Serum Vitamin D levels are associated with increased COVID-19 severity and mortality independent of visceral adiposity

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ABSTRACT (229 WORDS)

INTRODUCTION: Coronavirus disease (COVID-19) is a global pandemic. Vitamin D (25-OHD) deficiency has been associated with susceptibility to infectious disease. In this study, the association between COVID-19 outcomes and 25-OHD levels in patients attending a COVID-19 reference center in Mexico City are examined.

METHODS: Consecutive patients with confirmed COVID-19 were evaluated. All patients underwent clinical evaluation (including outcomes), laboratory measurements (including 25-OHD) and a thoracic computerized tomography (including the measurement of epicardial fat thickness). Low vitamin D was defined as levels <20ng/mL (<50nmol/L) and severely low (or deficient) 25-OHD as a level ≤12ng/mL (<30nmol/L)

RESULTS: Of the 551 patients included, low 25-OHD levels were present in 45.6% and severely low levels in 10.9%. Severely low 25-OHD levels were associated with mortality (HR 2.11, 95%CI 1.24-3.58, p=0.006) but not with critical COVID-19 (OR 0.97, 95%CI 0.94-0.99, p=0.042), adjusted for age, sex, body-mass index and epicardial fat. Using model-based causal mediation analyses the increased risk of COVID-19 mortality conferred by 25-OHD levels was partly mediated by its effect on D-dimer and cardiac ultrasensitive troponins. Notably, increased risk of COVID-19 mortality conferred by low vitamin D levels was independent of BMI and epicardial fat.

CONCLUSION: Vitamin D deficiency (≤12ng/mL or <30nmol/L), is independently associated with COVID-19 mortality after adjustment for visceral fat (epicardial fat thickness). Low 25-OHD may contribute to a pro-inflammatory and pro-thrombotic state, increasing the risk for adverse COVID-19 outcomes.

Keywords: Vitamin D, COVID-19, SARS-CoV-2, Adiposity, Severe disease

INTRODUCTION

Coronavirus Disease (COVID-19), caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a global pandemic. SARS-CoV-2 spreads primarily by close contact with respiratory droplets from infected individuals and contaminated surfaces (1-3). SARS-CoV-2 infects cells using the angiotensin converting enzyme-2 (ACE-2) receptor; infection can produce an interstitial pneumonia that may progress to acute respiratory distress syndrome (ARDS) and death (4-6).

Vitamin D (25-OHD) is a steroid hormone involved in essential physiological roles including preserving bone integrity, immunomodulation by stimulating innate immunity and tempering adaptive immunity, infectious disease prevention and cardiovascular health (7-9). It also acts on the renin angiotensin aldosterone (RAAS) system, inhibiting the angiotensin converting enzyme (ACE, 6). Several factors are known to influence vitamin D levels; lower levels are associated with ethnicity, variation in sun exposure due to higher latitudes, season, time of day, clothing, sunscreen use and skin pigmentation, age, lower sun exposure, obesity and chronic illnesses (10). The presence of obesity results in decreased bioavailability of vitamin D, which is probably related to sequestration into adipose tissue. Furthermore, higher visceral fat content has been shown to be related to a higher incidence of vitamin D deficiency (11).

Low levels of 25-OHD have been associated with increased susceptibility to infectious disease, particularly respiratory tract infections (12-14). Several studies have explored the relationship between COVID-19 and vitamin D levels (15-18); however, concerns regarding residual confounding and the lack of mechanistic interpretations for the association of low 25-OHD levels with adverse COVID-19 outcomes requires further studies. Obesity and ethnicity are important risk factors for severe disease, and are also known to modulate vitamin D levels. This may be particularly relevant in Mexico, where

high rates of diabetes and obesity have been associated with an increased risk of severe COVID-19 (19). In this study, the association between COVID-19 outcomes and 25-OHD levels in patients attending a COVID-19 reference center in Mexico City is evaluated. We aim to identify determinants of 25-OHD levels in COVID-19 patients and develop causal-mediation models to propose mechanisms by which Vitamin D may lead to increased COVID-19 mortality.

METHODS

Study population

This study included consecutive patients evaluated at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, a COVID-19 reference center in Mexico City between 17th March and 31st May 2020. Subjects were initially assessed at triage and required either ambulatory or in-hospital care for COVID-19 (confirmed with computerized tomography (CT) and/or via RT-qPCR test from nasopharyngeal swabs. All patients had moderate to severe disease as defined by National Institute of Health criteria (*Moderate Illness:* Evidence of lower respiratory disease during clinical assessment or imaging and who have saturation of oxygen (SpO_2) ≥94% on air. *Severe Illness:* SpO_2 <94% on air, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mm Hg, respiratory frequency >30 breaths/min, or lung infiltrates >50%). Subjects underwent a chest CT, and a radiologist determined the degree of pulmonary parenchymal disease and assessed epicardial fat thickness as a proxy for visceral fat. In addition, a medical history, anthropometric measurements and laboratory tests were obtained, including 25 hydroxy-vitamin D (25(OH)-D). The electronic files of each patient were reviewed to document the outcomes during hospitalization. All proceedings were approved by the research and ethics committee of the INCMNZ (Ref 3383) and informed consent was waived due to the nature of the study.

Laboratory and clinical measurements

Clinical variables and laboratory measures were obtained at the time of initial evaluation. Physical examination included: weight, height, body mass index (BMI, calculated as weight in kilograms divided by squared height in meters), pulse oximeter saturation (SpO2), respiratory rate (RR), temperature and arterial blood pressure (BP). Laboratory measurements included: full blood count and chemistry panel including liver function tests, C-reactive protein (CRP), fibrinogen, D-dimer, ferritin, troponin I (TPNI), erythrocyte sedimentation rate (ESR) and procalcitonin levels. The blood samples were processed in the central laboratory of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vitamin D (25-hydroxivitamin D) was measured by chemiluminescence using the Abbott Architect I2000 equipment. Low levels of vitamin D were defined as <20ng/mL (<50 nmol/L) and severely low (or deficiency) as a level <12ng/mL (<30nmol/L) (6-9).

Epicardial fat measurements

All patients underwent unenhanced CT scans including low-dose CT and two ultra-low-dose CT protocols with commonly reported imaging features of COVID-19 pneumonia. The thoracic CT was performed using a 64-slice scanner (GE MEDICAL SYSTEMS Revolution EVO). Epicardial adipose tissue (EAT) thickness was measured at 3 points (right atrioventricular fossa, left atrioventricular fossa, and anterior interventricular fossa) in the reformatted 4-chamber view using the multiplanar reconstruction (MPR) tool on the workstation (20, 21, 22). The maximum thickness of the EAT was determined from the surface of the myocardium to the pericardium (measured perpendicular to the pericardium). The measurements were made on 2 different occasions, obtaining a total of 6 measurements; the average of them was used for all statistical analyses. Pericardial adipose tissue (PAT) was quantified with the volume measurement tool with the Carestream system of the workstation. The thickness of the thoracic subcutaneous

adipose tissue (TscAT) was measured from the anterior border of the sternum to the skin, at the level of the mitral valve in the axial plane of the tomography. The 80th gender-specific percentile of EAT thickness was obtained and used as the threshold to define increased EAT thickness. In addition, chest CT findings were recorded and used to evaluate severity of COVID infection.

COVID-19 outcomes

Outcomes included mortality and critical disease (defined as the combination of mortality and need for mechanical ventilation /intubation). For time-to-event analyses, time from self-reported symptom onset prior to evaluation until last follow-up (censoring) or death, whichever occurred first was estimated.

Statistical analyses

Cases with low and severely low vitamin D levels were analyzed using Student's t-test or Mann-Whitney U according to the variable distribution (parametric or non-parametric) for continuous variables. The chi-squared test was applied for categorical variables. Logarithmic, squared root and cubic root transformations were carried out to ensure variable symmetry prior to modeling. All statistical analyses were conducted using R 4.0.2.

Prediction of mortality and severe COVID-19

Cox proportional risk regression analyses was used to investigate the association of vitamin D with mortality related to COVID-19. Univariate models and fully adjusted models were generated which included the following covariates: age, gender, BMI, C-reactive protein, D-dimer, ultrasensitive cardiac troponin, epicardial fat, T2D, CKD and oxygen saturation levels. An interaction effect was explored with BMI or BMI categories to rule out the differential impact of vitamin D adjusted for BMI. Model diagnostics were done using Schoenfeld residuals. Finally, the association of vitamin D with requirement for mechanical

ventilation or the composite of critical COVID-19 was explored using logistic regression analyses adjusted for the aforementioned covariates.

Predictors of vitamin D levels

Linear regression analyses were fitted to identify predictors of log-transformed vitamin D levels in patients with COVID-19, and model selection was carried out using minimization of the Bayesian Information Criterion (BIC). Logistic regression models were also fitted using a dummy variable which defined low and severely low vitamin D to identify predictors for these categories and again model selection was conducted using BIC. Finally, model diagnostics for linear regression were conducted using residual analyses and the Hosmer-Lemeshow test for logistic regression analyses.

Causal mediation models

Finally, to explore whether variables which are influenced by Vitamin D levels may act as a mediator of the risk conferred by Vitamin D on COVID-19 severity, model-based causal mediation analyses were developed: D-dimer and ultrasensitive cardiac troponins were proposed as mediators of the effect of Vitamin D on COVID-19 mortality. All mediation analyses were performed using the mediation R package; to permit inference to obtain a 95% confidence interval using bias-corrected accelerated non-parametric bootstrap. To demonstrate the sequential ignorability assumption, a sensitivity analysis was run to demonstrate residual confounding by varying the correlation between the residuals of both the outcome and the moderator models. Statistical analyses were performed using R software version 4.0.3. A p value <0.05 was considered statistically significant

RESULTS

Study population

This study included 551 patients with confirmed COVID-19 (with compatible computerized tomography findings and/or positive RT-qPCR test from nasopharyngeal swabs) and

vitamin D measurements. The mean age of participants was 51.92±13.74 years, with a male predominance (n=355, 64.4%), and a mean BMI of 30.05±5.72. Median follow-up was 15.0 days (IQR 10.0-20.0) and 445 patients required hospitalization (81.1%). Overall, 93 patients received invasive mechanical ventilation (16.88%) and 116 in-hospital deaths (21.1%) were recorded. Type 2 diabetes (T2D) was present in 146 patients (26.9%), 219 patients had obesity (42.7%) and 217 were overweight (42.4%). Mean vitamin D levels were 21.78±9.01 and vitamin D levels below 20ng/mL were present in 251 subjects (45.6%) (**Table 1**). Extremely low vitamin D levels (≤12ng/mL) were observed in 59 patients (10.7%) (**Table 1 supplementary material**).

Determinants of Vitamin D levels amongst patients with COVID-19

The pathophysiological adaptations to COVID-19 may be predictive of low vitamin D levels. To this end, determinants of low vitamin D in COVID-19 were sought in an attempt to develop a mechanistic explanatory model for this relationship. Subjects with low vitamin D levels (<20mmg/dl) were more likely to be female, have type 2 diabetes, higher HbA1c, D-dimer and ferritin levels and lower oxygen saturation, albumin and C-reactive protein. Using linear regression, a lower log-transformed vitamin D level was independently associated with female gender, higher log-transformed ultrasensitive cardiac troponin, higher log-transformed D-dimer, higher log-transformed epicardial fat area, and lower C-reactive protein levels (**Table 2**). When exploring a model to detect low vitamin D levels, there was a significantly higher odds for log-transformed D-dimer levels (OR 1.31, 95%CI 1.06-1.63); lower odds were associated with male gender (OR 0.45, 95%CI 0.31-0.65), higher oxygen saturation levels (OR 0.98, 95%CI 0.97-0.99) and higher C-reactive protein values (OR 0.75, 95%CI 0.61-0.92), adjusted for age, and log-transformed epicardial fat. There was no association between days from symptom onset and vitamin D levels at admission.

Vitamin D levels and COVID-19 mortality

Overall, vitamin D levels were significantly lower when comparing non-fatal to fatal COVID-19 cases (22.41±9.34 vs. 19.44±67.19, p<0.001). When assessing risk related to the association between mortality and vitamin D levels using Cox regression, a 1-unit increase in vitamin D levels was associated with a decreased risk of COVID-19 mortality. Interestingly, when stratifying cases according to gender, the difference in vitamin D levels between fatal and non-fatal cases was greater in women compared to men and lower in cases with obesity (**Figure 1**). When the mortality models were adjusted for age, gender, BMI, and C-reactive protein, CKD and T2D the observed association between vitamin D levels and a decrease in COVID-19 mortality persisted (**Table 3**). There was no significant interaction with BMI, (as a continuous variable or categorized) in normal weight, overweight and obese with vitamin D levels. Using post-estimation simulation to predict risk associated with changes in vitamin D levels using the simPH R package, there was a steady decrease in risk attributable to increasing vitamin D concentrations using vitamin D <20ng/mL and ≤12ng/mL as thresholds (**Figure 2**).

Vitamin D and critical COVID-19

When assessing the impact of vitamin D on the risk of invasive mechanical ventilation there was no significant association even after adjustment for age, gender, BMI, C-reactive protein, CKD or T2D status (OR 0.986, 95%CI 0.957-1.015, p=0.366). However, when assessing the composite of critical COVID-19 using logistic regression models, lower vitamin D levels were associated with critical COVID-19 (OR 0.97, 95%CI 0.94-0.99, p=0.042, adjusting by age, gender, BMI, C-reactive protein, D-dimer, CKD, SpO2 or T2D status).

Causal mediation models

Finally, model-based causal mediation models were developed to assess whether the effect of vitamin D (E) on increased mortality risk (Y) was mediated through changes in variables identified in **Table 2** (M), adjusted for age, gender, BMI and epicardial fat. The direct effect of vitamin D on increased mortality risk was significant ($\Delta_{E\rightarrow Y}$ -0.144, 95%CI -0.069, -0.010) and the indirect effect of vitamin D, mediated by increase D-dimer levels ($\Delta_{E\rightarrow MY}$ -0.035, 95%CI -0.164, -0.010), represented 19.3% (95%CI 9.5-77.0%) of the overall association of vitamin D on mortality. A similar scenario was observed for cardiac troponins, whereby both the direct effect of vitamin D on mortality ($\Delta_{E\rightarrow Y}$ -0.133, 95%CI -0.150, -0.020) and the indirect effect mediated by ultrasensitive cardiac troponins ($\Delta_{E\rightarrow MY}$ -0.047, 95%CI -0.085, -0.020), were significant and represented 26.2% (95%CI 14.9-73.0%) of the overall effect of vitamin D on mortality. Notably, there were no significant causal mediation models for either BMI or epicardial fat, here there was only a direct effect on mortality, independent of vitamin D levels.

DISCUSSION

In this study, the association between vitamin D levels and severity of COVID-19 was explored in a Mexican population. Severely low levels of vitamin D (deficiency) showed a clear association with mortality, even after adjusting for confounders, including epicardial fat as a proxy of visceral fat and BMI. A vitamin D level <20ng/mL (<50nmol/L), showed a strong negative predictive value, suggesting that when levels are adequate, the probability of mortality is low. Furthermore, the increased risk of mortality from COVID-19 was partly mediated by the effect of vitamin D on markers of disease severity, such as D-dimer and ultrasensitive cardiac troponins, independent of BMI and epicardial fat (these showed effects on COVID-19 mortality independent of vitamin D levels). This suggests that vitamin D may be a marker of an impaired response to infection within the pulmonary epithelium, in particular in those with severe deficiency (23).

Several studies have explored the relationship between COVID-19 and vitamin D levels (15-17, 24). These include those examining vitamin D levels and risk of infection and those examining an association with severity of COVID-19. Higher levels of IL-6 were observed in the vitamin D deficient group suggesting a greater inflammatory response in these patients (18). A recent systematic review and meta-analysis reported that vitamin D deficiency was not associated with increased risk of infection, but severe cases presented with greater vitamin D deficiency compared with mild cases. Vitamin D deficiency has been associated to increased hospitalization and mortality risk from COVID-19 (19).

Physiological mechanisms by which vitamin D exerts a protective function include enhanced innate immunity including augmentation of physical barriers to infection and optimization of adaptive immunity (25). Adequate vitamin D levels favor protective tissue maintenance, preserving tight, gap and adherens junctions, all of which are disturbed by viruses (26). Calcitriol (1,25 OH D) induces production of cathelicidins, LL-37 and beta-defensins, proteins associated with viral surface disruption and reduced viral replication rates, lower pro-inflammatory cytokine (e.g. tumor necrosis factor alpha, interleukin-6) accumulation in target tissues such as the lungs and increased anti-inflammatory cytokine concentrations that defend against viral infections (27). In addition, vitamin D enhances macrophage phagocytosis and efferocytosis (allowing removal of cellular debris preventing further inflammation), and regulation of the macrophage oxidative burst (eliciting a more potent and efficient oxidative surge) (24).

Vitamin D also has a modulatory effect on the inflammatory response caused by COVID-19. It curbs adaptive immunity by inhibiting B cell proliferation, differentiation and production of antibodies and plays a role in regulation the T cell phenotype (28). Vitamin D suppresses helper T cells (Th)1 proliferation, thus decreasing the synthesis of cytokines such as interferon-gamma, interleukin-2 and interleukin-10 (6) (27, 29) This reduction in

cytokines, suppresses antigen presentation on dendritic cells and diminishes T lymphocyte recruitment and proliferation. Thus, there is a shift in the adaptive immune response from Th1 to a more regulatory Th2 response, characterized by an increase in expression of Th2 associated cytokines (IL-4, IL-10) (30, 31). This may attenuate the quantity of pro-inflammatory cytokines that are associated with severe infection (32) In addition, vitamin D induces ACE 2 expression, and suppresses the angiotensin-renin system, thus reducing levels of proinflammatory angiotensin II. Hence, vitamin D deficiency, could potentiate the cytokine storm perpetuating a pro-inflammatory state and worsening pulmonary outcomes (33). Finally, thrombotic complications are common in such patients; vitamin D is also involved in the regulation of thrombotic pathways (29). In our study, low vitamin D levels in COVID-19 patients were related to inflammatory, pro-thrombotic and metabolic markers of severity, confirming observations from previous studies.

Adverse COVID-19 outcomes have been linked to the ethnic origin of the population under study; this has also been associated with the presence of vitamin D deficiency. Asians, African Americans, and ethnic minorities are at an increased risk of mortality from COVID-19 (34). This may partly be due to a decrease in the production of vitamin D dependent on UV rays. This is related to the skin levels of melanin present in these populations and on the unequal distribution of poverty and cardiometabolic disease rates across such ethnicities. This finding is relevant and may explain lower vitamin D levels and severity of COVID-19 in México in a previous study (35). Previous reports in similar populations, including Hispanics, have shown higher risk of SARS-CoV-2 infection, compared to Caucasian population (36).

Currently, routine vitamin D supplementation in hospitalized patients with COVID-19 is not recommended (37). A recent pilot study showed that administration of a high dose of calcifediol or 25-hydroxyvitamin D, significantly reduced the need for intensive care in

hospitalized patients (38). The authors speculate that calcifediol may reduce severity of the disease. Ideally, additional large randomized controlled trials are needed to properly assess this claim and whether vitamin D supplementation can significantly impact population risk.

This study has certain strengths and limitations. It included a large number of patients with heterogenous risk profiles in whom a variety of disease severity parameters were measured. In addition, a series of statistical tests were carried out to ensure minimal possibility of residual confounding. Nevertheless, some limitations must be acknowledged in order to properly interpret this study. First, a chemiluminescence immunoassay was used to assess vitamin D levels, this may lead to inconsistent results compared to other techniques including competitive binding protein - CBP, radioimmunoassay - RIA liquid chromatography - LC, UV detection with liquid chromatography and liquid chromatography mass spectrometry LCMS or tandem mass spectrometry. Since most patients were attended in the institution for the first time for COVID-19, historic vitamin D values were not available to assess the effect of vitamin D dynamics on infection risk or outcomes. Furthermore, given the disease course of COVID-19, severity profiles are highly heterogeneous even amongst hospitalized patients, which may influence vitamin D values on the basis of varying severity; control for this factor using propensity score matching was carried out, however, there remains a possibility for residual confounding. Notably, these results are from a COVID-19 reference center in Mexico City, this could reduce the representativeness of the findings. Further evidence in other regions of Mexico to confirm the role of vitamin D as a marker of disease severity and mortality in Mexicans with COVID-19 is needed.

Conclusions

Vitamin D levels lower or equal to 12 ng/ml (30nmol/L) are independently associated with COVID-19 mortality, even after adjusting for confounders (including measures of visceral and total body fat). No association was confirmed between vitamin D levels and the need for intubation. Vitamin D deficiency is more prevalent in women and patients with type 2 diabetes mellitus. Vitamin D supplementation may be considered in deficient patients, but evidence of benefit is required from double blind randomized controlled trials.

CONFLICT OF INTEREST/FINANCIAL DISCLOSURE: Nothing to disclose.

DATA AVAILABILITY: Data is available from the corresponding author upon reasonable request. Code for reproducibility of results available at: https://github.com/oyaxbell/covid_metabolism

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469 TABLES

470 **Table 1.** Clinical characteristics, imaging findings and severity scores in patients with
471 COVID-19, comparing cases with and without low vitamin D levels.

Parameter	Vitamin D [>20 ng/mL] (n=300)	Vitamin D [≤20 ng/mL] (n=251)	P-value
Age (years)	53.0 (±14.92)	51.0 (±12.60)	0.088
Male Sex (%)	219 (73%)	136 (54.2%)	<0.001
Low-Socioeconomic Status (%)	203 (67.66%)	172 (68.5%)	0.902
Critical outcome (%)	85 (28.3)	81 (32.3)	0.363
Intubation (%)	53 (17.6)	40 (15.9)	0.670
Mortality (%)	57 (19)	59 (23.5)	0.23
Arterial Hypertension (%)	83 (27.94%)	89 (35.9%)	0.058
Type 2 diabetes (%)	68 (22.9%)	78 (31.6%)	0.031
Time since diagnosis (years)	9.8 (±7.8)	9.20 (±7.5)	0.831
Obesity (%)	118 (39.9%)	111 (44.9%)	0.269
Smoking status (%)	15 (5.8%)	12 (5.8%)	0.789
CKD (%)	6 (2.0%)	12 (4.9%)	0.111

CVD (%)	6 (2.0%)	10 (4.0%)	0.258
Cirrhosis (%)	0 (0%)	3 (1.21%)	0.186
BMI (kg/m2)	29.83 (±4.92)	30.32 (±6.56)	0.343
Respiratory Rate (rpm)	28.52 (±12.40)	28.31 (±9.11)	0.821
Heart Rate (bpm)	102.31 (±18.2)	101.85 (±18.5)	0.771
Systolic Arterial Pressure (mmHg)	120 (110-131)	123 (110-135)	0.264
Diastolic Arterial Pressure (mmHg)	76 (70-80)	74 (67-81)	0.322
Oxygen saturation (%)	82.65 (±11.4)	79.89 (±13.4)	0.010
C-reactive protein	14.93 (9.02-23.20)	13.6 (6.37-21.76)	0.052*
Glucose levels (mg/dL)	149.7 (±85.73)	163.6 (±97.98)	0.0805*
HbA1c (%)	6.10 (5.8-7.05)	6.85 (6.0-9.6)	0.0040
Triglycerides (mg/dL)	149 (114-192)	140 (110-179)	0.2907
HDL-C (mg/dL)	32.7 (±15.25)	33.3 (±10.71)	0.9111
LDL-C (mg/dL)	95.9 (±57.38)	75.1 (±34.74)	0.2818
Total Cholesterol (mg/dL)	159.5 (±60.93)	135.7 (±44.23)	0.2658

Hemoglobin (%)	15.45 (1.85)	17.42 (22.99)	0.1794
Platelet Count ()	230.97 (90.89)	236.53 (103.83)	0.5089
Lymphocytes	8.91 (4.35)	9.16 (4.93)	0.5287
Neutrophils	6435.0 (4699.1)	6336.8 (5227.20)	0.8187
Serum Creatinine (mg/dL)	0.95 (0.79-1.15)	0.91 (0.74-1.16)	0.6451
Ferritin (mg/dl)	656.0 (323.3-1138.7)	553.3 (284.7-959.7)	0.06174*
D-Dimer	629 (401-1049)	821 (454-1376)	0.00071
Protrombin	11.4 (10.8-12.4)	11.4 (10.6-12.5)	0.3642
Fibrinogen	697.0 (556.5-854.5)	672 (482-789)	0.01406
BUN	18.1 (11.6)	20.7 (17.7)	0.0539*
AST	42.6 (30.5-62.5)	41.4 (30.1-64.7)	0.6787
ALT	35.9 (23.7-55-1)	33.50 (23.8-58.2)	0.6321
Albumin	3.8 (3.44-4.01)	3.6 (3.26-3.96)	0.0024
Lactate dehydrogenase	361 (291-466)	374 (278.5-498.5)	0.7565
CK	116 (64-236)	104.5 (55-225.3)	0.1976
Procalcitonin	0.270 (0.06-	0.32 (0.15-1.22)	0.2991

	0.57)		
Symptoms (n)	5 (3-5)	4 (3-5)	0.107
Comorbidities (n)	1 (0-1)	1 (0-2)	0.001
Time hospitalized (days)	6 (3-10)	6 (3-10)	0.995
CT findings			
Epicardial fat (%)	9.3 (7.3-11.7)	10 (8.2-12.2)	0.011
Pericardial fat (%)	185 (61.7)	145 (57.8)	0.407
Subthoracic fat (%)	15 (10-21)	17 (12-24)	0.010
Ground Glass Opacity (%)	297 (99)	248 (98)	0.710
Consolidations (%)	158 (53.6)	136 (54.2)	0.781
GGO + Consolidations (%)	158 (53.6)	136 (54.2)	0.721
Lobules afected 1 2 3	34 (11.3) 82 (32.7) 132 (52.6)	34 (11.3) 125 (41.7) 140 (46.6)	0.178
Hepatic Steatosis (%)	99 (33)	90 (35.9)	0.539
Severity Scores			
NEWS (pts)	8 (6-9)	8 (7-9)	0.733
QSOFA (pts)	1 (1-1)	1 (1-1)	0.329

CURB-65 (pts)	1 (0-2)	1 (0-2)	0.347

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473

474 **Table 2.** Multiple linear regression model to identify determinants of Vitamin D levels in
475 patients with COVID-19.

Model	Parameter	β-coefficient	95%CI	t	P-value
Vitamin D R^2=0.1091	Intercept	3.515	3.177-3.853	20.426	<0.001
	Male Sex	0.168	0.097 -0.238	4.668	<0.001
	Ultrasensitive troponin	-0.033	-0.063 - -0.002	-2.124	0.034
	D-dimer	-0.060	-0.100 - -0.020	-2.951	0.003
	C-reactive protein	0.065	0.026-0.103	3.265	0.001
	Epicardial fat	-0.126	-0.222 - -0.031	-2.599	0.010
	Type 2 diabetes	-0.072	-0.147 – 0.004	-1.871	0.062

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477

478 **Table 3.** Cox proportional risk regression models to predict mortality related to COVID-19
479 using Vitamin D levels adjusted for covariates. Model 1: Unadjusted, Model 2: Adjusted for
480 age, gender, body-mass index (BMI) and C-reactive protein (CRP), Model 3: Model 2
481 adjusted for chronic kidney disease (CKD), epicardial fat and type 2 diabetes (T2D.

Model	Parameter	β-coefficient	HR (95%CI)	P-value
Model 1 c-statistic=0.566	Vitamin D	-0.036	0.965 (0.942-0.988)	0.003
Model 2 c-statistic=0.694	Vitamin D	-0.043	0.938 (0.934-0.983)	0.001
	Age	0.037	1.037 (1.022-1.052)	<0.001
	Male gender	0.787	2.196 (1.373-3.512)	0.001
	BMI	0.038	1.039 (1.005-1.073)	0.024
	CRP	0.004	1.004 (1.002-1.007)	<0.001
Model 3 c-statistic=0.702	Vitamin D	-0.039	0.962 (0.935-0.989)	0.006
	CKD	0.301	0.749 (0.276-2.030)	0.570
	T2D	0.325	1.384 (0.882-2.170)	0.157
	Ultrasensitive cardiac troponin	0.170	1.185 (1.002-1.403)	0.048
	D-dimer	0.003	1.003 (0.804-1.252)	0.980

	Oxygen saturation	-0.026	0.975 (0.960-0.990)	0.001
	Epicardial fat thickness	0.669	1.952 (0.501-7.607)	0.335

482

483

484 **Table 4:** Causal mediation analyses predicting the effect of Vitamin D levels (E) mediated by elevated D-dimer, ultrasensitive cardiac
485 troponins or low SpO2 (M) on severe COVID-19 and mortality (Y), adjusted by gender, age, BMI and epicardial fat.
486 **Abbreviations:** ACME: average causal mediation effects; ADE: average direct effects; SpO2: Oxygen saturation levels.
487

Outcome (Y)	Efector (E)	Mediator (M)	ACME (95%CI)	ADE (95%CI)	Total Effect (95%CI)	Proportion Mediated (95%CI)
COVID-19 Mortality	Vitamin D	D-dimer	-0.035 (-0.069,-0.010)	-0.144 (-0.164, -0.10)	-0.179 (-0.188,-0.050)	19.3% (9.5-77.0%)
		Ultrasentivie cardiac troponins	-0.047 (-0.085, -0.020)	-0.133 (-0.150, -0.020)	-0.181 (-0.183, -0.060)	26.2% (15.0-73.0%)
		SpO2	-0.037 (-0.066, -0.010)	-0.144 (-0.159, -0.030)	-0.181 (-0.186,-0.050)	20.3% (9.7-64.0%)

FIGURE LEGENDS

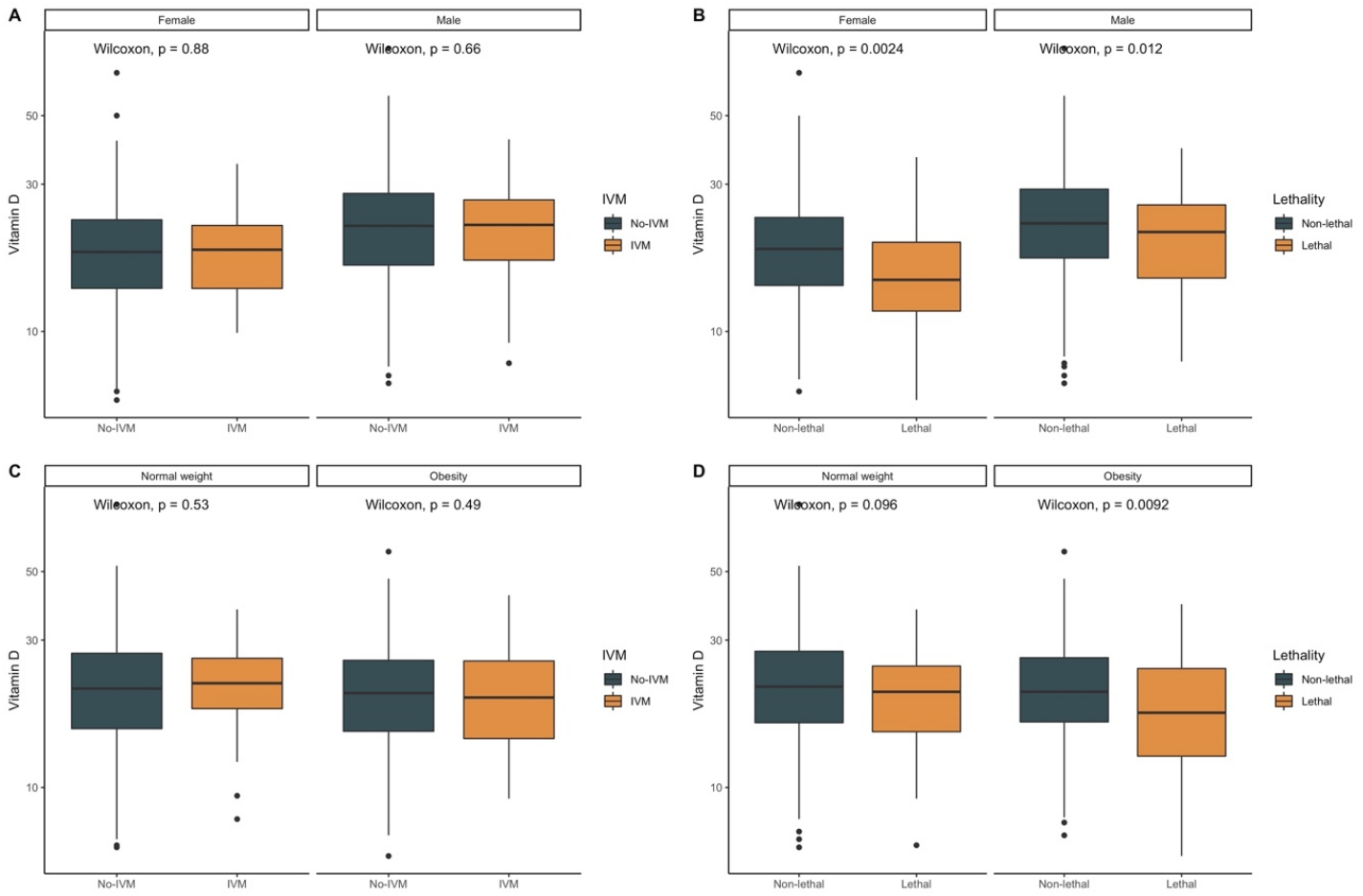

Figure 1. Boxplots comparing Vitamin D levels according to the need for invasive mechanical ventilation (IVM) or lethal COVID-19 stratified by gender (A-B) and by body-mass index categories (C-D).

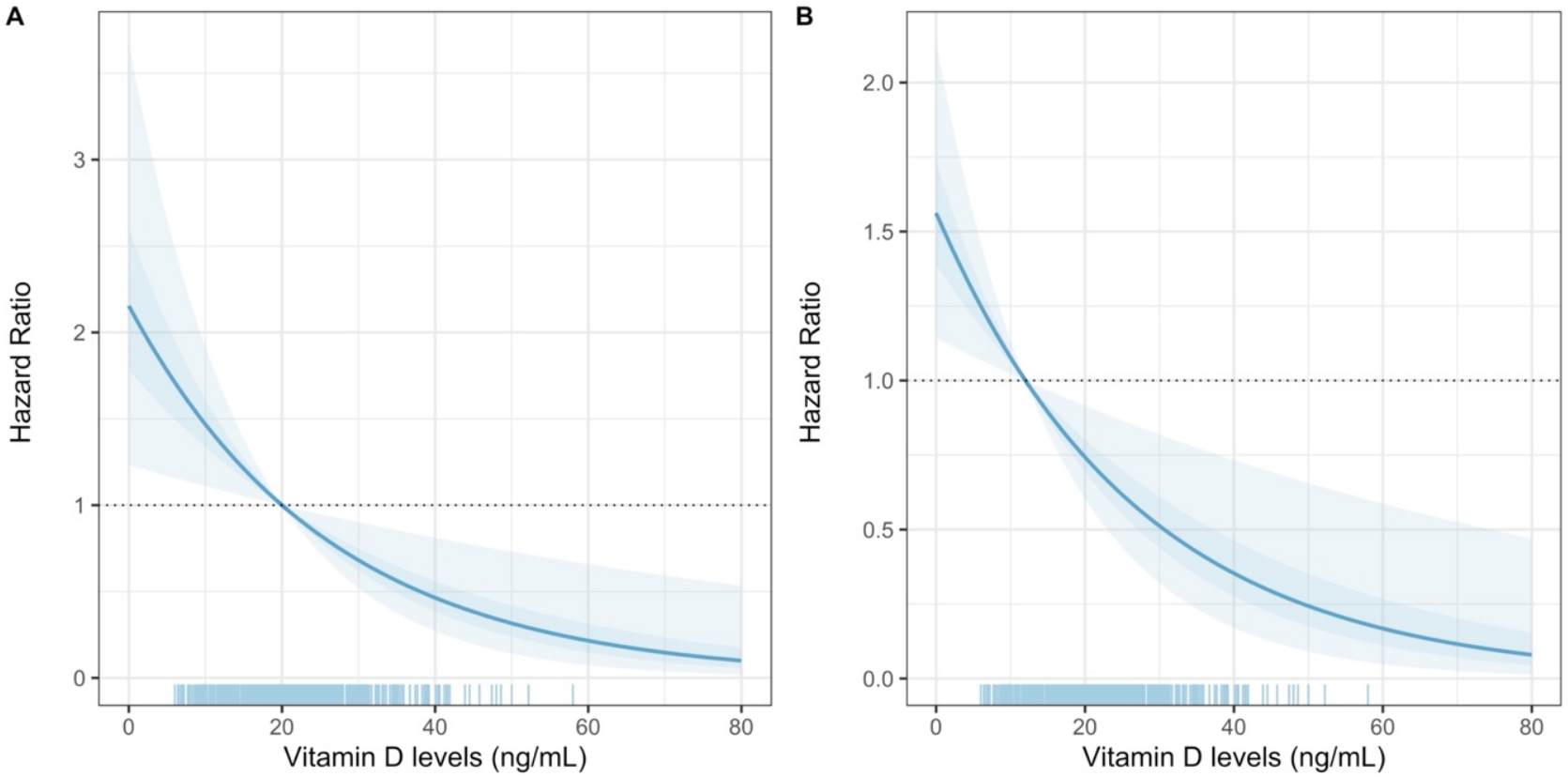

Figure 2. Post-estimation simulation of Vitamin D levels to predict COVID-19 lethality, adjusted for age, sex, BMI, C-reactive protein, epicardial fat, D-dimer, oxygen saturation, T2D, and CKD using Vitamin D cut-offs of <20ng/dL (A) and ≤12ng/mL

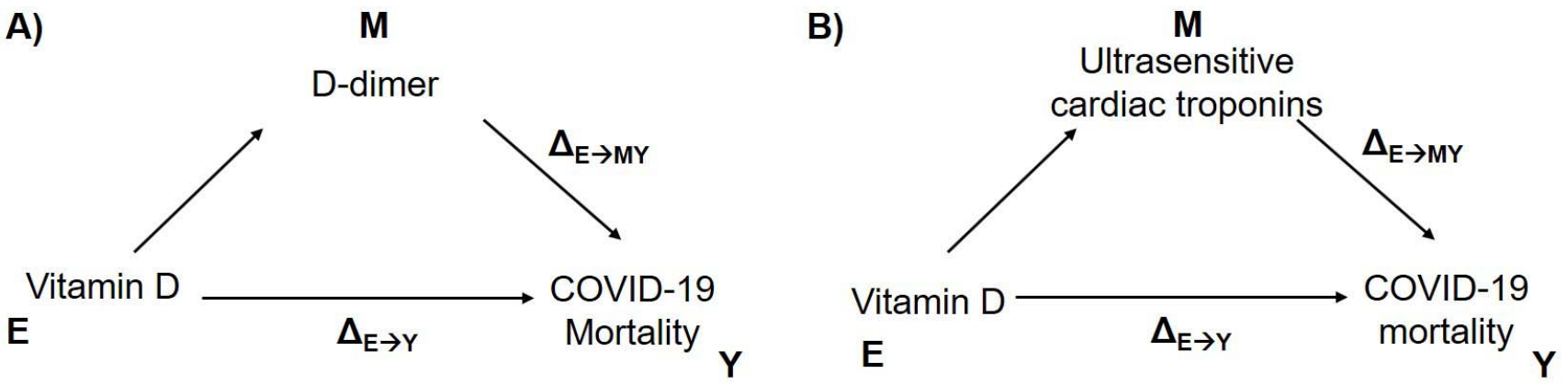

Figure 3. Model-based causal mediation analyses to investigate the role of Vitamin D on COVID-19 mortality mediated by D-dimer (A), ultrasensitive cardiac troponins (B)

Supplementary Material

Table 1. Clinical characteristics, imaging findings and severity scores in patients with COVID-19, comparing cases with and without severely low vitamin D levels (vitamin D deficiency).

Parameter	Vitamin D [>12 ng/mL] (n=494)	Vitamin D [≤12 ng/mL] (n=57)	P-value
Age (years)	51.6 (13.7)	54.9 (13.9)	0.0865
Male Sex (%)	327 (66.2)	28 (49.2)	**0.01626**
Severe outcome (%)	142 (28.7)	24 (42.1)	0.0537
Intubation (%)	83 (16.8)	10 (17.4)	0.981
Letality (%)	95 (19.23)	21 (36.8)	0.0035
Low-Socioeconomic Status (%)	336 (68)	39 (68.3)	0.921
Arterial Hypertension (%)	150 (30.7)	22 (38.6)	0.293
Type 2 diabetes (%)	124 (25.5)	22 (38.6)	0.0512
Time since diagnosis (years)	9.1 (7.14)	11.6 (9.78)	0.297
Obesity (%)	204 (41.9)	25 (44.6)	0.8008
Smoking status (%)	26 (6.25)	1 (1.75)	0.921
CKD (%)	15 (3.10)	3 (5.26)	0.637
CVD (%)	14 (2.88)	2 (3.51)	0.921
Cirrhosis (%)	2 (0.41)	1 (1.75)	0.7256
BMI (kg/m2)	30.0 (5.29)	30.5 (8.76)	0.7227
Respiratory Rate (rpm)	28.4 (11.4)	28.7 (6.9)	0.7946

Heart Rate (bpm)	102.5 (18.3)	98.8 (18.3)	0.1545
Systolic Arterial Pressure (mmHg)	122 (110-132)	120 (110-136)	0.3157
Diastolic Arterial Pressure (mmHg)	75 (70-80)	73 (66-80)	0.242
Oxygen saturation (%)	81.7 (12.2)	78.8 (13.8)	0.1302
C-reactive protein	14.7 (7.8-21.9)	12.8 (6.7-27.9)	0.8042

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Parameter	**Vitamin D [>12 ng/mL] (n=494)**	**Vitamin D [≤12 ng/mL] (n=57)**	**P-value**
Glucose levels (mg/dL)	152 (85.8)	185 (129.7)	0.0689
HbA1c (%)	6.3 (5.9-7.6)	8.0 (6.3-10.6)	0.00312
Triglycerides (mg/dL)	141 (111-183)	164 (118-237)	0.07487
HDL-C (mg/dL)	33 (13.4)	33 (7.78)	0.981
LDL-C (mg/dL)	84.7 (45.8)	80.3 (55.0)	0.887
Total Cholesterol (mg/dL)	147.7 (50.9)	135 (67.7)	0.7396
Hemoglobin	16.1 (12.3)	18.4 (28.9)	0.5584
Platelet Count	232.1 (95.5)	245.8 (108.9)	0.364
Leucocytes	8.96 (4.5)	9.62 (5.7)	0.4071

Neutrophils	6300.9 (4777.395)	7157.1 (6186.3)	0.3161
Serum Creatinine (mg/dL)	0.93 (0.77-1.15)	0.90 (0.75-1.19)	0.6636
Ferritin (mg/dl)	613 (287-1.069)	610 (389-984)	0.9173
D-Dimer	670 (413-1,178)	949 (567-1,803)	0.0025
Protrombin	11.4 (10.8-12.5)	11.4 (10.6-12.5)	0.4982
Fibrinogen	680 (526-820)	705 (464-896)	0.8438
BUN	18.8 (13.9)	23.9 (20.5)	0.0711
AST	41.9 (30.3-62.72)	42.6 (30.3-65.1)	0.6716
ALT	34.9 (23.9-56.8)	33.5 (22.3-53.6)	0.5808
Albumin	3.72 (3.38-4.00)	3.34 (2.93-3.77)	0.001
DHL	358 (283-476)	402 (316-521)	0.06721
CK	110 (61-229)	103 (55-250)	0.6239
Procalcitonina	0.27 (0.07-0.56)	3.045 (3.03-3.88)	0.034

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Parameter	**Vitamin D [>12 ng/mL] (n=494)**	**Vitamin D [≤12 ng/mL] (n=57)**	**P-value**
Symptoms (n)	4 (3-5)	4 (3-5)	0.162

Comorbid conditions (n)	1 (1-2)	2 (1-3)	0.042
Time hospitalized (days)	6 (3-10)	6 (3-8)	0.489
CT findings			
Epicardial fat (%)	9.7 (7.7-11.8)	10.2 (8.0-12.7)	0.105
Pericardial fat (%)	296 (59.4)	34 (59.6)	0.942
Subthoracic fat (%)	16 (11-22)	17 (12-25)	0.225
Ground Glass Opacity (%)	488 (98.8)	57 (100)	0.747
Consolidation (%)	258 (52.2)	36 (63.1)	0.162
GGO + Consolidation (%)	257 (52%)	36 (63.1)	0.154
Lobules afected 1 2 3	65 (13.1) 190 (38.5) 239 (48.4)	7 (12.3) 17 (29.8) 33 (57.8)	0.553
Hepatic Steatosis (%)	172 (34.8)	17 (29.8)	0.545
Severity Scores			
NEWS (pts)	8 (6-9)	8 (7-9)	0.694
QSOFA (pts)	1 (1-2)	1 (1-2)	0.785
CURB-65 (pts)	1 (0-2)	1 (0-2)	0.341