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# ASSOCIATION OF SERUM VITAMIN D WITH SEVERITY, DIETARY HABITS, INFLAMMATORY, AND METABOLIC BIOMARKERS IN COVID-19 PATIENTS: A HOSPITAL-BASED CROSS-SECTIONAL ANALYTICAL STUDY

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## Abstract
**Background:** Recent studies suggest a link between vitamin D deficiency and COVID-19 infection, but the conclusions are inconsistent. Therefore, we studied whether vitamin D deficiency could increase the risk for clinical severity and adverse clinical outcomes associated with COVID-19 patients.
**Methods:** We collected data from 400 patients, including demographic information, principal clinical symptoms, medical history, RT–PCR findings, laboratory findings, comorbidities, and dietary habits. **Results:** Among the patients, 76.3% had a mild and moderate infection, while 23.7% had a critical and severe infection, based on the CDC criteria. Approximately 29.5% were vitamin D sufficient, and the remaining 70.5% of patients were vitamin D insufficient. After adjustments for confounding factors, there was a significant (p=<0.05) association between vitamin D insufficiency and an increase in severity (OR=2.27), age (OR=3.24), BMI (OR=3.73), daily exercise (OR=0.63), sunbathing (OR=0.50), CRP (OR=3.24), serum ferritin (OR=1.73), IL-6 (OR=2.04), TNF-α (OR=2.38), HDL
(OR=0.52), triglycerides (OR=1.57), and cholesterol levels (OR=1.66) among COVID-19 patients. A significant association of vitamin D levels with the consumption of marine mammals and fish, vegetables and fruits, meat and eggs, and dairy products was observed.
**Conclusion:** Taken together, our findings suggest that serum vitamin D levels in the general population, especially hospitalized patients, are negatively associated with the severity of COVID-19 morbidity.

## Introduction
Coronavirus disease is a respiratory and systemic disorder coronavirus 2 (SARS-CoV-2) with a range of severity from mild respiratory illnesses to severe and critical lung injury, multiorgan failure, and death [1]. The World Health Organization (WHO) declared COVID-19 a global pandemic on 11th

March 2020 [2]. It is now affecting more than 219 countries and territories around the globe, with approximately more than 768.24 million confirmed COVID-19 cases; among those, more than 7 million died as of February 2024. Most patients with COVID-19 infection show mild to moderate respiratory illnesses and can recover without any special treatments [3]. Patients who are elderly or have underlying medical conditions, such as CVDs, autoimmune diseases, cancer, infections, and chronic respiratory disease, are at high risk of developing serious illnesses [4,5].

As a primary target, the virus infects erythrocytes and pneumocytes [6]. Spike proteins bind on the cell surface with angiotensin-converting enzyme 2 (ACE-2) to help viral entrance into the target cells [7]. As a regulator of the renin-angiotensin system, ACE-2 is found in many body tissues, including the lungs, gastrointestinal tract (GIT), kidneys, and cardiovascular system [8]. This is a reason for multiorgan failure in susceptible COVID-19 patients.

Several studies have confirmed an association between low serum vitamin D and upper respiratory tract diseases and higher mortality in COVID-19 patients [9,10]. Vitamin D deficiency increases inflammatory markers, which increases the severity and mortality of COVID-19 infection [11]. Individuals with vitamin D deficiency are more likely to be infected with COVID-19 [12]. A recent study revealed that elderly COVID-19 patients with vitamin D deficiency had worse clinical outcomes than those who were vitamin D sufficient [13].

In general, more than 80% of vitamin D is formed in the skin when humans are exposed to sunlight [5]. Vitamin D directly impacts macrophage modulation; it downregulates proinflammatory cytokines and upregulates Tregs (regulatory T cells), affecting innate and adaptive immune systems [14–16]. It also downregulates ACE-2 and acts as a modulator of the renin-angiotensin pathway [17]. Eventually, it reduces inflammation, acute respiratory distress pneumonia, and infection in the body, which is the common cause of mortality in COVID-19 patients [18]. A prospective study confirmed that high doses of vitamin D reduce hospital stays in COVID-19 infection [19]. A few studies have observed a significant association between vitamin D, sun exposure and severity, susceptibility to, and recovery from COVID-19 infection [20,21]. Subsequently, normal serum vitamin D level is linked with improved metabolic markers. Better vitamin D leads to reduced LDL, triglycerides, and cholesterol levels, while it elevates HDL levels [22].

In general, more than 80% of vitamin D is formed in the skin when humans are exposed to sunlight [5]. The ability of the human skin to synthesize vitamin D from sunlight decreases with age, which leads to vitamin D deficiency in the older population [23]. The above discussion suggests a link between vitamin D deficiency and COVID-19 infection, but the results are inconsistent. We hypothesize that vitamin D sufficiency would reduce the risk for severity, clinical outcomes, and inflammatory and metabolic markers in COVID-19 infection.

## Materials and methods

### Study design and study participants

This is a cross-sectional analytical study of the COVID-19 patient database in Hayatabad Medical Complex, a tertiary healthcare center in Peshawar-Pakistan [24]. Patients positive for COVID-19 and admitted to the hospital aged 18-80 years were included in this study. Patients were randomly selected for this study through an online random number generator. Informed verbal consent was sought from either the patient or his/her attendant. The confidentiality of participants was ensured in this study. Data were collected from September 2020 to March 2021. The ethical review board of Hayatabad Medical Complex, Peshawar-Pakistan approved this study under wide Ref. No.454/HEC/B&PSC/2020. The reporting of this study conforms to STROBE guidelines (The Strengthening the Reporting of Observational Studies in Epidemiology) [25].

### Data source

Hospital records were analyzed from the patient database of the Hayatabad Medical Complex COVID-19 Registry (HMC-Cov19R) [24]. HMC-Cov19R is an ongoing, prospective, hospital-based registry of patients diagnosed with COVID-19 presenting to the emergency department of Hayatabad

Medical Complex, Peshawar-Pakistan. In our study, we used the first laboratory findings of patients upon hospitalization. T framework of the methodological process of the study is shown in Figure 1.

**Patients and data collection**

Infectious disease specialists diagnosed the patients based on WHO guidelines and recommendations by the National Coordination Committee on COVID-19 of Pakistan [26]. Patients presenting symptoms of acute respiratory tract infection (e.g., fever, cough, and dyspnea) without any other etiology were selected. The diagnosis was confirmed by a real-time polymerase chain reaction (RT–PCR) test for COVID-19. The personal details of patients were de-identified.

CDC criteria were used for the disease prognosis and severity, including mild-moderate cases (fever and mild respiratory symptoms, 5 to 6 days after infection), severe cases (dyspnea, blood oxygen saturation ≤93%, respiratory frequency ≥30/minute and/or lung infiltrates >50% of the lung field within 1-2 days) and critical cases (respiratory failure, septic shock, and/or multiple organ failure/dysfunction). Multiple organ damage was considered for patients with at least two complications, including acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), acute cardiac injury (ACI), or acute liver injury. Patients who were pregnant, chronic kidney disease patients who were on dialysis, patients with chronic airway disease, and cancer patients who were on chemotherapy were excluded from our study to avoid confounding factor effects.

**Study measurements**

The following information was included in the data: demographic information, principal clinical symptoms, medical history, RT–PCR findings, radiological results, laboratory findings, comorbidities, progression of the disease, anthropometrics, and dietary habits.

A pretested questionnaire was used to collect data, including sociodemographic variables such as age, sex (male or female), population distribution (urban or rural), sunbathing (yes or no), daily exercise (yes or no), severity (mild to moderate or severe to critical) and comorbidities (yes or no).

All methods were carried out in accordance with relevant guidelines and regulations. For screening COVID-19 cases, RNA was extracted by a TANBead Nucleic Acid extraction kit [27], amplified by a Sansure detection kit using Mic qPCR biomolecular systems, and subjected to PCR according to the manufacturer's protocol [28].

Laboratory examination was carried out for COVID-19-positive patients at the time of admission, including a complete blood count, blood biochemistries (total serum 25-hydroxyvitamin D [25-OH]), sodium (Na), potassium (K), chloride ($Cl^-$), calcium (Ca), albumin, blood urea, creatinine, total bilirubin, alanine transaminase (ALT), alkaline phosphatase, C-reactive protein (CRP), serum ferritin, lactate dehydrogenase (LDH), D-dimer, TNF-α, IL-6, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and serum cholesterol levels.

Total serum 25(OH) D was measured on a COBAS E601 instrument by employing electrochemiluminescence immunoassay (ECLIA) (Abbott Architect), with a quantitative limit value of 2.2 ng/ml at 20% coefficient variation (CV). Metabolic biomarkers like HDL, LDL, Triglycerides, and cholesterol were detected through the immunoassays method by utilizing Architect c8000 Clinical Chemistry Analyzer (Abbott Architect). Inflammatory biomarkers were determined by the Enzyme-Linked Immunosorbent Assay method by using Hitachi Cobas c501 (Roche Diagnostics). For the operational definition of vitamin D sufficiency, a cutoff point of 30 ng/ml was used based on the Endocrine Society's Practice Guidelines for vitamin D, which defined vitamin D insufficiency and deficiency as circulating levels of 25(OH)D of 20–29 ng/mL and <20 ng/mL, respectively [29].

Anthropometric measurements (weight, height, and body mass index) were calculated as per WHO standards. BMI ranging from 18.5 to 24.9 kg/m$^2$ was considered normal, while BMI ≥25 kg/m$^2$ was considered overweight/obese [30].

Dietary habits were calculated by using the Food Frequency Questionnaire (FFQ) with minor modifications, with 60 different food items locally consumed in Pakistan. The questionnaire measured the consumption frequency as the number of times per day and the number of times per week. The individual food items were categorized manually into six different categories: marine mammals and fish (various fish and seafoods, etc.), dairy products (milk products excluding ice cream), meat and eggs (beef, mutton, poultry, eggs, etc.), vegetables and fruits, complex carbohydrates (roti, bread, cereals, etc.), and junk food (burger, pizza, ice cream, soda, sweets, fries, etc.). Each category comprises 5 to 20 food items.

**Statistical analysis**

The data were analyzed by using the statistical software SPSS version 21. Continuous variables are presented as the mean and standard deviation (mean±SD). The categorical variables were presented in the form of percentages, and the chi-square test ($\chi^2$) was used to identify differences in age, sex, severity of the disease, comorbidities, inflammatory markers, metabolic markers, and other laboratory findings in patients with and without vitamin D insufficiency. An independent t-test was used to determine the mean significant difference between the dietary frequency of the vitamin D-sufficient and vitamin D-insufficient groups.

A bivariate logistic regression model was used to measure the association between dependent and independent variables. All tests were performed at a confidence level of 95%.

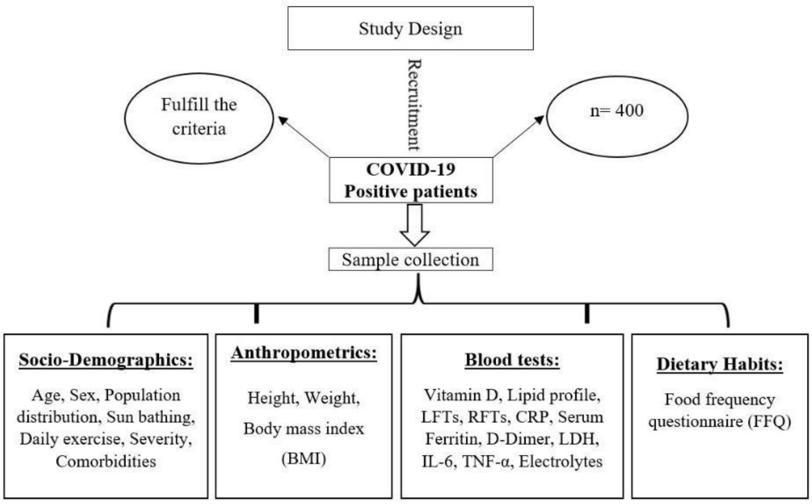


**Figure 1. A framework of the methodological process of the study.**

**Results**

A total of 400 COVID-19 patients were enrolled in this study, which consisted of more males (59%). Their mean age was 59.95±17.67 years, with 59% being over 40 years. The population distribution showed that more of the participants (63%) lived in rural areas. Furthermore, major routine activities such as daily exercise (57%) and sunbathing for at least 30 minutes/day (62.2%) were not performed by the majority. Additionally, based on the CDC criteria, 76.3% of the patients were classified as mild or moderate, while 23.7% were classified as severe or critical. The majority, 66.3%, of COVID-19 patients had no comorbidities, while 33.7% had chronic comorbidities. Last, the BMI calculated as per WHO standards showed that 64.5% had normal BMI, while 35.5% were either overweight/obese. The baseline characteristics of COVID-19 patients are shown in Table 1.

**Table 1. Demographic and general characteristics of the study population**

| Characteristics | | N | Frequency (%) |
|---|---|---|---|
| **Age (Mean±SD)** | | 400 | 59.95±17.67 |
| **Age** | Up to 40 years | 164 | 41 |
| | More than 40 years | 236 | 59 |
| **Sex** | Male | 220 | 55 |
| | Female | 180 | 45 |
| **Population Distribution** | Urban | 148 | 37 |
| | Rural | 252 | 63 |
| **Daily Exercise** | Yes | 159 | 39.75 |
| | No | 241 | 60.25 |
| **Sunbathing** | Yes | 151 | 37.8 |
| | No | 249 | 62.2 |
| **Severity** | Mild and moderate | 305 | 76.3 |
| | Severe and critical | 95 | 23.7 |
| **Comorbidities** | Yes | 135 | 33.7 |
| | No | 265 | 66.3 |
| **BMI** | Normal | 258 | 64.5 |
| | Overweight/Obese | 142 | 35.5 |

CDC criteria were used for the disease prognosis and severity. BMI; body mass index, BMI normal; <25 kg/m2, BMI overweight/obesity; ≥ 25 kg/m2, Sun Bathing; daily sun exposure for at least 30 minutes, comorbidities include diseases such as diabetes, chronic kidney disease, heart disease, stroke, or hypertension.

**Sociodemographic outcomes of COVID-19 patients based on vitamin D status**

A cutoff point equal to or higher than 30 ng/mL of 25(OH)D was used to define vitamin D sufficiency and vice versa. From this, 70.5% of the patients were vitamin D insufficient, as presented in Figure 2. The sociodemographic outcomes of COVID-19 patients based on vitamin D status are presented in Table 2. Patients aged over 40 years were more likely to be vitamin D insufficient (76.3% vs 23.7%, p=<0.001). Likewise, more patients with vitamin D insufficiency were not sunbathing routinely in comparison to vitamin D sufficiency patients (75.9% vs 24.1%, p=0.003). Consequently, there were more severe and critical COVID-19 patients in the vitamin D-insufficient group than in the vitamin D-sufficient group (82.1% vs 17.9%, p=<0.005). Similarly, there were significantly more overweight/obese patients in the vitamin D-insufficient group than in the vitamin D-sufficient group (85.9% vs 14.1%, p= <0.001). However, higher levels of D-dimer, LDH, blood urea, total bilirubin, ALT, and alkaline phosphatase were not significantly different across the vitamin D-insufficient and vitamin D-sufficient groups.

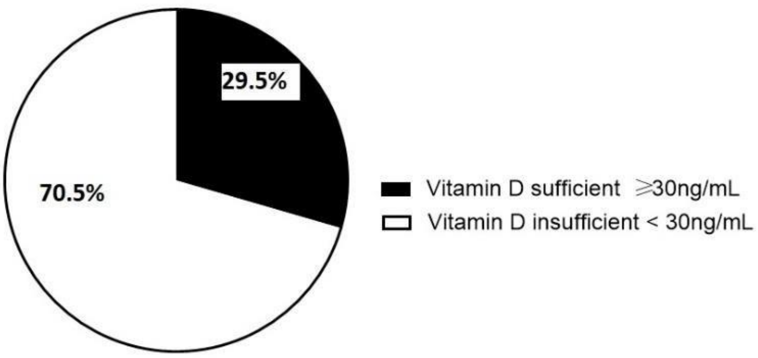


**Figure 2. Prevalence of vitamin D insufficiency among COVID-19 patients**

A cutoff point of 30 ng/ml was used based on the Endocrine Society's Practice Guidelines for Vitamin D, which defined vitamin D insufficiency as a circulating level of 25(OH)D of 20–29 ng/mL.

**Table 2. Sociodemographic outcomes of COVID-19 patients based on vitamin D status**

| Characteristics | | Vitamin D <30 ng/ml N=282 | Vitamin D ≥30 ng/ml N=118 | P value |
|---|---|---|---|---|
| **Sex** | Male | 150 (68.2) | 70 (31.8) | 0.262 |
| | Female | 132 (73.3) | 48 (26.7) | |
| **Age** | Up to 40 years | 102 (62.2) | 62 (37.8) | **<0.001** |
| | More than 40 years | 180 (76.3) | 56 (23.7) | |
| **Population Distribution** | Urban | 110 (74.3) | 38 (25.7) | 0.196 |
| | Rural | 172 (68.2) | 80 (31.8) | |
| **Daily Exercise** | Yes | 103 (64.8) | 56 (35.2) | **0.042** |
| | No | 179 (74.3) | 62 (25.7) | |
| **Sun Bathing** | Yes | 93 (61.6) | 58 (38.4) | **0.003** |
| | No | 189 (75.9) | 60 (24.1) | |
| **Severity** | Mild-moderate | 204 (66.9) | 101 (33.1) | **0.005** |
| | Severe-critical | 78 (82.1) | 17 (17.9) | |
| **Comorbidities** | Yes | 194 (73.2) | 71 (26.8) | 0.097 |
| | No | 88 (65.2) | 47 (34.8) | |
| **BMI** | Normal | 160 (62) | 98 (38) | **<0.001** |
| | Overweight/Obese | 122 (85.9) | 20 (14.1) | |

Values in bold indicate statistical significance (P<0.05), Vitamin D <30 ng/ml is vitamin insufficiency, and Vitamin D ≥30 ng/ml is vitamin D sufficiency. BMI; Body mass index, Sun Bathing; Daily sun exposure for at least 30 minutes, Comorbidities include diseases such as diabetes, chronic kidney disease, heart disease, stroke, or hypertension.

### Inflammatory biomarkers of COVID-19 patients based on vitamin D status

Inflammatory biomarkers of COVID-19 patients based on vitamin D status are presented in Table 3. The results showed that 82.5% of patients had CRP levels higher than normal (>0.5 mg/dL), whereas 17.5% of patients had normal CRP levels (<0.5 mg/dL). Higher CRP was more prevalent among patients in the vitamin D-insufficient group than in the vitamin D-sufficient group (74.2% vs 25.8%, p=0.001). Similarly, 73.3% of patients had serum ferritin levels greater than the normal range (>400 mg/dL), while 26.7% had normal serum ferritin levels (<400 mg/dL). The patients with vitamin D insufficiency had higher serum ferritin levels than those with vitamin D sufficiency (73.3% vs 26.7%, p=0.029). Subsequently, 63% of the patients had higher TNF-α levels (>24.47 pg/mL), whereas 37% of the patients had normal TNF-α levels (<24.47 pg/mL). There were more patients with higher TNF-α levels in the vitamin D-insufficient group than in the vitamin D-sufficient group (64.2% vs 35.8%, p<0.001). Additionally, 62.5% of the total patients had higher IL-6 levels, while 37.5% had normal IL-6 levels. A significant difference was found for higher levels of IL-6 between the vitamin D-insufficient and vitamin D-sufficient groups (65.2% vs 34.8%, p=0.003). Likewise, 43% of the patients had higher levels of creatinine, while 57% of the patients had normal creatinine levels. The findings revealed that there was a significantly higher creatinine level in patients in the vitamin D-insufficient group than in the vitamin D-sufficient group (76.7% vs 23.3%, p=0.025). On the other hand, higher levels of D-dimer, LDH, blood urea, total bilirubin, ALT, and alkaline phosphatase were not significantly different between the vitamin D-insufficient and vitamin D-sufficient groups.

**Table 3. Inflammatory biomarkers of COVID-19 patients based on vitamin D status**

| Characteristics | | Vitamin D <30 ng/ml N=282 | Vitamin D ≥30 ng/ml N=118 | P value |
|---|---|---|---|---|
| **Total Bilirubin** | <1 (mg/dL) | 238 (69.0) | 107 (31.0) | 0.086 |
| | >1 (mg/dL) | 44 (80.0) | 11 (20.0) | |
| **ALT** | <50 (U/L) | 192 (67.8) | 91 (32.2) | 0.066 |
| | >50 (U/L) | 90 (76.9) | 27 (23.1) | |
| **Alkaline Phosphatase** | <104 (U/L) | 169 (68.7) | 77 (31.3) | 0.316 |
| | >104 (U/L) | 113 (73.4) | 41 (26.6) | |
| **Creatinine** | <1.06 (mg/dL) | 150 (65.8) | 78 (34.2) | **0.025** |
| | >1.06 (mg/dL) | 132 (76.7) | 40 (23.3) | |
| **Blood urea** | <45 (mg/dL) | 162 (69.8) | 70 (30.2) | 0.729 |
| | >45 (mg/dL) | 120 (71.4) | 48 (28.6) | |
| **LDH** | <235 (U/L) | 52 (68.4) | 24 (31.6) | 0.660 |
| | >235 (U/L) | 230 (71.0) | 94 (29.0) | |
| **CRP** | <0.5 (mg/dL) | 37 (52.9) | 33 (47.1) | **0.001** |
| | >0.5 (mg/dL) | 245 (74.2) | 85 (25.8) | |
| **Serum ferritin** | <400 (ng/mL) | 57 (61.3) | 36 (38.7) | **0.029** |
| | >400 (ng/mL) | 225 (73.3) | 82 (26.7) | |
| **D Dimer** | <0.5 (ug/mL) | 43 (67.2) | 21 (32.8) | 0.526 |
| | >0.5 (ug/mL) | 239 (71.1) | 97 (28.9) | |
| **TNF-α** | <24.47 (pg/mL) | 120 (81.1) | 28 (18.9) | **<0.001** |
| | >24.47 (pg/mL) | 162 (64.2) | 90 (35.8) | |
| **IL-6** | <7.0 (pg/mL) | 119 (79.3) | 31 (20.7) | **0.003** |
| | >7.0 (pg/mL) | 163 (65.2) | 87 (34.8) | |

Values in bold indicate statistical significance (P<0.05), vitamin D <30 ng/ml is vitamin insufficiency, vitamin D ≥30 ng/ml is vitamin D sufficiency, LDH; lactate dehydrogenase, ALT; alanine transaminase, CRP; C-reactive protein, TNF-α; tumor necrosis factor alpha, IL-6; interleukin six, HDL; high-density lipoprotein, LDL; low-density lipoprotein.

### Metabolic biomarkers of COVID-19 patients based on vitamin D status

The metabolic biomarkers of COVID-19 patients based on vitamin D status are shown in Table 4. The analysis of the data indicated that approximately 59.75% of the patients had higher HDL levels (>35 mg/mL), while 40.25% of patients had lower HDL levels (<35 mg/mL). There were more patients in the vitamin D-insufficient group than in the vitamin D-sufficient group with lower HDL levels (<35 mg/mL) (78.3% vs 21.7%, p=0.006). Likewise, 56.25% of the patients had higher triglycerides (>200 mg/dL), while 43.75% of the patients had normal triglyceride levels (<200 mg/dL). There were more patients with higher triglyceride levels in the vitamin D-insufficient group than in the vitamin D-sufficient group (74.7% vs 25.3%, p=0.039). Consequently, 38.75% of patients had higher cholesterol levels (>200 mg/dL), whereas 61.25% of patients had normal cholesterol levels (<200 mg/dL). However, LDL levels were not significantly different between the vitamin D-insufficient and vitamin D-sufficient groups (p=0.334).

**Table 4. Metabolic biomarkers of COVID-19 patients based on vitamin D status**

| Characteristics | | Vitamin D <30 ng/ml N=282 | Vitamin D ≥30 ng/ml N=118 | P value |
|---|---|---|---|---|
| **HDL** | <35 (mg/mL) | 126 (78.3) | 35 (21.7) | **0.006** |
| | >35 (mg/mL) | 156 (65.3) | 83 (34.7) | |
| **LDL** | <150 (mg/dL) | 207 (69.2) | 92 (30.8) | 0.334 |
| | >150 (mg/dL) | 75 (74.3) | 26 (25.7) | |
| **Triglycerides** | <200 (mg/dL) | 114 (65.1) | 61 (34.9) | **0.039** |
| | >200 (mg/dL) | 168 (74.7) | 57 (25.3) | |
| **Serum cholesterol** | <200 (mg/dL) | 163 (66.5) | 82 (33.5) | **0.027** |
| | >200 (mg/dL) | 119 (76.8) | 36 (23.2) | |

Values in bold indicate statistical significance (P<0.05), Vitamin D <30 ng/ml is vitamin insufficiency, Vitamin D ≥30 ng/ml is vitamin D sufficiency, HDL; High-density lipoprotein, LDL; Low-density lipoprotein.

**Association of vitamin D insufficiency with age, severity, and BMI among COVID-19 patients**

The association of vitamin D insufficiency with age, severity, and BMI among COVID-19 patients is presented in Table 5. Our results reflect that age (more than 40 years), severe/critical illness, and overweight/obese COVID-19 patients were more likely to have vitamin D insufficiency. Age (more than 40 years) was significantly associated with vitamin D insufficiency among COVID-19 patients (OR=3.24, 95% CI 1.94-5.41, p=<0.001). Likewise, COVID-19 patients with low vitamin D levels were more likely to be severely/critically ill (OR=2.27, 95% CI 1.27-4.04, p=0.005) than those with normal vitamin D levels. In addition, patients in the vitamin D-insufficient group had higher odds (OR=3.73, 95% CI 2.18-6.38, p=<0.001) of being overweight or obese than patients in the vitamin D-sufficient group. Furthermore, patients with vitamin D insufficiency were less likely to routinely sunbath (OR=0.50, 95% CI 0.32-0.78, p=0.003) or exercise (OR=0.61, 95% CI 0.39-0.95, p=0.032) than patients with vitamin D sufficiency. The odds ratios are displayed in Figure 3.

**Table 5. Association of vitamin D insufficiency with age, severity, and BMI among COVID-19 patients**

| Characteristics | OR | 95% CI (lower, upper) | P value |
|---|---|---|---|
| **Age >40 years** | 3.24 | 1.94-5.41 | **<0.001** |
| **Sun Bathing** | 0.50 | 0.32-0.78 | **0.003** |
| **Daily Exercise** | 0.63 | 0.41-0.98 | **0.042** |
| **Severity (Severe-critical)** | 2.27 | 1.27-4.04 | **0.005** |
| **BMI (Overweight/Obese)** | 3.73 | 2.18-6.38 | **<0.001** |

Values in bold indicate statistical significance (P<0.05). BMI; Body mass index, Sun Bathing; Daily sun exposure for at least 30 minutes

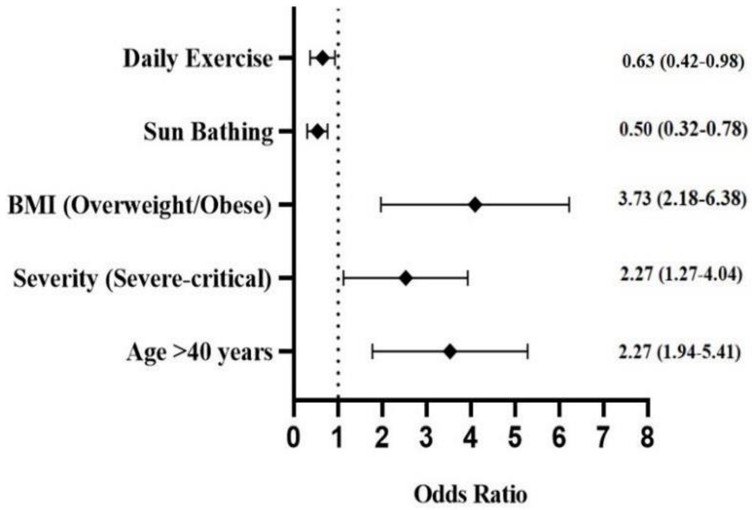


**Figure 3. Forest plot of age, severity, and BMI associated with vitamin D insufficiency based on odds ratio**.

**Association of vitamin D with inflammatory biomarkers among COVID-19 patients**

The association of vitamin D with inflammatory biomarkers among COVID-19 patients is shown in Table 6. The bivariate logistic regression analysis revealed that patients with vitamin D insufficiency had elevated creatinine levels (OR=1.65, 95% CI 1.05-2.58, p=0.025), higher CRP levels (OR=2.57, 95% CI 1.51-4.36, p=<0.001), higher serum ferritin (>400 ng/mL) (OR=1.73, 95% CI 1.06-2.82, p=0.029), elevated IL-6 (OR=2.04, 95% CI 1.27-3.28, p=0.003), and increased TNF-α (>24.47 pg/mL) (OR=2.38, 95% CI 1.46-3.86, p=<0.001) compared to those with vitamin D sufficiency. Consequently, patients with low vitamin D had increased CRP levels (OR=2.57, 95% CI 1.51-4.36, p=<0.001) in comparison to normal vitamin D patients. Similarly, increased odds (OR=1.73, 95% CI 1.06-2.82, p=0.029) were calculated for higher serum ferritin (>400 ng/mL) among the vitamin D-insufficient group in comparison to the vitamin D-sufficient group. Likewise, higher odds (OR=2.04, 95% CI 1.27-3.28, p=0.003) were measured for elevated IL-6 (>7.0 pg/mL) among patients with vitamin D insufficiency than among those with vitamin D sufficiency. Similarly, the patients with low vitamin D levels had greater odds (OR=2.38, 95% CI 1.46-3.86, p=<0.001) of having increased TNF-α (>24.47 pg/mL) than those with normal vitamin D levels. These results are reflected in Figure 4.

**Table 6. Association of vitamin D with inflammatory biomarkers among COVID-19 patients**

| Characteristics | OR | 95% CI (lower, upper) | P value |
|---|---|---|---|
| **Creatinine >1.06 mg/dL** | 1.65 | 1.05-2.58 | **0.025** |
| **CRP >0.5 mg/dL** | 2.57 | 1.51-4.36 | **<0.001** |
| **Serum ferritin >400 ng/mL** | 1.73 | 1.06-2.82 | **0.029** |
| **IL-6 >7.0 pg/mL** | 2.04 | 1.27-3.28 | **0.003** |
| **TNF-α >24.47 pg/mL** | 2.38 | 1.46-3.86 | **<0.001** |

Values in bold indicate statistical significance (P<0.05). LDH; Lactose dehydrogenase, ALT; alanine transaminase, CRP; C-reactive protein, TNF-α; Tumor necrosis factor-alpha, IL-6; Interleukin six

**Association of vitamin D with metabolic biomarkers among COVID-19 patients**

The association of vitamin D with metabolic biomarkers among COVID-19 patients is displayed in Table 7. The bivariate logistic regression analysis revealed that HDL levels (>35 mg/dL) were reduced (OR=0.52, 95% CI 0.33-0.82, p=0.006), while triglycerides (OR=1.57, 95% CI 1.02-2.43, p=0.039) and serum cholesterol (>200 mg/dL) (OR=1.66, 95% CI 1.05-2.62, p=0.027) were all increased among the patients with vitamin D insufficiency compared to those with vitamin D sufficiency. Additionally, the odds of elevated triglycerides were (OR=1.57, 95% CI 1.02-2.43, p=0.039) higher in the vitamin D-insufficient group than in the vitamin D-sufficient group. Moreover, higher odds (OR=1.66, 95% CI 1.05-2.62, p=0.027) were calculated for serum cholesterol (>200 mg/dL) patients with lower vitamin D levels in comparison to patients with normal vitamin D levels. However, LDL had no significant association with vitamin D levels among COVID-19 patients in our study.

**Table 7. Association of vitamin D with metabolic biomarkers among COVID-19 patients**

| Characteristics | OR | 95% CI (lower, upper) | P value |
|---|---|---|---|
| **HDL>35 mg/L** | 0.52 | 0.33-0.82 | **0.006** |
| **Triglycerides >200 mg/dL** | 1.57 | 1.02-2.43 | **0.039** |
| **Serum cholesterol >200 mg/dL** | 1.66 | 1.05-2.62 | **0.027** |

Values in bold indicate statistical significance (P<0.05). HDL; High-density lipoprotein, LDL; Low-density lipoprotein.

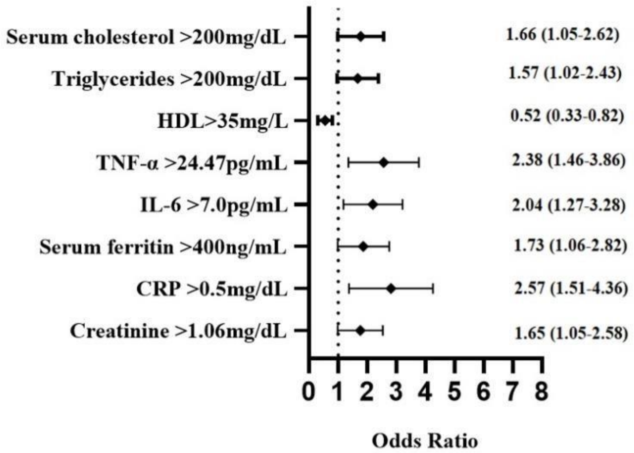


**Figure 4. Forest plot of inflammatory and metabolic biomarkers associated with vitamin D insufficiency based on odds ratios.**

**Association of vitamin D with dietary habits of COVID-19 patients**

The association of vitamin D with the dietary habits of COVID-19 patients is presented in Figure 5. A food frequency (FFQ) questionnaire was used to assess dietary habits. Approximately 60 food items were included in the local FFQ of Pakistan, and slight changes were made. The data were stratified into 2 groups: the vitamin D-sufficient group and the vitamin D-insufficient group. Upon analysis, the results showed that patients in the vitamin D-sufficient group (7±3.5) more frequently consumed marine mammals and fish than those in the vitamin D-insufficient group (3±3.0), with a p-value of 0.008. Similarly, patients with vitamin D sufficiency (23±5.3) consumed more meat and eggs than patients with vitamin D insufficiency (16±4.5), with a significant p-value (p<0.001). Similarly, a significant difference (p value=0.002) in the consumption of vegetables and fruit was observed between the vitamin D-sufficient and vitamin D-insufficient groups (16.0±3.0 vs 11±3.2). Subsequently, dairy products were consumed more frequently by patients with vitamin D sufficiency (12.0±2.5) than by those with vitamin D insufficiency (7±3.0), with a p-value of 0.003. On the other hand, consumption of complex carbohydrates (p value=0.072) and junk foods (p value=0.085) were not significantly different between vitamin D sufficient and vitamin D insufficient.

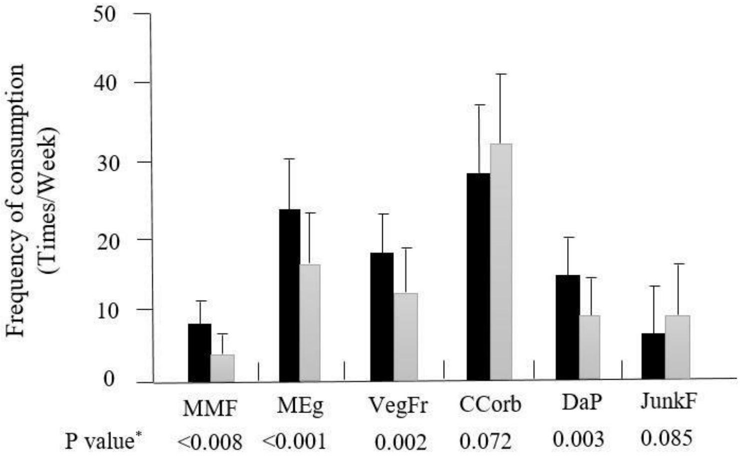


**Figure 5. Association of dietary habits with vitamin D levels in COVID-19 patients**
Vitamin D sufficient group ■ (n=118), vitamin D insufficient group ■ (n=282). MMF; Marine mammals and fish, Meg; Meat and eggs, VegFr; Vegetables and fruits, CCorb; Complex carbohydrates, DaP; Dairy products, JunkF; Junk foods

**Discussion**

This study assessed the association between vitamin D insufficiency and demographic variables and clinical and laboratory findings of COVID-19 patients. Our results confirmed that 25(OH)D insufficiency was associated with a significant increase in the age and severity of COVID-19 patients. Second, few participants had a sufficient level of vitamin D. It is worth mentioning that COVID-19 infection began in winter. Many characteristic features of the biology, physiology, and epidemiology of vitamin D point to its likely candidate for "seasonal stimulus" since the serum levels of 25(OH)D are lowest at the end of the winter season [31]. Additionally, according to the nationwide 1958 British birth cohort, Berry et al. [32] observed an independent association between serum vitamin D and seasonal infections. The authors assessed 25(OH)D, lung function, vital lung capacity, and respiratory diseases in 6789 study participants aged 45 years. They confirmed a strong association between the prevalence of respiratory diseases and the seasonal pattern of serum 25(OH)D concentration. They also established a linear association between vitamin D status and lung function and seasonal infections.

Indeed, the anti-inflammatory role of 1,25(OH)2D may explain the protective role of vitamin D in COVID-19 patients in the fight against immune hyperactivity and cytokine storm. A recent study also observed that C-reactive protein (CRP), a substitute for vitamin D status, was associated with the severity of COVID-19 infection [33]. They confirmed that a high CRP level associated with vitamin D deficiency was related to an increased risk for severe COVID-19 [33]. Their findings are parallel to our results. Our findings revealed that CRP levels were higher in patients with lower levels of serum 25(OH)D than in patients with a serum level of 25(OH)D >30 ng/mL. This finding can be clarified by the anti-inflammatory effect of 25(OH)D on reducing inflammatory markers such as CRP and serum ferritin observed in our study. This anti-inflammatory effect of 25(OH)D might prevent cytokine storms in COVID-19 infection and explain the reduced risk of severity observed in our patients with sufficient 25(OH)D levels [34]. A recent study found that CRP levels were positively correlated with lung lesions in the early stage of COVID-19 and could reflect disease severity [35]. Furthermore, at the initial stage of COVID-19, CRP levels increased significantly in severe cases [36]. Notably, consistent with our results, CRP levels associated with disease development predicted early severity of COVID-19 infection [36]. Other studies also found an inverse relationship between serum vitamin D and CRP levels [34,37–41], further strengthening our findings.

Patients with vitamin D insufficiency were 1.65 times more likely to have higher serum creatinine levels than patients with sufficient vitamin D. Importantly, consistent with our study, a negative correlation was found between serum creatinine level and vitamin D level [42]. Furthermore, it was found that patients with vitamin D insufficiency were more likely to have high triglyceride and cholesterol levels. A meta-analysis of 41 RCTs confirmed the association between vitamin D and the lipid profile. Their results established that vitamin D supplementation significantly improved serum triglyceride and cholesterol levels [43]. Individuals with obesity during the current pandemic may have vitamin D deficiency, which increases the severity of COVID-19 infection [44].

According to preliminary estimates of underlying health problems among COVID-19 patients in the United States, approximately 36% of patients had one or more underlying health problems or risk factors [45]. The percentage of at least one underlying health problem or risk factor was higher among COVID-19 patients (78%) admitted to the intensive care center (ICU) [45].

Age groups 40 and above as well as those with severe COVID-19 were more likely to be deficient in vitamin D levels. Consistent with our study, many studies have confirmed the association between 25(OH)D levels and susceptibility to COVID-19 and the severity of outcomes caused by COVID-19 infection [44,46–54]. An interventional study found that a high dose of vitamin D for COVID-19 patients reduced the need for admission into the intensive care unit (ICU) [55]. Furthermore, other studies also found that older COVID-19 patients with vitamin D deficiency were more likely to have worse clinical outcomes than patients with sufficient vitamin D [13,56–59]. The ability of the human skin to synthesize vitamin D from sunlight decreases with age, which leads to vitamin D deficiency in the older population [23].

Our study has some strengths and limitations. The strengths of our study include the large sample size (400) of the study participants and the current serum vitamin D status measured upon hospitalization. Some limitations of our study are worth mentioning. First, data were collected from a single tertiary care hospital. Second, our study is a cross-sectional study. Therefore, we cannot explain the cause-and-effect relationship between vitamin D insufficiency and the increased risk of severity of COVID-19 infection. Multicenter, prospective cohort studies, and randomized clinical trials (RCTs) need to evaluate the interaction between them.

## Conclusion

Our results concluded that sufficient serum vitamin D may play a vital role in COVID-19 infection. Based on the available literature and the results of our study, it may be proposed that serum vitamin D levels in the general population, especially hospitalized patients, are negatively associated with the severity of COVID-19 morbidity. Optimal levels of vitamin D may protect COVID-19 patients from elevated inflammatory markers and adverse metabolic markers.

## Declarations

### Ethics approval and consent to participate

The ethical review board of Hayatabad Medical Complex, Peshawar-Pakistan approved this study under wide Ref. No.454/HEC/B&PSC/2020. Informed consent was sought from either the patient or his/her attendant. The confidentiality of participants was ensured in this study. All methods were carried out in accordance with relevant guidelines and regulations.

### Consent for publication

Not applicable.

### Availability of data and materials

Data will be available upon request from the correspondence authors.

### Competing interests

The authors declare that they have no conflict of interest.

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## Authors' Contributions

S.A designed the study, collected and analyzed the data, and wrote the manuscript. A.N.W critically reviewed the manuscript. M.Z provided support in the methodology section. D.A.A contributed to the elaboration of the results. S.H edited the manuscript. Q.F proofread and finalized the manuscript.

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