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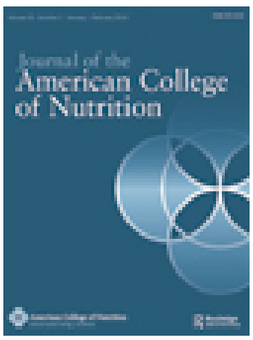

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Does Serum Vitamin D Level Affect COVID-19 Infection and Its Severity?-A Case-Control Study

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ABSTRACT

Background: As effective medication to treat COVID-19 is currently unavailable, preventive remedies may be particularly important.

Objective: To examine the relationship between serum 25-hydroxy vitamin D (25(OH)D) level and COVID-19 infection, its severity, and its clinical case characteristics.

Methods: This case-control study compared serum 25(OH)D levels and rates of vitamin D deficiency (VDD) between 80 healthy controls and 62 patients diagnosed with COVID-19 and admitted to Guangxi People's Hospital, China, 2/16/2020–3/16/2020. Cases were categorized into asymptomatic, mild/moderate, and severe/critical disease. Logistic regression analysis was conducted to examine the associations between 25(OH)D level, or VDD, and case status/severity of COVID-19 while controlling for demographics and comorbidities. A threshold level of vitamin D for conveying COVID-19 risk was estimated.

Results: Severe/critical COVID-19 cases were significantly older and had higher percentages of comorbidity (renal failure) compared to mild cases. The serum 25(OH)D concentration in COVID-19 patient was much lower than that in healthy control. And 25(OH)D level was the lowest in severe/critical cases, compared with mild cases. In further, significantly higher rates of VDD were found in COVID-19 cases (41.9%) compared to healthy controls (11.1%). And VDD was the greatest in severe/critical cases (80%), compared with mild cases (36%). These statistically significant associations remained even after controlling for demographics and comorbidities. A potential threshold of 25(OH)D (41.19 nmol/L) to protect against COVID-19 was identified.

Conclusion: Elderly and people with comorbidities were susceptible to severe COVID-19 infection. VDD was a risk factor for COVID-19, especially for severe/critical cases. While further confirmation is needed, vitamin D supplementation may have prevention or treatment potential for COVID-19 disease.

Abbreviations: COVID-19: Coronavirus Disease 2019; VDD: vitamin D deficient; OR: odds ratio; 95% CI: 95% confidence interval

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COVID-19; vitamin D deficiency; severity; risk factor

Introduction

The outbreak of Corona Virus Disease 2019 (COVID-19) is ongoing globally and poses a major public health challenge (1). As of September 5, 2020 there are 26,383,872 confirmed cases and 870,126 deaths worldwide (2). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes illness ranging from common cold to pneumonia (3). It has been reported that about 19% of patients with COVID-19 have severe symptoms and require hospitalization (3). Of those hospitalized, 3.37%–17% of patients develop acute respiratory distress syndrome and 5.0%–26.1% patients are admitted to intensive care units (4, 5). It has been reported that one in ten hospitalized patients progress rapidly and develop fatal multiple organ failure (4). In the early stages of

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the outbreak, the mortality rate in adults with cases of COVID-19 severe enough to require hospitalization has been reported to be as high as 28% (6). Limited case reports have reported that elderly, males, and people with comorbidities and excessive immune activation may be particularly susceptible to severe COVID-19 disease or progression to death (3, 6). No broadly effective treatment is currently available.

Vitamin D is a key hormone involved in the regulation of immune response, and prevention of inflammation and autoimmunity (7). Vitamin D deficiency (VDD) was defined as serum 25-hydroxyvitamin D (25(OH)D) concentration lower than 50 nmol/L (8). Numerous studies demonstrated that low 25(OH)D level was associated with the severity of infectious respiratory diseases, such as pharyngotonsillitis (9), bronchiolitis (10), pneumonia, and influenza (11). Vitamin D supplementation has been found to reduce illness severity in patients with respiratory infection in clinical trials (12). Clinical trials in adults with chronic obstructive pulmonary disease (COPD) also reveal that vitamin D supplementation could promote lung function recovery (13). In addition, vitamin D has been reported to be associated with risk factors of COVID-19, such as age and male (14), and vitamin D supplementation has shown therapeutic potential for risky comorbidities, such as obesity (15) and cardiovascular diseases (16). Therefore, vitamin D may modify or reduce the risk and susceptibility of COVID-19.

Several studies had reported the possible association between 25(OH)D level and COVID-19 (17, 18). While scientists are still seeking effective medication to treat COVID-19 cases, supplemental vitamin D may have potential to prevent healthy people from virus infection or reduce disease severity. No studies to date have evaluated vitamin D's potential effect on COVID-19 in Chinese population. To fill these knowledge gaps, we examined the association between 25(OH)D levels and COVID-19 disease, its severity, and its clinical characteristics in Chinese population.

Methods

Study design and study population

In this case-control study, the cases included all patients with COVID-19 disease, from February 16th, 2020 to March 16th, 2020, treated at the Yongwu Hospital of The People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China, the largest designated hospital to provide care to patients with COVID-19 in Guangxi Province. Generally, the patients came from all regions of Guangxi, and represented most of the confirmed cases in the region who were transferred to a hospital for treatment. The cases of COVID-19 were diagnosed according to the guidelines of the National Health Commission of China (19) and were confirmed by positive SARS-CoV-2 RNA with throat swab samples (Sansure Biotechnology, Changsha, Hunan, China). Controls were recruited from The Physical Examination Center of our Institution with no medical disorder (hypertention, diabetes, renal disease, and pneumonia) , and were frequency matched by sex and age as the cases. The ethics committee of The People's Hospital of Guangxi Zhuang Autonomous Region approved this study (No. KY-LW-2020-002). Informed consent was obtained from all participants.

Data collection

Demographic (sex and age) and clinical variables were extracted from medical records. Clinical variables include clinical symptoms (fever, cough, chest tightness, chest pain, and diarrhea) and comorbidities (hypertension, diabetes, COPD, asthma, liver injury, and renal failure). COPD and asthma were self-reported. Diabetes was considered as present whenever the use of antidiabetic drug treatment was reported. The patient who had a fasting blood sugar of greater than or equal to 126 mg/dl, or a 2 hour blood sugar of greater than or equal to 200 mg/dl, or an A1c greater than or equal to 6.5%, were diagnosed with diabetes. Hypertension was defined as mean systolic BP (SBP) $\geq$140 mmHg or mean diastolic BP (DBP) $\geq$90 mmHg, or the presence of antihypertensive medication. Liver injury was defined as increase of alanine aminotransferase or conjugated bilirubin by more than 2-fold, or increased aspartate aminotransferase, alkaline phosphatase as well as total bilirubin by at least 2-fold. Renal function was estimated based on glomerular filtration rate (GFR) using the CKD-EPI equation (20). Renal failure was defined as GRF $<$90 mL/min * 1.73 m^2.

Serum samples were collected for each patient at admission and stored at $-80\,^{\circ}C$ before measuring the concentration of 25-hydroxyvitamin D (25(OH)D) using an electrochemiluminescent immunoassay (ECLIA) with a Roche Elecsys 10100/201 system.

Disease severity and vitamin D deficiency classification

Severe COVID-19 case was defined according to the guidelines of the National Health Commission of China. Severe cases met at least one of the following criteria: 1) breathing rate $\geq$30/min; 2) pulse oximeter oxygen saturation (SpO_2) $\leq$93% when breathing ambient air; 3) ratio of partial oxygen pressure (PaO_2) to the fraction of inspired oxygen (FiO_2) $\leq$300 mmHg (1 mmHg = 0.133 kpa); and 4) lung imaging showing significant progression of $>$50% within 24 to 48 hours. Critical cases were defined as having at least one of the following: 1) respiratory failure (PaO_2 $<$60 mmHg when breathing ambient air); 2) hemodynamic shock (persisting hypotension requiring vasopressors to maintain MAP $\geq$65 mmHg and serum lactate level $>$2 mmol/L despite volume resuscitation; and 3) organ failure or admittance to intensive care unit (ICU). According to the Endocrine Society clinical practice guideline, vitamin D deficiency (VDD) was defined as a 25(OH)D $<$ 50 nmol/L, vitamin D insufficiency as 50 nmol/L $\leq$ 25(OH)D $<$ 75 nmol/L and vitamin D sufficiency as 25(OH)D $\geq$ 75 nmol/L (8).

Table 1. Demographic characteristics and 25(OH)D levels of the participants in Guangxi, China from February 16th, 2020 to March 16th, 2020.

	Healthy controls (n = 80)	All cases (n = 62)	Mild/moderate (n = 50)	Severe/critical (n = 10)
Gender				
Female	48 (60)	39 (63)	31 (62)	6 (60)
Male	32 (40)	23 (37)	19 (38)	4 (40)
Age, y				
Median [IQR]	42 [31–52]	43 [32–59]	39 [30–49]	65 [54–69][a]
Range	4–78	0.1–85	0.1–68	45–85
Area				
Urban	80 (100)	44 (71)	36 (72)	7 (70)
Rural	0	18 (29)	14 (28)	3 (30)
25(OH)D (nmol/L)	71.8 [57.6–83.7]	55.6 [41.9–66.1][b]	56.6 [44.6–66.4]	38.2 [33.2–50.5][a]
Vitamin D status				
Deficiency[c]	15 (19)	26 (42)	18 (36)	8 (80)
Non-deficiency	65 (81)	36 (58)	32 (64)	2 (20)
OR (95 CI)	3.13 (1.47–6.66)[b]		7.11 (1.36–37.16)[a]	

Data are present as n(%) or Median[IQR], unless indicated. IQR = interquartile range. OR = odds ratio. 95 CI = 95 confidence interval.
[a]Mild/moderate cases vs Severe/critical cases, $P < 0.05$.
[b]All cases vs Healthy controls, $P < 0.05$.
[c]Vitamin D deficiency is defined as 25(OH)D < 50 nmol/L.

Table 2. Clinical characteristics of COVID-19 patients stratified by disease severity.

	Mild/moderate (n = 50)	Severe/critical (n = 10)	*P* value
Onset Symptoms			
Fever	32 (64)	8 (80)	0.540
Cough	24 (48)	7 (70)	0.355
Chest tightness	4 (8)	0	..
Chest pain	2 (4)	0	..
Diarrhea	2 (4)	1 (10)	0.427[a]
Comorbidities			
Diabetes	3 (6)	2 (20)	0.190[a]
Hypertension	4 (8)	2 (20)	0.564
Liver injury	0	1 (10)	..
COPD	1 (2)	0	..
Asthma	0	0	..
Renal failure[b]	8 (16)	8 (80)	<0.001

Data are present as n(%). COPD = chronic obstructive pulmonary disease.
[a]Fisher's exact test.
[b]Renal failure is defined as GRF <90 mL/min * 1.73 m^2.

Confounding variables and statistical analysis

Potential confounding variables include age, sex, and comorbidities which are suspected risk factors of COVID-19 or thought to be associated with both COVID-19 infection and VDD according to previous limited studies. Continuous variables without normal distribution were analyzed using the Mann-Whitney U test. Continuous variables following normal distribution are presented as mean ± standard deviation (SD) and were analyzed using Students t-test. Categorical variables are presented as number and percentage and were analyzed using the chi-square test or Fishers exact test (when expected counts <1). Multivariate analysis was conducted using an unconditional logistic regression model, with all measured risk factors included as independent variables, and the dependent variable as either severe/critical disease or all COVID-19 cases vs controls. Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic characteristics of all participants

We identified 62 patients with laboratory-confirmed COVID-19 (median age of 43 years; 39 (63%) females; and 44 (71%) from urban areas) and enrolled 80 healthy individuals as controls (age and sex were frequency matched with cases). No patient died during hospitalization. As described in Table 1, severe/critical cases were significantly older than mild/moderate cases (median: 65 vs 39 years; $P < 0.001$).

Clinical features of COVID-19 patients stratified by illness severity

As presented in Table 2, the frequencies of certain symptoms, including fever (eight [80%]), cough (seven [70%]), and diarrhea (one [10%]), were higher in severe/critical COVID-19 cases than in mild/moderate cases (32 [64%], 24 [48%] and two [4%] respectively), but differences were not statistically significant. We also found higher rates of underlying conditions, including diabetes (two [20%]), hypertension (two [20%]), and renal failure (eight [80%]), in the severe/critical cases compared to mild/moderate cases (three [6%], four [8%], and eight [16%] respectively), yet only renal failure showed statistical significance.

The association between vitamin D and COVID-19/severity

As is shown in Table 1, the serum 25(OH)D levels in COVID-19 patients (55.6 nmol/L) were statistically lower than in healthy controls (71.8 nmol/L). Additionally, we found that 26 [41.9%] COVID-19 patients had VDD, while only 15 [18.8%] healthy individuals had VDD (OR, 3.13; 95% CI, 1.47–6.66)

The relationship between vitamin D and COVID-19 severity is described in Table 1. Serum 25(OH)D levels in severe/critical COVID-19 cases (38.2 nmol/L) were significantly lower than that in mild/moderate cases (56.6 nmol/L). Moreover, eight [80%] severe/critical cases had VDD, while only 18 [36.0%] mild/moderate diseases had VDD (OR, 7.11; 95% CI, 1.63–37.16).

Results of multivariate regression analysis (Table 3), including all potential risk factors as independent variables (including VDD, age, sex, renal failure, diabetes, and hypertension), indicate a statistically significant association

Table 3. Multivariate logistic regression analysis of all potential risk factors as predictors of severe/critical COVID-19.

	B	SE	Wald c	OR (95 CI)	*P* value
VDD	2.720	1.282	4.499	15.18 (1.23–187.45)	0.034
Age[a]	0.897	0.552	2.642	2.45 (0.83–7.23)	0.104
Gender (female)	1.437	1.392	0.302	4.21 (0.28–64.35)	0.429
Renal Failure	2.649	1.473	3.233	14.14 (0.79–253.9)	0.072
Hypertension	−0.651	1.612	0.163	0.52 (0.02–12.29)	0.687
Diabetes	−0.540	1.726	0.098	0.58 (0.02–18.17)	0.754

B = regression coefficient. SE = standard error. OR = odds ratio. 95 CI = 95 confidence interval. VDD = vitamin D deficiency.
[a]Every ten years per level (level 1-9).

between VDD and severe/critical disease (OR, 15.18; 95% CI, 1.23–187.45). The very large confidence interval indicates a small sample size being used. A separate linear regression analysis (data not shown) also showed an inverse association between serum 25(OH)D level as a continuous variable and severe/critical disease (OR per nmol/L, 0.91; 95% CI, 0.84–0.99).

Vitamin D status in different severity groups of COVID-19

To assess the association consistency, we also investigated the vitamin D status in relation to different severity groups and adverse events of COVID-19. As shown in Table 4, two (100%)asymptomatic cases were vitamin D sufficient, and eight (80%) severe cases were VDD (Mantel-Haenszel *P* value = 0.004). Additionally, all patients experiencing shock (ten [100%]) and requiring mechanical ventilation (ten [100%]) had VDD, while only 22 [37.9%] and 21 [36.8%] patients without shock or without mechanical ventilation were VDD (Table 4, P=0.025 and 0.012, respetively). Similar trends of VDD (three [75%] and four [80%]) were found among patients with FiO_2 ≤300 mmHg and lung infiltration ≥50%.

Predicting illness severity of COVID-19 with 25(OH)D level

A receiver operator characteristic curve was estimated to investigate the 25(OH)D level threshold for predicting illness severity of COVID-19 (mild/moderate vs severe/critical). As shown in Figure 1, the area under the curve is 0.778 (95% CI, 0.620–0.936; *P* = 0.006), and the cutoff point of 25(OH)D level is 41.19 nmol/L (sensitivity = 0.865; specificity = 0.7).

Discussion

Demographic factors or clinical symptoms of COVID-19

Consistent with previous studies, our study found that severe COVID-19 infection was significantly associated with older age. We also found significantly higher rate of comorbidity renal failure in the severe/critical COVID-19 cases compared to mild/moderate cases. A previous case-series study conducted by Guan and colleagues in China found that severe cases are older than non-severe cases (median; 52 vs 45 years old), and had a higher prevalence of symptoms, such as fever (82 [48%] vs 391 [43%]), cough (122 [70.5%] vs 623 [67.3%]), and diarrhea (ten [5.8%] vs 32 [3.5%]) and comorbidities, such as diabetes (28 [16.2%] vs 53 [5.7%]), hypertension (41 [23.7%] vs 124 [13.4%]), and chronic renal disease (three [1.7%] vs five [0.5%]) (21).

In the present study, the percentages of female in COVID-19 cases and severe cases were 39 [62.9%], and six [60%], respectively. However, in Guan's study, the percentage of females in COVID-19 cases and severe diseases was 459 [41.9%] and 73 [42.2%] (21). A retrospective case-series study of 1591 COVID-19 cases referred for ICU admission in Italy also demonstrated that the majority of cases were male (1304 [82%]), but they did not report the distribution of sex in all COVID-19 patients in the study (22). As sex in the control group was frequency matched with the case group, we were not be able to examine sex difference in this study.

Vitamin D deficiency and COVID-19 infection/severity

We found significantly higher rates of VDD among COVID-19 cases compared to healthy controls, as age and sex were matched between the two groups, the possible effect of these cofounding factors were excluded. The result is consistent with previous studies in respiratory diseases research. In Canada, McNally and colleagues conducted a case-control study including 46 pneumonia cases and 76 controls and found a higher rates of VDD in patients with pneumonia compared to a healthy population (14 [30%] vs 12 [16%]) (23). Another case control study conducted by Mamani and colleagues in Iran indicated that the percentage of VDD in community-acquired pneumonia (CAP) cases was 2.77 times higher than in healthy controls (24). These results suggest a possible association between VDD and respiratory tract infections.

Our study also showed a significantly greater rate of VDD in severe/critical COVID-19 cases than in mild/moderate cases. This finding was confirmed after adjusting for confounders, and was very consistent across all clinical indicators and by checking each critical COVID-19 adverse event. Numerous studies have demonstrated the association between vitamin D and the severity of respiratory tract infections (10, 24, 25), such as CAP and bronchiolitis. Mamani and colleagues found that the risk of severe CAP among patients with VDD was 3.15 (95% CI, 1.20–8.26) times higher than among those without VDD (24). A prospective study in CAP patients also indicated that patients with severe CAP had lower vitamin D concentrations than non-severe CAP (25). A large prospective cohort study of 1016 infants with bronchiolitis suggested that patients with VDD had higher risk of requiring intensive care (OR, 1.72; 95% CI, 1.12–2.64), and longer lengths of stay at hospital (OR, 1.39; 95% CI, 1.17–1.65) (10). To date, there are several studies which had investigated the association between vitamin D and COVID-19.Panagiotou et al. conducted a retrospect study including 134 patients, suggested that patients admitted to intense therapy unit (ITU) had greater

Table 4. Distribution of vitamin D level among different severity status.

	Sufficient (n = 10)	Insufficient (n = 26)	Deficient (n = 26)	*P* value
Clinical classification of severity				
Asymptomatic	2 (100)	0	0	0.004[a]
Mild/moderate	7 (14)	25 (50)	18 (36)	..
Severe/critical	1 (10)	1 (10)	8 (80)	..
Adverse events				
Shock				
Yes	0	0	4 (100)	0.025
No	10 (17)	26 (45)	22 (38)	..
Mechanical ventilation				
Yes	0	0	5 (100)	0.012
No	10 (17)	26 (46)	21 (37)	..
$FiO_2 \leq 300$ mmHg				
Yes	1 (12.5)	1 (12.5)	3 (75)	0.575
No	9 (16)	25 (44)	23 (40)	..
Lung infiltration ≥50				
Yes	0 (0)	1 (20)	4 (80)	0.099
No	10 (17)	25 (44)	22 (39)	..

Data are presented as n(%).
FiO_2=Fraction of inspired oxygen.
[a]The proportion was compared by using Mantel-Haenszel chi square test among different illness severity.

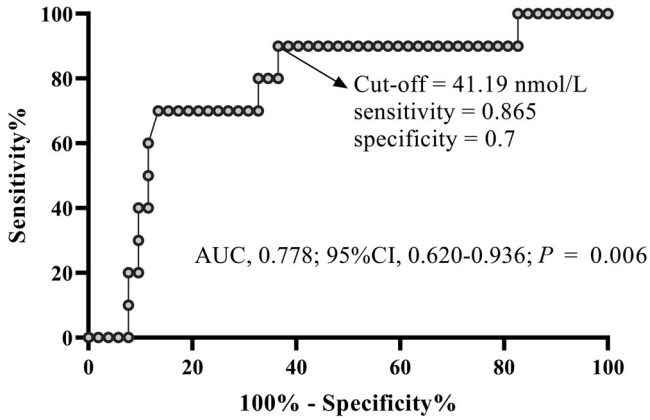

Figure 1. ROC of predicting illness severity of COVID-19 with 25(OH)D level.

prevalence of VDD than non-ITU patients (81% vs 60.9%, $P = 0.02$) (26), and Munshi et al. conducted a meta analysis including 1368 patients found that Patients with poor prognosis (N = 634) had significantly lower serum levels of vitamin D compared to those with good prognosis (N = 669) (18). A Israeli population-based study including 782 COVID-19 positive and 7025 COVID-19 negative, and found that low 25(OH)D level (<75nmol/L) was associated with the incidence and severity of COVID-19 (27). Furthermore, the ecological studies from Europe also reported the correlations between vitamin D status and COVID-19 (28, 29). These findings, along with our current results, suggest that VDD may be a common risk factor for illness severity of COVID-19 infection (17). The patients with lower serum vitamin D levels were susceptible to COVID-19 infection and progressed to severity (26). However, the results from these reports are still controvertial. A study from UK showed no such associations between vitamin D level and COVID-19 (30). Therefore, further investigation is still needed in future study.

Potential biological mechanisms

Although the exact biological mechanism is unknown, several studies had proposed that an excessive autoimmune response, or dysregulation of one's immune response, may contribute to COVID-19, these study also suggested that the host's innate immune response is a key determinant of COVID-19 severity (3, 31, 32). A study from Wuhan has revealed that the patients admitted to ICU had significantly higher neutrophils and lower lymphocytes than the patients not admitted to ICU (31), and the number of immune cells were closely associated with case severity and mortality (31). Another observational study from Wuhan has suggested that the patients in ICU had a robust increase of pro-inflammatory cytokines, including tumor necrosis factor-α, monocyte chemoattractant protein-1 and CXC motif chemokine 10 (33).

Vitamin D in the tissue might play a critical role in to regulate immunopathological inflammatory responses. Studies had found that vitamin D receptors exert an anti-inflammatory effect via downregulation of NF-κB-dependent (34). There is also evidence that vitamin D metabolites in immune cells could induce endogenous antimicrobial peptides and autophage, then support innate antiviral effector mechanisms in response to various infectious diseases (35). Therefore, vitamin D supplementation might be helpful in the treatment of severe COVID-19 by balancing the immune system and its response to infection. However, further studies that compare the immune status in subjects with VDD vs without VDD are needed to verify such a potential pathway.

Vitamin D levels among different groups and a potential effect threshold

We found that COVID-19 patients had lower levels of 25(OH)D, compared with healthy, non-infected individuals (median, 55.6 vs 71.8 nmol/L). In further, severe/critical COVID-19 patients have lower 25(OH)D concentrations than mild/moderate COVID = 19 (Median, 38.2 vs 56.6 nmol/L). Similarly, the rate of VDD was higher in COVID-19 patients compared with healthy, non-infected individuals, and VDD was the highest in severe/critical cases. These findings suggest that identifying an appropriate

level or threshold of vitamin D supplementation for preventing COVID-19 infection may be feasible and useful. Furthermore, evaluating serum 25(OH)D level at the early stage of disease may provide an index for predicting disease progression, or an indicator for possible vitamin D treatment in advance to reduce COVID-19 severity.

We have identified a potential protective threshold for COVID-19 illness progression. In particular, this study found that COVID-19 patients with a serum 25(OH)D concentration below 41.19 nmol/L tends to be more susceptible to become COVID-19 infection and also tends to have more severe or critical illness. Therefore, daily vitamin D supplementation may be administrated as add-on therapy for such patients. A randomized, placebo-controlled trial including 163 healthy white women with VDD (with mean 25(OH)D level of 39 nmol/L, which is close to our identified threshhold) has demonstrated that the effective dose of vitamin D_3 supplementation to achieve a 25(OH)D level >50 nmol/L is 800 IU/d (36). Whether this recommended dose of vitamin D_3 is effective for COVID-19 cases is unclear. Further studies are needed to evaluate the effect of vitamin D supplements on COVID-19.

Strengths and limitations

To our knowledge, the present study is one of the few studies using a healthy, non-infected control group to compare 25(OH)D levels among healthy people, cases with mild/moderate symptoms, and cases with severe/critical symptoms in order to address two important questions: 1) how do 25(OH)D levels differ between healthy people vs COVID-19 cases? and 2) how do clinical symptoms and VDD differ between mild/moderate cases vs severe/critical cases? Previous research has mostly used case-report and case-series designs, without use of a healthy control group as a reference.

Still, our findings must be interpreted with caution due to the following potential limitations. The first obvious weakness is our small sample size of the cases. The estimated statistical power of this study is 0.764, slightly lower than the standard for most epidemiologic studies of 0.80. Due to this limitation, we may not have had adequate power to detect the difference in some clinical symptoms and indicators between the mild and severe cases. Despite this lack of power, however, we still did detect strong and consistent relationships between low 25(OH)D and COVID-19 infection/severity, which may be a strong indication of the importance of VDD in the development of COVID-19 infection. Second, the categorization of vitamin D was defined according to Endocrine Society Clinical Practice Guideline in the present study, however, it is likely represent a null bias due to the small size, and the protective effect of serum 25(OH)D > 75nmol/l is quite possibly greater than observed here which could have been better demonstrated if a wider range of serum 25(OH)D was present in the controls. Third, selection bias is a common concern in observational studies. Both the cases and controls came from the same source population in Guangxi Zhuang Autonomous Region in Nanning, China. As the majority of participants (44 [71%]) came from urban areas, our findings may not be generalizable to populations in rural areas, or other regions of China. Forth, the controls were not tested for COVID-19. Therefore, there is the possibility of residual COVID-19 infection in the control group. The net effect of this would be a null bias that would tend to minimize any differences between the cases and controls. Lastly, serum 25(OH)D levels among the cases was determined only during hospital admission, after the onset of COVID-19 symptoms. However, prior research shows that blood vitamin D level is stable, and not affected by acute respiratory infection (37), so the 25(OH)D level at admission most probably reflects the level before infection.

The current study provides preliminary evidence to demonstrate an association between VDD and COVID-19 disease incidence and severity. Our analyses also show consistent findings of the protective effects of vitamin D against severe/critical symptoms and severe adverse events among the COVID-19 patients. In addition to social distancing, optimal vitamin D supplementation may provide a potential supplemental strategy for preventing COVID-19 or reducing its severity. Further studies with larger sample size and better representativeness are needed to validate the beneficial therapeutic effects of vitamin D on COVID-19 infection.

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Authors' contributions

K. Ye, S. Lin, F. Xu. Designed research; F.Tang, X.Liao, G. Huang, C. Chen, N. Tang conducted research; H. Xiao, X. Liao performed statistical analysis; F. Tang, X. Liao, M. Deng wrote the paper; Z. Qin, X. Peng, X. Liu, L. Ning, B. Wang, M. Li, J. Yang provided essential technical and material support; F. Xu, S. Lin, J. Yang, K. Ye had primary responsibility for final content. All authors read and approved the final manuscript.

Disclosure statement

All authors have no conflict of interest.

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References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8): 727–33. [cited Jan]. Available from: https://www.nejm.org/doi/pdf/10.1056/NEJMoa2001017?articleTools=true doi:10.1056/NEJMoa2001017.
2. Johns Hopkins University. Globle tracking map: COVID-19 cases and data visualization. 2020. [cited 2020 May 1]. Available from: https://coronavirus.jhu.edu/map.html.
3. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. Jama. 2020;323(13): 1239. [cited Feb 24]. Available from: http://jama.jamanetwork.com/Mobile/article.aspx?articleid=2762130 doi:10.1001/jama.2020.2648.
4. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet (London, England) 2020;395: 507–13. [cited Feb 15]. Available from: https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(20)30211-7.pdf
5. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. Jama. 2020;323(11):1061. [cited Feb 7]. Available from: https://jamanetwork.com/journals/jama/articlepdf/2761044/jama_wang_2020_oi_200019.pdf doi:10.1001/jama.2020.1585.
6. Weiss P, Murdoch DR. Clinical course and mortality risk of severe COVID-19. Lancet (London, England) 2020;395:1014–5. doi:10.1016/S0140-6736(20)30633-4.
7. Mangin M, Sinha R, Fincher K. Inflammation and vitamin D: the infection connection. Inflamm Res. 2014;63(10):803–19. doi:10.1007/s00011-014-0755-z.
8. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911–30. doi:10.1210/jc.2011-0385.
9. Esposito S, Lelii M. Vitamin D and respiratory tract infections in childhood. BMC Infect Dis. 2015; 15:487. doi:10.1186/s12879-015-1196-1.
10. Vo P, Koppel C, Espinola JA, Mansbach JM, Celedon JC, Hasegawa K, Bair-Merritt M, Camargo CA. Jr. Vitamin D status at the time of hospitalization for bronchiolitis and its association with disease severity. J Pediatr. 2018;203:416–22. doi:10.1016/j.jpeds.2018.07.097.
11. Gruber-Bzura BM. Vitamin D and influenza-prevention or therapy? Int J Mol Sci. 2018; 19:2419.
12. Laaksi I, Ruohola JP, Mattila V, Auvinen A, Ylikomi T, Pihlajamaki H. Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men. J Infect Dis. 2010;202(5): 809–14. doi:10.1086/654881.
13. Mathyssen C, Gayan-Ramirez G, Bouillon R, Janssens W. Vitamin D supplementation in respiratory diseases: evidence from randomized controlled trials. Pol Arch Intern Med. 2017; 127(11):775–84. doi:10.20452/pamw.4134.
14. AlQuaiz AM, Kazi A, Fouda M, Alyousefi N. Age and gender differences in the prevalence and correlates of vitamin D deficiency. Arch Osteoporos. 2018;13(1):49. doi:10.1007/s11657-018-0461-5.
15. Zhu XL, Chen ZH, Li Y, Yang PT, Liu L, Wu LX, Wang YQ. Associations of vitamin D with novel and traditional anthropometric indices according to age and sex: a cross-sectional study in central southern China. Eat Weight Disord 2019. [cited Nov 14]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/31728924.
16. Contreras-Manzano A, Villalpando S, Garcia-Diaz C, Flores-Aldana M. Cardiovascular risk factors and their association with Vitamin D deficiency in Mexican women of reproductive age. Nutrients. 2019;11(6):1211. doi:10.3390/nu11061211.
17. D'Avolio A, Avataneo V, Manca A, Cusato J, De Nicolò A, Lucchini R, Keller F, Cantù M. 25-hydroxyvitamin D concentrations are lower in patients with positive PCR for SARS-CoV-2. Nutrients. 2020;12(5):1359. doi:10.3390/nu12051359.
18. Munshi R, Hussein MH, Toraih EA, Elshazli RM, Jardak C, Sultana N, Youssef MR, Omar M, Attia AS, Fawzy MS, et al. Vitamin D insufficiency as a potential culprit in critical COVID-19 patients. J Med Virol. 2020;
19. China NHCo. The guidelines for the diagnosis and treatment of COVID-19 (Pilot 6rd version). 2018. [cited Feb 18]. Available from: http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2/files/b218cfeb1bc54639af227f922bf6b817.pdf.
20. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604–12. doi:10.7326/0003-4819-150-9-200905050-00006.
21. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020. [cited Feb 28]. Available from: https://www.nejm.org/doi/pdf/10.1056/NEJMoa2002032?articleTools=true.
22. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, Cereda D, Coluccello A, Foti G, Fumagalli R, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. Jama. 2020;323(16):1574. [cited Apr 6]. Available from: https://jamanetwork.com/journals/jama/fullarticle/2764365 doi:10.1001/jama.2020.5394.
23. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM. Vitamin D deficiency in young children with severe acute lower respiratory infection. Pediatr Pulmonol. 2009; 44(10):981–8. doi:10.1002/ppul.21089.
24. Mamani M, Muceli N, Ghasemi Basir HR, Vasheghani M, Poorolajal J. Association between serum concentration of 25-hydroxyvitamin D and community-acquired pneumonia: a case-control study. IJGM. 2017;10:423–9. doi:10.2147/IJGM.S149049.
25. Talebi F, Rasooli Nejad M, Yaseri M, Hadadi A. Association of vitamin D status with the severity and mortality of community-acquired pneumonia in Iran during 2016–2017: A Prospective Cohort Study. Rep Biochem Mol Biol. 2019; 8:85–90.
26. Panagiotou G, Tee SA, Ihsan Y, Athar W, Marchitelli G, Kelly D, Boot CS, Stock N, Macfarlane J, Martineau AR, et al. Low serum 25-hydroxyvitamin D (25[OH]D) levels in patients hospitalised with COVID-19 are associated with greater disease severity. Clin Endocrinol. 2020;93(4):508–11. doi:10.1111/cen.14276.
27. Merzon E, Tworowski D, Gorohovski A, Vinker S, Golan Cohen A, Green I, Frenkel Morgenstern M. Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. FEBS J. 2020.
28. Laird E, Rhodes J, Kenny RA. Vitamin D and inflammation: Potential implications for severity of Covid-19. Ir Med J. 2020; 113(5):81.
29. 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(7):1195–8. doi:10.1007/s40520-020-01570-8.
30. Hastie CE, Mackay DF, Ho F, Celis-Morales CA, Katikireddi SV, Niedzwiedz CL, Jani BD, Welsh P, Mair FS, Gray SR, et al. Vitamin D concentrations and COVID-19 infection in UK Biobank. Diabetes Metabolic Syndrome. 2020;14(4):561–5. doi:10.1016/j.dsx.2020.04.050.

31. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet (London, England) 2020;395. [cited Feb 15]. Available from: https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(20)30183-5.pdf.
32. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, Xu Y, Tian Z. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol. 2020;17(5):533–5. doi:10.1038/s41423-020-0402-2.
33. D'Antiga L. Coronaviruses and immunosuppressed patients. The facts during the third epidemic. Liver Transplant. 2020;26(6):832–4. doi:10.1002/LT.25756.
34. Wu S, Liao AP, Xia Y, Li YC, Li JD, Sartor RB, Sun J. Vitamin D receptor negatively regulates bacterial-stimulated NF-kappaB activity in intestine. Am J Pathol. 2010;177(2):686–97. doi:10.2353/ajpath.2010.090998.
35. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006;311(5768):1770–3. doi:10.1126/science.1123933.
36. Gallagher JC, Sai A, Templin T, 2nd, Smith L. Dose response to vitamin D supplementation in postmenopausal women: a randomized trial. Ann Intern Med. 2012;156(6):425–37. doi:10.7326/0003-4819-156-6-201203200-00005.
37. Haugen J, Chandyo RK, Ulak M, Mathisen M, Basnet S, Brokstad KA, Valentiner-Branth P, Shrestha PS, Strand TA. 25-Hydroxy-Vitamin D concentration is not affected by severe or non-severe pneumonia, or inflammation, in young children. Nutrients. 2017;9(1):52. doi:10.3390/nu9010052.