Vitamin D levels associate with blood glucose and BMI in COVID-19 patients predicting disease severity

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Abstract

Context: High prevalence of Vitamin-D (VD) deficiency in COVID-19 patients was reported and hypothesized to increase COVID-19 severity likely due to its negative impact on immune and inflammatory responses. Furthermore, clear associations between hypovitaminosis-D and fat body-mass excess and diabetes, factors associated with COVID-19 severity, have been widely recognized.

Objective: The aim of this study was to evaluate in COVID-19 patients the relationship between VD levels and inflammatory response, BMI, blood glucose and disease severity.

Design: Patients admitted to San Raffaele-Hospital for COVID-19 were enrolled in this study, excluding those with comorbidities and therapies influencing VD-metabolism. 25(OH)VD levels, plasma glucose levels, BMI and inflammatory parameters were evaluated at admission.

Results: A total of 88 patients were included. Median VD level was 16.3 ng/mL and VD-deficiency was found in 68.2% of patients. VD-deficiency was found more frequently in male patients and in those affected by severe COVID-19. Regression analyses showed a positive correlation between VD and PaO2/FiO2 ratio, and negative correlations between VD and plasma glucose, BMI, Neutrophil/Lymphocyte ratio, CRP and IL-6.

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Patients with both hypovitaminosis-D and diabetes mellitus, as well those with hypovitaminosis-D and overweight, were more frequently affected by a severe disease with worse inflammatory response and respiratory parameters, compared to those without or just one of these conditions.

Conclusions: We showed, for the first-time, a strict association of VD levels with blood glucose and BMI in COVID-19 patients. VD-deficiency might be a novel common pathophysiological mechanism involved in the detrimental effect of hyperglycemia and adiposity on disease severity.

Keywords: Vitamin D; plasma glucose; body mass index; COVID-19; SARS-CoV-2

Introduction

COVID-19 clinical manifestations are characterized by widely varying respiratory and extra-respiratory features. Pulmonary manifestations range from asymptomatic forms to acute respiratory distress syndromes with high mortality risk, and extra-respiratory manifestations include cardiovascular, thrombotic, neurological, gastrointestinal and endocrine features (1-5).

Risk factors recognized for COVID-19 severe forms characterized by hyperinflammatory response and dramatic pulmonary and systemic complications include older age, presence of concomitant comorbidities such as cardiovascular disease, diabetes and cancer, male sex and obesity (1-9). In addition, several findings have recently showed a novel role of an endocrine osteo-metabolic phenotype suggested to possibly influence COVID-19 severity and clinical outcomes (10-17). This phenotype is typically characterized by a widespread prevalence of acute hypocalcemia and chronic hypovitaminosis D and a highly prevalence of vertebral fractures (10-17).

A widespread prevalence of Vitamin D (VD) deficiency in COVID-19 patients was reported by several studies and, since VD is proved to be involved in immune response and immunocompetence, VD deficiency has been hypothesized to predispose to SARS-CoV-2 infection and to increase COVID-19 severity, although not all researches confirmed these findings (18-25).

Furthermore, clear associations between hypovitaminosis D and overweight, obesity and diabetes mellitus, factors known to increase COVID-19 severity risks, have been widely recognized (26-29). Several pathophysiological mechanisms have been hypothesized to explain the associations between fat body mass excess and hypovitaminosis D, including lower dietary intake of VD, decreased outdoor physical activity with poorer skin exposure to

sunlight, impaired hydroxylation in adipose tissue, VD accumulation in fat and alterations in VD-receptors in patients with body fat excess (26,27).

It was widely reported that patients with diabetes mellitus had low VD levels, likely due to impaired hepatic and renal metabolism of VD, decreased dietary VD intake and reduced intestinal absorption of VD due to diabetic autonomic neuropathy (28-30). Moreover, low circulating VD levels were reported to be associated with poor glycemic control in diabetic patients and large prospective studies suggested that VD deficiency may predispose to a higher risk of developing impaired fasting glucose and diabetes (31-33), although the influence of VD supplementation on the diabetes onset risk is still unknown and, in a recent prospective randomized trial, VD supplementation did not result in a significantly lower risk of new-onset diabetes than placebo in prediabetic subjects (34).

To date, only few studies tried to investigate the impact of hypovitaminosis D on inflammatory-immune response in COVID-19 (19-21), and no data are available about the relationship between VD and blood glucose (GLU) and body mass index (BMI) in these patients.

The aim of this study was to evaluate in COVID-19 patients the relationship between VD levels and inflammatory response, BMI, blood GLU and disease severity.

Methods

Study design

This was a retrospective sub-study of the COVID-BioB study, a large prospective observational investigation performed at San Raffaele University Hospital, a tertiary health-care hospital in Milan, Italy (35). The study protocol complies with the Declaration of

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Helsinki, was approved by the Hospital Ethics Committee (protocol no. 34/int/2020) and was registered on ClinicalTrials.gov (NCT04318366). Full description of patient management and clinical protocols were previously published (35). Signed informed consent was obtained from all patients participating in this study. Adult patients (age ≥18 years) admitted to San Raffaele University Hospital for COVID-19 during the first Italian wave of the pandemic (March 18th to May 5th, 2020) were enrolled in the COVID-BioB study. Confirmed COVID-19 was defined as positive real-time reverse-transcriptase polymerase chain reaction (RT-PCR) from a nasal and/or throat swab together with signs, symptoms, and/or radiological findings suggestive of COVID-19 pneumonia. Patients admitted for other reasons and subsequently diagnosed with superimposed SARS-CoV- 2 infection were excluded.

Data collection

Data were collected from medical chart review or directly by patient interview and entered in a dedicated electronic case record form (eCRF) specifically developed for the COVID-BioB study. Prior to the analysis, data were cross-checked with medical charts and verified by data managers and clinicians for accuracy. As part of the COVID-BioB protocol, blood samples from all enrolled patients were collected and stored in the COVID-19 biobank of our institution according to appropriate quality control systems (35).

For this study the following variables were collected: age, sex, BMI (calculated as the ratio of weight in kilograms [kg] divided by height in squared metres), vitamin 25-OHD3 (ng/mL), PaO2/FiO2 ratio (calculated as the ratio between the arterial partial pressure of oxygen measured on arterial blood gas analysis and the fraction of inspired oxygen), estimated glomerular filtration rate (eGFR, as estimated by the CKD-EPI equation and expressed as ml/min/1.73 m^2), lymphocyte and neutrophil counts (x10^9/L), lactate dehydrogenase (LDH,

U/L), high-sensitivity C-reactive protein (CRP, mg/dL), plasma GLU (mg/dL) on admission to the Emergency Department (ED), comorbidities (including history of hypertension, diabetes mellitus, coronary artery disease [CAD], and active malignancy), and clinical outcomes (discharge from ED or hospital ward, hospitalization, needs for non-invasive mechanical ventilation, admission to intensive care unit [ICU] and mortality). Severe COVID-19 disease was defined as need for high-flow oxygen therapy and/or non-invasive mechanical ventilation, admission to ICU and/or death for COVID-19 complications.

Patients with comorbidities and concomitant active therapies influencing VD metabolism were excluded from the study analyses, such as chronic kidney disease, osteoporosis, patients on glucocorticoids and antiepileptic drugs, VD/calcium, loop/thiazide diuretics, and patients with an eGFR≤30 mL/min/1.73m^2 using creatinine levels at initial evaluation.

Definitions and cut offs

VD deficiency was defined as 25OHVD level below 20 ng/mL, according to the last consensus report by Sempos et al. (36). Overweight (OW) was defined as a BMI above 25 kg/m^2, accordingly to WHO classification (37). Hyperglycemia was defined as a plasma GLU above 125 mg/dL, according to American Diabetes Association cut off values (38).

Assays

VD measurements were performed on a Roche COBAS 8000 (Roche, Basel, Switzerland) using electrochemiluminescence immunoassays (ECLIA). Multiplex immunoassays (Bio-Rad) based on Luminex technology were used for the quantification of interleukin 1beta (IL-1β), interleukin 6 (IL-6), interferon gamma (IFN-γ) and interleukin 18 (IL-18) in human samples, according to the manufacturer's instructions. Data were measured on Bio-Plex 200 System and calculated using Bio-Plex Manager 6.0 and 6.1 software.

Statistical analysis

Descriptive statistics were obtained for all study variables. Categorical variables were summarised as counts and percentages. Kolmogorov-Smirnov normality test was performed (p<0.05) and continuous variables were expressed as medians and interquartile range (IQR) [25th – 75th percentile]. Fisher exact test or χ2 test and the Wilcoxon signed-rank test or the Kruskal–Wallis test were employed to determine the statistical significance of differences in proportions and medians, respectively. Linear regression analyses were used to correlate continuous variables. All statistical tests were two-sided. A p-value of <0.05 was considered statistically significant. Statistical analysis was conducted using IBM SPSS Statistics (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.).

Results

Clinical Presentations and Outcomes

A total of 111 patients were initially included in the study but, subsequently, twenty-three of them were excluded for the following exclusion criteria: eleven patients were affected by chronic kidney disease and seven of them were on VD supplements, three patients were affected by osteoporosis and three by osteopenia, and were on VD supplements, and, finally, six patients were on VD supplements alone.

Finally, a total of 88 COVID-19 patients were included in the study. Approximately two-thirds of patients were male (67%), and the median age was 56.8 years (49.2-67.5). Patients demographic, inflammatory and disease characteristics upon hospital admission are summarized in Table 1.

The main comorbidities were history of arterial hypertension (35.2%), followed by diabetes mellitus (20.5%) and CAD (10.2%). Sixty-two patients (70.5%) were hospitalized after initial evaluation and 28 (31.8%) were admitted to the ICU during hospitalization, while 17 (19.3%) patients died. Severe COVID-19 was found in 50 (56.8%) patients.

Vitamin D and Disease Severity

On initial hospital admission, median VD level was 16.3 ng/mL [11.2–23.9] and VD deficiency was found in 60 (68.2%) patients.

VD deficiency was found more frequently in male patients compared to female group (77% vs 48%, p=0.007) and no differences regarding age and comorbidities distribution were found between VD deficiency patients and VD non-deficient ones (Table 2). Male patients presented a median VD level of 15.9 ng/mL [10.4-18.5] compared to female patients presenting a median VD level of 20.4 ng/mL [13.2-27.8] (p=0.017).

Linear regression analyses showed a positive correlation between VD levels and PaO2/FiO2 ratio (p=0.019, r=0.25), and negative correlations between VD levels and Neutrophil/Lymphocyte (N/L) ratio (p=0.04; r=-0.19), CRP levels (p=0.047, r=-0.18) and IL-6 levels (p=0.038, r=-0.22) (Figure 1).

Lower VD levels were found in patients affected by severe disease than non-severe patients (13.4 ng/mL vs 18.45 ng/mL, p=0.007). Moreover, patients with VD deficiency had higher levels of CRP, LDH, IL-6, IFN-γ (p=0.04, p=0.01, p=0.002, p=0.04; respectively), lower PaO2/FiO2 and higher N/L ratios (p=0.008, p=0.004; respectively), and higher rate of severe disease (65% vs 39%, p=0.02), compared to VD non-deficient ones (Table 2).

No differences regarding clinical outcomes were found between VD deficiency patients and VD non-deficient ones (Table 2).

Vitamin D and Glucose levels

On initial hospital admission, median GLU level was 112.5 mg/dL [97.2–137.5] and hyperglycemia, defined as a GLU level above 125 mg/dL, was found in 29 (32.9%) patients.

Linear regression analyses showed a negative correlation between GLU and VD levels (p=0.03, r=-0.23) (Figure 2, panel a).

In hyperglycemic patients we found lower levels of VD compared to normo-glycemic patients (13.3 ng/mL vs 18.2 ng/mL; p=0.006). Furthermore, VD deficiency patients were characterized by a higher GLU levels compared to normal VD group (118 mg/dL vs 100 mg/dL; p=0.004) (Table 2) and were more frequently hyperglycemic (41.6% vs 14.3%; p=0.01).

Severe COVID-19 was found in 7/24 (29%) of patients with normal GLU and normal VD, in 24/39 (61%) of those with hyperglycemia or hypovitaminosis D, and in 19/25 (76%) of patients with both hyperglycemia and VD deficiency (p=0.003) (Table 3). No statistical differences were found regarding age in these three groups (Table 3).

Higher PaO2/FiO2 and lower N/L ratio, LDH, CRP, IL-1β and IL-6 levels were found in patients without hyperglycemia and VD deficiency compared to those with one or both conditions (Table 3). Higher rates of hospitalization, ICU admission and NIV requirement were found in patients with hyperglycemia and hypovitaminosis D compared to those with one or none conditions (Table 3).

In order to evaluate the effects of hypovitaminosis D or hyperglycemia alone in patients with only one of these conditions we compared baseline characteristics, inflammatory parameters and disease outcomes of patients with hypovitaminosis D and normoglycemia vs. those with hyperglycemia and normal VD levels (Table 4). Hyperglycemic patients were significantly older compared to hypovitaminosis D patients (73 [54-78] vs 52 [48-63] yrs.; p=0.042) (Table 4) and required more frequently NIV support and ICU admission (Table 4).

Vitamin D and Body Mass Index

BMI was available in 59 patients. In these patients median VD level was 16.4 ng/mL and VD deficiency was found in 66.1% of patients. Median BMI was 27 and OW was found in 43/59 (72.9%) patients, of whom 20 were obese (33.9%).

Linear regression analysis showed a negative correlation between BMI and VD levels (p=0.042, r=-0.26) (Figure 2, panel b).

In OW patients we found lower levels of VD compared to normal-weight patients (16.2 ng/mL vs 20.2 ng/mL; p=0.044). Furthermore, VD deficiency patients were characterized by a higher BMI compared to normal VD group (28.4 vs 25.6; p=0.043) and were more frequently OW (82% vs 55%; p=0.027).

Severe COVID-19 was found in 3/9 (33%) of patients with normal weight and normal VD, in 11/18 (61%) of those with OW or hypovitaminosis D, and in 23/32 (72%) of patients with both OW and VD deficiency (p=0.1) (Table 5). In particular, comparing OW patients with hypovitaminosis D vs normal-weight patients with normal VD, severe COVID-19 was found more frequently in the first group (33% vs 72%; p=0.047). Furthermore, higher PaO2/FiO2 ratio and lower IL-6 levels were found in patients without OW and VD deficiency compared

to those with one or both conditions (371 vs 243 vs 211; p=0.03) (8.7 vs 24.3 vs 43.8 pg/mL; p=0.046) (Table 5).

In order to evaluate the effects of hypovitaminosis D or overweight alone in patients with only one of these conditions we compared baseline characteristics, inflammatory parameters and disease outcomes of patients with hypovitaminosis D and normal weight vs. those with overweight and normovitaminosis D (Table 6). Hypovitaminosis D patients were affected by a worse inflammatory response with higher levels of N/L, CRP and LDH compared to overweight patients (Table 6); no statistically differences were found in baseline characteristics and disease outcomes (Table 6).

Discussion

From the beginning of COVID-19 spread in Europe, we have proposed that VD deficiency could have been involved in increased SARS-CoV-2 infection susceptibility and negative outcomes of COVID-19 (39).

VD role is well known to be crucial for the skeletal homeostasis in physiological and disease states, but it also has many systemic extra-skeletal functions, including immunomodulation and immunocompetence both regarding innate and adaptive immunity (40). This immunomodulatory role is consistent with several findings showing low levels of VD in hospitalized patients with COVID-19 and with reported correlations between VD levels and disease clinical severity and outcomes (14).

A very recent systematic reviews and meta-analyses assessing the impact of VD status on COVID-19 infection and related mortality concluded that low VD levels seem associated with increased SARS-CoV-2 infection risk, COVID-19 severity and associated mortality,

although these analyses were conducted with available evidences to-date obtained from largely not high-quality observational studies (41-43).

In our study we found clear associations between lower VD levels and worse inflammatory and clinical parameters in COVID-19 patients.

PaO2/FiO2 ratio, representing a reliable clinical indicator of hypoxemia, is one of the most useful parameters used in patients with respiratory diseases in order to identify patients at higher risk of severe disease (44). In our study VD levels were found positively associated with PaO2/FiO2 ratio and in VD deficiency patients lower PaO2/FiO2 ratio values were found. Moreover, inflammatory parameters, such as CRP and LDH, and cytokines immune markers, such as IL-6 and IFN-γ, typically increased and associated with severe COVID-19 (45) were found higher in VD deficiency patients, and some of these parameters were found negatively correlated with lower VD levels. Furthermore, the N/L ratio, which proved to be the one of the mostly effective biochemical markers able to predict severe COVID-19 representing an immune dysregulated response with lymphopenia and neutrophilia (46,47), was found higher in VD deficient patients and negatively associated with VD.

From the beginning of the COVID-19 pandemic several studies showed clearly how male patients were at higher risk of a more severe disease as compared to women with a similar pattern in different countries (9). In a recent study on patients with COVID-19, the majority of VD-deficient subjects on hospital admission were males (48), and our data are in agreement with these previous results. Therefore, these findings confirm that underdiagnosis of mineral metabolism diseases in male patients remains a critical medical issue with several systemic implications, including higher infectious risks and dysregulated inflammatory response.

History or newly diagnosed diabetes mellitus and abnormalities of plasma GLU, including worsened hyperglycemia and euglycemic ketosis, rapidly emerged as one of the most relevant medical condition negatively influencing COVID-19 outcomes (49,50). Several studies reported diabetic patients at higher risk of hospitalization, severe pulmonary involvement, and mortality compared to non-diabetic subjects (49,50). These findings suggest that glycemic control may improve outcomes in patients with COVID-19, although, a potential caveat in this regard may be represented by the finding that in ICU patients with critical illness a too tight GLU control could be rather harmful increasing mortality risk (51). Interestingly, it was previously reported that type 2 diabetic patients are characterized by low VD levels (52). In fact, post-menopausal diabetic women were cross-sectionally reported to have significantly higher prevalence of severe hypovitaminosis D as compared to controls (39 vs 25%, respectively) (53). Decreased circulating VD levels were also suggested to be involved in the pathophysiology of diabetes-related skeletal fragility (31) which is also prevalent in males (54) and very relevant to COVID-19 patients as by us recently shown (15).

Confirming these previous findings that showed strict relationships between glycemic status and VD levels, we found a negative correlation between GLU and VD levels in COVID-19 patients on admission in ED. VD deficiency was found more frequently in hyperglycemic patients compared to normo-glycemic subjects and GLU levels were found significantly higher in patients with VD deficiency compared to VD non-deficient ones.

Furthermore, for the first-time, we showed that patients affected by both VD deficiency and hyperglycemia were at higher risk of severe COVID-19 as compared to those without or with only one of these two conditions; in particular, the VD deficient and hyperglycemic patient group was characterized by worse respiratory exchanges, higher inflammatory response and

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worse disease outcomes, although hyperglycemia alone appears to have more severe negative consequences than hypovitaminosis D alone at least in part due to the older age of the first vs the latter group. Since low VD also characterizes diabetic patients with retinopathy (55) it can be hypothesized that hypovitaminosis D may worsen the predisposition of patients with diabetes to the microvascular damage typical of COVID-19.

Interestingly, recent critical analysis of randomized trials reported that supplementation with VD in diabetes may improve glyco-metabolic control, as assessed by fasting blood glucose and by glycated hemoglobin, likely through decreased in insulin resistance and stimulated beta cell function (56,57) particularly in patients with poor glycemic control at baseline (34). A very recent cross-sectional study showed that serum VD was statistically inversely associated with HOMA-IR, but this association was found only in female population and not in males (58). This sex-dependent correlation was explained by the authors by the differential sex steroid hormones effects on pancreatic beta cell and different distribution and metabolic effects of body fat tissue between men and women (58).

Moreover, some studies suggested that VD treatment may slow the progression to diabetes in either patients at high risk of diabetes or with prediabetes, specifically in those with low baseline VD levels (59).

Based on the findings of our study, it can be hypothesized for the first time that low VD may be a predisposing detrimental or even causal factor in the bidirectional relationship between diabetes and COVID-19 increasing synergistically the vulnerability of diabetic patients to the infection as well as facilitating the diabetogenic action of COVID-19.

Furthermore, body mass index and altered body composition with increased adiposity are reported as independent risk factors for greater disease severity and poor prognosis in COVID-19 patients (60,61). Low levels of VD were frequently reported in obese and OW patients being inversely related to BMI and adiposity (26,62), influencing negatively skeletal and muscle health with a resulting increased predisposition to obese osteo-sarcopenic phenotype (63,64). In fact, BMI has also been reported to predict resistance to VD (65).

A possible direct relationship between VD status, body fat, age and SARS-CoV-2 infection and COVID-19 severity has been previously hypothesized. In fact, aging and fat accumulation may decrease VD bioavailability and action (66). In our cohort we found a very high prevalence of OW and hypovitaminosis D and, for the first time, we showed a negative correlation between BMI and VD values in COVID-19 patients. Furthermore, VD deficiency prevalence was found higher in OW patients and those affected by both the conditions presented more frequently a severe disease with higher IL-6 and lower PaO2/FiO2 ratio levels compared to those without VD deficiency and OW and those with one of the two conditions. Interestingly, among these latter groups we found that patients with hypovitaminosis D alone had worse biochemical inflammatory parameters compared to patients with OW alone further underlining the importance of the synergistic negative impact of hypovitaminosis D and OW as unfavourable prognostic factor in patients with COVID-19.

In fact, based on our data it can be thought that low VD levels in obese subjects may be associated with more severe COVID-19 and eventually worsen the prognosis in those subjects admitted to ICU likely due to enhanced baseline inflammatory state. In fact, VD may exert a protective effect in obese individuals, by reducing systemic inflammation (67).

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Moreover, VD has been suggested to play a role in modulating fat distribution and activity (68). Thus, adequate VD status may also be key in preserving body composition also in the post COVID-19 recovery (69).

Limitations of this study were first its retrospective and cross-sectional nature, which did not allow us to evaluate the longitudinal modifications of biochemical and clinical parameters during disease progression and recovery; secondly, the relatively limited number of patients enrolled due to the strict and rigorous inclusion and exclusion criteria used which on the other hand allowed us to analyse our findings without the need to consider the potential impact of several confounding factors; third, we assessed 25-hydroxyvitamin D which is universally accepted as the best marker of the individual VD status (70,71). In this specific setting, it could have been useful to assess also active or free VD (72,73). However, being these parameters not routinely available and due to the known methodological issues in their assays (36) they were not included in the study protocol.

To our knowledge, this is the first study providing evidence of a strict association of VD levels with male sex, GLU levels and BMI in COVID-19 patients in predicting disease severity. Our study has limitations and future larger studies and the results of ongoing interventional randomized clinical trials could be conclusive in clarifying the therapeutic role of VD supplementation in preventing SARS-CoV-2 infection and hard clinical endpoints including mortality in metabolically comorbid patients with COVID-19 (74-76). Moreover, since VD supplementation has been reported to be effective in preventing respiratory infections (77) it could be interesting to extend our observation to non COVID-19 patients in order to understand if hypovitaminosis D may associate with diabetes (78) and obesity in predisposing to negative outcomes of pneumonia in the non COVID setting.

In conclusion, we found high prevalence of low VD levels in our cohort of COVID-19 patients admitted to ED, which was associated with an increased disease severity likely through an excessive immune-inflammatory response. Moreover, we showed, for the first time, a significant association between VD levels and male sex, GLU levels and BMI in COVID-19 and since male sex, hyperglycemia and adiposity are largely recognized as a risk factors for worse disease, VD deficiency might be identified as a novel pathophysiological mechanism involved as common denominator of the endocrine phenotype (79,80) that negatively influence COVID-19 patients outcomes (14).

Data Availability: All data generated and analysed during this study are included in this published

article.

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Tables and Figures Legends

Table 1. Baseline Characteristics of COVID-19 Patients.

Table 2. Clinical-Biochemical Parameters and Disease Outcomes in Patients with Vitamin D Deficiency compared to Vitamin D non-deficient ones.

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Table 4. Clinical-Biochemical Parameters and Disease Outcomes comparisons between Patients with Vitamin D Deficiency or Hyperglycemia alone.

Table 5. Clinical-Biochemical Parameters and Disease Outcomes comparison between Patients with VD Deficiency and Overweight and those with One or None Conditions.

Table 6. Clinical-Biochemical Parameters and Disease Outcomes comparisons between Patients with Vitamin D Deficiency or Overweight alone.

Figure 1. Vitamin D correlations with Respiratory and Inflammatory Parameters.

Figure 2. Vitamin D correlations with Plasma Glucose and BMI.

Table 1. Baseline Characteristics of COVID-19 Patients.

Baseline Characteristics of Patients With COVID-19	
	N. (%) - median (IQR)
Demographic informations	
Total Patients	88 (100%)
Age, yrs	56.8 (49.2-67.5)
Male	59 (67%)
BMI, kg/m^2	27 (24.7-30.5)
Normal-weight	16 (28.2%)
Over-weight	43 (72.8%)
Missing	*29*
Comorbidities	
Hypertension	31 (35.2%)
Coronary artery disease	9 (10.2%)
Diabetes Mellitus	18 (20.5%)
Malignancy	2 (2.3%)
Clinical and laboratory parameters at admission	
PaO$_2$/FiO$_2$ ratio	288 (190-366)
Neutrophil-Lymphocyte Ratio	5.3 (3.7-8)
CRP, mg/dL	102 (26-161)
LDH, U/L	403 (262-538)
eGFR, mL/min/1.73m^2	96.5 (79.5-107)
IL-1β, pg/mL	3 (0.6-5.3)
IL-6, pg/mL	25.2 (6.9-77.4)

IFN-γ, pg/mL	11.6 (3.7-33.7)
IL-18, pg/mL	719 (521-1156)

Abbreviations: n., number; IQR, interquartile range; yrs, years; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; LDH, lactate dehydrogenase; PaO2/FiO2 ratio, ratio between the arterial partial pressure of oxygen measured on arterial blood gas analysis and the fraction of inspired oxygen; IL-1β, interleukin 1beta; IL-6, interleukin 6; IFN-γ, interferon gamma; IL-18, interleukin 18.

Table 2. Clinical-Biochemical Parameters and Disease Outcomes in Patients with Vitamin D

Deficiency compared to Vitamin D non-deficient ones.

Vitamin D Status and Clinical-Biochemical Parameters and Outcomes			
	VD levels <20 ng/mL (n.60)	**VD levels ≥20 ng/mL (n.28)**	*p value*
Age, yrs.	56 (50-67)	60 (48-70)	*0.75*
BMI	29 (26-32)	25.6 (23.8-29.7)	***0.043***
Hypertension	23 (38%)	8 (28%)	*0.37*
Coronary artery disease	4 (6.6%)	5 (18%)	*0.13*
Diabetes Mellitus	12 (20%)	6 (21%)	*0.87*
Malignancy	1 (1.6%)	1 (3.6%)	*0.54*
PaO$_2$/FiO$_2$ ratio	271 (178-331)	356 (230-415)	***0.008***
Neutrophil-Lymphocyte Ratio	6 (4.1-8.6)	3.7 (2-5.8)	***0.004***
CRP, mg/dL	119 (44-172)	74 (8-153)	***0.04***
LDH, U/L	444 (312-563)	307 (221-470)	***0.01***
Plasma Glucose, mg/dL	118 (106.2-143.5)	110 (90.2-112.5)	***0.004***
IL-1β, pg/mL	3.3 (0.3-6.6)	2.1 (0.8-5)	*0.4*
IL-6, pg/mL	32.4 (13.9-88)	8.2 (0.4-35.4)	***0.002***
IL-18, pg/mL	847 (451-1222)	706 (533-1335)	*0.8*
IFN-γ, pg/mL	13.8 (5.3-39.8)	6.8 (0.7-30.3)	***0.04***
Hospitalized, n. (%)	45 (75%)	17 (60.7%)	*0.17*
Severe Disease, n. (%)	39 (65%)	11 (39%)	***0.02***
NIV, n. (%)	25 (41.7%)	9 (31.1%)	*0.33*
ICU, n. (%)	22 (36.7%)	6 (21.4%)	*0.15*

Death, n. (%)	12 (20%)	5 (17.8%)	*0.8*

Abbreviations: n., number; CRP, C-reactive protein; LDH, lactate dehydrogenase; PaO2/FiO2 ratio, ratio between the arterial partial pressure of oxygen measured on arterial blood gas analysis and the fraction of inspired oxygen; IL-1β, interleukin 1beta; IL-6, interleukin 6; IFN-γ, interferon gamma; IL-18, interleukin 18; NIV, non-invasive ventilation; ICU, intensive care unit. P values reported in bold are those statistically significant.

Table 3. Clinical-Biochemical Parameters and Disease Outcomes comparisons between Patients with Vitamin D Deficiency and Hyperglycemia and those with One or None Conditions.

Clinical-Biochemical Parameters and Outcomes distribution in different VD and Glycemic Status			
	Normo-GLU AND Normo-VD (n.24)	**Hyper-GLU OR Hypo-VD (n.39)**	**Hyper-GLU AND Hypo-VD (n.25)**	*p value*
Age, yrs.	58 (47-77)	65 (55-69)	57 (51-64)	*0.2*
PaO$_2$/FiO$_2$ ratio	371 (288-413)	271 (164-316)	223 (147-310)	***0.001***
Neutrophil-Lymphocyte Ratio	4.6 (2-8.5)	5.6 (3.7-9.8)	5.9 (3.9-7.8)	***0.003***
CRP, mg/dL	100 (9.3-166)	103 (41-163)	109 (46-169)	***0.019***
LDH, U/L	283 (195-610)	363 (327-535)	444 (312-581)	***0.003***
IL-1β, pg/mL	2 (0.5-10.6)	1.3 (0.2-9.2)	2.9 (0.2-4.7)	***0.045***
IL-6, pg/mL	8.7 (0.4-37.1)	24 (7.5-54.8)	38.9 (19.3-151)	***0.002***
IL-18, pg/mL	876 (514-2048)	609 (456-1142)	702 (522-1036)	*0.4*
IFN-γ, pg/mL	32 (2.2-42.8)	10 (4.8-30.5)	16.6 (6.9-43)	*0.09*
Hospitalized, n. (%)	13 (54%)	28 (71.7%)	21 (84%)	*0.07*
Severe Disease, n. (%)	7 (29%)	24 (61%)	19 (76%)	***0.003***
NIV, n. (%)	5 (20.8%)	15 (39%)	14 (58.3%)	***0.026***
ICU, n. (%)	2 (8.3%)	14 (39%)	12 (48%)	***0.009***

| Death, n. (%) | 3 (12.5%) | 7 (17.9%) | 7 (28%) | *0.37* |

Abbreviations: GLU, plasma glucose; VD, vitamin D; n., number; CRP, C-reactive protein; LDH, lactate dehydrogenase; PaO2/FiO2 ratio, ratio between the arterial partial pressure of oxygen measured on arterial blood gas analysis and the fraction of inspired oxygen; IL-1β, interleukin 1beta; IL-6, interleukin 6; IFN-γ, interferon gamma; IL-18, interleukin 18; NIV, non-invasive ventilation; ICU, intensive care unit. P values reported in bold are those statistically significant.

Table 4. Clinical-Biochemical Parameters and Disease Outcomes comparisons between

Patients with Vitamin D Deficiency or Hyperglycemia alone.

Clinical-Biochemical Parameters and Outcomes distribution in patients with VD deficiency or Hyperglycemia alone		
	Hyper-GLU <u>AND</u> Normo-VD (n.4)	**Hypo-VD <u>AND</u> Normo-GLU (n.35)**	*p value*
Age, yrs.	73 (54-78)	52 (48-63)	***0.042***
PaO$_2$/FiO$_2$ ratio	209 (53-316)	304 (228-333)	*0.07*
Neutrophil-Lymphocyte Ratio	10.4 (5.4-11.8)	5.9 (3.9-8.1)	*0.4*
CRP, mg/dL	160 (141-285)	98 (43-154)	*0.06*
LDH, U/L	541 (411-610)	439 (299-505)	*0.14*
IL-1β, pg/mL	1.6 (1.4-10.6)	3.7 (1.6-7.7)	*0.52*
IL-6, pg/mL	35 (8.4-189)	34.8 (10-81)	*1*
IL-18, pg/mL	1962 (1053-2999)	676 (480-1160)	***0.013***
IFN-γ, pg/mL	19 (2-80.6)	14 (4.7-42)	*0.9*
Hospitalized, n. (%)	4 (100%)	24 (65%)	*0.3*
Severe Disease, n. (%)	4 (100%)	20 (57%)	*0.14*
NIV, n. (%)	4 (100%)	11 (32%)	***0.02***
ICU, n. (%)	4 (100%)	10 (29%)	***0.01***
Death, n. (%)	2 (50%)	5 (14%)	*0.14*

Abbreviations: GLU, plasma glucose; VD, vitamin D; n., number; CRP, C-reactive protein;

LDH, lactate dehydrogenase; PaO2/FiO2 ratio, ratio between the arterial partial pressure of

oxygen measured on arterial blood gas analysis and the fraction of inspired oxygen; IL-1β,

interleukin 1beta; IL-6, interleukin 6; IFN-γ, interferon gamma; IL-18, interleukin 18; NIV,

non-invasive ventilation; ICU, intensive care unit. P values reported in bold are those

statistically significant.

Table 5. Clinical-Biochemical Parameters and Disease Outcomes comparison between

Patients with VD Deficiency and Overweight and those with One or None Conditions.

Clinical-Biochemical Parameters and Outcomes distribution in different VD and BMI Status			
	Normal Weight <u>AND</u> Normo-VD (n.9)	**Overweight <u>OR</u> Hypo-VD (n.18)**	**Overweight <u>AND</u> Hypo-VD (n.32)**	*p value*
Age, yrs.	58 (46-67)	53 (49-65)	61 (53-68)	*0.3*
PaO$_2$/FiO$_2$ ratio	371 (253-418)	292 (220-332)	211 (111-311)	***0.03***
Neutrophil-Lymphocyte Ratio	3.2 (2-5.2)	5.9 (4.1-8.1)	6.8 (4.2-10.5)	*0.62*
CRP, mg/dL	37 (6.4-118)	117 (46-159)	122 (38-236)	*0.9*
LDH, U/L	275 (199-360)	440 (309-526)	495 (323-672)	*0.35*
IL-1β, pg/mL	2.1 (0.4-5)	2.2 (0.3-3.5)	3.7 (1.6-7.7)	*0.84*
IL-6, pg/mL	7.3 (0.4-24.7)	26.7 (18.4-177)	34.8 (10-81.4)	***0.04***
IL-18, pg/mL	717 (433-1101)	693 (510-1229)	765 (641-1128)	*0.5*
IFN-γ, pg/mL	13.8 (5.3-39.8)	6.8 (0.7-30.3)	16.1 (6.24-33.4)	*0.55*
Hospitalized, n. (%)	5 (55%)	15 (83%)	27 (84%)	*0.14*
Severe Disease, n. (%)	3 (33%)	11 (61%)	23 (72%)	*0.1*
NIV, n. (%)	3 (33%)	9 (50%)	14 (43.7%)	*0.71*
ICU, n. (%)	2 (22%)	6 (33%)	11 (34%)	*0.78*

Death, n. (%)	1 (11%)	3 (16.6%)	4 (12.5%)	*0.89*

Abbreviations: VD, vitamin D; n., number; CRP, C-reactive protein; LDH, lactate dehydrogenase; PaO2/FiO2 ratio, ratio between the arterial partial pressure of oxygen measured on arterial blood gas analysis and the fraction of inspired oxygen; IL-1β, interleukin 1beta; IL-6, interleukin 6; IFN-γ, interferon gamma; IL-18, interleukin 18; NIV, non-invasive ventilation; ICU, intensive care unit. P values reported in bold are those statistically significant.

Table 6. Clinical-Biochemical Parameters and Disease Outcomes comparisons between Patients with Vitamin D Deficiency or Overweight alone.

| Clinical-Biochemical Parameters and Outcomes distribution in patients with VD deficiency or Overweight alone		
	Overweight AND Normo-VD (n.11)	**Hypo-VD AND Normal Weight (n.7)**	*p value*
Age, yrs.	63 (48-69)	67 (57-79)	*0.25*
PaO$_2$/FiO$_2$ ratio	253 (190-345)	178 (54-242)	*0.06*
Neutrophil-Lymphocyte Ratio	4.3 (2.8-5.8)	9.1 (6.6-13)	***0.02***
CRP, mg/dL	75 (28-113)	143 (100-293)	***0.04***
LDH, U/L	344 (299-419)	508 (363-566)	***0.04***
IL-1β, pg/mL	1.6 (0.3-5.5)	0.3 (0.27-14.6)	*1*
IL-6, pg/mL	10 (6.9-36.4)	45 (16-81)	*0.18*
IL-18, pg/mL	603 (442-1145)	633 (461-1142)	*0.83*
IFN-γ, pg/mL	7.4 (3.2-23.8)	16 (7-81.7)	*0.2*
Hospitalized, n. (%)	9 (81%)	6 (86%)	*0.83*
Severe Disease, n. (%)	6 (54%)	5 (71%)	*0.6*
NIV, n. (%)	4 (36%)	5 (70%)	*0.3*
ICU, n. (%)	3 (27%)	3 (43%)	*0.62*
Death, n. (%)	2 (18%)	1 (15%)	*1*

Abbreviations: VD, vitamin D; n., number; CRP, C-reactive protein; LDH, lactate dehydrogenase; PaO2/FiO2 ratio, ratio between the arterial partial pressure of oxygen measured on arterial blood gas analysis and the fraction of inspired oxygen; IL-1β, interleukin 1beta; IL-6, interleukin 6; IFN-γ, interferon gamma; IL-18, interleukin 18; NIV, non-invasive ventilation; ICU, intensive care unit. P values reported in bold are those statistically significant.

Figure 1. Vitamin D correlations with Respiratory and Inflammatory Parameters.
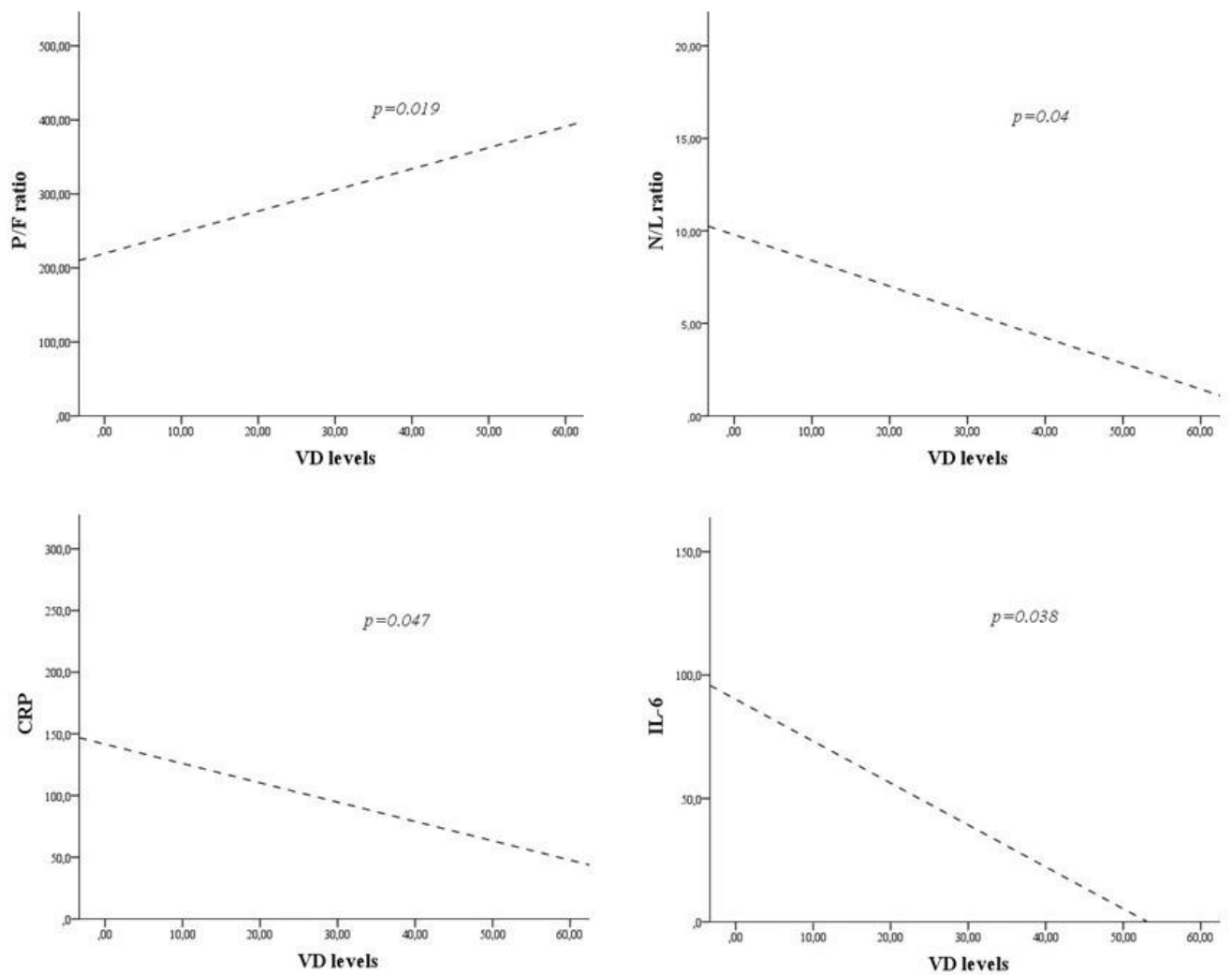
Abbreviations: VD, Vitamin D; P/F, ratio between the arterial partial pressure of oxygen measured on arterial blood gas analysis and the fraction of inspired oxygen; N/L, Neutrophil-Lymphocyte Ratio; CRP, C-reactive protein; IL-6, interleukin 6.

Figure 2. Vitamin D correlations with Plasma Glucose and BMI.
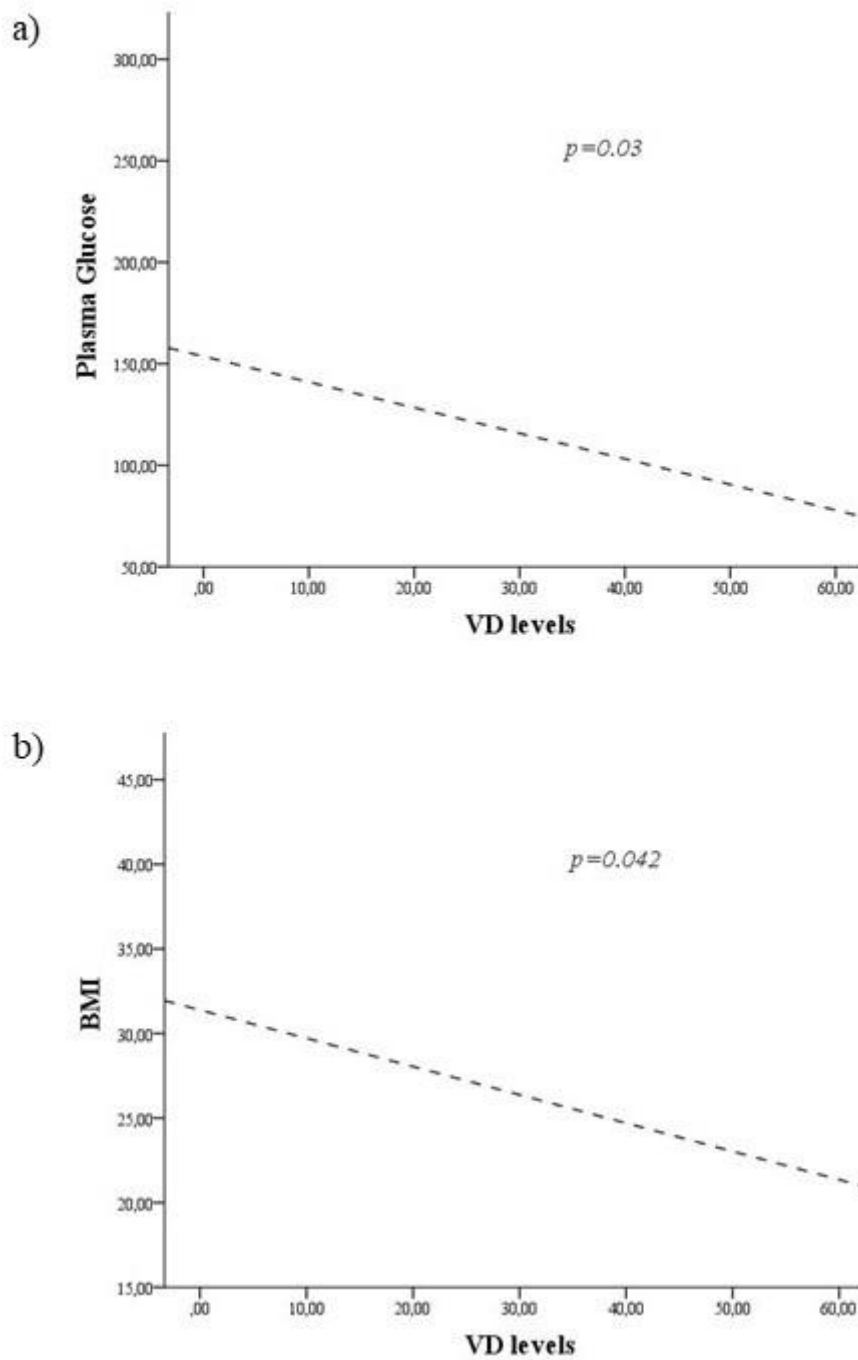
Abbreviations: VD, Vitamin D; BMI, body mass index.