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Relationship Between COVID-19 Clinical Severity and Serum 25(OH)D Levels

COVID-19 Klinik Özellikleri ile Serum 25(OH)D Düzeyleri Arasındaki İlişkinin Değerlendirilmesi

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Abstract

Introduction: This study aimed to evaluate the relationship between serum 25-hydroxyvitamin D [25(OH)D] levels and clinical course and outcome of Coronavirus disease-2019 (COVID-19).
Materials and Methods: In this single-center, prospective study, patients with COVID-19 were divided into three groups with uncomplicated disease, mild pneumonia, and severe pneumonia. Cases were compared in terms of clinical and laboratory findings as well as serum 25(OH)D levels on hospital admission. Patients were also grouped according to their COVID-19-related outcomes and then compared in terms of 25(OH)D levels.
Results: The median serum 25(OH)D levels of the COVID-19 and control groups were 16 (11-23) ng/ml and 21.5 (18-25) ng/ml, respectively (p<0.001). No significant differences were found in the serum 25(OH)D levels between noncomplicated, mild pneumonia, and severe pneumonia groups 16.5 (16-25.2), 15 (11-21), and 16 (11.5-26.5) ng/ml, respectively, p=0.521. When compared in terms of COVID-19-related outcomes, no significant difference was found in vitamin D levels.
Conclusion: Patients with vitamin D deficiency may have higher risk for COVID-19 infections. However, no relationship was found between serum vitamin D levels and clinical severity and outcomes of COVID-19.
Keywords: COVID-19, vitamin D deficiency, clinical severity, outcomes

Öz

Giriş: Bu çalışmada serum 25(OH) vitamin D [25(OH)D] düzeyleri ile Koronavirüs hastalığı-2019'un (COVID-19) klinik seyri arasındaki ilişkinin değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntem: Bu tek merkezli, ileriye dönük çalışmada, COVID-19 hastaları komplikasyonsuz hastalık, hafif pnömoni ve şiddetli pnömoni olarak üç gruba ayrılmıştır. Bu olgular, klinik ve laboratuvar bulguları ile hastaneye yatıştaki serum 25(OH)D düzeyleri açısından karşılaştırılmıştır. Ayrıca hastalar yoğun bakım destek gereksinimi ve mortalite gelişimi açısından gruplandırılmış ve 25(OH)D vitamini düzeyleri açısından karşılaştırılmıştır.
Bulgular: COVID-19 ve kontrol gruplarının medyan serum 25(OH)D düzeyleri 16 (11-23) sırasıyla ng/ml ve 21,5 (18-25) ng/ml (p<0,001) olarak saptanmıştır. Komplike olmayan, hafif pnömoni ve şiddetli pnömoni gruplarında 25(OH)D düzeyleri sırasıyla 16,5 ng/ml (16 ila 25,2), 15 (11 ila 21) ve 16 ng/ml (11,5 ila 26,5) olarak saptanmış ve gruplar arasında istatistiksel anlamlı farklılık saptanmamıştır (p=0,521). COVID-19 ile ilgili sonuçlar açısından karşılaştırıldığında, D vitamini düzeylerinde anlamlı bir fark saptanmamıştır (p=0,411, p=0,438).
Sonuç: D vitamini eksikliği olan hastalar, COVID-19 enfeksiyonları açısından daha yüksek riske sahip olabilirler. Bununla birlikte, serum D vitamini seviyeleri ile COVID-19'un klinik şiddeti ve sonucu arasında bir ilişki gösterilememiştir.
Anahtar Kelimeler: COVID-19, D vitamini eksikliği, klinik önem, sonuçlar

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Introduction

Vitamin D is a fat-soluble vitamin that plays an important role in calcium homeostasis and bone metabolism. In addition to the classical vitamin D effects on calcium metabolism, increasing evidence suggests the role of vitamin D in the regulation of the immune response and epithelial differentiation[1]. The association of vitamin D deficiency with many other clinical conditions, such as chronic metabolic, cardiovascular, and neoplastic diseases, is still investigated[2]. Studies have reported that a low vitamin D level is associated with increased susceptibility to infectious diseases, particularly upper respiratory tract infections[3,4]. Several studies have also shown that a low serum 25-hydroxyvitamin D [25(OH)D] level increases the risk of community-acquired pneumonia[5-7]. Moreover, 25(OH)D levels of 20-30 ng/ml, decrease the risk for acute respiratory infection (ARI)[8,9]. In a cohort study, every 4 ng/ml increase in serum 25(OH)D level was shown to decrease the ARI risk by 7%[10]. A similar relationship was previously defined between vitamin D and seasonal/epidemic influenza infections[8,11]. These data lead to a hypothesis on the possible relationship between COVID-19 infection and low serum vitamin D levels.

The documented antiviral and modulatory effects of vitamin D on immune system, renin-angiotensin (RAS), and coagulation system may affect the pathogenesis and clinical course of COVID-19[12]. Besides, comorbidities associated with COVID-19 severity and mortality, such as advanced age, ethnicity, obesity, and hypertension, are also risk factors for vitamin D deficiency. This suggests that there may be a relationship between COVID-19 severity and vitamin D deficiency[12,13].

In this study, we aimed to evaluate the relationship between serum 25(OH)D levels and clinical course and outcome of COVID-19.

Materials and Methods

This single-center, prospective study was conducted on patients hospitalized for COVID-19 between April 1, 2020, and July 31, 2020. This study was approved by the Gazi University Clinical Research Ethics Committee (decision no: 654, 10.05.2020).

Study Population, Groups, and Definitions

Patients hospitalized for COVID-19 with confirmed Severe Acute Respiratory Syndrome Coronavirus-2 polymerase chain reaction (SARS-CoV-2 PCR), (Bio-Speedy Direct RT-qPCR SARS-CoV-2, Bioeksen R&D Technologies Ltd., İstanbul, Turkey) positivity in oropharyngeal and nasopharyngeal samples were included in the study. Patients with uncomplicated COVID-19 were hospitalized for isolation. Patients aged <18 years, pregnant women, patients with a positive SARS-CoV-2 antibody test without a positive PCR test, and patients taking vitamin D supplements upon admission were excluded from the study. Healthy volunteers with similar demographic characteristics to patients with COVID-19 were included in the study as a control group. The control group was selected from healthy volunteers who presented to the checkup center of our hospital between April and August 2019 and whose serum samples were stored by the biochemistry laboratory for other studies.

Patients with COVID-19 were categorized into three groups as noncomplicated disease (mild disease), pneumonia (moderate disease), and severe pneumonia (severe disease)[14]. Pneumonia was diagnosed based on chest X-ray and/or thorax computed tomography findings[14]. Patients without imaging findings were allocated to the uncomplicated COVID-19 group[14]. Patients who have pneumonia with an oxygen saturation <90% or PaO_2/FiO_2 <300 or respiratory rate >30/min were allotted to the severe pneumonia group[14]. Patients with pneumonia who do not meet the criteria for severe pneumonia were assigned to the mild pneumonia group[14].

Study Protocol

Patients were included in the study according to the order of hospitalization. Demographic and clinical features, radiologic findings, routine blood tests (such as hemogram, biochemistry tests, coagulation parameters, ferritin, creatine phosphokinase test, cardiac enzymes, C-reactive protein, procalcitonin, and urinalysis), and outcomes of patients with COVID-19 were recorded. Demographic features of control patients were also recorded. In addition to blood sampling for routine laboratory analysis, 5-10 ml of venous blood samples were obtained from all patients and healthy volunteers for the measurement of serum 25(OH)D levels on initial admission.

Measurement of Serum 25(OH)D Levels

All serum samples were centrifuged for 15 min at 3000 cycles. The serum samples were separated and placed in Eppendorf tubes, labeled with a sequence number, and kept at -80 °C until the working day. Serum 25(OH)D levels were determined using commercially available immunoassay kits (Beckman Coulter Inc., Brea, CA, USA) in the Beckman Coulter AU5800 autoanalyzer (Beckman Coulter Inc.). TThe total (within-laboratory) coefficient of variation was 7.4% for clinical decision levels (20-30 ng/ml). Additionally, bias was 8.1% (in cutoff values) according to external quality control results of Vitamin D External Quality Assessment Scheme. Serum vitamin D level of <12 ng/ml was considered severe deficiency, 12-20 ng/ml as mild–moderate deficiency, 21-30 ng/ml as insufficiency, and >30 ng/ml as normal.

Statistical Analysis

Data were analyzed using IBM Statistical Package for the Social Sciences statistics version 22.0 (IBM Corp., Armonk, N.Y., USA) package program. Categorical variables were presented as number-percentage and compared using the chi-square test. The suitability of continuous variables to normal distribution was evaluated by the Shapiro-Wilk test, histogram, and Q-Q plots. Normally distributed continuous variables were presented as mean and standard deviation, and those that are not distributed normally were presented as median and interquartile range of 25-75%. Non-parametric variables were compared using Mann-Whitney U and parametric variables using Student's t-test. A p-value of <0.05 was considered significant.

Results

This study included 98 patients with COVID-19 and 98 healthy volunteers. The median ages of the COVID-19 and control groups were 42.5 (31-59.25) and 42.0 (31-46) (p=0.124), respectively. Moreover, 39.8% of the patients in the COVID-19 group and 49% of those in the control group were men (p=0.196). The median serum 25(OH)D levels of the COVID-19 and control groups were 16 (11-23) ng/ml and 21.5 (18-25) ng/ml, respectively (p<0.001). Furthermore, 33.6% (33/98) of the control patients and 65.3% (64/98) of patients with COVID-19 had a serum 25(OH)D level below 20 ng/ml (p<0.001).

Among patients with COVID-19, 13 (13.3%) had severe pneumonia, 35 (35.7%) had mild pneumonia, and 50 (51%) had noncomplicated disease. Demographics, clinical characteristics, and laboratory results of these patients are shown in Table 1. The serum 25(OH)D levels of patients with COVID-19 were compared according to the disease severity, presence of pneumonia, and outcomes (Table 2).

Discussion

In this study, serum vitamin D levels were lower in patients with COVID-19 than in healthy controls. In the literature, serum vitamin D levels <20 ng/ml are defined as vitamin D deficiency, and according to this definition, 65.3% of the patients with COVID-19 in our study had vitamin D deficiency[15]. This suggests that serum vitamin D concentrations <20 ng/ml may cause increased susceptibility to COVID-19. Clinical studies have presented contradictory results on this subject[16]. Some studies conducted during this pandemic period have shown that a negative correlation may exist between the mean vitamin D levels and incidence of COVID-19[16-18]. Meltzer et al.[19] showed an approximately 1.7-fold increase in the incidence of COVID-19 infection in patients with vitamin D deficiency. Similarly, in clinical studies, serum 25(OH)D levels were low in patients with COVID-19, and low serum 25(OH)D levels (11.9-19.00 ng/ml) were shown to increase the risk of COVID-19 infection[20,21]. However, a study conducted in the United Kingdom did not find a relationship between serum vitamin D levels and the risk of COVID-19 after adjustment of confounding factors, and the authors concluded that measurement of serum vitamin D levels would not be useful in clinical practice[22].

$1,25(OH)_2D$ vitamin D has antiviral activity in the early viremic phase and immunomodulatory activity in the late hyperinflammatory period due to the modulation toll-like receptor expression and NK cell activity and suppression of T helper 1 cell responses and proinflammatory cytokines such as tumor necrosis factor-alpha, interferon-gamma, interleukin (IL)-1-beta, and IL-6[13,23,24]. Besides its immune-modulatory effect, vitamin D has been shown to be related to cardiometabolic diseases (e.g., hypertension, diabetes, and obesity), which are risk factors for severe COVID-19[25]. Vitamin D is known to reduce angiotensin-converting enzyme 2 expression, which mediates SARS-CoV-2 infection by modulating the RAS[23,26]. This modulatory effect is protective against acute lung injury and acute respiratory distress syndrome, and vitamin D deficiency has been shown to cause acute lung injury/acute respiratory distress syndrome[24,27,28]. A study also revealed that vitamin D is involved in the regulation of the coagulation cascade, which plays an important role in the pathogenesis of COVID-19, and its deficiency is associated with increased incidence of thrombosis[5]. These data suggest that serum 25(OH)D levels and vitamin D repletion may be associated with the clinical severity of COVID-19[3,5,29]. Clinical studies have supported this hypothesis[16,30,31]. These studies have stated that serum vitamin D levels are lower in severe COVID-19 cases, and this deficiency causes an increase in the risk of acute respiratory failure and COVID-19-associated mortality[16,18,30-34]. Alipio et al.[10] found a significant difference in vitamin D levels between patients with mild and severe COVID-19 and emphasized that serum vitamin D levels were associated with outcomes of COVID-19. Yajia Li et al.[35] showed that 1° of the latitude to the north leads to a 4.4% increase in mortality in the first four months of the epidemic and stated that sunlight, with its vitamin D activation effect, may have a protective influence on COVID-19-related mortality. Unlike these studies, the present study did not find a difference in vitamin D levels among patients with COVID-19 with different clinical severities, and serum vitamin D levels were not correlated with the presence of pneumonia and COVID-19-related mortality. However, these results may be associated with the small number of patients in our study. Because of the small sample size, especially in the severe pneumonia group, our results need to be verified by other studies before they can be generalized. A recent review emphasized that not enough evidence supported the association of vitamin D levels with

Table 1. Demographics, clinical characteristics, and laboratory results of patients with Coronavirus disease-2019 according to clinical severity

	Severe pneumonia (n=13)	Mild pneumonia (n=35)	Non-complicated (n=50)	p value
Age, median (IQR: 25-75%)	75 (69 to 78)	47 (34 to 60)	33 (28 to 43)	<0.001
Male sex, n (%)	9 (69.2)	24 (68.6)	15 (30)	0.001
BMI, kg/m^2 median (IQR: 25-75%)	**26 (24.8 to 28.7)**	**24.2 (23.8 to 24.5)**	**23.8 (21.6 to 25.6)**	**0.027**
Comorbidities, n (%)				
Diabetes	3 (23.1)	4 (11.4)	3 (6.0)	0.153
Hypertension	8 (61.5)	8 (22.9)	1 (2.0)	<0.001
COPD	3 (23.1)	0	0	N/A
CVD	4 (30.8)	1 (2.9)	1 (2.0)	0.003
Malignancy	4 (30.8)	2 (5.7)	0	N/A
ACE/ARB inhibitors	5 (38.5)	4 (11.4)	1 (2.0)	<0.001
Symptoms on admission, n (%)				
Fever >38 °C	5 (38.5)	18 (51.4)	2 (4.0)	<0.001
Cough	5 (38.5)	19 (54.3)	2 (4.0)	<0.001
Dyspnea	10 (76.9)	9 (25.7)	2 (4.0)	<0.001
Sputum	4 (30.8)	2 (5.7)	1 (2.0)	0.006
Headache	1 (7.7)	4 (11.4)	0	N/A
Nasal congestion	0	2 (5.7)	1 (2.0)	N/A
Sore throat	2 (15.4)	5 (14.3)	2 (4.0)	0.120
Myalgia and arthralgia	2 (15.4)	14 (40.0)	1 (2.0)	<0.001
Diarrhea	1 (7.7)	2 (5.7)	0	N/A
Laboratory findings on admission, median (IQR: 25-75%)				
White blood cell, mm^3 (RI: 3.91-10.9 mm^3)	4.94±1.50	3.90±1.90	4.48±1.62	0.003
Lymphocyte, mm^3 (RI: 1.26-3.35 mm^3)	0.96±0.73	1.52±0.81	2.24±0.75	<0.001
Hemoglobin, g/dl, (RI: 13-16.9 g/dl)	12.2 (11.7 to 13.4)	14.2 (2.9 to 15.0)	13.6 (12.7 to 14.9)	0.07
Platelet, ×10^3/mm^3 (RI: 166-308 mm^3)	233.300±106.246	203.714±69.600	259.440±66.863	0.004
Creatinine, mg/dl, (RI: 0.67-1.17 mg/dl)	1.07 (0.84 to 1.64)	0.86 (0.71 to 0.97)	0.67 (0.58 to 0.79)	<0.001
AST, U/l (RI: 0-50 U/l)	36 (28 to 52)	29 (24 to 39)	21.5 (17 to 24)	<0.001
LDH, U/l (RI: 0-248 U/l)	338 (287 to 548)	237 (184 to 293)	199 (171 to 230)	<0.001
CK, U/l (RI: 0-171 U/l)	186 (62 to 342)	108 (76 to 149)	82 (58 to 128)	0.093
INR (RI: 0.8-1.12)	1.06 (0.89 to 1.19)	1.0 (0.96 to 1.10)	1.03 (0.97 to 1.10)	0.452
D-dimer, µg/ml (RI: 0-0.5 µg/ml)	1.0 (0.65 to 1.88)	0.40 (0.25 to 1.17)	0.22 (0.17 to 0.37)	<0.001
Ferritin, ng/ml (RI: 23.9-336.2 ng/ml)	229 (143 to 582)	197 (66 to 356)	19 (10 to 39)	<0.001
Fibrinogen, mg/dl (RI: 200-400 mg/dl)	554 (508 to 676)	404 (310 to 471)	294 (254 to 341)	<0.001
CRP, mg/l (RI: 0-5 mg/l)	93.4 (51.9 to 132)	10.7 (5.3 to 17.9)	2.31 (1.72 to 4.10)	<0.001
Procalcitonin, ng/ml (RI: 0-0.5 ng/ml)	0.17 (0.10-1.0)	0.66 (0.04 to 0. 9)	0.21 (0.02 to 0.032)	<0.001
25(OH)D, ng/ml	16 (11.5 to 26.5)	15 (11 to 21)	16.5 (16 to 25.2)	0.521

IQR: Interquartile range, BMI: Body mass index, COPD: Chronic obstructive pulmonary disease, CVD: Cardiovascular disease, ACE: Angiotensin-converting enzyme, ARB: Angiotensin receptor blocker, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, CK: Creatine kinase, INR: International normalized ratio, CRP: C-reactive protein, RI: Reference interval, 25(OH)D: 25-hydroxyvitamin D

Table 2. Serum 25-hydroxyvitamin D levels of patients with Coronavirus disease-2019 according to the disease severity, presence of pneumonia, and outcome

	n (%)	25(OH)D levels, median, IQR (25-75%), ng/ml	p value
Clinical severity			
Severe pneumonia	13 (13.3%)	16 (11.5 to 26.5)	0.521
Mild pneumonia	35 (35.7%)	15 (11 to 21)	
Non-complicated	50 (51.0 %)	16.5 (16 to 25.2)	
COVID-19-related pneumonia			
Pneumonia	48 (49.0%)	15 (11 to 20.7)	0.348
Non-pneumonia	50 (51.0%)	16.5 (12 to 25.2	
COVID-19-related pneumonia			
Mild pneumonia	**35 (35.7%)**	**15 (11 to 21)**	**0.554**
Severe pneumonia	**13 (13.3%)**	**16 (11.5 to 26.5)**	
Requiring ICU support			
ICU	11 (11.2%)	15 (8 to 20)	0.411
Non-ICU	87 (88.8%)	16 (11 to 24)	
Mortality (28th days)			
Mortality	5 (5.1%)	18 (4.5 to 20)	0.438
No mortality	93 (94.9%)	16 (11 to 21.5)	

IQR: Interquartile range, ICU: Intensive care unit, COVID-19: Coronavirus disease-2019, 25(OH)D: 25-hydroxyvitamin D

COVID-19 severity and mortality[16]. Therefore, randomized controlled trials and new large cohort studies are necessary to test this hypothesis.

Our study has some limitations. First, the levels of parathormone that regulates the conversion of 25(OH)D to 1,25 dihydroxyvitamin D, serum calcium, and phosphorus were not measures[36]. Second, the small sample size, particularly in the severe COVID-19 group (severe pneumonia, intensive care unit, and mortality), increases the probability of type 2 error during statistical evaluation. Third, in our study population, all subgroups, including the control group, had vitamin D deficiency. This makes it difficult to evaluate the vitamin D level and COVID-19 relationship. Fourth, data such as comorbid diseases and body mass index could not be obtained because the control group was determined from the pre-COVID-19 pandemic period. Therefore, the possibility that the difference in serum vitamin D levels between the COVID-19 group and the control group was due to other reasons could not be completely ruled out.

Conclusion

In this study, we found lower serum vitamin D levels in patients with COVID-19 than in healthy controls. This suggests that serum vitamin D concentrations may be associated with COVID-19. Our findings showed no relationship between vitamin D levels and clinical severity and outcome of COVID-19. Large-scale prospective clinical studies are needed on this subject.

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Ethics

Ethics Committee Approval: This study was approved by the Gazi University Clinical Research Ethics Committee (decision no: 654, 10.05.2020).

Informed Consent: Written informed consent was obtained from the patients included in the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.T., Ö.G., Design: H.S.Ö., P.A.Y., Ö.G., K.H., Data Collection or Processing: P.A.Y., M.Y., M.B., Analysis or Interpretation: H.S.Ö., Literature Search: H.S.Ö., P.A.Y., Ö.G., K.H., Writing: H.S.Ö., P.A.Y., Y.Y., Ö.G., K.H.

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References

1. Fraser WD. Bone and mineral metabolism. In: Rifai N HA, Wittwer CT (eds). Tietz textbook of clinical chemistry and molecular diagnostics. 6th edition. St. Louise: Elsevier, 2018:1460-1.

2. Rosen CJ, Adams JS, Bikle DD, Black DM, Demay MB, Manson JE, Murad MH, Kovacs CS. The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. Endocr Rev. 2012;33:456-92.

3. Mitchell F. Vitamin-D and COVID-19: do deficient risk a poorer outcome? Lancet Diabetes Endo. 2020;8:570.

4. Carotti M, Salaffi F, Sarzi-Puttini P, Agostini A, Borgheresi A, Minorati D, Galli M, Marotto D, Giovagnoni A. Chest CT features of coronavirus disease 2019 (COVID-19) pneumonia: key points for radiologists. Radiol Med. 2020;125:636-46.

5. Kenneth Weir E, Thenappan T, Maneesh B, Chen Y. Does vitamin D deficiency increase the severity of COVID-19? Clin Med (Lond). 2020;20:e107-8.

6. Fabbri A, Infante M, Ricordi C. Editorial - Vitamin D status: a key modulator of innate immunity and natural defense from acute viral respiratory infections. Eur Rev Med Pharmacol Sci. 2020;24:4048-52.

7. Jolliffe DA, Griffiths CJ, Martineau AR. Vitamin D in the prevention of acute respiratory infection: systematic review of clinical studies. J Steroid Biochem Mol Biol. 2013;136:321-9.

8. Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, Bhattoa HP. Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths. Nutrients. 2020;12:988.

9. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, Esposito S, Ganmaa D, Ginde AA, Goodall EC, Grant CC, Griffiths CJ, Janssens W, Laaksi I, Manaseki-Holland S, Mauger D, Murdoch DR, Neale R, Rees JR, Simpson S, Jr., Stelmach I, Kumar GT, Urashima M, Camargo CA, Jr. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ. 2017;356:i6583.

10. Alipio M. Vitamin D Supplementation Could Possibly Improve Clinical Outcomes of Patients Infected with Coronavirus-2019 (COVID-2019). SSRN Electronic Journal. 2020.

11. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML. Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults. PLoS One. 2010;5:e11088.

12. Bilezikian JP, Bikle D, Hewison M, Lazaretti-Castro M, Formenti AM, Gupta A, Madhavan MV, Nair N, Babalyan V, Hutchings NJ, Napoli N, Accili D, Binkley N, Landry DW, Giustina A. Mechanisms in endocrinology: Vitamin D and COVID-19. Eur J Endocrinol. 2020;183:R133-47.

13. Martineau AR, Forouhi NG. Vitamin D for COVID-19: a case to answer? Lancet Diabetes Endocrinol. 2020;8:735-6.

14. Neill Adhikari JAA, Balkhair A. Interim Guidance. World Health Organization. 27 May 2020;Clinical management of COVID-19. Available from: https://apps.who.int/iris/bitstream/handle/10665/332196/WHO-2019-nCoV-clinical-2020.5-eng.pdf?sequence=1&isAllowed=y

15. Holick MF. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. Rev Endocr Metab Disord. 2017;18:153-65.

16. Ali N. Role of vitamin D in preventing of COVID-19 infection, progression and severity. J Infect Public Health. 2020;13:1373-80.

17. 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;32:1195-8.

18. Demir M, Demir F, Aygun H. Vitamin D deficiency is associated with COVID-19 positivity and severity of the disease. J Med Virol. 2021;93:2992-9.

19. Meltzer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of Vitamin D Deficiency and Treatment with COVID-19 Incidence. medRxiv. 2020;2020.05.08.20095893. Preprint

20. Merzon E TD, Gorohovski A, Vinker S, Cohen AG, Green I, Morgenstern MF. Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. FEBS J 2020;3693-702.

21. D'Avolio A, Avataneo V, Manca A, Cusato J, De Nicolo A, Lucchini R, Keller F, Cantu M. 25-Hydroxyvitamin D Concentrations Are Lower in Patients with Positive PCR for SARS-CoV-2. Nutrients. 2020;12:1359.

22. Hastie CE, Mackay DF, Ho F, Celis-Morales CA, Katikireddi SV, Niedzwiedz CL, Jani BD, Welsh P, Mair FS, Gray SR, O'Donnell CA, Gill JM, Sattar N, Pell JP. Vitamin D concentrations and COVID-19 infection in UK Biobank. Diabetes Metab Syndr. 2020;14:561-5.

23. Charoenngam N, Holick MF. Immunologic effects of vitamin D on human health and Disease. Nutrients. 2020;12:2097.

24. Malek Mahdavi A. A brief review of interplay between vitamin D and angiotensin-converting enzyme 2: implications for a potential treatment for COVID-19. Rev Med Virol. 2020;30:e2119.

25. de Lucena TMC, da Silva Santos AF, de Lima BR, de Albuquerque Borborema ME, de Azevedo Silva J. Mechanism of inflammatory response in associated comorbidities in COVID-19. Diabetes Metab Syndr. 2020;14:597-600.

26. Cui C, Xu P, Li G, Qiao Y, Han W, Geng C, Liao D, Yang M, Chen D, Jiang P. Vitamin D receptor activation regulates microglia polarization and oxidative stress in spontaneously hypertensive rats and angiotensin II-exposed microglial cells: Role of renin-angiotensin system. Redox Biol. 2019;26:101295.

27. Xu J, Yang J, Chen J, Luo Q, Zhang Q, Zhang H. Vitamin D alleviates lipopolysaccharideinduced acute lung injury via regulation of the reninangiotensin system. Mol Med Rep. 2017;16:7432-8.

28. Dancer RC, Parekh D, Lax S, D'Souza V, Zheng S, Bassford CR, Park D, Bartis DG, Mahida R, Turner AM, Sapey E, Wei W, Naidu B, Stewart PM, Fraser WD, Christopher KB, Cooper MS, Gao F, Sansom DM, Martineau AR, Perkins GD, Thickett DR. Vitamin D deficiency contributes directly to the acute respiratory distress syndrome (ARDS). Thorax. 2015;70:617-24.

29. Goddek S. Vitamin D3 and K2 and their potential contribution to reducing the COVID-19 mortality rate. Int J Infect Dis. 2020;99:286-90.

30. Maghbooli Z, Sahraian MA, Ebrahimi M, Pazoki M, Kafan S, Tabriz HM, Hadadi A, Montazeri M, Nasiri M, Shirvani A, Holick MF. Vitamin D sufficiency, a serum 25-hydroxyvitamin D at least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection. PLoS One. 2020;15:e0239799.

31. Munshi R, Hussein MH, Toraih EA, Elshazli RM, Jardak C, Sultana N, Youssef MR, Omar M, Attia AS, Fawzy MS, Killackey M, Kandil E, Duchesne J. Vitamin D insufficiency as a potential culprit in critical COVID-19 patients. J Med Virol. 2021;93:733-40.

32. Carpagnano GE, Di Lecce V, Quaranta VN, Zito A, Buonamico E, Capozza E, Palumbo A, Di Gioia G, Valerio VN, Resta O. Vitamin D deficiency as a predictor of poor prognosis in patients with acute respiratory failure due to COVID-19. J Endocrinol Invest. 2021;44:765-71.

33. 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;25:189-96.

34. Kerget B, Kerget F, Kiziltunc A, Kocak AO, Araz O, Yilmazel Ucar E, Akgun M. Evaluation of the relationship of serum vitamin D levels in COVID-19 patients with clinical course and prognosis. Tuberk Toraks. 2020;68:227-35.

35. Yajia Li QL, Zhang N, Liu Z. Sunlight and vitamin D in the prevention of coronavirus disease (COVID-19) infection and mortality in the United States. Nutrition Dietetics. 2020.

36. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol. 2014;21:319-29.