). The immune response is mainly mediated by lymphocytes in viral infections (37). The mechanism of lymphopenia in COVID-19 is associated with the destruction of lymphatic tissues and T-cell apoptosis induced by cytokines (37). With increased proinflammatory cytokines in severe COVID-19, T-cell lymphopenia leads to a predisposition to the cytokine storm. This causes an increase in NLR and PLR (7). The present study found that NLR, PLR, and CRP were important in determining the severity of the disease, which was consistent with literature (4, 7, 37, 38). In addition, NLR was found to be the best predictor of severe/critical COVID-19.

The study had some limitations. Firstly, the study was retrospective and further prospective studies should be conducted to determine the factors affecting vitamin D levels. Secondly, although the measurement of vitamin D levels right before the coronavirus infection is a challenging undertaking, it could be meaningful as it would eliminate the seasonal change, and effect of inflammation on these levels. Thirdly, the study should have been conducted with a larger number of patients.

Conclusion

This study found no relationship between vitamin D levels and disease severity. On the contrary, CRP, PLR, and NLR values were associated with disease severity, among which NLR was an independent predictor of severe/critical course of COVID-19. Additionally, we could not detect a relationship between inflammatory markers and 25-OH vitamin D levels in COVID-19.

Learning points

- Vitamin D deficiency may aggravate pro-inflammatory immune responses.
- Inflammation plays a key role in the pathogenesis of COVID-19. CRP is an easily measured inflammatory marker. Recently, NLR and PLR have also been used as inflammatory markers in different diseases, including infections.
- The study included 300 PCR test-positive patients whose 25-OH vitamin D levels were measured in the past 6 months.
- There is no relationship between vitamin D levels and disease severity. Inflammatory parameters were associated with disease severity, among which NLR was an independent predictor of severe/critical course of COVID-19. Additionally, there was no relationship between inflammatory markers and 25-OH vitamin D levels in COVID-19.

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