Araştırma Makalesi *Research Article*

Pamukkale Tıp Dergisi
Pamukkale Medical Journal

doi:https://dx.doi.org/10.31362/patd.1080912

Does the plasma vitamin D level affect the severity of infection in COVID-19 patients of different age groups?

Plazma D vitamini düzeyi farklı yaş gruplarındaki COVID-19 hastalarında enfeksiyonun şiddetini etkiler mi?

Dilek Güvenç, Aynur Atilla, Bahattin Avcı, Vedide Rezan Uslu, Sedat Gökmen, Fatih Temoçin, Tuba Kuruoğlu

Gönderilme tarihi:01.03.2022 Kabul tarihi:06.04.2022

Abstract

Purpose: SARS-CoV-2 has caused an on-going global pandemic of COVID-19 disease. Vitamin-D has an immunomodulatory effect on the disease by suppressing the adaptive immune system which can lead to a cytokine storm, and boosting the innate immune system. This study evaluated the relationship between both the clinical characteristics of COVID-19 patients and the severity of their infections, and their serum Vitamin D levels.

Material and methods: Forty COVID-19 patients from the period April to July, 2020, and 46 healthy subjects from a similar period in 2019, were included. Serum Vitamin-D level, clinical findings, comorbidities, chest computed tomography findings, hematological and serum biochemistry analyzes of the patients were evaluated.

Results: COVID-19 patients had a significantly lower mean serum 25(OH) Vitamin-D level (12.86±6.27 ng/mL) than healthy subjects (25.4±12.7 ng/mL) (p<0.001). The prevalence of Vitamin D deficiency in COVID-19 patients was high, not only in the elderly, but also in middle age and young patients. Ground-glass opacification and paving stone sign were the most frequent patterns observed in chest-computed tomography (CT) images. There was a significant negative relationship between Vitamin-D deficiency and C-reactive protein (CRP) level (p=0.0243). In addition, a high CRP level was associated with abnormal CT findings (p=0.001).

Conclusion: The authors conclude that determining the Vitamin-D level in COVID-19 patients and administering it at the appropriate dosage can reduce the severity and progression of COVID-19 disease by contributing to the regulation of the cytokine storm and pulmonary inflammatory response.

Key words: Computed tomography, COVID-19, vit D, Laboratory findings.

Guvenc D, Atilla A, Avci B, Uslu VR, Gokmen S, Temocin F, Kuruoglu T. Does the plasma vitamin d level affect the severity of infection in COVID-19 patients of different age groups? Pam Med J 2022;15:499-512.

Öz

Amaç: SARS-CoV-2, devam eden küresel COVID-19 pandemisine sebep olmuştur. D vitamini, sitokin fırtınasına yol açabilen adaptif immun yanıtı baskılayarak ve doğuştan gelen immun yanıtı güçlendirerek immunmodulatör etki oluşturur. Bu çalışmada, COVID-19 hastalarının klinik özellikleri, enfeksiyonlarının şiddeti ve serum D vitamini düzeyleri arasındaki ilişki değerlendiridi.

Gereç ve yöntem: Nisan-Temmuz 2020 döneminde 40 COVID-19 hastası ve 2019'daki aynı döneme ait 46 sağlıklı denek çalışmaya dahil edildi. Hastaların serum D vitamini düzeyi, klinik bulgular, komorbiditeler, akciğere ait bilgisayarlı tomografi bulguları, hematolojik ve serum biyokimya analizleri değerlendirildi.

Bulgular: COVID-19 hastalarının ortalama serum 25(OH) D vitamini düzeyi (12,86±6,27 ng/mL) sağlıklı deneklere (25,4±12,7 ng/mL) göre anlamlı derecede düşük belirlenmiştir (p<0,001). COVID-19 hastalarında D vitamini eksikliği prevalansı sadece yaşlılarda değil orta yaş ve genç hastalarda da yüksek bulunmuştur. Göğüs bilgisayarlı tomografi (BT) görüntülerinde en sık gözlenen paternler buzlu cam opaklaşması ve kaldırım taşı görüntüsü olmuştur. Vitamin D eksikliği ile C-reaktif protein (CRP) düzeyi arasında negatif ilişki belirlenmiştir (p=0,0243). Ayrıca yüksek CRP düzeyinin anormal BT bulguları ile ilişkili olduğu saptanmıştır (p=0,001).

Sonuç: COVID-19 hastalarında D vitamini seviyesinin belirlenmesi ve uygun dozda uygulanmasının sitokin fırtınasının ve pulmoner inflamatuar yanıtın düzenlenmesine katkıda bulunarak COVID-19 hastalığının şiddetini ve ilerlemesini azaltabileceği sonucuna varılmıştır.

Dilek Güvenç, Assoc. Prof. Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ondokuz Mayis, Samsun, Turkey, e-mail: dguvenc@omu.edu.tr (https://orcid.org/0000-0003-0036-0914) (Corresponding Author)

Aynur Atilla, Assoc. Prof. Department of Infectious Disease and Clinical Microbiology, Faculty of Medicine, University of Ondokuz Mayis, Samsun, Turkey, e-mail: aynura@omu.edu.tr (https://orcid.org/0000-0001-8027-1991)

Bahattin Avcı, Prof. Department of Biochemistry, Faculty of Medicine, University of Ondokuz Mayis, Samsun, Turkey, e-mail: b.avci@omu.edu.tr (https://orcid.org/0000-0001-6471-6495)

Vedide Rezan Uslu, Prof. Department of Statistics, Faculty of Art and Sciences, University of Ondokuz Mayis, Samsun, Turkey, e-mail: rezzanu@omu.edu.tr (https://orcid.org/0000-0003-2468-4956)

Sedat Gökmen, MSc, Department of Laborant and Veterinary Health, Suluova Vocational School, Amasya University, Amasya, Turkey, e-mail: sedat.gokmen@amasya.edu.tr (https://orcid.org/0000-0002-4793-3030)

Fatih Temoçin, Assoc. Prof. Department of Infectious Disease and Clinical Microbiology, Faculty of Medicine, University of Ondokuz Mayis, Samsun, Turkey, e-mail: fatih.temocin@omu.edu.tr (https://orcid.org/0000-0002-4819-8242)

Tuba Kuruoğlu, Ass. Prof. Department of Infectious Disease and Clinical Microbiology, Faculty of Medicine, University of Ondokuz Mayis, Samsun, Turkey, e- mail: tuba.kuruoglu@omu.edu.tr (https://orcid.org/0000-0003-3805-367X)

Anahtar kelimeler: Bilgisayarlı tomografi, COVID-19, vit D, laboratuvar bulguları.

Güvenç D, Atilla A, Avcı B, Uslu VR, Gökmen S, Temoçin F, Kuruoğlu T. Plazma D vitamini düzeyi farklı yaş gruplarındaki COVID-19 hastalarında enfeksiyonun şiddetini etkiler mi? Pam Tıp Derg 2022;15:499-512.

Introduction

The variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, COVİD-19), particularly Delta and Omicron, have emerged and the epidemic still continues with different symptoms [1]. Complications that cause patients to be hospitalised are primary acute respiratory distress syndrome (ARDS), severe pneumonia, acute respiratory injury and acute renal failure [2].

A weak innate immune system response can lead to a higher load of COVID-19 virus. Consequently, the adaptive immune system is over-activated resulting in a 'cytokine storm' [3, 4].

Vitamin D (VD) deficiency is a significant problem for global public health, with a reported VD deficiency prevalence of 63% in Turkey, and 50% in the city of Samsun [5, 6]. There is a relationship between low VD levels and increased susceptibility to respiratory infections [7]. *In vitro* and clinical trials have shown that VD is effective against many respiratory viruses [8, 9]. The antiviral activity of VD occurs via interference with viral replication, by down-regulating the receptors for the virus in host cells, and showing immunomodulatory and anti-inflammatory effects [10]. VD has an immunomodulatory effect by suppressing the adaptive immune system, which can lead to cytokine storm, and enhancing the innate immune system [11, 12]. In addition, VD is involved in increasing the production of antimicrobial peptides such as cathelicidin and defensin β2, which act as barriers to inhibit pathogen invasion in the respiratory epithelium [4].

The SARS-CoV-2 enters the cell through the angiotensin converting enzyme 2 (ACE2) receptor, which is regulates the renin-angiotensin system (RAS) [13, 14]. The protective effect of VD on the lungs by modulation of RAS , regulation of ACE2 expression, decreasing pulmonary vascular permeability and preventing alveolar capillary damage was previously reported [15, 16]. Previous retrospective studies that measured VD levels of patients a long time before contracting COVID-19 may not reflect their current VD levels, so "real time" data is required [17].

Based on that, we aimed to investigate whether there is a relationship between clinical characteristics, severity of infection and the VD level of patients diagnosed with COVID-19, regardless of age, in a city north of Turkey.

Materials and methods

Study population

This single-center prospective study was done in Ondokuz Mayis University (OMU), Faculty of Medicine, Infectious Diseases and Clinical Microbiology Department in Samsun, Turkey between April 11 and July 3, 2020. Forty patients were divided equally amongst gender numbers and normal or abnormal CT findings. No patient was in intensive care. The diagnosis of COVID-19 was confirmed by real-time reverse transcriptase–polymerase chain reaction (RT-PCR) of nasopharyngeal or nasal swabs. The serum 25-hydroxy VD levels of 46 healthy COVID-19 free individuals (22 male, 24 female, mean age 43.63), whose data were obtained from an electronic health record database at the same faculty in a similar period in 2019, were used as the control group.

Ethics committee approval was granted by the Ondokuz Mayis University Clinical Research Ethics Committee and was conducted in accordance with the principles of the Declaration of Helsinki. Measurement of levels of 25(OH) VD.

Blood samples were centrifuged at 3000 x g for 10 minutes and the sera were stored at -80^{0}C. The concentrations of VD in all serum samples were analyzed with liquid chromatography tandem mass spectrometry (LC-MS/MS), a Thermo Scientific TSQ Ouantum Access Max Triple Quadrupole Mass Spectrometer (USA) device and 25-OH-Vitamin D2/D3 assay kits (Recipe® Chemicals ClinMass® LC-MS/MS, Ref. No. MS7000, Munich, Germany). The limit of detection and the lower limit of quantification of the test are 0.7 ng/mL and 2.3 ng/mL,

respectively. Intra- and inter-assay precisions of the test are 4.7/3.3% and 6.6/4.2%, respectively.

Hematological and serum biochemistry analysis

A Sysmex Automated Hematology Analyzer XN-1000 (Japan) was used for hematological analysis. Serum D-dimer levels were determined with an Afias-6 Boditech analyzer (Korea) and other serum parameters were determined with a Roche Hitachi Cobas 8000 (Japan) device in combination with Roche Diagnostics GmbH kits. All assays were conducted according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed by usi ng SPSS Version 25.0 (SPSS Inc., Chicago, Illinois, USA) for Windows. Differences between groups were tested with the two sample-t test (independent test) and Chi-square test was used to determine the relationship between categorical variables. Before applying the statistical tests, we checked whether the assumptions required by the particular test were satisfied by the data. Data were transformed by using the Box-Cox transformation methodology to ensure a normal distribution. To compare the data for non-parametric variables, we used the Mann-Whitney-U test that corresponds to the two sample t test. All data were expressed as the mean ± standard deviation (SD). The statistical significance was set at $p<0.05$.

Results

Participants' characteristics

Patients were divided into three groups based on their serum VD status; VD level <20 ng/mL (deficient), 21-29 ng/mL (insufficient), and 30-60 ng/mL (sufficient). The demographics and baseline characteristics of the patients with respect to VD groups are shown in Table 1.

Table 1. Demographics and baseline characteristics of the COVID-19 patients in vitamin D groups

	All Patients (n=40)	Vit D Deficiency (n=32)	Vit D Insufficient (n=6)	Vit D Sufficient (n=2)
Age (years)	42.35	42.03125	48.166667	30
20-30	13 (32.5%)	12 (37.5%)		1 (50%)
30-60	20 (50%)	14 (43.15%)	5 (83.33%)	1 (50%)
60-85	7 (17.5%)	6 (18.75%)	1 (16.67%)	
Gender				
Female	20 (50%)	17 (53.125%)	1 (16.67%)	2 (100%)
Male	20 (50%)	15 (46.875%)	5 (83.33%)	
Symptoms and Signs				
Cough	26 (65%)	20 (62.5%)	4 (66.67%)	2 (100%)
Fever	19 (47.5%)	15 (46.875%)	3 (50%)	1 (50%)
Headache	17 (42.5%)	15 (46.875%)	2 (33.33%)	
Sore Throat	16 (40%)	15 (46.75%)	1 (16.67%)	
Muscle & Joint Pain	16 (40%)	14 (43.75%)	2 (33.33%)	
Shortness of Breath	11 (27.5%)	9 (28.125%)	2 (33.33%)	1 (50%)
Diarrhea	8 (20%)	7 (21.825%)		1 (50%)
Smell and Taste Dysfunction	5 (12.5%)	3 (9.375%)	2 (33.33%)	
Other Symptoms	3 (7.5%)	2 (6.25%)	1 (16.67%)	
Comorbidities				
Hypertension	7 (17.5%)	6 (18.75%)	1 (16.67%)	
Diabetes	4 (10%)	4 (15.5%)		
Hypothyroidism	3 (7.5%)	3 (9.375%)		
Cardiovascular Disease	2 (5%)	1 (3.125%)	1 (16.67%)	
Pulmonary Disease	2 (5%)	2 (6.25%)		
Immune system disease	2 (5%)	1 (3.125%)		1 (50%)

Table 1. Demographics and baseline characteristics of the COVID-19 patients in vitamin D groups

Osteoporosis	1 (2.5%)	1 (3.125%)		
Obesity	1 (2.5%)	1 (3.125%)		
Cancer	1 (2.5%)		1 (16.67%)	
Chest-Computed Tomography				
Normal chest tomography	20 (50%)	15 (46.875%)	5 (83.33%)	
Abnormal chest tomography	20 (50%)	17 (53.125%)	1 (16.67%)	2 (100%)
Multiple Ground-glass Opacities	11 (27.5%)	9 (28.125%)	1 (16.67%)	1 (50%)
Unique Ground-glass Opacities	9 (22.5%)	8 (25%)		1 (50%)
Paving Stone Sign	9 (22.5%)	7 (21.875%)	1 (16.67%)	1 (50%)
Consolidation	7 (17.5%)	6 (18.75%)		1 (50%)
Bilateral Lung Involvement	5 (12.5%)	5 (15.625%)		
Unilateral Lung Involvement	1 (2.5%)	1 (3.125%)		

Serum 25(OH) VD level

The mean levels of serum VD in the study and control groups were 12.86 (±6.27) ng/mL and 25.4 (±12.7) ng/mL, respectively (p<0.001). VD deficiency was very common (80%) among patients but there was no significant difference in the VD status for gender (p=0.115). Specifically, mean serum VD levels for males and females were 15.56 ng/mL and 13.62 ng/mL, respectively.

Clinical findings and comorbidities

All patients were discharged after receiving an average of 4.85 days of hospitalization (range 1-12 days) and no deaths occurred. All patients received standard drug treatments (hydroxychloroquine, chloroquine, favipiravir, azithromycin and enoxaparin), following the guidelines for COVID-19 [18], taking into account the patient's general health status and comorbidity situation.

Chest computed tomography evaluation

The most common CT findings were multiple ground-glass opacities, unique ground-glass opacities and paving stone (Table 1). A large proportion of patients with abnormal CT findings (17; 85%) had VD deficiency while only 10% had VD at the 'sufficient' level. Most of the patients had bilateral lung involvement. Abnormal CT findings were only seen in patients with VD deficiency. The majority of patients with consolidation had VD deficiency. Despite that, there was no significant difference in CT chest scans among different VD status groups (p=0.189). However, we found significant differences between abnormal chest CT findings and plasma CRP concentration (p=0.001) and age (p=0.007). CT images of the patients with the lowest and highest levels of CRP are presented in Figure 1.

Laboratory findings for COVID-19 patients

There were numerous differences in hematological and biochemical data among patients (Table 2). We found a significant difference in the serum level of CRP between the VD deficient group and the VD insufficient group (Table 3, p=0.0243). Moreover, there were significant correlations between the CT scan data of patients and serum calcium, albumin, LDH, CRP, AST and ALT levels, and age (Table 4).

Discussion

The main findings of our study are that there is a high prevalence of VD deficiency in COVID-19 patients, and the severity of infection increase as VD levels decrease from insufficient to deficient. Unlike most data presented to date, VD deficiency poses a risk to young and middle-aged people as well as elders with COVID-19. The relationship between COVID-19 positivity, risk of hospitalization, mortality and low VD corresponds to similar studies in different countries [19-23]. In contrast, Hastie et al. [24] (2020) reported that there is no link between

Table 2. Laboratory findings for COVID-19 patients in Vit D groups

	Normal Range		All patients (n=40)	Vit D Deficiency (n=32)	Vit D Insufficient (n=6)	Vit D Sufficient (n=2)
	Female	**Male**				
Hemoglobin (g/dL)	11.90-14.60	13.50-6.90				
Decreased			13 (32.5%)	11 (34.375%)	1 (16.67%)	1 (50%)
Normal			27 (67.5%)	21 (64.625%)	5 (83.33%)	1 (50%)
White Blood Cells (x10^3/µl)	4.5-12.7	3.9-10.9				
Decreased			9 (22.5%)	7 (21.875%)	2 (33.33%)	
Normal			31 (77.5%)	25 (78.125%)	4 (12.5%)	2 (100%)
Red Blood Cells (x10^6/µl)	3.9-5.1	4.4-5.6				
Decreased			7 (17.5%)	5 (15.625%)	1 (16.67%)	1 (50%)
Normal			33 (82.5%)	27 (84.375%)	5 (83.33%)	1 (50%)
Platelets (x10^3/µl)	173-390	166-308				
Decreased			5 (12.5%)	4 (12.5%)	1 (3.125%)	
Normal			34 (85%)	27 (84.375%)	5 (83.33%)	2 (100%)
Increased			1 (2.5%)	1 (3.125%)		
Lymphocytes (x10^3/µl)	1.26-3.5					
Decreased			12 (30%)	10 (31.25%)	1 (16.67%)	1 (50%)
Normal			28 (70%)	22 (68.75%)	5 (83.33%)	1 (50%)
Increased						
Total Serum Calcium (mg/dL)	18-60 age 8.6-10	60-90 age 8.8-10.2				
Decreased			7 (20.59%)	7 (26.92%)		
Normal			23 (67.65%)	16 (61.54%)	5 (83.33%)	2 (100%)
Increased			4 (11.76%)	3 (50%)	1 (50%)	

Serum Phosphorus (mg/dL)	2.3-4.7						
Decreased			1 (10%)	1 (10%)			
Normal			9 (90%)	9 (90%%)			
Albumin (g/dL)	3.5-5						
Decreased			2 (9.09%)	2 (11.11%)			
Normal			20 (90.91%)	16 (88.89%)	3 (100%)	1 (100%)	
Serum Creatinine (mg/dL)	0.5-0.9	0.7-1.2					
Decreased			2 (5.128%)	2 (6.451%)			
Normal			31 (79.487%)	24 (77.419%)	5 (83.33%)	2 (100%)	
Increased			6 (15.384%)	5 (16.129%)	1 (16.67%)		
D Dimer (ng/mL)	0-499						
Normal			28 (73.684%)	23 (76.666%)	4 (66.67%)	1 (50%)	
Increased			10 (26.315%)	7 (23.333%)	2 (33.33%)	1 (50%)	
LDH (U/L)	135-214	135-225					
Decreased			2 (5.555%)	2 (6.896%))			
Normal			22 (61.111%)	16 (55.172%)	5 (100%)	1 (50%)	
Increased			12 (33.333%)	11 (37.931%)		1 (50%)	
Ferritin (ng/mL)	4.6-204	21.8-274.7					
Decreased			1 (2.564%)	1 (3.225%)			
Normal			29 (74.358%)	21 (67.741%)	6 (100%)	2 (100%)	
Increased			9 (23.076%)	9 (29.032%)			
Blood Urea Nitrogen (mg/dL)	5.0-24						
Decreased			2 (5%)	2 (6.25%)			
Normal			38 (95%)	30 (93.75%)	6 (100%)	2 (100%)	
Increased							

C Reactive Protein (mg/L)	0-5						
Normal			20 (50%)	13 (40.625%)	6 (100%)	1 (50%)	
Increased			20 (50%)	19 (59.375%)		1 (50%)	
AST (U/L)	8.0-46						
Decreased			1 (2.5%)	1 (3.125%)			
Normal			36 (90%)	28 (87.5%)	6 (100%)	2 (100%)	
Increased			3 (7.5%)	3 (9.375%)			
ALT (U/L)	0-35						
Normal			35 (87.5%)	27 (84.375%)	6 (100%)	2 (100%)	
Increased			5 (12.5%)	5 (15.625%)			

Table 3. The mean laboratory findings, age and hospitalization duration for deficient and insufficient Vit D groups

	Levels of 25(OH) Vit D		Test for mean difference
	Deficient	Insufficient	*p* value
	Mean (±SD) n		
Age	42 (±17.9) 32	48.2 (±15) 6	0.436
Duration of hospitalization	4.94 (±2.47) 32	4.67 (±3.93) 6	0.824
Hemoglobin (g/dL)	12.8 (±2.62) 32	13.78 (±1.49) 6	0.438
WBC (1 000/µl)	6.12 (±1.91) 32	6.37 (±1.83) 6	0.77
RBC (1 000 000/µl)	4.55 (±0.52) 32	4.66 (±0.54) 6	0.784
Serum Creatinine (mg/dL)	0.897 (±0.36) 31	1.07 (±0.24) 6	0.138
Total serum Calcium (mg/dL)	9.15 (±0.61) 26	9.48 (±0.51) 6	0.224
Blood Urea Nitrogen (mg/dL)	15.07 (±8.71) 32	11.45 (±1.83) 6	0.295
Ferritin (ng/mL)	215 (±301) 31	124.7 (±33.3) 6	0.378
Albumin (g/dL)	3.83 (±0.47) 18	4.1 (±0.53) 3	0.373
Lactate Dehydrogenase (U/L)	237 (±105) 29	182.2 (±27.5) 5	0.278
C Reactive Protein (mg/L)	22.7 (±32.7) 32	2.58 (±1.37) 6	0.0243*
D-Dimer (ng/mL)	466 (±644) 30	395 (±166) 6	0.167
Platelets (1 000/µl)	215 (±76.4) 32	235.8 (±31.4) 6	0.193
Lymphocytes (10^3/µl)	1.624 (±0.66) 32	1.855 (±0.55) 6	0.426
AST (U/L)	26.9 (±18.9) 32	22.83 (±7.78) 6	0.814
ALT (U/L)	21.4 (±13.5) 32	17.83 (±8.33) 6	0.818

* Indicates statistical significance

Table 4. The relationships of age and hematological parameters to normal and abnormal CT findings in COVID-19 patients

	CT findings		Test for mean difference
	Normal	Abnormal	*p* value
	Mean (±SD) n		
Age	36 (±13.7) 20	50.8 (±18) 18	0.007*
Hemoglobin (g/dL)	13.31 (±2.81) 20	12.57 (±2.08) 18	0.163
WBC (1 000/µl)	6.1 (±1.94) 20	6.22 (±1.86) 18	0.856
RBC (1 000 000/µl)	4.66 (±0.49) 20	4.46 (±0.43) 18	0.227
Serum Phosphorus (mg/dL)	3.03 (±0.55) 3	2.983 (±0.55) 7	0.897
Serum Creatinine (mg/dL)	0.87 (±0.21) 19	0.98 (±0.45) 18	0.545
Total Serum Calcium(mg/dL)	9.57 (±0.44) 15	9.89 (±0.54) 17	0.001*
Blood Urea Nitrogen (mg/dL)	12.37 (±2.61) 20	16.9 (±11.2) 18	0.175
Ferritin (ng/mL)	108.8 (±99.3) 19	297 (±364) 18	0.152
Albumin (g/dL)	4.26 (±0.17) 5	3.48 (±0.47) 16	0.029*
Lactate Dehydrogenase (U/L)	176.1 (±24.8) 16	277 (±117) 18	0.001*
C Reactive Protein (mg/L)	5.91 (±5.75) 20	34.6 (±39.8) 18	0.001*
D-Dimer (ng/mL)	420 (±637) 18	488 (±556) 18	0.466
25(OH) vitamin D (ng/mL)	14.11 (±7.66) 20	11.48 (±4.02) 18	0.189
Platelets (1 000/µl)	223.3 (±69.9) 20	212.8 (±74.4) 18	0.657
Lymphocytes (1 000/µl)	1.52 (±0.57) 20	1.82 (±0.69) 18	0.156
AST (U/L)	19.75 (±6.26) 20	33.6 (±22.9) 18	0.008*
ALT (U/L)	16.55 (±9.28) 20	25.67 (±14.7) 18	0.027*

* Indicates statistical significance

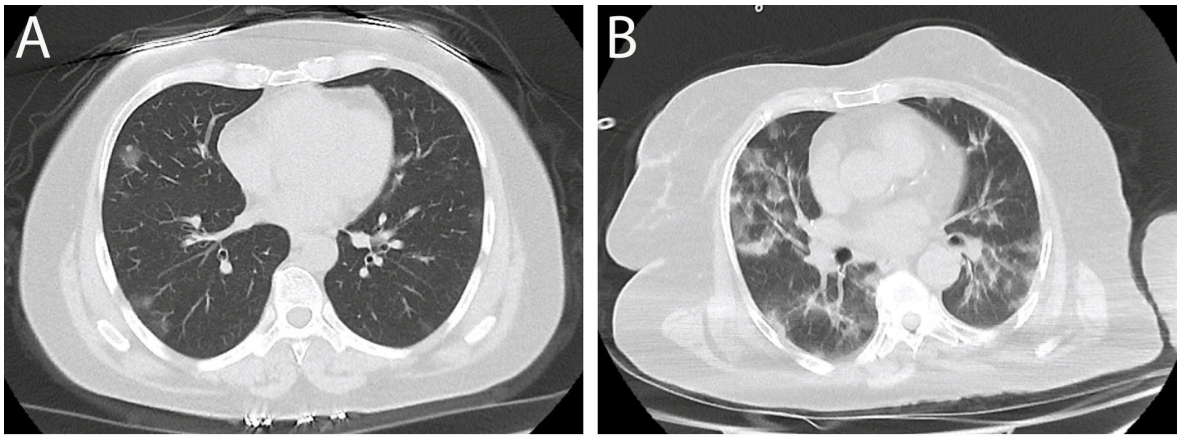

Figure 1. Chest CT images of the COVID-19 patients with the highest and lowest levels of CRP

(A): A 51-year-old woman with the lowest CRP level (1 mg/dL) and 20.6 ng/mL Vit-D; the chest CT scan shows patches of predominantly peripheral ground-glass opacity in both lungs
(B): An 80-year-old woman with the highest CRP level (161 mg/dL) and 11.4 ng/mL Vit-D; the CT scan shows bilateral, predominantly peripheral and irregular ground-glass opacities and consolidation in both lungs

VD concentration and the risk of COVID-19 infection as a result of their evaluation of UK biobank data. The VD data of the participants in that study were quite old, having been obtained between 2006 and 2010. A patient's VD levels may change drastically in ten years since the half-life of VD is a matter of days in the body.

In the present study, the VD level was significantly different in COVID-19 patients in comparison to the control group (p<0.001). Our results showed that although there was no relationship between VD level and age, there was a relationship between the presence of abnormal CT findings and patient age. Most patients (80%) were in the deficient VD level group, 82% were under 60 years of age and half of all patients were middle-aged (30-60).

There is conflicting information about the association of age group and VD deficiency related to COVID-19. A deficient VD level was detected in 17 critically ill COVID-19 patients with an average age of 64 years in Barcelona, Spain Ojeda et al [25] and Zhao et al. [26] (2020) reported an age range between 20 to 90, and the largest percentage (31%) of these patients were in the middle age group. Another study reports a positive correlation between VD deficiency and increased age [27].

Governmental policies, lifestyle differences, dietary regimens and patient numbers may have caused the variation between previous study results. Our results revealed that only two COVID-19 patients (5%) had VD levels regarded as sufficient. In a different study, the risk of COVID-19 positivity continued to decrease until the serum VD level reached 55 ng/mL. Furthermore, persons with VD value of 55 ng/mL had a lower COVID-19 positivity rate than persons in the 'sufficient' (30-34 ng/mL) VD range [28]. Finding only two COVID-19 positive individuals with serum VD levels in the 'sufficient' range is unsurprising considering previous data.

In our study, no significant differences were found between the VD deficient group and VD insufficient group regarding hematological parameters, except for the CRP level, which was almost 9 times higher. Viral infections increase CRP levels [29, 30]. It is produced via cytokine storm that is regulated by interleukin-6 and can be increased by interleukin-1β [31]. Many studies have shown that there is an inverse correlation between VD and CRP levels in inflammatory and non-inflammatory diseases [32, 33]. Active metabolites of VD that are formed in T and B lymphocytes suppress T-cell mediated inflammation. VD stimulates the proliferation of T-regulator cells, which has a suppressive effect on T-cell activity, by increasing the production of IL-10 in dendritic cells [34-36].

Cytokine storm can cause diffuse alveolar damage and fibrosis, progressive respiratory failure and multiple organ dysfunction [37]. VD receptors in heart, lungs and kidneys, can prevent multiple organ damage by reducing cytokine levels [38]. In a comparative analysis of cytokine storm and associated mortality in COVID-19 patients in the USA, France, Iran,

and the UK, an association between high CRP and low levels of VD was identified. Moreover, VD may play a role in reducing complications due to irregular inflammation and cytokine storm [3].

In a study of COVID-19 patients with acute respiratory failure and high serum inflammation markers, VD hypovitaminosis was detected in 81%. Also severe VD deficiency was reported to decrease survival rate [39]. While the previous study reported no statistical significance between VD levels and CRP, the findings were similar. The difference in their study may be due to the greater mean age of included patients.

The abnormal chest findings recorded in our study, were much more common in VD deficient patients than in VD insufficient patients. However, difference was not significant (p=0.189), which is probably due to the low number of patients in each group. The extent of patched ground glass opacities and consolidation in both lungs was related to the severity of the COVID-19 disease [40].

De Smet et al. [41] stated that advanced radiological stage was correlated with more profound VD deficiency in male COVID-19 patients. More specifically, while the prevalence of VD deficiency was 55.2% in patients with early-stage radiological findings (ground-glass opacities), this prevalence was 74% in patients with advanced stage (consolidation) findings (p=0.0010). In the present study, we determined significant changes in some hematological parameters, such as serum calcium, albumin, LDH, CRP, AST and ALT in patients, with CT findings (p<0.05) (Table 4). Mardani et al. [42] reported that ALT, CRP, and LDH are among the laboratory findings with high accuracy in predicting RT-PCR positive COVID-19 cases. COVID-19 patients had blood biochemical abnormalities such as low albumin and high LDH levels that indicate multi-organ dysfunction and disease severity [43]. Decreased albumin, and increased LDH, ALT, AST and CRP levels, are some of the main laboratory abnormalities that indicate the poor progression of the COVID-19 disease [44].

Our results showed significant relationships between many blood parameters and the presence of abnormal CT findings when the patients were divided into groups (p<0.05) (Table 4). We determined a statistically significant relationship only for the CRP level when patients were divided into groups based on their VD level (p=0.0243) (Table 3). This may have been due to the uneven distribution of the number of patients in the VD groups whereas the number of patients in the CT groups was approximately equal. We also determined in this study that the CRP level was associated not only with VD groups but also the presence of CT findings. These results strengthen support for the proposition that VD level is associated with the severity of infection.

It has been reported that VD treatment in patients with COVID-19 reduces the need for intensive care [45, 46].

In addition to the effects of VD on the immune system, it has important roles that include the regulation of insulin expression, adipogenesis and blood pressure in cardiometabolic diseases such as diabetes, hypertension and obesity, which worsen the condition of COVID-19 patients [47, 48].

The main limitations of our study was the small number of patients, having patients from a single center, heterogenous distribution of VD levels and the lack of follow-up data in terms of CRP and CT findings. Large-scale clinical studies should be conducted with VD supplementation and with control groups and in which a homogeneous distribution is achieved across age groups. Patients should then be followed-up over a long period. Separately, the reasons for the high prevalence of VD deficiency should be investigated in terms of pharmacogenetic and environmental factors.

In conclusion, in this study the prevalence of Vit D deficiency in COVID-19 non-ICU patients was high in the young and middle age group, as well as in the elderly. Additionally, there was a negative relationship between Vit D deficiency and the CRP level. A high CRP level is associated with the presence of lung damage and consequently abnormal CT findings. In the light of these findings, determining the plasma level of Vit D in COVID-19 patients and adding it to the treatment protocol at the appropriate dosage may reduce the severity and progression of COVID-19 disease by contributing to the regulation of pulmonary inflammatory response and cytokine storm.

Conflict of interest: No conflict of interest was declared by the authors.

References

1. Wang C, Han J. Will the COVID-19 pandemic end with the Delta and Omicron variants? Springer; Environmental Chemistry Letters 2022:1-11.
2. Al Quteimat OM, Amer AM. The impact of the COVID-19 pandemic on cancer patients. Am J Clin Oncol 2020;43:452-455. https://doi.org/10.1097/COC.0000000000000712
3. Daneshkhah A, Agrawal V, Eshein A, Subramanian H, Roy H, Backman V. Evidence for possible association of vitamin D status with cytokine storm and unregulated inflammation in COVID-19 patients. Aging Clin Exp Res 2020;32:2141-2158. https://doi.org/10.1007/s40520-020-01677-y
4. Zdrenghea MT, Makrinioti H, Bagacean C, Bush A, Johnston SL, Stanciu LA. Vitamin D modulation of innate immune responses to respiratory viral infections. Rev Med Virol 2017;27:e1909. https://doi.org/10.1002/rmv.1909
5. Alpdemir M, Alpdemir MF. Vitamin D deficiency status in Turkey: a meta-analysis. Int J Med Biochem 2019;2:118-131. https://doi.org/10.14744/ijmb.2019.04127
6. Çubukçu M, Recai A, Müderrisoğlu S. Samsun ilinde D vitamini düzeylerinin yaş, cinsiyet ve mevsimsel özelliklere göre değerlendirilmesi. Ankara Med J 2019;19:769-775. https://doi.org/10.17098/amj.652002
7. Hughes D, Norton R. Vitamin D and respiratory health. Clin Exp Immunol 2009;158:20-25. https://doi.org/10.1111/j.1365-2249.2009.04001.x
8. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr 2010;91:1255-1260. https://doi.org/10.3945/ajcn.2009.29094
9. Loeb M, Dang AD, Thiem VD, et al. Effect of vitamin D supplementation to reduce respiratory infections in children and adolescents in Vietnam: a randomized controlled trial. Influenza Other Respi Viruses 2019;13:176-183. https://doi.org/10.1111/irv.12615
10. Teymoori Rad M, Shokri F, Salimi V, Marashi SM. The interplay between vitamin D and viral infections. Rev Med Virol 2019;29:e2032. https://doi.org/10.1002/rmv.2032
11. Aranow C. Vitamin D and the immune system. J Investig Med 2011;59:881-886. http://dx.doi.org/10.2310/JIM.0b013e31821b8755
12. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients 2013;5:2502-2521. https://doi.org/10.3390/nu5072502
13. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;395:565-574. https://doi.org/10.1016/S0140-6736(20)30251-8
14. Burrell LM, Johnston CI, Tikellis C, Cooper ME. ACE2, a new regulator of the renin–angiotensin system. Trends Endocrinol Metab 2004;15:166-169. https://doi.org/10.1016/j.tem.2004.03.001
15. Parekh D, Dancer RCA, Scott A, et al. Vitamin D to prevent lung injury following esophagectomy—a randomized, placebo-controlled trial. Crit Care Med 2018;46: 1128-1135. https://doi.org/10.1097/CCM.0000000000003405
16. Dancer RCA, Parekh D, Lax S, et al. Vitamin D deficiency contributes directly to the acute respiratory distress syndrome (ARDS). Thorax 2015;70:617-624. http://dx.doi.org/10.1136/thoraxjnl-2014-206680
17. Mitchell F. Vitamin-D and COVID-19: do deficient risk a poorer outcome? Lancet Diabetes Endocrinol 2020;8:570. https://doi.org/10.1016/S2213-8587(20)30183-2
18. Ministry of Health. COVID-19 (SARS-CoV-2 Infection) Treatment for Adult Patients 2020. Available from: https://covid19.saglik.gov.tr/Eklenti/39061/0/covid-19rehberieriskinhastatedavisipdf.pdf. Accessed October 14, 2020
19. Singh S, Kaur R, Singh RK. Revisiting the role of vitamin D levels in the prevention of COVID-19 infection and mortality in European countries post infections peak. Aging Clin Exp Res 2020;32:1609-1612. https://doi.org/10.1007/s40520-020-01619-8
20. Meltzer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of vitamin D status and other clinical characteristics with COVID-19 test results. JAMA Netw Open 2020;3:2019722. https://doi.org/10.1001/jamanetworkopen.2020.19722
21. Ilie PC, Stefanescu S, Smith L. The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality. Aging Clin Exp Res 2020;32:1195-1198. https://doi.org/10.1007/s40520-020-01570-8
22. Merzon E, Tworowski D, Gorohovski A, et al. Low plasma 25 (OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. FEBS J 2020;287:3693-3702. https://doi.org/10.1111/febs.15495
23. Karahan S, Katkat F. Impact of serum 25 (OH) vitamin D level on mortality in patients with COVID-19 in Turkey. J Nutr Health Aging 2021;25:189-196. https://doi.org/10.1007/s12603-020-1479-0
24. Hastie CE, Mackay DF, Ho F, et al. Vitamin D concentrations and COVID-19 infection in UK Biobank. Diabetes Metab Syndr 2020;14:561-565. https://doi.org/10.1016/j.dsx.2020.04.050

25. Ojeda A, Cuñat T, Calvo A. Diagnosis of vitamin D deficiency in patients admitted in ICU with COVID 19 disease. J Biomed Res Rev 2020;3:1-4. https://doi.org/
26. Zhao J, Gao HY, Feng ZY, Wu QJ. A retrospective analysis of the clinical and epidemiological characteristics of COVID-19 patients in Henan Provincial People's Hospital, Zhengzhou, China. Front Med 2020;7:286. https://doi.org/10.3389/fmed.2020.00286
27. D'Avolio A, Avataneo V, Manca A, et al. 25-hydroxyvitamin D concentrations are lower in patients with positive PCR for SARS-CoV-2. Nutrients 2020;12:1359. https://doi.org/10.3390/nu12051359
28. Kaufman HW, Niles JK, Kroll MH, Bi C, Holick MF. SARS-CoV-2 positivity rates associated with circulating 25-hydroxyvitamin D levels. PLoS One 2020;15:e0239252. https://doi.org/10.1371/journal.pone.0239252
29. Coster D, Wasserman A, Fisher E, et al. Using the kinetics of C-reactive protein response to improve the differential diagnosis between acute bacterial and viral infections. Infection 2020;48:241-248. https://doi.org/10.1007/s15010-019-01383-6
30. Wang G, Wu C, Zhang Q, et al. C-reactive protein level may predict the risk of COVID-19 aggravation. Open Forum Infect Dis 2020:7:ofaa153. https://doi.org/10.1093/ofid/ofaa153
31. Black S, Kushner I, Samols D. C-reactive protein. J Biol Chem 2004;279:48487-48490. https://doi.org/10.1074/jbc.R400025200
32. Kruit A, Zanen P. The association between vitamin D and C-reactive protein levels in patients with inflammatory and non-inflammatory diseases. Clin Biochem 2016;49:534-537. https://doi.org/10.1016/j.clinbiochem.2016.01.002
33. Liefaard MC, Ligthart S, Vitezova A, et al. Vitamin D and C-reactive protein: a Mendelian randomization study. PLoS One 2015;10:e0131740. https://doi.org/10.1371/journal.pone.0131740
34. Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ 2017;356:i6583. https://doi.org/10.1136/bmj.i6583
35. Chun RF, Liu PT, Modlin RL, Adams JS, Hewison M. Impact of vitamin D on immune function: lessons learned from genome-wide analysis. Front Physiol 2014;5:151. https://doi.org/10.3389/fphys.2014.00151
36. Adorini L, Penna G. Dendritic cell tolerogenicity: a key mechanism in immunomodulation by vitamin D receptor agonists. Hum Immunol 2009;70:345-352. https://doi.org/10.1016/j.humimm.2009.01.016
37. Xiong Y, Liu Y, Cao L, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect 2020;9:761-770. https://doi.org/10.1080/22221751.2020.1747363
38. Aygun H. Vitamin D can prevent COVID-19 infection-induced multiple organ damage. Naunyn Schmiedebergs Arch Pharmacol 2020;393:1157-1160 https://doi.org/10.1007/s00210-020-01911-4
39. Carpagnano GE, Di Lecce V, Quaranta VN, et al. Vitamin D deficiency as a predictor of poor prognosis in patients with acute respiratory failure due to COVID-19. J Endocrinol Invest 2021;44:765-771. https://doi.org/10.1007/s40618-020-01370-x
40. Liu KC, Xu P, Lv WF, et al. CT manifestations of coronavirus disease-2019: a retrospective analysis of 73 cases by disease severity. Eur J Radiol 2020;126:108941. https://doi.org/10.1016/j.ejrad.2020.108941
41. De Smet D, De Smet K, Herroelen P, Gryspeerdt S, Martens GA. Serum 25(OH)D level on hospital admission associated with COVID-19 stage and mortality. Am J Clin Pathol 2021;155:381-388. https://doi.org/10.1093/ajcp/aqaa252
42. Mardani R, Vasmehjani AA, Zali F, et al. Laboratory parameters in detection of COVID-19 patients with positive RT-PCR; a diagnostic accuracy study. Arch Acad Emerg Med 2020;8:43.
43. Deng X, Liu B, Li J, Zhang J, Zhao Y, Xu K. Blood biochemical characteristics of patients with coronavirus disease 2019 (COVID-19): a systemic review and meta-analysis. Clin Chem Lab Med 2020;58:1172-1181. https://doi.org/10.1515/cclm-2020-0338
44. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med 2020;58:1131-1134. https://doi.org/10.1515/cclm-2020-0198
45. Tan CW, Ho LP, Kalimuddin S, et al. Cohort study to evaluate the effect of vitamin D, magnesium, and Vitamin B_{12} in combination on progression to severe outcomes in older patients with coranavirus (COVID-19). Nutrition 2020;79-80:111017. https://doi.org/10.1016/j.nut.2020.111017
46. Castillo ME, Costa LME, Barrios JMV, et al. Effect of calcifediol treatment and best available therapy versus best available therapy on intensive care unit admission and mortality among patients hospitalized for COVID-19: a pilot randomized clinical study. J Steroid Biochem Mol Biol 2020;203:105751. https://doi.org/10.1016/j.jsbmb.2020.105751
47. Sassi F, Tamone C, D'Amelio P. Vitamin D: nutrient, hormone, and immunomodulator. Nutrients 2018;10:1656. https://doi.org/10.3390/nu10111656
48. Szymczak Pajor I, Śliwińska A. Analysis of association between vitamin D deficiency and insulin resistance. Nutrients 2019;11:794. https://doi.org/10.3390/nu11040794

Acknowledgement: The authors thank Dr Gregory T. Sullivan for proofreading an earlier version of this manuscript.

Ethical approval: The procedures involving human subjects were approved by the Ondokuz Mayis University Clinical Research Ethics Committee (Decision No. OMU KAEK 2020/422).

Authors' contributions

D.G. and A.A. designed and planned the study, acquired the data and critically revised the manuscript for important intellectual content. B.A., S.G., F.T. and T.K. analysed the data, drafted the manuscript and had primary responsibility for final content. V.R.U. analysed the data and statistical analysis. All authors read and approved the final manuscript.