RESEARCH ARTICLE

REVISED The association between vitamin D deficiency and the clinical outcomes of hospitalized COVID-19 patients [version 4; peer review: 2 approved, 2 not approved]

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Abstract

Background

Vitamin D deficiency is an emerging public health problem that affects more than one billion people worldwide. Vitamin D has been shown to be effective in preventing and reducing the severity of viral respiratory diseases, including influenza. However, the role of vitamin D in COVID-19 infection remains controversial. This study aimed to analyze the association of vitamin D deficiency on the clinical outcome of hospitalized COVID-19 patients.

Methods

A prospective cohort study was conducted among hospitalized COVID-19 patients at two COVID-19 referral hospitals in Indonesia from October 2021 until February 2022.

Results

The median serum 25(OH)D level in 191 hospitalized COVID-19 patients was 13.6 [IQR=10.98] ng/mL. The serum 25(OH)D levels were significantly lower among COVID-19 patients with vitamin D deficiency

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who had cardiovascular disease (p-value=0.04), the use of a ventilator (p-value=0.004), more severe COVID-19 cases (p-value=0.047), and mortality (p-value=0.002). Furthermore, serum 25(OH)D levels were significantly different between patients with mild and severe COVID-19 cases (p-value=0.019). Serum 25(OH)D levels in moderate and severe COVID-19 cases were significantly different (p-value=0.031). Lower serum 25(OH)D levels were significantly associated with an increased number of comorbidities (p-value=0.03), the severity of COVID-19 (p-value=0.002), and the use of mechanical ventilation (p-value=0.032). Mortality was found in 7.3% of patients with deficient vitamin D levels. However, patients with either sufficient or insufficient vitamin D levels did not develop mortality.

Conclusions

COVID-19 patients with vitamin D deficiency were significantly associated with having cardiovascular disease, mortality, more severe COVID-19 cases, and the used of mechanical ventilation. Lower serum 25(OH)D levels were associated with an increased number of comorbidities, COVID-19 severity, and the use of mechanical-ventilation. Thus, we suggest hospitalized COVID-19 patients to reach a sufficient vitamin D status to improve the clinical outcome of the disease.

Keywords
Vitamin D, 25(OH)D, clinical outcome, COVID-19

This article is included in the Emerging Diseases and Outbreaks gateway.

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REVISED **Amendments from Version 3**

The reviewer did raise a very important concern which will improve our manuscript.

-The title was updated.
-We have included additional information in the discussion section.
-We have included several factors that are considered to be limitations of our study.
-We added more references to enhance the quality of our paper.

Any further responses from the reviewers can be found at the end of the article

Introduction

Coronavirus Disease-2019 (COVID-19) is a rapidly spreading pandemic disease caused by Severe Acute Respiratory Syndrome Corona-Virus-2 (SARS-CoV-2), the seventh coronavirus that infect humans. This highly contagious virus spreads through phonation and breathing droplets or through direct contact with an infected person.[1–3] The disease can exhibit a wide range of symptoms, from asymptomatic to dramatic, such as hypoxia and multiorgan failure.[1–5] There is a lack of evidence-based data about the risk factors for the infection, as well as the most effective treatments. Current hospital-based management is focused on the excessive inflammatory response and respiratory support due to the fact that targeted antiviral therapies have not been widely accessible.[6]

Vitamin D is a versatile steroid hormone that plays multiple roles in the body, including the regulation of bone and calcium metabolism.[7,8] It also supports the innate and adaptive immune systems against respiratory viruses.[7,9] It controls the innate immune system by stimulating the synthesis of antimicrobial peptides such as IL-37, cathelicidins, and defensivins.[1,10,11] Vitamin D also modulates adaptive immunity by regulating the formation of inflammatory T helper type 17 (Th17) cells toward the anti-inflammatory regulatory T cells and altering the primary pro-inflammatory cytokines, such as interferon-γ, TNF-α and IL-6.[1,7,10–12] This regulation is considered to be less effective in cases of vitamin D deficiency, although it might be obtained if vitamin D had reached a sufficient level.[1]

Deficient vitamin D is a global health crisis, affecting over a billion people.[7,13–17] Vitamin D deficiency was widespread across Southeast Asian countries, despite extensive exposure to sunlight.[18] Based on current evidence, vitamin D helps prevent and mitigate the severity of viral respiratory diseases, such as influenza.[4,7,19,20] However, the role of vitamin D in COVID-19 infection remains unclear.[4,7]

Furthermore, this is the first study that analyzes the International Severe Acute Respiratory and Emerging Infections Consortium Coronavirus Clinical Characterisation Consortium (ISARIC-4C) score in a group of patients with vitamin D deficiency. The ISARIC 4C mortality score provides an approach for evaluating the risk of mortality upon admission by utilizing demographic and physiological parameters. This scoring system is derived from a comprehensive population cohort study conducted at the national level in the United Kingdom.[21] Understanding the clinical course of COVID-19 is crucial until a viable vaccination becomes widely accessible, due to the lack of specific therapies and the tremendous health and economic impact of the pandemic.[1,22] In this situation, deficient vitamin D is a modifiable risk factor due to its safety and affordability.[1,23,24] The aim of this study was to assess the association of vitamin D deficiency on the clinical outcome of hospitalized COVID-19 patients. Thus, a comprehensive understanding can be obtained as a promising strategy for evaluating the prognosis and treatment for COVID-19 patients.

Methods

Study design

This study was a cross-sectional study conducted at two COVID-19 referral hospitals in Jakarta, Indonesia (National Emergency Hospital Wisma Atlet Kemayoran and Dr. Cipto Mangunkusumo General Hospital), from October 2021 until February 2022. The included subjects were COVID-19 positive (confirmed by reverse transcription-polymerase chain reaction [RT-PCR]) and admitted to the hospital; aged 18 years and older. The exclusion criteria were COVID-19 patients with clinically asymptomatic and severely affected COVID-19 patients who arrived using mechanical ventilation prior to admission. This study specifically involved subjects registered with mild, moderate, or severe disease according to WHO interim guidance and Indonesian government policy at admission.[25] The STROBE guidelines (Strengthening the Reporting of Observational Studies in Epidemiology) were followed for this study.

Data collection

The SARS-CoV-2 infection was confirmed through positive RT-PCR obtained from nasal and oropharyngeal swabs collected.[26] The examination was carried out in the Biosafety Level 3-facility (BSL-3) with Biological Safety Cabinet Class II (BSC-II).

During the admission, each patient had 3–5 mL of blood collected in an acid citrate dextrose tube from a cuffed venous sample. The samples were transported to the laboratory in a cold chain for the measurement of vitamin D. Vitamin D status was evaluated by measuring serum 25(OH) D or 25-hydroxyvitamin D levels. The results were gathered using Roche Diagnostics' Cobas e411, a competitive electrochemiluminescent protein binding assay.

According to Endocrine Society Clinical Practice Guideline, a serum 25(OH) D level of less than 20 ng/mL (50 nmol/L) was considered as deficient.[27] In this study, we divided serum 25(OH) D level into three categories, subjects with serum 25(OH) D levels ≤ 20 ng/mL (≤50 nmol/L) were considered as deficient, serum 25(OH) D levels 21-29 ng/mL (51-74 nmol/L) were considered as insufficient, and serum 25(OH) D levels ≥ 30 ng/mL (≥75 nmol/L) were considered as sufficient.

ISARIC-4C Score

Characteristics examined in the ISARIC-4C mortality score were obtained from each included patient during their admission, as defined by Knight et al.[21] The determinant factors include sex, age, respiratory rate (RR), peripheral oxygen saturation (%), Glasgow Coma Scale (GCS) score, urea serum (mmol/L), and C-reactive protein (mmol/L; CRP).[6,31] The total scores were categorized into low risk (score 0–3), intermediate risk (score 4–8), high risk (score 9–14), and very high risk (score ≥ 15).[21]

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 27 for Macintosh was used to analyze the data that was collected. The serum 25(OH) D levels between two subgroups were analyzed with either Mann-Whitney U test for 2 subgroups and Kruskal-Wallis test for more than 2 subgroups.

Ethical approval

Ethical approval for this study was granted by Ethics Committee of the Faculty of Medicine, Universitas Indonesia (ethical approval number: KET533/UN2.F1/ETIK/PPM.00.01/2021) and by the Ethics Committee of Wisma Atlet Hospital Jakarta (029/KERSDCWA/2021). The Declaration of Helsinki was implemented during this study.

Results

This cross-sectional study included 191 subjects. Before being enrolled, each participant signed a written consent form. The characteristics of the included subjects can be observed in Table 1. From the 191 subjects, 54.5% were female.

Table 1. Subject characteristics.

Variables	N = 191
Age, median [IQR]	42 [28]
Serum 25(OH) D level, median [IQR], in ng/mL	13.6 [10.98]
Sex, N (%)	
Female	104 (54.5)
Male	87 (45.5)
Body mass index (BMI), median [IQR]	22.66 [4.13]
COVID-19 categories, n (%)	
Mild	93 (48.7)
Moderate	67 (35.1)
Severe – critical	31 (16.2)
Number of comorbid, N (%)	
None	72 (37.7)
1	40 (20.9)
2	79 (41.4)

Table 1. *Continued*

Variables	N = 191
Type of comorbidities	
Type 2 DM, N (%)	63 (32.9)
Hypertension, N (%)	60 (31.5)
Cardiovascular disease, N (%)	17 (8.9)
Chronic liver disease, N (%)	6 (3.2)
Chronic kidney failure, N (%)	19 (9.9)
Cerebrovascular disease, N (%)	12 (6.3)
Malignancy, N (%)	19 (9.9)
HIV, N (%)	1 (0.6)
Autoimmune diseases, N (%)	10 (5.2)
COPD, N (%)	2 (1.1)
Vaccination status, n (%)	
Unvaccinated	60 (31.5)
One dose	2 (1)
Two doses	128 (67.1)
Three doses	1 (0.5)
Simple oxygenation, n (%)	61 (31.9)
ISARIC-4C Score, N (%)	
Low risk	109 (57)
Intermediate risk	39 (20.4)
High risk	31 (16.2)
Very high risk	12 (6.3)

Abbreviations: COPD, chronic obstructive pulmonary disease; Type 2 DM, Type 2 Diabetes Mellitus.

Subjects who had a history of diabetes mellitus, peripheral vascular disease, stroke or transient ischaemic index, cardiovascular disease, chronic obstructive pulmonary disease, chronic liver disease, and chronic kidney disease were considered to have comorbidities according to the Charlson Comorbidity Index (CCI).[28]

Table 2 provided the significance levels of serum 25(OH) D level across all included subgroups using the chi-square (χ^2) analysis. Vitamin D deficiency was found in 74.4% of COVID-19 patients, including 65.4% of patients under the age of 60 and 11% of patients over the age of 60. Lower serum 25(OH) D levels were associated with an increased number of

Table 2. The categorized levels of serum 25(OH) D levels based on the influencing factors.

Variables	Categories		Serum 25(OH) D Level			*p*-value
		Total	Deficient (≤20 ng/mL)	Insufficient (21-29 ng/mL)	Sufficient (≥30 ng/mL)	
Age						0.928
	≤60 years old	164 (85.9%)	125 (65.4%)	30 (15.7%)	9 (4.7%)	
	>60 years old	27 (14.1%)	21 (11.0%)	5 (2.6%)	1 (0.5%)	
ISARIC-4C score						0.135
	Low risk	109 (57.07%)	77 (40.3%)	23 (12%)	9 (4.7%)	
	Intermediate risk	39 (20.42%)	30 (15.7%)	8 (4.2%)	1 (0.5%)	
	High risk	31 (16.23%)	27 (14.1%)	4 (2.1%)	0 (0.0%)	
	Very high risk	12 (6.28%)	12 (6.3%)	0 (0.0%)	0 (0.0%)	

Table 2. *Continued*

Variables	Categories		Serum 25(OH) D Level			*p*-value
		Total	Deficient (≤20 ng/mL)	Insufficient (21-29 ng/mL)	Sufficient (≥30 ng/mL)	
Number of comorbidities						**0.03**
	0	72 (37.70%)	42 (24.1%)	17 (8.9%)	9 (4.7%)	
	1	40 (20.90%)	33 (17.3%)	6 (3.1%)	1 (0.5%)	
	2	79 (41.40%)	67 (35.1%)	12 (6.3%)	0 (0.0%)	
Type of comorbidities						
DM type II	No	128 (67.0%)	93 (48.7%)	25 (13.1%)	10 (5.2%)	0.051
	Yes	63 (33.0%)	53 (27.7%)	10 (5.2%)	0 (0.0%)	
Hypertension	No	131 (68.6%)	96 (50.3%)	25 (13.1%)	10 (5.2%)	0.072
	Yes	60 (31.4%)	50 (26.2%)	10 (5.2%)	0 (0.0%)	
Cardiovascular disease	No	174 (91.1%)	133 (69.6%)	31 (16.2%)	10 (5.2%)	0.534
	Yes	17 (8.9%)	13 (6.8%)	4 (2.1%)	0 (0.0%)	
Chronic liver disease	No	185 (96.9%)	140 (73.3%)	35 (18.3%)	10 (5.2%)	0.385
	Yes	6 (3.1%)	6 (3.1%)	0 (0.0%)	0 (0.0%)	
Chronic kidney disease	No	172 (90.1%)	128 (67.0%)	34 (17.8%)	10 (5.2%)	0.136
	Yes	19 (9.9%)	18 (9.4%)	1 (0.5%)	0 (0.0%)	
Malignancy	No	172 (90.1%)	128 (67.0%)	34 (17.8%)	10 (5.2%)	0.136
	Yes	19 (9.9%)	18 (9.4%)	1 (0.5%)	0 (0.0%)	
COPD	No	189 (99.0%)	145 (75.9%)	34 (17.8%)	10 (5.2%)	0.497
	Yes	2 (1.0%)	1 (0.5%)	1 (0.5%)	0 (0.0 %)	
HIV	No	190 (99.5%)	145 (75.9%)	35 (18.3%)	10 (5.2%)	0.856
	Yes	1 (0.5%)	1 (0.5%)	0 (0.0%)	0 (0.0%)	
Autoimmune diseases	No	181 (94.8%)	139 (72.8%)	32 (16.8%)	10 (5.2%)	0.498
	Yes	10 (5.2%)	7 (3.7%)	3 (1.6%)	0 (0.0%)	
BMI						0.435
	Underweight	103 (53.93%)	84 (44%)	13 (6.8%)	6 (3.1%)	
	Normoweight	8 (4.19%)	6 (3.1%)	2 (1.0%)	0 (0.0%)	
	Overweight	34 (17.80%)	25 (13.1%)	8 (4.1%)	1 (0.5%)	
	Obesity grade I	46 (24.08%)	31 (16.2%)	12 (6.3%)	3 (1.6%)	
Mortality						0.097
	No	177 (92.7%)	132 (69.1%)	35 (18.3%)	10 (5.2%)	
	Yes	14 (7.3%)	14 (7.3%)	0 (0.0%)	0 (0.0%)	
COVID-19 severity						**0.002**
	Mild	93 (48.69%)	62 (32.5%)	21 (11.0%)	10 (5.2%)	
	Moderate	67 (35.07%)	55 (28.8%)	12 (6.3%)	0 (0%)	
	Severe	31 (16.24%)	29 (15.2%)	2 (1.0%)	0 (0%)	
Vaccine doses						0.339
	0	60 (31.4%)	52 (27.7%)	7 (3.7%)	1 (0.5%)	
	1	2 (1.0%)	2 (1.0%)	0 (0.0%)	0 (0.0%)	
	2	128 (67.0%)	91 (47.6%)	28 (14.7%)	9 (4.7%)	
	3	1 (0.5%)	1 (0.5%)	0 (0.0%)	0 (0.0%)	

Table 2. *Continued*

Variables	Categories		Serum 25(OH) D Level			*p*-value
		Total	Deficient (≤20 ng/mL)	Insufficient (21-29 ng/mL)	Sufficient (≥30 ng/mL)	
HFNC or ventilator use						**0.032**
	No	171 (89.53%)	126 (66%)	35 (18.3%)	10 (5.2%)	
	Yes	20 (10.50%)	20 (10.5%)	0 (0.0%)	0 (0%)	
Total		**191 (100%)**	**146 (76.4%)**	**35 (18.4%)**	**10 (5.2)**	

Abbreviations: BMI, body mass index; HFNC, High-flow nasal canule; DM type II, diabetes mellitus type II; HIV, human immunodeficiency virus; COPD, chronic obstructive pulmonary diseaseBold value denotes statistical significance.

comorbidities, the severity of COVID-19, and the use of mechanical ventilation. Among 191 patients, mortality was found in 7.3% of patients with deficient vitamin D levels. However, subjects with either sufficient or insufficient vitamin D levels did not develop mortality.

Table 3 showed that serum 25(OH) D levels were significantly lower among COVID-19 patients with vitamin D deficiency who had cardiovascular disease, the use of a ventilator, more severe COVID-19 cases, and mortality. Mortality was found in 9.59% of COVID-19 patients with vitamin D deficiency.

Table 3. The effect of vitamin D deficiency among each subgroup.

Variables	Categories	N (%)	Serum 25(OH) D Level	
			Mean ± SD or median [IQR], in ng/mL	*p*-value
Age groups	≤60 years old	125 (85.62%)	11.94 [7.69]	0.186[a]
	>60 years old	21 (14.38%)	9.74 ± 5.15	
ISARIC-4C score	Low risk	77 (52.74%)	12.07 ± 4.55	0.067[b]
	Intermediate risk	30 (20.55%)	10.75 ± 4.73	
	High risk	27 (18.49%)	9.22 ± 5.09	
	Very high risk	12 (8.22%)	10.97 ± 6.15	
Number of comorbidities	0	46 (31.51%)	12.30 [8.06]	0.133[c]
	1	33 (22.60%)	10.90 ± 4.60	
	2	67 (45.89%)	10.52 ± 5.12	
Type of comorbidities				
DM type II	No	93 (63.7%)	11.05 ± 4.79	0.675[d]
	Yes	53 (36.30%)	11.41 ± 5.12	
Hypertension	No	96 (65.75%)	11.21 ± 4.84	0.914[d]
	Yes	50 (34.25%)	11.12 ± 5.05	
Cardiovascular disease	No	133 (91.1%)	11.44 ± 4.85	**0.040**[d]
	Yes	13 (8.90%)	8.52 ± 4.72	
Chronic kidney disease	No	128 (87.7%)	11.49 ± 4.72	0.051[a]
	Yes	18 (12.3%)	7.75 [9.56]	
Malignancy	No	128 (87.67%)	11.37 ± 4.93	0.211[d]
	Yes	18 (12.33%)	9.83 ± 4.56	
BMI	Underweight	84 (57.53%)	11.73 [8.09]	0.082[c]
	Normoweight	6 (4.11%)	7.97 ± 3.54	
	Overweight	25 (17.12%)	12.47 ± 4.90	
	Obesity grade I	31 (21.23%)	9.93 ± 4.94	

Table 3. *Continued*

Variables	Categories	N (%)	Serum 25(OH) D Level	
			Mean ± SD or median [IQR], in ng/mL	*p*-value
Mortality rate	No	132 (90.41%)	11.58 ± 4.80	**0.002**[d]
	Yes	14 (9.59%)	7.44 ± 4.26	
COVID-19 severity	Mild	62 (42.47%)	11.78 ± 4.62	**0.047**[c]
	Moderate	55 (37.67%)	12.04 [6.64]	
	Severe	29 (19.86%)	7.37 [8.84]	
HFNC or ventilator use	No	126 (86.30%)	11.66 ± 4.70	**0.004**[a]
	Yes	20 (13.70%)	6.39 [7.99]	

Abbreviations: BMI, body mass index; DM type II, diabetes mellitus type II; HFNC, High-flow nasal canule.
Bold value denotes statistical significance.
[a]Analyzed using Mann-Whitney U test.
[b]Analyzed using ANOVA test.
[c]Analyzed using Kruskal-Wallis test.
[d]Analyzed using t-test.

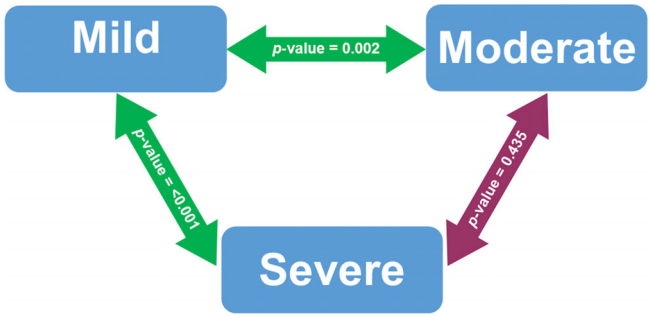

Figure 1. The bivariate analysis of serum 25(OH) D levels based on COVID-19 severity among deficient vitamin D subjects after adjusted with Bonferroni correction. Analyzed using Mann-Whitney U test. Significant at p-value < 0.05. The green double-arrow denotes statistically significant difference. The red double-arrow denotes non-statistically significant difference.

Figure 1 presented the bivariate analysis performed on COVID-19 patients who were vitamin D deficiency. Serum 25(OH) D levels were significantly different between patients with mild and severe COVID-19 cases (p-value < 0.001). Serum 25(OH) D levels in mild and moderate COVID-19 cases were also significantly different (p-value 0.002).

Discussion

Prior studies indicates that Indonesia has a high prevalence of deficient vitamin D (60%), despite being located in a tropical zone where sunlight is abundant all year around.[30–32] Whereas the skin's absorption of sunlight is established as the primary source of vitamin D, other variables, including age, comorbidities, and skin pigmentation, may alter the vitamin D level.[15,32] Based on the skin's sensitivity to ultraviolet (UV) light, the majority of Indonesians have either Fitzpatrick skin phototype IV (with medium to dark brown) or phototype V (dark brown). A lower vitamin D is associated with darker skin pigmentation due to the higher melanin present in darker skin.[18,32,33] Other factors, including haze, altitude, and air pollution, also alter the ultraviolet B radiation.[32,34]

The beneficial effect of vitamin D to reduce the severity of respiratory tract infection remains controversial.[4,7] Prior studies by Luigi et al. have shown that insufficient levels of vitamin D may have a detrimental effect on the prognosis of acute COVID-19, as well as on the development of Long-COVID and the long-term immune response to anti-SARS-CoV-2 vaccination.[35–37] The current study investigated the association of vitamin D deficiency to the clinical outcome of hospitalized patients at two COVID-19 referral hospitals in Indonesia. We found that compared to insufficient and sufficient, those with deficient vitamin D status had more number of comorbidities (Table 2). In COVID-19 patients with deficient vitamin D were significantly associated with cardiovascular disease (Table 3). Our findings was supported by de la Guía-Galipienso *et al.*, that revealed vitamin D deficiency may play a critical role in the initiation of inflammation, myocardial calcification, and endothelial dysfunction, which are risk factors for cardiovascular disease.[27,38] The vitamin

D receptor (VDR) and the enzyme 1α-hydroxylase, which are necessary for the formation of vitamin D's active form, are expressed in cardiomyocytes, vascular endothelial cells, fibroblasts, and smooth muscle cells.[39–42] Left ventricular hypertrophy, vascular dysfunction, and arterial stiffness have been associated with vitamin D deficiency. A deficiency of the vitamin D receptor causes an increase in left ventricular mass and elevated levels of atrial natriuretic peptide, as well as cardiac metalloproteases and disturbances in homeostasis. Furthermore, the development of fibrotic extracellular matrix induces left ventricular dilation.[27,43,44]

Vitamin D has been shown to have a number of beneficial effects on the cardiovascular system, including natriuretic peptide secretion, inhibition of the renin-angiotensin-aldosterone system (RAAS), anti-hypertrophic effects, and inhibition of cardiomyocyte proliferation.[39,45,46] Calcitriol and its analogues activate VDR, which directly suppresses angiotensin I expression and local angiotensin II synthesis in myocardial, kidney tissue, and renal arteries.[39,47] Studies have revealed that vitamin D enhances the anti-hypertensive effects of angiotensin 1–7 by inducing the production of angiotensin-converting enzyme 2 (ACE2).[39,48,49] MiR-106b-5p, which acts on juxtaglomerular cells to boost renin synthesis, has been shown to be directly influenced by VDR-deficient immune cells.[39,49]

Moreover, vitamin D affects the progression of HF through modulating the production of metalloproteinases. Evidence strongly suggests that vitamin D has an anti-inflammatory effect by preventing nuclear factor kappa B (NF-κB) and promoting the production of IL-10, which have a significant role in the progression of CVD.[39,50,51] Vitamin D deficiency induces arterial stiffness and endothelial dysfunction in blood vessels, which in turn leads to enhanced inflammation, endothelial cell malfunction, and atherogenesis.[27,52]

The severity of COVID-19 were significantly associated with the lower serum 25(OH) D levels (Tables 2, 3 and Figure 1). Vitamin D is an immunomodulatory hormone with antibacterial and anti-inflammatory properties, and it plays a crucial role in the immune system. Vitamin D has been reported to exert its effects against COVID-19 by limiting the viral transmission, diminishing viral replication, and optimizing viral clearance.[27,53] Vitamin D boosts the innate immune response and protects against excessive inflammation, by increasing anti-inflammatory IL-10 and decreasing pro-inflammatory cytokines and tumor necrosis factor alpha (TNF-α).[29,53–57] According to the research of Daneshkhah *et al.*, a lack of vitamin D raises C-reaction protein (CRP) levels, which in turn elevates the risk of a cytokine storm.[27,58] The protective effects of vitamin D on the coagulation pathway led to a reduced risk of acute respiratory distress syndrome and thrombosis.[53,59–61] Thus, increasing vitamin D levels to adequate levels may help to prevent COVID-19 infection and complications.[27,53,54,59,62]

Futhermore, Sabico *et al.* that conducted a multi-center randomized clinical trial in Middle East, a region with high prevalence of vitamin D deficiency, revealed that a daily oral supplementation of 5000 IU vitamin D3 for 2 weeks reduced the recovery time for gustatory sensory loss and cough among patients with mild to moderate COVID-19 symptoms and sub-optimal vitamin D status.[63]

To the best of our knowledge, this is the first study that analyse the ISARIC-4C score in a group of patients with vitamin D deficiency. We found that serum 25(OH) D levels had no significant association with ISARIC-4C Score (Tables 2, 3). In contrast with study by Wellbelove *et al.* that concluded the ISARIC-4C mortality score is good predictors for 30-day mortality in COVID-19 (AUROC of 0.74–0.88).[64] The ISARIC-4C consortium established the ISARIC-4C Mortality Score to predict the mortality of hospitalized COVID-19 patients. Multicentre cohort study was conducted among 74,944 participants at 260 different hospitals. However, the ISARIC-4C has been internally validated but not externally validated. Hence, further study is warranted to fully understand the potential of ISARIC-4C as a prognostic tool to classify patients into specific management groups.[6,29,65]

Serum 25(OH) D levels were significantly lower among subjects that used the ventilator (Tables 2, 3). Among all patients, mortality was found in 7.3% of patients with deficient vitamin D levels. However, patients with either sufficient or insufficient vitamin D levels did not develop mortality (Table 2). Serum 25(OH) D levels in vitamin D deficiency subjects were significantly lower in the COVID-19 patients with mortality status (Table 3). Our findings were consistent with the cohort study by Angelidi *et al.* and the single-center retrospective study by Alguwaihes *et al.*, which discovered that lower 25(OH) D levels were associated with increased mechanical ventilation needs and mortality risk among hospitalized patients.[66,67]

Prior studies have revealed that vitamin D deficiency has been correlated to a 58% increased risk of acute respiratory infection, prolonged mechanical ventilation, and a 10-fold increase in mortality risk.[66,68,69]

In contrast, a single-center prospective study conducted in the Indian subcontinent, a region with a high prevalence of deficient vitamin D, demonstrated no statistically significant difference in the median length of stay (LOS) between

patients with sufficient vitamin D and deficient vitamin D (p-value = 0.176). The LOS for patients with deficient vitamin D was 12 days (95% CI: 10, 12 days), and the LOS for patients with sufficient vitamin D was 11 days (95% CI: 10, 13 days). They also showed that deficient vitamin D (defined as 25(OH) D < 30 ng/mL) in patients with COVID-19 was not associated with the length of hospital stay, the need for mechanical ventilation, or the mortality rate.[70] These different results could be explained by different cut-offs to define deficient vitamin D. In our study, a serum 25(OH) D level of less than 20 ng/mL (50 nmol/L) was considered deficient according to the Endocrine Society Clinical Practice Guidelines.[27] On the other hand, most hospitalized patients with COVID-19 have numerous comorbidities, and this population tends to have lower vitamin D levels. Another cohort study by Luigi et al., which excluded the effect of reverse causality and the concomitant comorbidities, demonstrated that vitamin D levels upon admission to the hospital can be used to prospectively predict worse outcomes for both severe and non-severe COVID-19.[71] Thus, vitamin D levels in such a setting should be interpreted with caution.[70]

As a steroid hormone, vitamin D interacts with the vitamin D receptor located in the nucleus of cells to have physiologic effects.[17,66] The interaction of 25(OH) D with other steroid hormone receptors may have physiological effects similar to glucocorticoids.[66,72] Although the underlying mechanisms of vitamin D's protection against severe COVID-19 are unknown, it is established that vitamin D reduces the production of proinflammatory cytokines such as Th1, TNF-α, interferon-β, IL-6, and promotes the production of anti-inflammatory responses such as T regulatory cells and Th2.[55,66,73–75] There are several explanations for vitamin D's beneficial effects on critically ill patients. Initially, critically ill patients who are given vitamin D supplements will have their plasma vitamin D concentrations restored. Furthermore, vitamin D regulates the synthesis of immune system effector molecules such as β-defensin and cathelicidin, which are both antimicrobial peptide.[55,66,76,77] Cathelicidin enhances the production of anti-inflammatory cytokines while decreasing the synthesis of pro-inflammatory cytokines. As a result, vitamin D deficiency could increase the risk of sepsis and inflammation in severely ill patients by diminishing the immune response and modulatory effects on innate immunity.[78–83]

Furthermore, several COVID-19 pandemic studies contained some substantial biases and were not representative of the real-world conditions of the COVID-19 pandemic.[84,85] In regions where authorities implemented home isolation and social distancing measures, individuals with mild to moderate cases of COVID-19 were generally not hospitalized.[84,86] In contrast, in urban areas experiencing a high prevalence of COVID-19 cases and facing constraint intensive care resources, particularly mechanical ventilation, the majority of the hospital admissions were primarily for the individuals with severe or critical cases of COVID-19.[84,87–89] Hospitalized people from a single center or a few centers were unlikely to represent the distribution of COVID-19 cases in an area. Also, there were evident selection biases in why and where people were hospitalized in these studies.[84,90,91] Multiple studies have reported the presence of censoring among subjects who were still hospitalized leading to potential biases in the interpretation of the findings.[84,87,92] On the contrary, we were able to identify every COVID-19 patient in our area, all of whom were admitted to the hospital. We also conducted a comprehensive follow-up for all the patients. The characteristics of our subjects were more similar to they who were exposed to the SARS-CoV-2 infection in a non-epidemic setting. As the result, our study provided a significant potential for widespread applicability and reflected the real-world conditions of the COVID-19 pandemic.[84,93–95]

The strength of this study lies in the fact that it is the first study to analyze the ISARIC-4C Score in COVID-19 patients with deficient vitamin D. The majority of this study's data were collected during the Omicron variation's development and can be utilized to make comparisons to the Delta variant or any other variants. However, this study has several limitations that should be considered to improve the further research. First, this study did not include a healthy control group as a reference population. Second, after patients were discharged, serum 25(OH) D levels were not measured. Third, the observational design and small sample size could potentially miss an important finding in this present study. Fourth, we did not collect any information on the use of vitamin D supplementation. Fifth, it was not possible to determine the impact of disease on vitamin D levels due to reverse causality. Furthermore, considering the established associations between vitamin D levels and comorbidities, and relationship between comorbidities and COVID-19 outcomes, it is difficult not to consider the possibility that vitamin D may become an epiphenomenon typically present in those with a more severe disease.

Hence, despite these limitations, our study clearly showed that lower vitamin D levels at admission represent a strong and reliable factor predicting worse outcomes. We demonstrates that hospitalized COVID-19 patients with vitamin D deficiency had a higher risk of using mechanical ventilation and mortality from respiratory failure and other complications. Additionally, a prior meta-analysis revealed that people with a deficient vitamin D level had an increased risk of SARS-CoV-2 infection and COVID-19-related hospitalization. Our data are consistent with the findings of recent pilot studies and a meta-analysis showing that a sufficient vitamin D status is able to reduce COVID-19 severity, indicating that it may be beneficial in minimizing the clinical and economic burden associated with COVID-19.[1,96–98]

Conclusion

We found that lower serum 25(OH) D levels were associated with an increased number of comorbidities, COVID-19 severity, and the use of mechanical ventilation. COVID-19 patients with vitamin D deficiency status were significantly associated with having cardiovascular disease, mortality, more severe COVID-19 cases, and the used of high-flow nasal canule (HFNC) or ventilator. This study doesn't diminish the significance of the continuing vaccine effort against the health-economic burden of SARS-CoV-2 infection. As a result, we strongly suggest achieving sufficient vitamin D status, which may serve as an important adjuvant strategy to improve clinical outcomes before vaccines become widely available.

Data availability

Underlying data

Figshare: Impact of Vitamin D Deficiency in Relation to the Clinical Outcomes of Hospitalized COVID-19 Patients, DOI: https://doi.org/10.6084/m9.figshare.22145768.v2.[99]

This project contains the following data:

- The data here is only for research paper validation of corresponding author Andhika Rachman entitled: "Impact of Vitamin D Deficiency in Relation to the Clinical Outcomes of Hospitalized COVID-19 Patients". The raw data consists of subjects characteristics and the levels of serum 25-hydroxy-vitamin D of hospitalized COVID-19 patients.

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References

1. Chiodini I, Gatti D, Soranna D, *et al.*: **Vitamin D Status and SARS-CoV-2 Infection and COVID-19 Clinical Outcomes.** *Front. Public Health.* 2021 Dec 22; **9**.
PubMed Abstract | Publisher Full Text | Free Full Text

2. Zhou M, Zhang X, Qu J: **Coronavirus disease 2019 (COVID-19): a clinical update.** *Front. Med.* 2020 Apr 2; **14**(2): 126–135.
PubMed Abstract | Publisher Full Text | Free Full Text

3. Hu B, Guo H, Zhou P, *et al.*: **Characteristics of SARS-CoV-2 and COVID-19.** *Nat. Rev. Microbiol.* 2021 Mar 6; **19**(3): 141–154.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Kalichuran S, van Blydenstein SA, Venter M, *et al.*: **Vitamin D status and COVID-19 severity.** *S. Afr. J. Infect. Dis.* 2022 Apr 26; **37**(1).
Publisher Full Text

5. Huang C, Soleimani J, Herasevich S, *et al.*: **Clinical Characteristics, Treatment, and Outcomes of Critically Ill Patients With COVID-19: A Scoping Review.** *Mayo Clin. Proc.* 2021 Jan; **96**(1): 183–202.
PubMed Abstract | Publisher Full Text | Free Full Text

6. Mohamed RAE, Abdelsalam EM, Maghraby HM, *et al.*: **Performance Features and Mortality Prediction of the 4C Score Early in COVID-19 Infection: A Retrospective Study in Saudi Arabia.** *J. Investig. Med.* 2022 Feb 5; **70**(2): 421–427.
PubMed Abstract | Publisher Full Text | Free Full Text

7. Hafez W, Saleh H, Arya A, *et al.*: **Vitamin D Status in Relation to the Clinical Outcome of Hospitalized COVID-19 Patients.** *Front. Med. (Lausanne).* 2022 Mar 29; **9**.
Publisher Full Text

8. Gruber-Bzura BM: **Vitamin D and Influenza—Prevention or Therapy?** *Int. J. Mol. Sci.* 2018 Aug 16; **19**(8): 2419.
PubMed Abstract | Publisher Full Text | Free Full Text

9. Teymoori-Rad M, Shokri F, Salimi V, *et al.*: **The interplay between vitamin D and viral infections.** *Rev. Med. Virol.* 2019 Mar; **29**(2): e2032.
PubMed Abstract | Publisher Full Text

10. Bouillon R, Marcocci C, Carmeliet G, *et al.*: **Skeletal and Extraskeletal Actions of Vitamin D: Current Evidence and Outstanding Questions.** *Endocr. Rev.* 2019 Aug 1; **40**(4): 1109–1151.
PubMed Abstract | Publisher Full Text | Free Full Text

11. Quesada-Gomez JM, Entrenas-Castillo M, Bouillon R: **Vitamin D receptor stimulation to reduce acute respiratory distress syndrome (ARDS) in patients with coronavirus SARS-CoV-2 infections.** *J. Steroid Biochem. Mol. Biol.* 2020 Sep; **202**: 105719.
PubMed Abstract | Publisher Full Text | Free Full Text

12. Mohan M, Cherian JJ, Sharma A: **Exploring links between vitamin D deficiency and COVID-19.** *PLoS Pathog.* 2020 Sep 18; **16**(9): e1008874.
PubMed Abstract | Publisher Full Text | Free Full Text

13. van Schoor NM, Lips P: **Worldwide vitamin D status.** *Best Pract. Res. Clin. Endocrinol. Metab.* 2011 Aug; **25**(4): 671–680.
Publisher Full Text

14. Palacios C, Gonzalez L: **Is vitamin D deficiency a major global public health problem?** *J. Steroid Biochem. Mol. Biol.* 2014 Oct; **144**: 138–145.
PubMed Abstract | Publisher Full Text | Free Full Text

15. Siddiqee MH, Bhattacharjee B, Siddiqi UR, *et al.*: **High prevalence of vitamin D deficiency among the South Asian adults: a systematic review and meta-analysis.** *BMC Public Health.* 2021 Dec 9; **21**(1): 1823.
PubMed Abstract | Publisher Full Text | Free Full Text

16. Nair R, Maseeh A: **Vitamin D: The "sunshine" vitamin.** *J. Pharmacol. Pharmacother.* 2012 Apr; **3**(2): 118–126.
PubMed Abstract | Publisher Full Text

17. Holick MF, Vitamin D: **Deficiency.** *N. Engl. J. Med.* 2007 Jul 19; **357**(3): 266–281.
Publisher Full Text

18. Nimitphong H, Holick MF: **Vitamin D status and sun exposure in southeast Asia.** *Dermatoendocrinology.* 2013 Jan 27; **5**(1): 34–37.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Bergman P, Lindh ÅU, Björkhem-Bergman L, *et al.*: **Vitamin D and Respiratory Tract Infections: A Systematic Review and Meta-**

Analysis of Randomized Controlled Trials. *PLoS One.* 2013 Jun 19; **8**(6): e65835.
PubMed Abstract | Publisher Full Text | Free Full Text

20. Cannell JJ, Vieth R, Umhau JC, *et al.*: **Epidemic influenza and vitamin D.** *Epidemiol. Infect.* 2006 Dec 7; **134**(6): 1129–1140.
PubMed Abstract | Publisher Full Text | Free Full Text

21. Crocker-Buque T, Myles J, Brentnall A, *et al.*: **Using ISARIC 4C mortality score to predict dynamic changes in mortality risk in COVID-19 patients during hospital admission.** *PLoS One.* 2022 Oct 12; **17**(10): e0274158.
PubMed Abstract | Publisher Full Text | Free Full Text

22. Godlee F: **Covid-19: Transparency and communication are key.** *BMJ.* 2020 Dec 10; m4764.
Publisher Full Text

23. Reid D, Talwar D, Toole B, *et al.*: **PP434 ACUTE CHANGES IN THE SYSTEMIC INFLAMMATORY RESPONSE IS ASSOCIATED WITH TRANSIENT DECREASES IN CIRCULATING 25-HYDROXYVITAMIN D CONCENTRATIONS FOLLOWING ELECTIVE KNEE ARTHROPLASTY.** *Clin. Nutr. Suppl.* 2010 Jan; **5**(2): 193.
Publisher Full Text

24. Louw JA, Werbeck A, Louw MEJ, *et al.*: **Blood vitamin concentrations during the acute-phase response.** *Crit. Care Med.* 1992 Jul; **20**(7): 934–941.
Publisher Full Text

25. Susanto AD, Rozaliyani A, Prasetyo B, *et al.*: **Epidemiological and Clinical Features of COVID-19 Patients at National Emergency Hospital Wisma Atlet Kemayoran, Jakarta, Indonesia.** *Kesmas.* 2021; **16**(1): 11–16.
Publisher Full Text

26. Torretta S, Zuccotti G, Cristofaro V, *et al.*: **Diagnosis of SARS-CoV-2 by RT-PCR Using Different Sample Sources: Review of the Literature.** *Ear Nose Throat J.* 2021 Apr; **100**(1).
Publisher Full Text

27. de la Guía-Galipienso F, Martínez-Ferran M, Vallecillo N, *et al.*: **Vitamin D and cardiovascular health.** *Clin. Nutr.* 2021 May; **40**(5): 2946–2957.
PubMed Abstract | Publisher Full Text | Free Full Text

28. Ludvigsson JF, Appelros P, Askling J, *et al.*: **Adaptation of the Charlson Comorbidity Index for Register-Based Research in Sweden.** *Clin. Epidemiol.* 2021 Jan; **13**: 21–41.
PubMed Abstract | Publisher Full Text | Free Full Text

29. Gupta RK, Harrison EM, Ho A, *et al.*: **Development and validation of the ISARIC 4C Deterioration model for adults hospitalised with COVID-19: a prospective cohort study.** *Lancet Respir. Med.* 2021 Apr; **9**(4): 349–359.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Pinzon RT, Angela PAW, Pradana AW: **Vitamin D deficiency among patients with COVID-19: case series and recent literature review.** *Trop. Med. Health.* 2020 Dec 20; **48**(1): 102.
PubMed Abstract | Publisher Full Text | Free Full Text

31. Alathari BE, Aji AS, Ariyasra U, *et al.*: **Interaction between Vitamin D-Related Genetic Risk Score and Carbohydrate Intake on Body Fat Composition: A Study in Southeast Asian Minangkabau Women.** *Nutrients.* 2021 Jan 23; **13**(2): 326.
PubMed Abstract | Publisher Full Text | Free Full Text

32. Pulungan A, Soesanti F, Tridjaja B, *et al.*: **Vitamin D insufficiency and its contributing factors in primary school-aged children in Indonesia, a sun-rich country.** *Ann. Pediatr. Endocrinol. Metab.* 2021 Jun 30; **26**(2): 92–98.
PubMed Abstract | Publisher Full Text | Free Full Text

33. Sawicki CM, van Rompay MI, Au LE, *et al.*: **Sun-Exposed Skin Color Is Associated with Changes in Serum 25-Hydroxyvitamin D in Racially/Ethnically Diverse Children.** *J. Nutr.* 2016 Apr; **146**(4): 751–757.
PubMed Abstract | Publisher Full Text | Free Full Text

34. Laing EM, Lewis RD: **New Concepts in Vitamin D Requirements for Children and Adolescents: A Controversy Revisited.** 2018; 42–65.

35. di Filippo L, Frara S, Terenzi U, *et al.*: **Lack of vitamin D predicts impaired long-term immune response to COVID-19 vaccination.** *Endocrine.* 2023; **82**(3): 536–541.
PubMed Abstract | Publisher Full Text | Free Full Text

36. di Filippo L, Frara S, Giustina A: **Response to the Letter to the Editor From Min et al: Low Vitamin D Levels are Associated With Long COVID Syndrome in COVID-19 Survivors.** *J. Clin. Endocrinol. Metab.* 2023; **109**(1): e438–e439.
PubMed Abstract | Publisher Full Text

37. di Filippo L, Frara S, Nannipieri F, *et al.*: **Low Vitamin D Levels Are Associated With Long COVID Syndrome in COVID-19 Survivors.** *J. Clin. Endocrinol. Metab.* 2023; **108**(10): e1106–e1116.
PubMed Abstract | Publisher Full Text | Free Full Text

38. Liss Y, Frishman WH: **Vitamin D.** *Cardiol. Rev.* 2012 Jan; **20**(1): 038–044.
Publisher Full Text

39. Cosentino N, Campodonico J, Milazzo V, *et al.*: **Vitamin D and Cardiovascular Disease: Current Evidence and Future Perspectives.** *Nutrients.* 2021 Oct 14; **13**(10): 3603.
PubMed Abstract | Publisher Full Text | Free Full Text

40. Franczyk A, Stolarz-Skrzypek K, Wesołowska A, *et al.*: **Vitamin D and Vitamin D Receptor Activators in Treatment of Hypertension and Cardiovascular Disease.** *Cardiovasc. Hematol. Disord. Drug Targets.* 2014 Feb 28; **14**(1): 34–44.
Publisher Full Text

41. Ni W, Watts SW, Ng M, *et al.*: **Elimination of Vitamin D Receptor in Vascular Endothelial Cells Alters Vascular Function.** *Hypertension.* 2014 Dec; **64**(6): 1290–1298.
PubMed Abstract | Publisher Full Text | Free Full Text

42. Khammissa RAG, Fourie J, Motswaledi MH, *et al.*: **The Biological Activities of Vitamin D and Its Receptor in Relation to Calcium and Bone Homeostasis, Cancer, Immune and Cardiovascular Systems, Skin Biology, and Oral Health.** *Biomed. Res. Int.* 2018; **2018**: 1–9.
PubMed Abstract | Publisher Full Text | Free Full Text

43. Weishaar RE, Simpson RU: **Involvement of vitamin D3 with cardiovascular function. II. Direct and indirect effects.** *Am. J. Physiol. Endocrinol. Metab.* 1987 Dec 1; **253**(6): E675–E683.
PubMed Abstract | Publisher Full Text

44. Mancuso P, Rahman A, Hershey SD, *et al.*: **1,25-Dihydroxyvitamin-D3 Treatment Reduces Cardiac Hypertrophy and Left Ventricular Diameter in Spontaneously Hypertensive Heart Failure-prone (cp/+) Rats Independent of Changes in Serum Leptin.** *J. Cardiovasc. Pharmacol.* 2008 Jun; **51**(6): 559–564.
PubMed Abstract | Publisher Full Text

45. Pilz S, Tomaschitz A: **Role of vitamin D in arterial hypertension.** *Expert. Rev. Cardiovasc. Ther.* 2010 Nov 10; **8**(11): 1599–1608.
Publisher Full Text

46. Hiemstra TF, Lim K, Thadhani R, *et al.*: **Vitamin D and Atherosclerotic Cardiovascular Disease.** *J. Clin. Endocrinol. Metab.* 2019 Sep 1; **104**(9): 4033–4050.
PubMed Abstract | Publisher Full Text | Free Full Text

47. Freundlich M, Li YC, Quiroz Y, *et al.*: **Paricalcitol Downregulates Myocardial Renin-Angiotensin and Fibroblast Growth Factor Expression and Attenuates Cardiac Hypertrophy in Uremic Rats.** *Am. J. Hypertens.* 2014 May 1; **27**(5): 720–726.
PubMed Abstract | Publisher Full Text | Free Full Text

48. Xu J, Yang J, Chen J, *et al.*: **Vitamin D alleviates lipopolysaccharide-induced acute lung injury via regulation of the renin-angiotensin system.** *Mol. Med. Rep.* 2017 May; **16**(5): 7432–7438.
PubMed Abstract | Publisher Full Text | Free Full Text

49. Oh J, Matkovich SJ, Riek AE, *et al.*: **Macrophage secretion of miR-106b-5p causes renin-dependent hypertension.** *Nat. Commun.* 2020 Sep 23; **11**(1): 4798.
PubMed Abstract | Publisher Full Text | Free Full Text

50. Roffe-Vazquez DN, Huerta-Delgado AS, Castillo EC, *et al.*: **Correlation of Vitamin D with Inflammatory Cytokines, Atherosclerotic Parameters, and Lifestyle Factors in the Setting of Heart Failure: A 12-Month Follow-Up Study.** *Int. J. Mol. Sci.* 2019 Nov 19; **20**(22): 5811.
PubMed Abstract | Publisher Full Text | Free Full Text

51. Wöbke TK, Sorg BL, Steinhilber D: **Vitamin D in inflammatory diseases.** *Front. Physiol.* 2014 Jul 2; **5**.
Publisher Full Text

52. Antonopoulos AS, Sanna F, Sabharwal N, *et al.*: **Detecting human coronary inflammation by imaging perivascular fat.** *Sci. Transl. Med.* 2017 Jul 12; **9**(398).
PubMed Abstract | Publisher Full Text

53. Benskin LL: **A Basic Review of the Preliminary Evidence That COVID-19 Risk and Severity Is Increased in Vitamin D Deficiency.** *Front. Public Health.* 2020 Sep 10; **8**.
PubMed Abstract | Publisher Full Text | Free Full Text

54. Hribar CA, Cobbold PH, Church FC: **Potential Role of Vitamin D in the Elderly to Resist COVID-19 and to Slow Progression of Parkinson's Disease.** *Brain Sci.* 2020 May 8; **10**(5): 284.
PubMed Abstract | Publisher Full Text | Free Full Text

55. Grant W, Lahore H, McDonnell S, *et al.*: **Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths.** *Nutrients.* 2020 Apr 2; **12**(4): 988.
PubMed Abstract | Publisher Full Text | Free Full Text

56. Boucher BJ: **The problems of vitamin d insufficiency in older people.** *Aging Dis.* 2012 Aug; **3**(4): 313–329.
PubMed Abstract

57. Sabetta JR, DePetrillo P, Cipriani RJ, *et al.*: **Serum 25-Hydroxyvitamin D and the Incidence of Acute Viral Respiratory Tract Infections in Healthy Adults.** *PLoS One.* 2010 Jun 14; **5**(6): e11088.
PubMed Abstract | Publisher Full Text | Free Full Text

58. Daneshkhah A, Agrawal V, Eshein A, *et al.*: **The Possible Role of Vitamin D in Suppressing Cytokine Storm and Associated Mortality in COVID-19 Patients.** 2020.
Publisher Full Text

59. Tian Y, Rong L: **Letter: Covid-19, and vitamin D. Authors' reply.** *Aliment. Pharmacol. Ther.* 2020 May; **51**(10): 995–996.
PubMed Abstract | Publisher Full Text | Free Full Text

60. Mansur JL: **Letter: low population mortality from COVID-19 in countries south of latitude 35 degrees North supports vitamin D as a factor determining severity.** *Aliment. Pharmacol. Ther.* 2020 Jul; **52**(2): 411–412.
PubMed Abstract | Publisher Full Text | Free Full Text

61. Mohammad M, Mishra A, Ashraf MZ: **Emerging Role of Vitamin D and its Associated Molecules in Pathways Related to Pathogenesis of Thrombosis.** *Biomolecules.* 2019 Oct 24; **9**(11): 649.
PubMed Abstract | Publisher Full Text | Free Full Text

62. Cao Z, Wu Y, Faucon E, *et al.*: **SARS-CoV-2 & Covid-19: Key-Roles of the 'Renin-Angiotensin' System/Vitamin D Impacting Drug and Vaccine Developments.** *Infect. Disord. Drug Targets.* 2020 Jul 20; **20**(3): 348–349.
PubMed Abstract | Publisher Full Text

63. Sabico S, Enani MA, Sheshah E, *et al.*: **Effects of a 2-Week 5000 IU versus 1000 IU Vitamin D3 Supplementation on Recovery of Symptoms in Patients with Mild to Moderate Covid-19: A Randomized Clinical Trial.** *Nutrients.* 2021; **13**(7): 2170.
Publisher Full Text

64. Wellbelove Z, Walsh C, Perinpanathan T, *et al.*: **Comparing the 4C mortality score for COVID-19 to established scores (CURB65, CRB65, qSOFA, NEWS) for respiratory infection patients.** *J. Infect.* 2021 Mar; **82**(3): 414–451.
PubMed Abstract | Publisher Full Text | Free Full Text

65. Knight SR, Ho A, Pius R, *et al.*: **Risk stratification of patients admitted to hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: development and validation of the 4C Mortality Score.** *BMJ.* 2020 Sep 9; **370**: m3339.
PubMed Abstract | Publisher Full Text | Free Full Text

66. Angelidi AM, Belanger MJ, Lorinsky MK, *et al.*: **Vitamin D Status Is Associated With In-Hospital Mortality and Mechanical Ventilation: A Cohort of COVID-19 Hospitalized Patients.** *Mayo Clin. Proc.* 2021 Apr; **96**(4): 875–886.
PubMed Abstract | Publisher Full Text | Free Full Text

67. Alguwaihes AM, Sabico S, Hasanato R, *et al.*: **Severe vitamin D deficiency is not related to SARS-CoV-2 infection but may increase mortality risk in hospitalized adults: a retrospective case–control study in an Arab Gulf country.** *Aging Clin. Exp. Res.* 2021; **33**: 1415–1422.
Publisher Full Text

68. Manson JE, Bassuk SS: **Commentary. Eliminating vitamin D deficiency during the COVID-19 pandemic: A call to action.** *Metabolism.* 2020 Nov; **112**: 154322.
PubMed Abstract | Publisher Full Text | Free Full Text

69. Al-Daghri NM, Amer OE, Alotaibi NH, *et al.*: **Vitamin D status of Arab Gulf residents screened for SARS-CoV-2 and its association with COVID-19 infection: a multi-centre case–control study.** *J. Transl. Med.* 2021; **19**: 166.
Publisher Full Text

70. Dhar A, Mir H, Koul PA. **Vitamin D Levels and Length of Hospitalization in Indian Patients With COVID-19: A Single-Center Prospective Study.** *Cureus.* 2022 Jul 9; **14**(7): e26704.
PubMed Abstract | Publisher Full Text | Free Full Text

71. di Filippo L, Uygur M, Locatelli M, *et al.*: **Low vitamin D levels predict outcomes of COVID-19 in patients with both severe and non-severe disease at hospitalization.** *Endocrine.* 2023; **80**(3): 669–683.
PubMed Abstract | Publisher Full Text | Free Full Text

72. Demer LL, Hsu JJ, Tintut Y: **Steroid Hormone Vitamin D.** *Circ. Res.* 2018 May 25; **122**(11): 1576–1585.
PubMed Abstract | Publisher Full Text | Free Full Text

73. Lemire JM, Adams JS, Kermani-Arab V, *et al.*: **1,25-Dihydroxyvitamin D3 suppresses human T helper/inducer lymphocyte activity *in vitro*.** *J. Immunol.* 1985 May; **134**(5): 3032–3035.
PubMed Abstract | Publisher Full Text

74. Cantorna M, Snyder L, Lin YD, *et al.*: **Vitamin D and 1,25(OH)2D Regulation of T cells.** *Nutrients.* 2015 Apr 22; **7**(4): 3011–3021.
PubMed Abstract | Publisher Full Text | Free Full Text

75. Jeffery LE, Burke F, Mura M, *et al.*: **1,25-Dihydroxyvitamin D3 and IL-2 Combine to Inhibit T Cell Production of Inflammatory Cytokines and Promote Development of Regulatory T Cells Expressing CTLA-4 and FoxP3.** *J. Immunol.* 2009 Nov 1; **183**(9): 5458–5467.
PubMed Abstract | Publisher Full Text | Free Full Text

76. Liu PT, Stenger S, Li H, *et al.*: **Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response.** *Science (1979).* 2006 Mar 24; **311**(5768): 1770–1773.

77. Adams JS, Ren S, Liu PT, *et al.*: **Vitamin D-Directed Rheostatic Regulation of Monocyte Antibacterial Responses.** *J. Immunol.* 2009 Apr 1; **182**(7): 4289–4295.
PubMed Abstract | Publisher Full Text | Free Full Text

78. Shen H, Mei Y, Zhang K, *et al.*: **The Effect of Vitamin D Supplementation on Clinical Outcomes for Critically Ill Patients: A Systemic Review and Meta-Analysis of Randomized Clinical Trials.** *Front. Nutr.* 2021 May 4; **8**.
PubMed Abstract | Publisher Full Text | Free Full Text

79. Wang TT, Nestel FP, Bourdeau V, *et al.*: **Cutting Edge: 1,25-Dihydroxyvitamin D3 Is a Direct Inducer of Antimicrobial Peptide Gene Expression.** *J. Immunol.* 2004 Sep 1; **173**(5): 2909–2912.
PubMed Abstract | Publisher Full Text

80. Quraishi SA, de Pascale G, Needleman JS, *et al.*: **Effect of Cholecalciferol Supplementation on Vitamin D Status and Cathelicidin Levels in Sepsis.** *Crit. Care Med.* 2015 Sep; **43**(9): 1928–1937.
PubMed Abstract | Publisher Full Text | Free Full Text

81. Dürr UHN, Sudheendra US, Ramamoorthy A: **LL-37, the only human member of the cathelicidin family of antimicrobial peptides. Biochimica et Biophysica Acta (BBA).** *Biomembranes.* 2006 Sep; **1758**(9): 1408–1425.
Publisher Full Text

82. Mora JR, Iwata M, von Andrian UH: **Vitamin effects on the immune system: vitamins A and D take centre stage.** *Nat. Rev. Immunol.* 2008 Sep; **8**(9): 685–698.
PubMed Abstract | Publisher Full Text | Free Full Text

83. Kankova M, Luini W, Pedrazzoni M, *et al.*: **Inhibition of cytokine production by vitamin D3 deficiency.** *Int. J. Immunopharmacol.* 1991 Jan; **13**(6): 773.
Publisher Full Text

84. Xu J, Wang W, Ye H, *et al.*: **A predictive score for progression of COVID-19 in hospitalized persons: a cohort study.** *NPJ Prim. Care Respir. Med.* 2021 Jun 3; **31**(1): 33.
PubMed Abstract | Publisher Full Text | Free Full Text

85. Wynants L, Van Calster B, Collins GS, *et al.*: **Prediction models for diagnosis and prognosis of covid-19: systematic review and critical appraisal.** *BMJ.* 2020; **369**.
PubMed Abstract | Publisher Full Text | Free Full Text

86. Gandhi RT, Lynch JB, del Rio C: **Mild or Moderate Covid-19.** *N. Engl. J. Med.* 2020 Oct 29; **383**(18): 1757–66.
Publisher Full Text

87. Galloway JB, Norton S, Barker RD, *et al.*: **A clinical risk score to identify patients with COVID-19 at high risk of critical care admission or death: An observational cohort study.** *J. Infect.* 2020 Aug; **81**(2): 282–8.
PubMed Abstract | Publisher Full Text | Free Full Text

88. Di Bella S, Cesareo R, De Cristofaro P, *et al.*: **Neck circumference as reliable predictor of mechanical ventilation support in adult inpatients with COVID-19: A multicentric prospective evaluation.** *Diabetes Metab. Res. Rev.* 2021 Jan 14; **37**(1): e3354.
PubMed Abstract | Publisher Full Text | Free Full Text

89. Li X, Xu S, Yu M, *et al.*: **Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan.** *J. Allergy Clin. Immunol.* 2020 Jul; **146**(1): 110–118.
PubMed Abstract | Publisher Full Text | Free Full Text

90. Liang WH, Guan WJ, Li CC, *et al.*: **Clinical characteristics and outcomes of hospitalised patients with COVID-19 treated in Hubei (epicentre) and outside Hubei (non-epicentre): a nationwide analysis of China.** *Eur. Respir. J.* 2020 Jun; **55**(6): 2000562.
PubMed Abstract | Publisher Full Text | Free Full Text

91. Bialek S, Bowen V, Chow N, *et al.*: **Geographic Differences in COVID-19 Cases, Deaths, and Incidence — United States, February 12–April 7, 2020.** *MMWR Morb. Mortal. Wkly. Rep.* 2020 Apr 17; **69**(15): 465–471.
Publisher Full Text

92. Gong J, Ou J, Qiu X, *et al.*: **A Tool for Early Prediction of Severe Coronavirus Disease 2019 (COVID-19): A Multicenter Study Using the Risk Nomogram in Wuhan and Guangdong, China.** *Clin. Infect. Dis.* 2020 Jul 28; **71**(15): 833–40.
PubMed Abstract | Publisher Full Text | Free Full Text

93. Verity R, Okell LC, Dorigatti I, *et al.*: **Estimates of the severity of coronavirus disease 2019: a model-based analysis.** *Lancet Infect.*

Dis. 2020 Jun; **20**(6): 669–677.
PubMed Abstract | Publisher Full Text | Free Full Text

94. Wu JT, Leung K, Bushman M, *et al.*: **Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China.** *Nat. Med.* 2020 Apr 1; **26**(4): 506–510.
PubMed Abstract | Publisher Full Text | Free Full Text

95. Bialek S, Boundy E, Bowen V, *et al.*: **Severe Outcomes Among Patients with Coronavirus Disease 2019 (COVID-19) — United States, February 12–March 16, 2020.** *MMWR Morb. Mortal. Wkly. Rep.* 2020 Mar 27; **69**(12): 343–346.
Publisher Full Text

96. McCullough PJ, Lehrer DS, Amend J: **Daily oral dosing of vitamin D3 using 5000 TO 50,000 international units a day in long-term hospitalized patients: Insights from a seven year experience.** *J. Steroid Biochem. Mol. Biol.* 2019 May; **189**: 228–239.
PubMed Abstract | Publisher Full Text

97. Pal R, Banerjee M, Bhadada SK, *et al.*: **Vitamin D supplementation and clinical outcomes in COVID-19: a systematic review and meta-analysis.** *J. Endocrinol. Investig.* 2022 Jan 24; **45**(1): 53–68.
PubMed Abstract | Publisher Full Text | Free Full Text

98. Griffin G, Hewison M, Hopkin J, *et al.*: **Vitamin D and COVID-19: evidence and recommendations for supplementation.** *R. Soc. Open Sci.* 2020 Dec; **7**(12): 201912.
PubMed Abstract | Publisher Full Text | Free Full Text

99. Rachman A: **Raw Data - Impact of Vitamin D Deficiency in Relation to the Clinical Outcomes of Hospitalized COVID-19 Patients. figshare.** *Journal Contribution.* 2023.
Publisher Full Text

Open Peer Review

Current Peer Review Status: ✔ ✘ ✔ ✘

Version 4

Reviewer Report 27 February 2024

https://doi.org/10.5256/f1000research.162135.r243971

✔ **Luigi di Filippo**
Institute of Endocrine and Metabolic Sciences, Università Vita-Salute San Raffaele, IRCCS Ospedale San Raffaele, Milan, Italy

Approved

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: endocrine diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 3

Reviewer Report 19 January 2024

https://doi.org/10.5256/f1000research.155718.r233579

✘ **Guo-Xun Chen**
[1] Department of Nutrition, University of Tennessee, Knoxville, USA
[2] College of Food Science and Technology at Huazhong Agricultural University, Huazhong Agricultural University, Wuhan, Hubei, China

The association studies like this does not ensure that there is a role of vitamin D (VD) deficiency in

COVID-19 outcomes. VD deficiency has been associated with many diseases. However, its supplementation appears to lack conclusions. It will be helpful to show that VD supplementation will help outcomes of COVID-19. Without it, the value of this study is limited.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biochemistry and nutrition.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 04 January 2024

https://doi.org/10.5256/f1000research.155718.r233590

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Luigi di Filippo
Institute of Endocrine and Metabolic Sciences, Università Vita-Salute San Raffaele, IRCCS Ospedale San Raffaele, Milan, Italy

Dear Editor,
thanks for the opportunity to revise the manuscript “Impact of vitamin D deficiency in relation to the clinical outcomes of hospitalized COVID-19 patients” submitted by Dr. Rachman et al.
The authors aimed to evaluate the impact of vitamin D deficiency on COVID-19 outcomes and

reported lower vitamin D as potential risk factor, consistently with the most available literature.
The authors had the novelty to use ISARIC-4C Score in assessing COVID-19 severity.
The main limitation of the study is in assessment of vitamin D levels at admission in hospital where a proper exclusion of effects of disease in lowering vitamin D levels as an effect of reverse causality was not possible. Moreover, given the known associations and relationships between vitamin D levels and comorbidities, and, at the same time, comorbidities and COVID-19 outcomes, it is not possible to exclude that vitamin D should be represent only an epiphenomenon typically present in those with a more severe disease.
Besides these limitations, that should be mentioned in the paper, the authors clearly show that lower vitamin D levels at admission represent a strong and reliable factor predicting worse outcomes. In this light, authors should mention that vitamin D levels, evaluated at admission in hospital, were previously demonstrated to prospectively predict worse outcomes in both those with severe and non-severe disease (therefore, excluding the reverse causality effect) also in multivariate analyses (therefore, excluding the effect of concomitant comorbidities) (DOI: 10.1007/s12020-023-03331-9).
In addition, should be of interest for the readers, to mention the novelties regarding vitamin D role in Long COVID and COVID-19 vaccination (DOI: 10.1210/clinem/dgad207; DOI: 10.1210/clinem/dgad327 ; DOI: 10.1007/s12020-023-03481-w).
Thanks.

References

1. di Filippo L, Frara S, Terenzi U, Nannipieri F, et al.: Lack of vitamin D predicts impaired long-term immune response to COVID-19 vaccination.*Endocrine*. 2023; **82** (3): 536-541 PubMed Abstract | Publisher Full Text
2. di Filippo L, Uygur M, Locatelli M, Nannipieri F, et al.: Low vitamin D levels predict outcomes of COVID-19 in patients with both severe and non-severe disease at hospitalization.*Endocrine*. 2023; **80** (3): 669-683 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: endocrine diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 17 October 2023

https://doi.org/10.5256/f1000research.155718.r205417

Parvaiz Koul
Department of Pulmonary Medicine, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India

While the authors have made changes and updated the references, the moot question of hospitalising mild cases of COVID remains unanswered and that in my opinion is a significant enough reason for me to recommend 'Not Approved' for the revision too.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Version 2

Reviewer Report 29 August 2023

https://doi.org/10.5256/f1000research.148441.r197568

Parvaiz Koul
Department of Pulmonary Medicine, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India

The manuscript provides an analysis of vitamin D levels in 189 patients with COVID-19 in Indonesia and correlates the vitamin D levels with the outcomes of illness using ISARIC-4C mortality score among others. While the concept of the study is good in a tropical country with high sunlight exposure, there are some issues in design that make me uncomfortable for the interpretation of the results.

1. The study has been put forth as a 'cohort' study. However it obviously is not a longitudinal one and hence needs to be classified as an observational cross sectional study.

2. The biggest issue in design is as to why were patients with mild COVID 19 illness included and hospitalized. No guidelines recommend hospital admission of mild cases of COVID 19 and inclusion of these cases involves a serious issue in design which would clearly affect the outcomes of the patients and the results of the study. Inclusion and the very hospitalization of such cases is as such flawed and seriously affects the results.

3. The details of the ISARIC-$C Mortality score should normally be a part of the Methods section. While the authors mention this as the first usage in COVID-19 studies, they have not unfortunately described this in the appropriate section of the manuscript.

4. An important study from India is missing from the 'Discussion' section. The study is also from an area where we expect abundance of sunlight too. In this study (Dhar A, Mir H, Koul PA. Vitamin D Levels and Length of Hospitalization in Indian Patients With COVID-19: A Single-Center Prospective Study. Cureus. 2022 Jul 9;14(7):e26704. doi: 10.7759/cureus.26704. PMID: 35959182; PMCID: PMC9359910.) of 200 patients, there was no statistically significant difference in the length of hospital stay between patients with normal serum vitamin D (VDS) and those with VDD, median LOS being 12 days (95% CI: 10, 12 days) in VDD cases and 11 days (95% CI: 10,13 days) in VDS cases (p = 0.176). The authors concluded that In Indian patients, baseline vitamin D levels are not associated with the length of hospital stay, need for mechanical ventilation, or mortality.

   Such discrepant studies need to be part of the discussion of a study like the one that authors have conducted and the possible reasons for such dichotomy proposed.

References
1. Dhar A, Mir H, Koul PA: Vitamin D Levels and Length of Hospitalization in Indian Patients With COVID-19: A Single-Center Prospective Study.*Cureus*. 2022; **14** (7): e26704 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pulmonary Medicine

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 06 Sep 2023

Andhika Rachman

Dear Prof. Parvaiz Koul,
We sincerely appreciate the time and consideration you have provided in reviewing our manuscript.

We would like to confirm that we have made revisions to the manuscript using the “track changes” system.

If you have any further suggestion, please don’t hesitate to contact us.

Sincerely,
Andhika Rachman, PhD
Medical staff, Division of Hematology and Medical Oncology, Department of Internal

Medicine, Faculty of Medicine, Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital

The manuscript provides an analysis of vitamin D levels in 189 patients with COVID-19 in Indonesia and correlates the vitamin D levels with the outcomes of illness using ISARIC-4C mortality score among others. While the concept of the study is good in a tropical country with high sunlight exposure, there are some issues in design that make me uncomfortable for the interpretation of the results.

1. The study has been put forth as a 'cohort' study. However it obviously is not a longitudinal one and hence needs to be classified as an observational cross sectional study.

Response:
The reviewer did raise a very important concern which will improve our manuscript. We agree with the reviewer's constructive comments that our study is an observational cross sectional study.

2. The biggest issue in design is as to why were patients with mild COVID 19 illness included and hospitalized. No guidelines recommend hospital admission of mild cases of COVID 19 and inclusion of these cases involves a serious issue in design which would clearly affect the outcomes of the patients and the results of the study. Inclusion and the very hospitalization of such cases is as such flawed and seriously affects the results.

Response:
We appreciate your constructive suggestions. We have provided additional explanations regarding the inclusion criteria in both the methods and discussion sections.

During the initial phase of the pandemic, Indonesia encountered a challenging situation as it emerged as the first and enduring epicenter. The incidence of positive cases and deaths has experienced a significant surge, leading to the highest numbers in the Southeast Asian region. The Indonesian government has established a national policy that emphasizes emergency hospitals, which are intended to isolate mild cases and stop community transmission. This Indonesian government strategy has been proven to approach disease containment successfully in Wuhan, China, which was reflected by a significantly reduced COVID-19 mortality rate. Thus, this study includes patients who have been registered with mild, moderate, or severe disease in accordance with the policy established by the Indonesian government.[82]

Furthermore, several COVID-19 pandemic studies contained some substantial biases and were not representative of the real-world conditions of the COVID-19 pandemic.[84,85] In regions where authorities implemented home isolation and social distancing measures,

individuals with mild to moderate cases of COVID-19 were generally not hospitalized.[84,86] In contrast, in urban areas experiencing a high prevalence of COVID-19 cases and facing constraint intensive care resources, particularly mechanical ventilation, the majority of the hospital admissions were primarily for the individuals with severe or critical cases of COVID-19.[84,87,88,89] Hospitalized people from a single center or a few centers were unlikely to represent the distribution of COVID-19 cases in an area. Also, there were evident selection biases in why and where people were hospitalized in these studies.[84,90,91] Multiple studies have reported the presence of censoring among subjects who were still hospitalized leading to potential biases in the interpretation of the findings.[84,87,92] On the contrary, we were able to identify every COVID-19 patient in our area, all of whom were admitted to the hospital. We also conducted a comprehensive follow-up for all the patients. The characteristics of our subjects were more similar to they who were exposed to the SARS-CoV-2 infection in a non-epidemic setting. As the result, our study provided a significant potential for widespread applicability and reflected the real-world conditions of the COVID-19 pandemic.[84,93,94,95]

Reference:

82. Susanto AD, Rozaliyani A, Prasetyo B, Agustin H, Baskoro H, Arifin AR, et al. Epidemiological and Clinical Features of COVID-19 Patients at National Emergency Hospital Wisma Atlet Kemayoran, Jakarta,Indonesia. Kesmas: National Public Health Journal. 2021;16(1):11-16. 10.21109/kesmas.v0i0.5233
83. Dhar A, Mir H, Koul PA. Vitamin D Levels and Length of Hospitalization in Indian Patients With COVID-19: A Single-Center Prospective Study. Cureus. 2022 Jul 9;14(7):e26704. 10.7759/cureus.26704
84. Xu J, Wang W, Ye H, Pang W, Pang P, Tang M, et al. A predictive score for progression of COVID-19 in hospitalized persons: a cohort study. NPJ Prim Care Respir Med. 2021 Jun 3;31(1):33.
85. Wynants L, Van Calster B, Collins GS, Riley RD, Heinze G, Schuit E, et al. Prediction models for diagnosis and prognosis of covid-19: systematic review and critical appraisal. BMJ. 2020. 10.1136/bmj.m1328
86. Gandhi RT, Lynch JB, del Rio C. Mild or Moderate Covid-19. New England Journal of Medicine. 2020 Oct 29;383(18):1757–66. 10.1056/NEJMcp2009249
87. Galloway JB, Norton S, Barker RD, Brookes A, Carey I, Clarke BD, et al. A clinical risk score to identify patients with COVID-19 at high risk of critical care admission or death: An observational cohort study. Journal of Infection. 2020 Aug;81(2):282–8. 10.1016/j.jinf.2020.05.064
88. Di Bella S, Cesareo R, De Cristofaro P, Palermo A, Sanson G, Roman-Pognuz E, et al. Neck circumference as reliable predictor of mechanical ventilation support in adult inpatients with COVID-19: A multicentric prospective evaluation. Diabetes Metab Res Rev. 2021 Jan 14;37(1). [1]
89. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. Journal of Allergy and Clinical Immunology. 2020 Jul;146(1):110–8. 10.1016/j.jaci.2020.04.006
90. Liang W hua, Guan W jie, Li C chen, Li Y min, Liang H rui, Zhao Y, et al. Clinical characteristics and outcomes of hospitalised patients with COVID-19 treated in Hubei (epicentre) and outside Hubei (non-epicentre): a nationwide analysis of China. European

Respiratory Journal. 2020 Jun;55(6):2000562. 10.1183/13993003.00562-2020
91. Bialek S, Bowen V, Chow N, Curns A, Gierke R, Hall A, et al. Geographic Differences in COVID-19 Cases, Deaths, and Incidence — United States, February 12–April 7, 2020. MMWR Morb Mortal Wkly Rep. 2020 Apr 17;69(15):465–71.
92. Gong J, Ou J, Qiu X, Jie Y, Chen Y, Yuan L, et al. A Tool for Early Prediction of Severe Coronavirus Disease 2019 (COVID-19): A Multicenter Study Using the Risk Nomogram in Wuhan and Guangdong, China. Clinical Infectious Diseases. 2020 Jul 28;71(15):833–40. 10.1093/cid/ciaa443
93. Verity R, Okell LC, Dorigatti I, Winskill P, Whittaker C, Imai N, et al. Estimates of the severity of coronavirus disease 2019: a model-based analysis. Lancet Infect Dis. 2020 Jun;20(6):669–77
94. Wu JT, Leung K, Bushman M, Kishore N, Niehus R, de Salazar PM, et al. Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China. Nat Med. 2020 Apr 1;26(4):506–10.
95. Bialek S, Boundy E, Bowen V, Chow N, Cohn A, Dowling N, et al. Severe Outcomes Among Patients with Coronavirus Disease 2019 (COVID-19) — United States, February 12–March 16, 2020. MMWR Morb Mortal Wkly Rep. 2020 Mar 27;69(12):343–6.

3. The details of the ISARIC-$C Mortality score should normally be a part of the Methods section. While the authors mention this as the first usage in COVID-19 studies, they have not unfortunately described this in the appropriate section of the manuscript.

Response:
Thank you for your helpful suggestions. We have added and revised the details of the ISARIC-4C Mortality Score in the Method section of the manuscript.
Characteristics examined in the ISARIC-4C mortality score were obtained from each included patient during their admission, as defined by Knight et al.[81] The determinant factors include sex, age, respiratory rate (RR), peripheral oxygen saturation (%), Glasgow Coma Scale (GCS) score, urea serum (mmol/L), and C-reactive protein (mmol/L; CRP).[6,27] The total scores were categorized into low risk (score 0–3), intermediate risk (score 4–8), high risk (score 9–14), and very high risk (score $\geq$ 15).[81]

Reference:
6. Mohamed RAE, Abdelsalam EM, Maghraby HM, et al.: Performance Features and Mortality Prediction of the 4C Score Early in COVID-19 Infection: A Retrospective Study in Saudi Arabia. *J. Investig. Med.* 2022 Feb 5;70(2):421–427. 34836890 10.1136/jim-2021-001940 PMC8635889
27. Gupta RK, Harrison EM, Ho A, et al.: Development and validation of the ISARIC 4C Deterioration model for adults hospitalised with COVID-19: a prospective cohort study. *Lancet Respir. Med.* 2021 Apr;9(4):349–359. 33444539 10.1016/S2213-2600(20)30559-2 PMC7832571
81. Crocker-Buque T, Myles J, Brentnall A, Gabe R, Duffy S, Williams S, et al. Using ISARIC 4C mortality score to predict dynamic changes in mortality risk in COVID-19 patients during hospital admission. PLoS One. 2022 Oct 12;17(10):e0274158

4. An important study from India is missing from the 'Discussion' section. The study is also from an area where we expect abundance of sunlight too. In this study (Dhar A, Mir H, Koul PA. Vitamin D Levels and Length of Hospitalization in Indian Patients With COVID-19: A Single-Center Prospective Study. Cureus. 2022 Jul 9;14(7):e26704. doi: 10.7759/cureus.26704. PMID: 35959182; PMCID: PMC9359910.) of 200 patients, there was no statistically significant difference in the length of hospital stay between patients with normal serum vitamin D (VDS) and those with VDD, median LOS being 12 days (95% CI: 10, 12 days) in VDD cases and 11 days (95% CI: 10,13 days) in VDS cases (p = 0.176). The authors concluded that In Indian patients, baseline vitamin D levels are not associated with the length of hospital stay, need for mechanical ventilation, or mortality.

Such discrepant studies need to be part of the discussion of a study like the one that authors have conducted and the possible reasons for such dichotomy proposed.

Response:
Thank you for your valuable suggestions. We have improved our paper by citing the prospective study in the Indian subcontinent by Dhar et al. We have also provided the explanations for the possible reasons underlying the observed dichotomy in the discussion section.

Interestingly, a single-center prospective study conducted in the Indian subcontinent, a region with a high prevalence of deficient vitamin D, demonstrated no statistically significant difference in the median length of stay (LOS) between patients with sufficient vitamin D and deficient vitamin D (p-value=0.176). The LOS for patients with deficient vitamin D was 12 days (95% CI: 10, 12 days), and the LOS for patients with sufficient vitamin D was 11 days (95% CI: 10, 13 days). They also showed that deficient vitamin D (defined as 25(OH)D < 30 ng/mL) in patients with COVID-19 was not associated with the length of hospital stay, the need for mechanical ventilation, or the mortality rate.[83] These different results could be explained by different cut-offs to define deficient vitamin D. In our study, a serum 25(OH)D level of less than 20 ng/mL (50 nmol/L) was considered deficient according to the Endocrine Society Clinical Practice Guidelines.[25]On the other hand, most hospitalized patients with COVID-19 have numerous comorbidities, and this population tends to have lower vitamin D levels. Thus, vitamin D levels in such a setting should be interpreted with caution.[83]

Reference:
25. de la Guía-Galipienso F , Martínez-Ferran M, Vallecillo N, et al.: Vitamin D and cardiovascular health. *Clin. Nutr.*2021 May;40(5):2946–2957. 33397599 10.1016/j.clnu.2020.12.025 PMC7770490
83. Dhar A, Mir H, Koul PA. Vitamin D Levels and Length of Hospitalization in Indian Patients With COVID-19: A Single-Center Prospective Study. Cureus. 2022 Jul 9;14(7):e26704. 10.7759/cureus.26704

If you have any suggestions, we would greatly appreciate hearing them. Thanks for your attention. We eagerly await your response.

Competing Interests: The authors declare that we have no conflict of interest.

Reviewer Report 14 June 2023

https://doi.org/10.5256/f1000research.148441.r178400

Shaun Sabico
Department of Biochemistry College of Science, King Saud University, Riyadh, Riyadh Province, Saudi Arabia

I commend the authors for satisfactorily addressing the comments. I have no further suggestions.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nutrition, medical sciences, vitamin D, metabolism

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 11 May 2023

https://doi.org/10.5256/f1000research.145122.r169546

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Shaun Sabico
Department of Biochemistry College of Science, King Saud University, Riyadh, Riyadh Province, Saudi Arabia

The present prospective study by Rachman and colleagues analyzed the impact of vitamin D deficiency among covid-19 patients and found that those who had more comorbidities and had more severe manifestations of covid-19 were more likely to be vitamin D deficient. Although the data itself is not new, this is one of the very few studies coming from Southeast Asia where vitamin D deficiency is not expected to be as pronounced in other regions. The use of ISARIC-4C mortality score is also an added novelty. Despite this fact, their findings largely echo previous observations. I have several comments:

Major:

1. Please add more details how covid-19 was diagnosed with reference. Was it through nasopharyngeal swab? And where was vitamin D measured? Was it in a BSL3 facility?

2. The statistics could have been expanded to control for confounders such as age and BMI. These were not explicitly mentioned in the data analysis. Furthermore, with the number of variables measured, the p-value should have been Bonferroni adjusted unless the authors prove this wasn't necessary.

3. Lastly, the study design appears to be cross-sectional and not prospective. The observational design and small sample size have limited the findings to at best, suggestive. The limitation section could have been expanded taking into consideration the points raised.

Minor

1. Several studies that support your findings from the Middle East where deficiency is very pronounced are suggested to be added including clinical trials on vitamin D and covid-19. Similar findings from different regions and ethnic groups reinforce the role of vitamin D in severity of covid-19.

References

1. Sabico S, Enani MA, Sheshah E, Aljohani NJ, et al.: Effects of a 2-Week 5000 IU versus 1000 IU Vitamin D3 Supplementation on Recovery of Symptoms in Patients with Mild to Moderate Covid-19: A Randomized Clinical Trial.*Nutrients*. 2021; **13** (7). PubMed Abstract | Publisher Full Text
2. Al-Daghri NM, Amer OE, Alotaibi NH, Aldisi DA, et al.: Vitamin D status of Arab Gulf residents screened for SARS-CoV-2 and its association with COVID-19 infection: a multi-centre case-control study.*J Transl Med*. 2021; **19** (1): 166 PubMed Abstract | Publisher Full Text
3. Alguwaihes AM, Sabico S, Hasanato R, Al-Sofiani ME, et al.: Severe vitamin D deficiency is not related to SARS-CoV-2 infection but may increase mortality risk in hospitalized adults: a retrospective case-control study in an Arab Gulf country.*Aging Clin Exp Res*. 2021; **33** (5): 1415-1422 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nutrition, medical sciences, vitamin D, metabolism

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 18 May 2023

Andhika Rachman

Response To The Reviewer:

Dear Dr. Shaun Sabico,
We would like to thank you for your time and consideration in handling our manuscript.

We want to confirm that we have revised the manuscript with the tracked changes system.

If you have any suggestions, please don't hesitate to contact us.

Sincerely,
Andhika Rachman, PhD
Medical staff, Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital

Major:

1. Please add more details how covid-19 was diagnosed with reference. Was it through nasopharyngeal swab? And where was vitamin D measured? Was it in a BSL3 facility?

Thank you for your construction feedback. We have added more details on the methods.
The SARS-CoV-2 infection was confirmed through positive RT-PCR obtained from nasal and oropharyngeal swabs collected.[77] The examination was carried out in the Biosafety Level 3-facility (BSL-3) with Biological Safety Cabinet Class II (BSC-II).
During the admission, each patient had 3–5 mL of blood collected in an acid citrate dextrose tube from a cuffed venous sample. The samples were transported to the laboratory in a cold chain for the measurement of vitamin D.

Reference:
77. Torretta S, Zuccotti G, Cristofaro V, Ettori J, Solimeno L, Battilocchi L, D'Onghia A, Bonsembiante A, Pignataro L, Marchisio P, Capaccio P. Diagnosis of SARS-CoV-2 by RT-PCR Using Different Sample Sources: Review of the Literature. Ear Nose Throat J. 2021 Apr;100(2_suppl):131S-138S. doi: 10.1177/0145561320953231.

1. The statistics could have been expanded to control for confounders such as age and BMI. These were not explicitly mentioned in the data analysis. Furthermore, with the number of variables measured, the p-value should have been Bonferroni adjusted unless the authors prove this wasn't necessary.

The reviewer did raise a very important concern which will improve our manuscript. We strongly agree that the reviewer's suggestion should be considered for further researches regarding this issue. The confounders such as age and BMI could not be controlled due to the minimum sample size of the subgroups and the fact that the number of samples is unequally distributed, which has not fulfilled the criteria to conduct the analysis. Thus, we have raised the point that the small sample size has become our limitation. We plan to improve the design and participants selection in the next study.

Furthermore, we have added Figure 1 (a bivariate analysis that has been adjusted with the Bonferoni correction). Figure 1 presents the bivariate analysis performed on COVID-19 patients with vitamin D deficiency. Serum 25(OH)D levels were significantly different between patients with mild and severe COVID-19 cases (p-value < 0.001). Serum 25 (OH) D levels in mild and moderate COVID-19 cases were also significantly different (p-value 0.002).

1. Lastly, the study design appears to be cross-sectional and not prospective. The observational design and small sample size have limited the findings to at best, suggestive. The limitation section could have been expanded taking into consideration the points raised.

We have added the points of observational design and small sample size as limitations of our study. Additionally, we want to verified that our study's design is cohort prospective. Even if the risk variables (vitamin D levels) are measured upon admission, the outcome is followed and observed until the patient leaves the hospital, becomes worse, requires a ventilator, or passes away. The person had not used a ventilator when first recruited.

Minor

1. Several studies that support your findings from the Middle East where deficiency is very pronounced are suggested to be added including clinical trials on vitamin D and covid-19. Similar findings from different regions and ethnic groups reinforce the role of vitamin D in severity of covid-19.

Thank you for your advice. We have improved our paper by citing the multi-center randomized clinical trial in the Middle East that conducted by Sabico et al. We have also cited the studies carried out by Al-Daghri et al. and Alguwaihes et al.

Please don't be hesitant to contact us if you have any concerns or suggestions about the revised manuscript. If you have any suggestions, we would greatly appreciate hearing them. Thanks for your attention. We eagerly await your response.

Competing Interests: The authors declare that we have no conflict of interest.