

The Impact of Vitamin D Deficiency on the Severity of Symptoms and Mortality Rate among Adult Patients with Covid-19: A Systematic Review and Meta-Analysis

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Abstract

Introduction: We aimed to study the prevalence of vitamin D deficiency (VDD) in patients with COVID-19 infection and evaluate the impact of vitamin D levels on the severity of symptoms and the case fatality rate. **Evidence Acquisition:** A comprehensive literature search was performed up to December 20, 2020, using the following databases: MEDLINE, PubMed, EMBASE, SCOPUS, Web of Science, and preprint databases (BioRxiv and MedRxiv). Any individual observational study related to the prevalence and impact of vitamin D deficiency/insufficiency (VDD/VDI) on the severity of COVID-19 symptoms and mortality rates was included. No language restrictions were applied, and both published and non-published studies were included. **Evidence Synthesis:** Two of the authors independently performed the literature search and assessed the eligibility of studies. The quality of studies included was assessed using the Newcastle–Ottawa Scale. Data were analyzed using the Review Manager Software (version 5) and Comprehensive Meta-analysis Software (version 3). A total of 43 studies were included with a sample size of 254,963 patients with COVID-19. Pooled analysis showed a higher prevalence of VDD and VDI in patients with COVID-19 (59.0% and 40.1%, respectively). Moreover, a significant association was noticed between vitamin D levels and severity of symptoms (odds ratio [OR] = 3.38, 95% confidence interval [CI]: 1.94–5.87, $P < 0.0001$), as well as the case fatality rate (OR = 2.30, 95% CI: 1.47–3.59, $P < 0.00001$). **Conclusions:** VDD is highly prevalent in patients with COVID-19 infection. Lower vitamin D levels correlate with disease severity and poor prognosis although most of the data have been derived from moderate-quality observational studies.

Keywords: Vitamin D; 25-hydroxyvitamin D; 25-OH vitamin D; coronavirus; COVID-19

INTRODUCTION

There has been renewed interest in the possible role of vitamin D in the prevention and treatment of acute respiratory infection in light of the ongoing COVID-19 pandemic. 1,25-hydroxyvitamin D, the active metabolite of vitamin D, is produced in the kidneys via hydroxylation of the inactive form, 25-hydroxyvitamin D.^[1] 1,25-hydroxyvitamin D exerts its actions by binding to vitamin D receptors (VDRs) that are nuclear, ligand-dependent transcription factors involved in the regulation of over 900 genes essential for maintaining a wide array of physiological functions.^[1]

Active metabolites of vitamin D have been shown to stimulate innate as well as acquired immune responses.^[2,3] Evidence derived from experimental studies suggests 1,25-hydroxyvitamin D levels have an

inverse correlation with pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF α), which are the key inflammatory markers linked to the development of adult respiratory distress syndrome (ARDS).^[4,5] The vitamin D-induced upregulation of cathelicidins and β defensins, which are potent antimicrobial peptides, is considered to be an important factor in mitigating the severity of acute respiratory infections.^[6]

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The biological plausibility of the immunomodulatory role of vitamin D can be extrapolated as one of the important causal factors linked to an increased incidence of acute respiratory tract infections in geographic regions with vitamin D deficiency (VDD) or vitamin D insufficiency (VDI), especially during the winter months. There is also a higher prevalence rate of VDD in the elderly, obese, darker skin ethnicities, and hospitalized patients,^[7-9] with these factors emerging as strong predictors of adverse clinical outcomes in patients with COVID-19.

A number of epidemiological and observational studies have reported an inverse correlation between vitamin D levels and COVID-19 severity and case-fatality rates. Besides, the seasonal dependence of the COVID-19 pandemic indicates an inverse correlation with solar ultraviolet dose and thereby vitamin D production.^[10,11] One of the ecological studies has underlined higher rates of COVID-19 cases and deaths in countries such as Italy and Spain, which have an overall lower mean concentration of 25-hydroxyvitamin D. In contrast, countries, such as Finland and Norway, which have implemented a vitamin D food fortification policy, have reported a significantly lower number of COVID-19 cases and deaths.^[12]

An analysis of 186 patients with COVID-19 confirmed an inverse correlation between vitamin D levels and disease severity based on radiological findings.^[13] A similar finding was confirmed by another large retrospective population-based study that substantiated a 1.6% lower risk for SARS-CoV-2 positivity with each increment of 1 ng/mL of vitamin D level.^[14] In contrast, a study based on participants recruited from the UK Biobank did not observe any association between VDD and the risk of developing COVID-19 in a multivariate analysis.^[15,16] However, these findings might be influenced by the fact that the COVID-19 polymerase chain reaction (PCR) test results were available only in 1,474 of the 348,598 individuals recruited, resulting in underestimation of the true prevalence of COVID-19 in this cohort.^[15] In addition, the results of this study need to be interpreted cautiously, as the baseline vitamin D levels of biobank participants were measured over a decade ago (2006–2010).^[15,16]

A meta-analysis of 11,321 participants supported the benefit of vitamin D supplements in preventing acute respiratory tract infections, although uncertainty exists about the generalization of these observations to patients with COVID-19 as well.^[17] Overall, it is biologically plausible that active metabolites of vitamin D levels have an impact on COVID-19 disease severity although the evidence that has emerged so far from observational studies has been conflicting and of moderate quality.

We have carried out this systematic review and meta-analysis to ascertain the prevalence of VDD in patients with COVID-19. We have also evaluated whether there is any correlation between vitamin D levels with disease severity and case-fatality rate in adult patients with COVID-19.

Evidence acquisition

Study design

A systematic review and meta-analysis of observational studies (cross-sectional, cohort, case-control studies, and case series) related to the prevalence of VDD/VDI in patients with COVID-19 and the association of low vitamin D levels with disease severity and mortality rates in patients with COVID-19 were conducted. The PRISMA guidelines were followed in this study.^[18] This systematic review is registered at PROSPERO NIH; registration number is CRD42020196502.

Search strategy

A literature search was conducted using the following databases: MEDLINE, PubMed, EMBASE, SCOPUS, Web of Science, and preprint databases (BioRxiv and MedRxiv). Moreover, manual searching for references that were cited in the retrieved articles was performed and the authors were contacted if deemed necessary. The literature search was carried out until December 20, 2020. Search terms included “Vitamin D” or “25-hydroxyvitamin D” or “25-OH vitamin D” and “coronavirus” or “SARS-CoV-2” or “COVID-19.”

Inclusion and exclusion criteria

Inclusion criteria included i) any individual observational studies (prospective or retrospective) related to the prevalence and impact of VDD/VDI on the symptoms severity of COVID-19 and mortality rates, ii) adult human studies, and iii) published and unpublished. Notably, no language restrictions were applied in this review.

Exclusion criteria included i) *in vitro* studies, ii) experimental studies, iii) ecological studies, review articles, case reports, and editorial articles unless they contain information relevant to the unpublished studies, and iv) studies performed on the pediatric population.

Primary outcomes

The three primary outcomes in the current review include i) the prevalence of VDD/VDI in patients with COVID-19, measured by determining the number of events in three sub-groups: subgroup A: VDD <20 ng/mL, subgroup B: VDI: 20–30 ng/mL, and subgroup C: normal vitamin D >30 ng/mL) in patients with COVID-19; ii) the severity of COVID-19, which is defined by the number of patients with COVID-19 (3 subgroups: A: VDD, B: VDI, and C: normal range) who required admission to intensive care units (ICU) and/or required invasive oxygen therapy or ventilatory support or developed acute respiratory distress syndrome (ARDS) or severe radiological changes; and iii) the case-fatality rate, measured by dividing the number of deaths that occurred in recruited participants by the total sample size.

Data extraction

Two of the authors independently performed the literature search (title and abstract) and assessed the eligibility of studies. Studies that fulfilled the inclusion criteria were included in the current systematic review. Any disagreements between the assessments were resolved by consensus.

The following data were retrieved from the eligible studies: author names and year of publication, country, study design, sample size, the mean age of recruited subjects, the cut-off value of VDD/VDI, odds ratio (OR) for dichotomous data, and mean for continuous variables, confidence intervals, *P* value, and summary of findings. Notably, any adjustment for confounders (i.e., comorbidities) was reported.

Quality assessment

Two of the authors independently evaluated the quality of included studies using the Newcastle–Ottawa Scale (NOS) for case–control and cohort studies.^[19] Moreover, the modified NOS was used to assess the quality of cross-sectional studies.^[20] NOS is a reliable and validated tool that assesses three essential parameters of observational studies: selection, comparability, and outcome ascertainment.^[19] It is scored from 0 to 9 for case–control and cohort studies and 0 to 10 for cross-sectional studies. Generally, a score of less than 5 indicates a high risk of bias. Any disagreement between the authors was resolved by consensus.

Evidence synthesis

Statistical analysis

Data from the meta-analysis were analyzed using the Review Manager Software (version 5) and Comprehensive Meta-analysis Software (version 3). Pooled data were presented as the events rate for the prevalence of VDD/VDI in patients with COVID-19 and the OR was reported for the association between VDD/VDI and disease severity and mortality. Findings that were reported as the median and interquartile range in some of the included studies were converted to the mean and standard deviation and another pooled analysis of mean vitamin D level (ng/mL) and severity of symptoms of COVID-19 was produced.^[21] A *P* value of less than 0.05 was considered statistically significant. Sensitivity analysis was performed to evaluate the effects of individual studies on overall pooled results and heterogeneity. Furthermore, publication bias was assessed using the funnel plot and Egger's regression intercept.

Heterogeneity between studies was assessed using the *I*² statistics. A value of >25% indicates considerable heterogeneity between studies. The random-effects model was used in the current analysis in view of a wide range of studies included and we intended to generalize the findings beyond the current sample.^[22]

Ethical approval

The work was completed in partial fulfilment of an MSc in endocrinology from the University of South Wales, UK

RESULTS

Following a thorough search in the aforementioned databases, 7,106 references were initially retrieved, of which 63 studies were eligible after removing the duplicates and reviewing the titles and abstracts. Forty-three studies were included in the systematic review after full-text assessment (39 were

published studies and 4 studies were at a preprint stage.^[23–26] Figure 1 depicts the PRISMA flow diagram of study selection. The sample size of the studies included ranged from 10 to 191,779, with 254,963 participants with COVID-19. For the meta-analysis, studies conducted in the UK Biobank^[15,16,23–24,27] were excluded because vitamin D testing was performed a decade ago. Additionally, other studies that included patients with VDD who were tested during the preceding 5 to 10 years prior to COVID-19 diagnosis were excluded from the meta-analysis.^[28,29] Twenty-nine studies addressed the prevalence of VDD/VDI in patients with COVID-19, whereas 17 studies identified the association between VDD/VDI and severity of symptoms and mortality rate. Table 1 summarizes the characteristics of studies included in the systematic review.

Prevalence of VDD/VDI in patients with COVID-19

There were different cut-off values for VDD and VDI among the recruited studies. A cut-off value <20 ng/mL was used to define vitamin D deficiency in the majority of studies,^[13,14,31,33,34,36,38,39,41,43,44,46–48,53,54,57,58,61] whereas vitamin D deficiency was defined in five studies^[36,39,46,48,61] if the level was <30 nmol/L, which is equivalent to 12 ng/mL and <25 nmol/L in another study.^[56] Moreover, Lau *et al.* (2020),^[25] Maghbooli *et al.* (2020),^[45] Ferrari *et al.* (2020),^[51] and Abdollahi *et al.* (2020)^[55] used a cut-off value <30 ng/mL to define VDI, which therefore also included those with VDD. For the sake of this meta-analysis, we included these studies in the insufficiency group. Furthermore, Radujkovic *et al.* (2020),^[39] Panagiotou *et al.* (2020),^[42] and Pizinni (2020)^[32] defined VDI as a level <50 nmol/L, which is equivalent to <20 ng/mL. For consistency with other studies, we categorized this cut-off value as VDD. In addition, the study conducted by D'Avolio *et al.* (2020)^[30] did not report any event rates of VDD/VDI among patients with COVID-19, and data were presented by inter-quartile range. However, the author was contacted via email and the events rate was provided. Also, Abrishami *et al.*^[35] defined VDD if the level is less than 25 ng/mL. Lastly, the cut-off value to define VDD was not mentioned in the two studies and therefore excluded from the meta-analysis.^[28,50] With the exception of a study conducted by Meltzer *et al.* (2020),^[31] no studies accounted for the possibility of vitamin D treatment initiation or adjustment after testing in defining VDD/VDI. Meltzer *et al.* (2020)^[31] labeled patients as likely to be vitamin D deficient if they had low vitamin D (<20 ng/mL) and there was no increase in the dose of vitamin D treatment after testing. Although observational studies on the pediatric population were excluded from the current analysis, recruited individuals in a population study by Merzon *et al.* (2020)^[40] ranged in age from 2 months to 103 years. However, only a minority (6%) were aged between 0 and 20 years and we believe that such a low percentage will not affect our findings. Similarly, 4% of recruited participants were under the age of 18 years in another study.^[28] Furthermore, SARS-CoV-2 PCR testing was performed to confirm COVID-19 in the majority of the studies with the exception of one study that identified the association

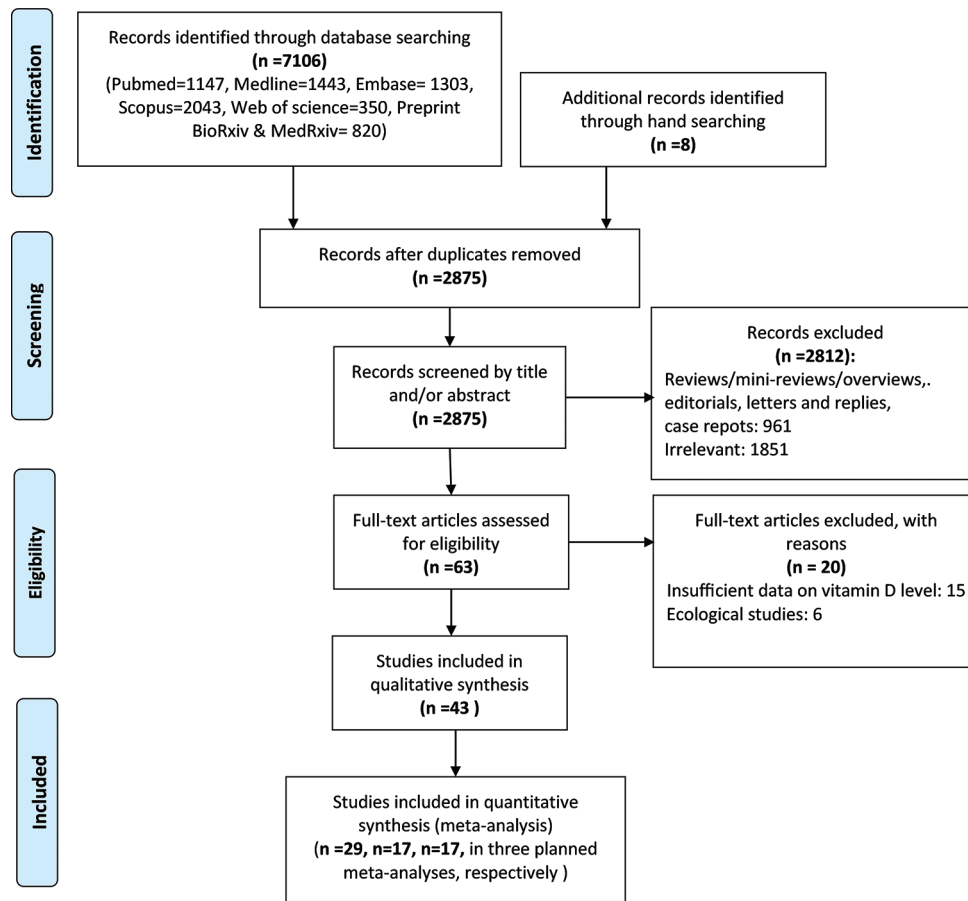


Figure 1: PRISMA flow diagram showing the study selection

between VDD and positive seroconversion of COVID-19.^[61] For the sake of consistency in the method used to diagnose COVID-19, this study was excluded from our meta-analysis. The pooled analysis underlined a higher prevalence of VDD and VDI in patients with COVID-19 (59.0% and 40.1%, respectively). Figure 2 shows the forest plot of the events rate of VDD/VDI in patients with COVID-19.

VDD/VDI and the severity of symptoms (outcomes) in patients with COVID-19

Two analyses were carried out to determine the association between VDD/VDI and severe outcomes in patients with COVID-19. Subjects were divided into the following groups: 1a) COVID-19 and VDD (ordinary outcome/mild), 1b) COVID-19 and VDD (severe outcome), 2a) COVID-19 and VDI (ordinary outcome/mild), 2b) COVID-19 and VDI (severe outcome).

Considerable differences were found when defining the criteria of the severity of COVID-19 within the included studies. As mentioned above, any patients with COVID-19 who developed ARDS, severe radiological changes, or required ICU admission or invasive oxygen therapy or ventilator support, were considered to have severe COVID-19. Smet *et al.* (2020)^[13] determined the association between VDD and severity of COVID-19 using radiological changes on computed

tomography (CT) chest. Patients were sub-categorized into three groups, with the third group indicating a more progressive and advanced stage. In our meta-analysis, group three was considered a severe outcome, whereas group one was included under the ordinary/mild category. Similarly, Abrishami *et al.* (2020)^[35] used the radiological worsening of the lungs as an indicator of severe outcome. Also, in studies conducted by Carpagnano *et al.* (2020)^[33] and Kerget *et al.* (2020),^[52] patients who developed ARDS or were admitted to an ICU, respectively, were analyzed under severe outcomes. In a study conducted by Baktash *et al.* (2020),^[48] patients who required ventilator support and a high dependency unit admission were included in the severe category. Finally, in a study conducted by Karonova *et al.*,^[37] the authors were contacted for more information regarding the criteria used to define severe cases of COVID-19 and it was stated that severe cases were those who were admitted to the ICU. In accordance with the definition of severe outcome in the present study and given the inadequate information about the severity of COVID-19 among hospitalized patients in a population-based study conducted by Merzon *et al.* (2020),^[40] the hospitalization rate was not considered as a severe outcome and not included in the current analysis. Besides, only 31.06% of recruited subjects had SARS-CoV-2 PCR testing in a study conducted by Maghbooli *et al.* (2020),^[45] which might have affected the

Table 1: Characteristics of studies included in the systematic review

Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Hastie <i>et al.</i> ^[15]	UK Biobank	Retrospective cross-sectional analysis	348,598 (449 with COVID-19, 348,149 without COVID-19)	Age range: 37-73 years	-Diabetes: 18874 (5.4%), obesity: 82,928 (23.8%)	VDD: 25(OH) D <25 nmol/L. Time: 2006-2010. Method: immunoassay (Diasorin)	0.92 (0.71-1.21), P=0.564 (multivariate analysis)	No significant association between vitamin D concentration and COVID-19 risk	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)
Hastie <i>et al.</i> ^[16]		Retrospective cross sectional analysis	341,484 participants (656 with COVID-19, 203 deaths)	Age range: 37-73 years		VDD: 25(OH) D <25 nmol/L. Time: 2006-2010. Method: immunoassay (Diasorin)	Vitamin D level and mortality rate: univariate analysis: HR 0.92; 95% CI 0.86-0.98; P=0.016. Multivariate analysis: HR 0.98; 95% CI=0.91-1.06; P=0.696	No significant association between VD concentration and COVID-19 severity and mortality rate	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)
Darling <i>et al.</i> ^[23] Preprint		Retrospective analysis	1303 (580 with COVID-19, 723 without COVID-19)	COVID-19 positive: 57.5 (±8.7). COVID-19 negative: 57.9 (±8.7)	Obesity: n=458 (36.2%)	* Used quartile rather than individual vitamin D level. Q1: bottom 25%, Q2, Q3, Q4: top 25%. Time: 2006-2010. Method: immunoassay (Diasorin)	COVID-19 and VD level: Q1: OR=1. Q2: OR=0.93, CI=0.67-1.28, P=0.65. Q3: OR=1.03, CI=0.74-1.44, P=0.84. Q4: OR=1.11, CI=0.79-1.56, P=0.54	No significant association between VD level and COVID-19 risk.	Selection: *, comparability: **, outcome: **, Total: 6/10 (moderate quality)
Li <i>et al.</i> ^[24] Preprint		Retrospective analysis	1,746 patients with COVID-19, 399 deaths. Controls=494,034	COVID-19 cases: 68.76 (±9.18), Controls: 68.44 (±8.11)	BMI: Cases: 27.53 (±4.88) kg/m ² , control: 27.42 (±4.79) kg/m ²	VDD: 25(OH) D <25 nmol/L. Time: 2006-2010. Method: immunoassay (Diasorin)	VD level and mortality rate: 1.00 (0.99-1.01), P=0.687. (Multivariate analysis)	No significant association between VD level and COVID-19 risk and severity of symptoms	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)
Raisi-Estabragh <i>et al.</i> ^[27]		Retrospective analysis	4510 participants (COVID-19 positive=1326, COVID-19 negative=3184)	COVID-19 positive: 68.11 (±9.23). COVID-19 negative: 68.91 (±8.72)	COVID-19 positive: BMI=28.04 [± 6.47] kg/m ² , diabetes: 217 (16.4%), hypertension: 624 (47.1%), COVID-19 negative: BMI=27.41 [± 6.37], diabetes: 449 (14.1%), hypertension: 1,457 (45.8%)	VDD: 25(OH) D <25 nmol/L. Time: 2006-2010. Method: immunoassay (Diasorin)	COVID-19 and VD level: OR 1.00 [1.00, 1.00]. P=0.7185. (Multivariate analysis)	Risk of COVID-19 among men and BAME was not explained by vitamin D level.	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)

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Table 1: Contd...

Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Kaufman <i>et al.</i> ^[14]	Columbia (USA)	Retrospective population based analysis	191779 with COVID-19	median: 54 years (40.4-64.7)	Not mentioned.	Normal: vit D \geq 30 ng/mL. Insufficiency: 30 > Vit. D >20 ng/mL. Deficiency: Vit. D <20 ng/mL. Time: within the preceded one year. Method: chemiluminescent immunoassay (DiaSorin) or liquid chromatography/tandem mass spectrometry.	Vitamin D level and SARS-CoV-2 positivity: adjusted OR 0.984 per ng/mL increment, 95% CI: 0.983-0.986; $P < 0.001$. The result was adjusted to race/ethnicity, gender, and latitude. Importantly, vitamin D level was adjusted for seasonality.	Strong inverse relationships between vitamin D level and positivity for SARS-CoV-2.	Selection: ***, comparability: *, outcome: ***, Total: 7/10 (moderate quality)
Israel <i>et al.</i> ^[29]	Israel	large observational population study	52405 with COVID-19, 524,050 matched control. Test: RT-PCR	Median: cases (32.00, 18.00-50.00), controls (32.00, 18.00-50.00)	Adjusted Clinical Group ACG comorbidity score (median [IQR]): Cases (0.44, 0.17-1.67), controls (0.44, 0.17-1.67).	VDD <30 nmol/L (equal to 12 ng/mL). Time: 2010-2019. Method: not documented	Vitamin D level <30 nmol/L and COVID-19: OR 1.27 (95% CI: 1.199-1.355, P value=0.000). Vitamin D level 30-50 nmol/L and COVID-19: OR 1.183 (95% CI: 1.118-1.251, $P=0.000$). (Multivariate analysis)	Significant association between VDD and COVID-19 risk.	Selection: **, comparability: **, outcome: ***, Total: 7/10 (moderate quality)
Katz <i>et al.</i> ^[28]	Florida (USA)	Cross-sectional study	887 with COVID-19	35 (4%) were less than 18 years.	No demographic statistics	Not documented. Time: during the preceded five years. Method: not documented	VDD and COVID-19 positivity: OR=5.155, 95%CI: 3.974-6.688, $P < 0.001$ (adjusted for age)	Significant association between VDD and COVID-19 risk	Selection: **, comparability: **, outcome: ***, Total: 7/10 (moderate quality)
D'Avolio. <i>et al.</i> ^[30]	Switzerland	Retrospective cohort study	107 (27 with COVID-19, 80 without COVID-19). Test: PCR	median: 73 years (IQR 63-81)		The level of vitamin D was illustrated by median and interquartile range IQR. Time: within 7 weeks of PCR result. Method: liquid chromatography coupled with a tandem mass spectrometry (LC-MS/MS)	VD level and COVID-19 risk: $P=0.004$, No OR CI were documented. Data were displayed as (25 th -75 th percentiles, interquartile range (IQR)		

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Table 1: Contd...

Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Smet <i>et al.</i> ^[13]	Belgium	Retrospective observational study	2903 (186 with COVID-19, 2717 diseased control). Test: PCR	Median: 69 (52-80)	Chronic lung disease: 28 (15.1%), coronary artery disease: 110 (59.1%), diabetes: 26 (14.0%)	VDD: 25(OH) D <20 ng/mL. Time: on admission. Method: liquid chromatography- tandem mass spectrometry	VDD in cases versus control: 109 (58.6%) vs 1227 (45.2%), <i>P</i> <0.05. VDD rates increased from 52.2% in stage 1 CT to 62.4% in stage 3 (<i>P</i> <0.05). Confounding factors were dealt with by stratification. - VDD and mortality rate: adjusted OR: 1.09 (CI: 1.03-1.24), <i>P</i> =0.0014. (Multivariate analysis)	A significant association between VDD and COVID-19 severity and mortality rate	Selection: ***, comparability: *, outcome: **, Total: 5/9 (moderate quality)
Lau <i>et al.</i> ^[25] Preprint	New Orleans	Retrospective cross sectional analysis	20 patients (13 ICU patients, 7 floor patients)	65.2±16.2 years	Hypertension: 15 (75.0%), diabetes: 7 (35.0%)	VDI: 25(OH) D of <30 ng/mL. Time: on admission. Method: immunoassay	ICU: 11 (84.6%) had VDI. Mean value=19.2±10.8 ng/mL. Floor: 4 (57.1%) had VDI. Mean value=29.8±13.3 ng/mL. <i>P</i> =0.29	Vitamin D insufficiency is prevalent in patients with severe COVID-19	Selection: *, comparability: *, outcome: ***, Total: 5/10 (moderate quality)
Meltzer <i>et al.</i> ^[31]	Chicago, USA	Retrospective cohort study	489 patients were tested for COVID-19 and vitamin D. 71 patients (15%) were positive for COVID-19. 124 (25%) patients were likely deficient.	49.2 (18.4)	Hypertension: 261 (53%), diabetes: 137 (28%), chronic pulmonary disease: 117 (24%), chronic kidney disease: 116 (24%), depression: 119 (24%).	25(OH) D of <20 ng/mL. When combined with the treatment, the following definitions were used: -Likely Deficient: VDD and treatment not increased. -Likely sufficient: VD sufficient and treatment not decreased. Time: within one year. Method: not documented	Likely VDD: RR=1.77 (1.12-2.81), <i>P</i> <0.02 (multivariate analysis)	Significant association between VDD and COVID-19 risk	Selection: ***, comparability: *, outcome: **, Total: 6/9 (moderate quality)

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Table 1: Contd...

Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Pizzini et al. ^[32]	Austria	A prospective multicenter observational study	287 (59%) patients were likely sufficient. 78 (15%) patients were uncertain. Test: PCR 109 patients with COVID-19 (22 outpatient +87 hospitalised). Test: RT-PCR	Median: 58±14	Cardiovascular disease: 44 (40%), endocrine diseases: 49 (45%), hypertension: 32 (29%), hypercholesterolemia: 24 (22%), type 2 diabetes mellitus: 20 (18%), Chronic kidney disease: 7 (6%)	VDD <30 nmol/L (equal to 12 ng/mL), VDI 30-50 nmol/L (equal to 12-20 ng/mL). Time: 8 weeks post onset of COVID-19. Method: not documented	Mean vitamin D level in mild COVID-19 versus severe COVID-19 patients with COVID-19 vs. 50±24 nmol/L, P=0.116).	Low vitamin D level is prevalent among patients with COVID-19 but not significantly associated with poor outcomes.	Selection: **, comparability: **, outcome: ***, Total: 5/10 (moderate quality)
Carpagnano et al. ^[33]	Italy	Retrospective observational analysis	42 patients with acute respiratory failure due to COVID-19 were admitted to the respiratory intermediate care unit (RICU), not requiring intubation or intensive ventilation. Test: RT-PCR	65±13	BMI: 28.5 (± 5) kg/m ² . Hypertension: 26 (61.9%), cardiovascular disease: 16 (38.1%), chronic kidney disease: 16 (38.1%), diabetes: 11 (26.2%)	Normal: vit D ≥30 ng/mL. Insufficiency: 30 >Vit. D >20 ng/mL. Moderate deficiency: 20 > Vit. D >10. Severe deficiency: <10 ng/mL. Time: within 12 hours of admission. Method: chemiluminescence immunoassay	Prevalence of hypovitaminosis (vit D <30 ng/mL) =34/42 (81%). -No OR, CI or P value was mentioned.	No significant association between VDD and severity of COVID-19. However, a significantly higher mortality rate among patients with severe VDD.	Selection: **, comparability: **, outcome: ***, Total: 6/10 (moderate quality)
Im et al. ^[34]	South Korea,	Prospective analysis	200 patients (50 with COVID-19 and 150 without COVID-19. Test: PCR	COVID-19 positive: 52.2±20.7. Control: 52.4±20.2	Not documented	VDD: 25(OH) D of <20 ng/dl. Time: within 7 days of admission. Method: liquid chromatography-tandem mass spectrometry	Prevalence of VDD: COVID-19 cases versus control: 38/50 (76.0%) vs 65/150 (43.3%). P=0.003. Prevalence of VDD in patients with COVID-19 requiring mechanical ventilation: 80%	Vitamin D insufficiency is prevalent in patients with severe COVID-19	Selection: **, comparability: **, outcome: ***, Total: 5/10 (moderate quality)

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Table 1: Contd...

Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Abrishami et al. ^[35]	Iran	Retrospective analysis	73 patients with COVID-19. Test: RT-PCR	55.18±14.98 years	Diabetes: 11 (15.1%), hypertension: 18 (24.7%), ischemic heart disease: 13 (17.8%), chronic kidney disease: 16 (21.9%), asthma/COPD: 7 (9.6%),	VDD: 25(OH) D <25 ng/mL. Time: within 3 days of initial CT scan. Method: immunoassay	Vitamin D level and severe lung involvement in COVID-19: (OR=0.96, 95% CI 0.93-0.98, P=0.04). Mean vitamin D level in survived versus deceased: 38.41±18.51 versus 13.83±12.53, P<0.001. COVID-19: VDD and mortality rate: OR 6.84 (1.55-30.19), P=0.01. (Multivariate analysis)	A significant association between vitamin D deficiency and severity of COVID-19 and mortality rate.	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)
Faul et al. ^[36]	Ireland	Retrospective study	33 patients with COVID-19 related pneumonia. Test: RT-PCR	ARDS: 60±15, No ARDS: 56±14	None had cancer, diabetes, cardiovascular disease or received immunosuppressive therapy	VDD: 25(OH) <30 nmol/L (equal to 12 ng/mL). Time: at baseline. Method: not documented.	Hazard ratio for intubation in patients with VDD: 3.19 (1.05-9.7), P=0.03	Low vitamin D level is associated with the severity of symptoms in patients with COVID-19.	Selection: -, comparability: *, outcome: ***, Total: 4/10 (low quality)
Karonova et al. ^[37]	Russia	Retrospective analysis	80 hospitalized patients with COVID-19 pneumonia.	53.2±15.7	Obesity: 18 (22.5%), ischemic heart disease: 18 (22.5%), diabetes: 12 (15%)	VDI: 30 > Vit. D >20 ng/mL. VDD: Vit. D <20 ng/mL. Time: baseline. Method: chemiluminescence immunoassay	VDD and severity of symptoms (OR 5.0, [95% CI: 1.06-23.66]. VDD and fatality (OR 5.87, [95% CI: 0.72-48.04]. Obesity effect was adjusted.	A positive association between VDD and severity of symptoms of COVID-19 and mortality rate.	Selection: **, comparability: *, outcome: **, Total: 6/10 (moderate quality)
Tort et al. ^[38]	Mexico	Cross sectional study	172 hospitalised patients with COVID-19	51.44±14.21	Not documented	NVD: vit D ≥30 ng/mL. VDI: 30 > Vit. D >20 ng/mL. VDD: Vit. D <20 ng/mL.	VDD and deaths: 27 (77.1%), VDI and death: 8 (22.9%), normal vitamin D and death: 0 (0.0%), P value=0.256. Patients with vitamin D level of less than 8 ng/mL were 3.69 times risk of dying (P=0.001, CI: 1.62-8.37).	VDD is prevalent in patients hospitalized with COVID-19. A significant association between very low vitamin D levels (<8 ng/mL) and fatality from COVID-19.	Selection: **, comparability: **, outcome: **, Total: 5/10 (moderate quality)

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Table 1: Contd...

Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Radujkovic <i>et al.</i> ^[39]	Germany	Prospective observational study	185 patients with COVID-19 (93 hospitalized and 92 outpatients. Test: RT-PCR	Median 60 (49-70)	Cardiovascular disease: 58 (31%), diabetes: 19 (10%), Chronic kidney disease: 8 (4%), chronic lung disease: 15 (8%), active or history of malignancy: 17 (9%)	VDD: <12 ng/mL. VDI: <20 ng/mL. Time: at admission. Method: immunoassay	VDD was associated with a higher risk of severe outcomes (invasive mechanical ventilation/death and death (HR 6.12 and 14.73, respectively) (multivariate analysis)	A significant association between VDD and the severity of COVID-19 and mortality rate.	Selection: **, comparability: **, outcome: **, Total: 8/10 (high quality)
Merzon <i>et al.</i> ^[40]	Israel	Population based retrospective analysis	7,807 individuals (782 were tested positive for COVID-19, 7,025 were tested negative for COVID-19). Test: 2019-nCoV Assay (Allplex™)	COVID-19 positive: 35.58. COVID-19 negative: 47.35	COVID-19 positive: Obesity: 235 (30.05%), diabetes: 154 (19.69%), hypertension: 174 (22.25%), cardiovascular disease: 78 (9.97%), chronic lung disorder: 66 (8.44%). COVID-19 negative: Obesity: 1,900 (27.05%), diabetes: 1,578 (22.46%), hypertension: 1,962 (27.93%), cardiovascular disease: 1,172 (16.68%), chronic lung disorder: 935 (13.31%).	NVD: vit D ≥30 ng/mL. VDI: 30>Vit. D >20 ng/mL. VDD: Vit. D <20 ng/mL. Time: within 2 months prior to COVID-19 onset. (author was contacted) Method: chemiluminescence assay (DiaSorin)	low vitamin D (<30 ng/mL) and COVID-19: OR 1.50 (95 CI: 1.13-1.98), P=0.001 -Low vitamin D (<30 ng/mL) and hospitalization rate in patients with COVID-19: OR 1.95 (95 CI: 0.99-4.78), P=0.056 (Multivariate analysis)	Low vitamin D level was an independent risk factor for COVID-9 infection.	Selection: *, comparability: **, outcome: **, Total: 6/10 (moderate quality)
Macaya <i>et al.</i> ^[41]	Spain	Retrospective analysis	80 patients with COVID-19. Test: RT-PCR	Severe COVID-19: 75 years (66-84). Non-severe COVID-19: 63 years (50-72)	Hypertension: 50 (62.5%), diabetes mellitus: 32 (40%), cardiac disease: 19 (23.8%), advanced chronic kidney disease: 26 (32.5%), obesity: 23 (28.8%), smoking: 13 (16.3%)	VDD: 25(OH) D of <20 ng/mL. Time: on admission or within 3 months prior to COVID-19 onset. Method: chemiluminescent immunoassay	VDD in severe versus non-severe COVID-19: 20 (65%) versus 24 (49%), P value=0.236. Multivariate analysis: the OR for VDD was 3.2 (95% CI: 0.9-11.4), P=0.070.	A significant association between VDD and severe outcomes in patients with COVID-19 who were under 67 years of age. No significant association was found in those who were older than 67 years old.	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)

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Table 1: Contd...

Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Panagiotou et al. ^[42]	UK	Cross-sectional	160 inpatients with COVID-19 (extended dataset per author); Intensive therapy unit	ITU: 61.1±11.8, Non-ITU: 76.4±14.9	ITU: Hypertension (24, 68.6%), Diabetes: (14, 40%), Obesity: (9, 25.7%), Non-ITU wards: Hypertension (32, 40.5%), Diabetes: (24, 30.4%), Obesity: (5, 6.3%)	VDI <50 nmol/L (equal to 20 ng/mL), NVD ≥50 nmol/L (20 ng/mL). Time: at admission. Method: immunoassay	ITU: prevalence of VDD was 35 (81.4%), VD level and mortality rate: OR 0.97 (95% CI: 0.42-2.23), P=0.94.	VDD is more common among patients admitted to ITU. No significant association between vitamin D level and mortality rate.	Selection: *, comparability: **, outcome: **, Total: 6/10 (moderate quality)
Jain et al. ^[43]	India	Prospective observational study	154 patients with COVID-19: 91 asymptomatic and 63 severe (admitted to ICU). Test: RT-PCR	Asymptomatic group: 42.34±6.41. Severe group: 51.41±9.12	Patients with chronic obstructive airway disease; chronic kidney disease on dialysis, or on chemotherapy were excluded from the study. BMI: asymptomatic versus severe group: 27.23±3.45 kg/m ² vs 26.83±5.81	VDD: 25(OH) D of <20 ng/mL. Time: at diagnosis. Method: immunoassay	VDD in asymptomatic versus severe group: 29 (31.9%) vs. 61 (96.8%). The fatality rate was 21% among patients with VDD.	VDD is more common among patients with severe COVID-19 and associated with a higher mortality rate.	Selection: ***, comparability: *, outcome: **, Total: 7/10 (moderate quality)
Hernández et al. ^[44]	Spain	Case-control study	216 patients with COVID-19 (19 of them were on vitamin D supplement and were excluded from the analysis). Control: 197. Test: RT-PCR	Cases versus control: 61.0 (47.5-70.0) vs 61.0 (56.0-66.0)	Cases versus control: BMI (29.2±4.7 vs 28.9±4.0), hypertension (76 (38.6%) vs 87 (44.2%)), diabetes vs 31 (15.7%), cardiovascular disease (21 (10.7%) vs 22 (11.2%)).	VDD: 25(OH) D of <20 ng/mL. Time: at admission. Method: chemiluminescence assay	VDD and combined severity endpoints of COVID-19: adjusted OR 1.13, 95% CI 0.27-4.77; P=0.865. (Multivariate analysis)	No significant association between VDD and combined severity endpoints of COVID-19	Selection: ***, comparability: **, outcome: **, Total: 7/10 (moderate quality)
Maghbooli et al. ^[45]	Iran	Cross sectional study	235 hospitalized patients. RT-PCR or radiological	58.7 years±15.2 SD.	BMI (mean): 27.41±4.55 kg/m ² , diabetes: 36.6%, hypertension: 44.4%, immunological disorders: 1.3%, COPD: 1.3%, heart disorders: 22.1%, malignancy: 0.9%, lung disorders: 5.5%, asthma: 4.3%	VDI: 25(OH) D of <30 ng/mL. Time: at admission. Method: electrochemiluminescence	Low vitamin D (<30 ng/mL) and severity of COVID-19: OR 1.59, 95% CI: 1.05-2.41, P=0.02 -Low vitamin D (<30 ng/mL) and mortality in patients with COVID-19: OR 1.12, 95% CI: 1.0-1.26, P=0.04 (multivariate analysis)	A significant association between VDD and severity of symptoms and fatality from COVID-19. -However, only 31.06% of recruited subjects had SARS-CoV-2 PCR testing.	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)

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Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Luo <i>et al.</i> ^[46]	China	Cross-sectional study	335 hospitalised patients with COVID-19. Test: PCR	56.0 years (IQR: 43.0-64.0)	Comorbidities: 147 (43.9%). BMI: 23.5±3.13. Smoking: 45 (13.4)	VDD: 25(OH) D of <30 nmol/L (equal to 12 ng/mL). Time: at admission. Method: chemiluminescence immunoassay	VDD and severe COVID-19 (OR: 2.72; 95% CI: 1.23-6.01, P value=0.014). (multivariate analysis)	Significant association between VDD and severity of COVID-19.	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)
Ye <i>et al.</i> ^[47]	China	Case-control study	62 patients with COVID-19 and 80 healthy controls (matched with cases by age and sex). Test: PCR	Median: 43 [32-59]	Diabetes: 5 (83.3%), hypertension: 6 (10.0%), liver injury: 1 (1.7%), COPD: 1 (1.7%), asthma: 0 (0.0%), renal failure: 16 (26.7%)	Normal: vit D ≥30 ng/mL, VDI: 30 >Vit. D >20 ng/mL. VDD: Vit. D <20 ng/mL. Time: at admission. Method: electro chemiluminescent immunoassay	VDD and severe/critical COVID-19: OR 5.18, 95% CI: 1.23-187.45, P=0.034 (multivariate analysis)	Significant association between VDD and severe/critical course of COVID-19	Selection: ***, comparability: **, outcome: **, Total: 7/10 (moderate quality)
Baktash <i>et al.</i> ^[48]	UK	prospective cohort study	105 patients: 70 patients were COVID-19 positive, 35 were COVID-19 negative. Test: RT-PCR	81 years, range 65-102	Hypertension: 34 (48.6%), diabetes: 26 (37.0%), ischemic heart disease: 15 (21.4%), chronic kidney disease: 16 (22.9%)	VDD: <30 nmol/l (12 ng/mL). Normal VD: ≥ 30 nmol/l (12 ng/mL). Time: at admission.	VDD and ventilatory requirement: RR: 4.15, 95%CI: 1.05-16.34, P=0.042. VDD and mortality rate: RR: 1.40, 95% CI: 0.36-5.47, P=0.50	A significant association between VDD and severity of symptoms. No significant association between VDD and mortality rate.	Selection: ***, comparability: **, outcome: **, Total: 7/9 (high quality)
Mardani <i>et al.</i> ^[49]	Iran	Cross sectional	123 patients: 63 patients were COVID-19 positive; 60 patients were COVID-19 negative. Test: RT-PCR	COVID-positive: 43.3 years, COVID-negative: 40.1 years.	Not documented	VDD: <10 ng/mL, VDI: 10-30 ng/mL, Normal VD: 30-100 ng/mL. Time: at admission. Method: enzyme-linked immunosorbent analysis (ELISA)	Mean vitamin D level in COVID-19-positive versus negative patients: 18.5 ng/dL vs. 30.2 ng/dL	Patients with COVID-19 had low vitamin D levels, neutrophil to lymphocyte ratio (NLR), and high ACE levels.	Selection: ***, comparability: *, outcome: **, Total: 6/10 (moderate quality)
Arvinte <i>et al.</i> ^[50]	Colorado (USA)	Pilot study	21 patients with COVID-19 in the ICU	Median: 61 years (20-94)	BMI: 31.6 (±7.3) kg/m ² , HbA1c: 7.6% (±2.0).	Definition: not documented. Time: at admission.	Mean vitamin D in overall sample: 22.0 (±9.5) ng/mL. Vitamin D level in survivor (n=11) versus non-survivor (n=10): 21.3 (±11.3) vs. 22.8 (±7.7) ng/mL, P=0.72.	overall low level of vitamin D in most patients with COVID-19	Selection: comparability: **, outcome: **, Total: 5/10 (moderate quality)

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Table 1: Contd...

Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Ferrari <i>et al.</i> ^[51]	Italy	Retrospective analysis	347 patients (128 COVID-19 positive, and 219 COVID-19 negative). Test: RT-PCR	COVID-19 positive: 65.0±15.0. COVID-19 negative: 58.7±20.2	Not documented	VDD <30 ng/mL. Time: near or during the time of COVID-19 onset. Method: immunoassay	Vitamin D level in patients with severe COVID-19 (death) versus non-severe COVID-19: (19.1±10.0 ng/mL vs. 22.2±16.6 ng/mL, <i>P</i> >0.05).	No significant association between vitamin D level and COVID-19 risk and death	Selection: **, comparability: * , outcome: **, Total: 6/10 (moderate quality)
Kerget <i>et al.</i> ^[52]	Turkey	Prospective observational study	88 patients with COVID-19 and 20 patients without COVID-19 (control). Test: RT-PCR	Cases: 49.1±21.1. Control: 35.2±6.9		Definition: not documented. Time: after admission. Method: enzyme-linked immunosorbent analysis (ELISA)	Vitamin D level in patients with COVID-19 who developed ARDS vs no ARDS: (16.8±10.5 vs. 21.8±15.8). Vitamin D level in patients with COVID-19 who died versus survived: (7.48±3 ng/mL vs. 21.1±14.2 ng/mL).	Vitamin D level was significantly lower in patients with severe COVID-19 compared to mild cases	Selection: ***, comparability: * , outcome: **, Total: 5/9 (moderate quality)
Cereda <i>et al.</i> ^[53]	Italy	Prospective observational cohort study	129 hospitalized patients with COVID-19. Test: RT-PCR	Median: 77 (65.0-85.0)	BMI: median 24.7(22.5-27.6) kg/m ² , diabetes: 39(30.7%), hypertension: 89 (70.1%), ischemic heart disease: 52 (40.9%), chronic kidney disease: 24 (18.9%)	NVD: vit. D ≥30 ng/mL. VDI: 30 >Vit. D >20 ng/mL. VDD: Vit. D <20 ng/mL. Time: within 48 hours of admission. Method: chemiluminescence immunoassay	VDD and in-hospital mortality: 1.73 (1.11-2.69, <i>P</i> =0.016). (Multivariate analysis)	No significant association between VDD and severe outcome.	Selection: ***, comparability: **, outcome: *, Total: 7/9 (high quality)
Vassiliou <i>et al.</i> ^[54]	Greece	Prospective observational study	30 patients with COVID-19 admitted to the ICU. Test: RT-PCR	65±11	Hypertension: 15 (50%), diabetes: 5 (16.7%), dyslipidemia: 9 (30%), coronary artery disease: 4 (13.3%), asthma: 1 (3.3%), COPD: 1 (3.3%), smoking: 3 (10%)	NVD: vit D ≥30 ng/mL. VDI: 30 >Vit. D >20 ng/mL. VDD: Vit. D <20 ng/mL. Time: at admission. Method: electrochemiluminescence immunoassay (ECLIA)	Patients with low versus high vitamin D level and 28-ICU mortality: 5 (33%) vs. 0 (0.0%).	A significant association was found between low vitamin D levels and mortality rate among ICU patients with COVID-19	Selection: **, comparability: * , outcome: **, Total: 5/9 (moderate quality)

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Table 1: Contd...

Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Abdollahi et al. ^[55]	Iran	Case-control study (matched)	Cases: 201 patients with COVID-19. Controls: 201 patients without COVID-19. Test: RT-PCR	Cases: 48 (±16.95). Controls: 46.34 (±13.5)	Cases versus controls: Diabetes 42 (20.89%) vs 19 (9.45%), heart failure and hypertension: 20 (9.95%) vs 15 (7.46%), respiratory infections: 14 (6.96%) vs 8 (39.80%)	NVD: vit. D ≥30 ng/mL. VDI: 30 > Vit. D >10 ng/mL. VDD: Vit. D <10 ng/mL. Time: at admission. Method: enzyme-linked immunosorbent assay method	Insufficient VD level in cases versus controls: 162 (80.5%) vs 132 (65.7%), P=0.001.	Low vitamin D level is prevalent in patients with COVID-19	Selection: ***, comparability: **, outcome: **, Total: 7/10 (moderate quality)
Anjum et al. ^[56]	Pakistan	Prospective observational study	140 patients with COVID-19. Test: RT-PCR	42±14.73.	BMI: mean 23.48±3.62. Other comorbidities were not documented	VDD: 25(OH) D <25 nmol/L (equal to 10 ng/mL). Time: at admission. Method: not documented	Mortality rate in VDD versus non-VDD groups: 16 (26.67%) vs 6 (7.5%), P=0.02. Prevalence of VDD: 116 (65.9%).	A significant association between severe VDD and mortality rate	Selection: **, comparability: *, outcome: *, Total: 4/9 (low quality)
Conçalves et al. ^[57]	Brazil	Cross-sectional study	176 patients with COVID-19. Test: RT-PCR	72.9±9.1	Hypertension: 127 (72.2), diabetes: 72 (40.9), heart disease: 48 (27.3), lung diseases: 48 (27.3)	VDD: 25(OH) D of <20 ng/mL. Time: at admission. Method: liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)		VDD is prevalent in ICU patients with COVID-19	Selection: **, comparability: **, outcome: **, Total: 6/10 (moderate quality)
Karahan et al. ^[58]	Turkey	Retrospective study	149 hospitalized patients with COVID-19. Test: RT-PCR	63.5±15.3	Diabetes: 61 (40.9%), hypertension: 85 (57.0%), coronary artery disease: 32 (21.5%), chronic kidney disease: 29 (19.5%)	NVD: vit D ≥30 ng/mL. VDI: 30 > Vit. D >20 ng/mL. VDD: Vit. D <20 ng/mL. Time: not documented. Method: electrochemiluminescence method	Mean vitamin D level in severe-critical versus moderate cases: 10.1±6.2 vs. 26.3±8.4 ng/mL. VD level and mortality rate: OR 0.927 (CI: 0.875-0.982), P=0.010 (multivariate analysis)	A significant association between VDD and severity and mortality rate in patients with COVID-19	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)
Ling et al. ^[59]	UK	Cross-sectional study (multi-center)	403 patients with COVID-19 in the primary analysis. Test: RT-PCR	Median: 74 (63-83)		VDD: 25(OH) D <25 nmol/L. Time: within 12 weeks prior to admission. Method: immunoassay	VDD and invasive mechanical ventilation: OR 2.73 (CI: 0.94-7.93), P=0.065. VDD and mortality rate: OR 0.80 (CI: 0.41-1.54), P=0.496 (multivariate analysis)	No significant association between vitamin D level and COVID-19 mortality.	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)

Table 1: Contd...

Author and year of study	Country	Study design	Sample size	Age (mean, years)	Comorbidities (n.%)	Definition of VDD, time, and method of testing	OR, CI, P	Summary of findings	NOS quality assessment
Sun <i>et al.</i> ^[60]	China	Retrospective study	241 patients with COVID-19, vitamin D testing was done in 26 patients only. Test: RT-PCR	Median: 65 (IQR, 55-72)	Not documented	Definition: not documented. Time: within 24 hours of admission. Method: not documented.	VD level: median 10.20 (IQR, 8.20-12.65) ng/mL.	Patients with COVID-19 had low vitamin D and serum calcium levels.	Selection: **, comparability: **, outcome: **, Total: 5/10 (moderate quality)
Fanii <i>et al.</i> ^[61]	UK	Cross-sectional study	392 health care workers (214 (55%) had seroconversion, 178 (45%) had no seroconversion. Test: serology	Median: 41 (30-50)	BMI: 25.9 (22.9-30.1) kg/m ² ; one or more comorbidities: 152 (39%), hypertension: 34 (9%), asthma 26 (7%)	VDD <30 nmol/l (equal to 12 ng/mL). Time: at baseline. Method: mass spectrometry	VDD and positive seroconversion: OR 2.6, 95%CI 1.41-4.80; P=0.002	VDD was an independent risk factor for the development positive seroconversion of COVID-19	Selection: **, comparability: **, outcome: **, Total: 7/10 (moderate quality)
Pinzon <i>et al.</i> ^[26] Preprint	Indonesia	Case series	10 hospitalized patients with COVID-19. Test: either RT-PCR or serology	49.6 years.	Hypertension: 4 (40.0%), diabetes: 1 (10.0%), COPD: 1 (10.0%), post stroke: 1 (10.0%)	NVD: vit. D ≥30 ng/mL. VDI: 30 >Vit. D >20 ng/mL. VDD: Vit. D <20 ng/mL. Time: not documented. Method: enzyme-linked immunosorbent assay method	VDD: 9/10 patients (90%). VDI: 1/10 patients (10%)	VDD is prevalent in patients with COVID-19	

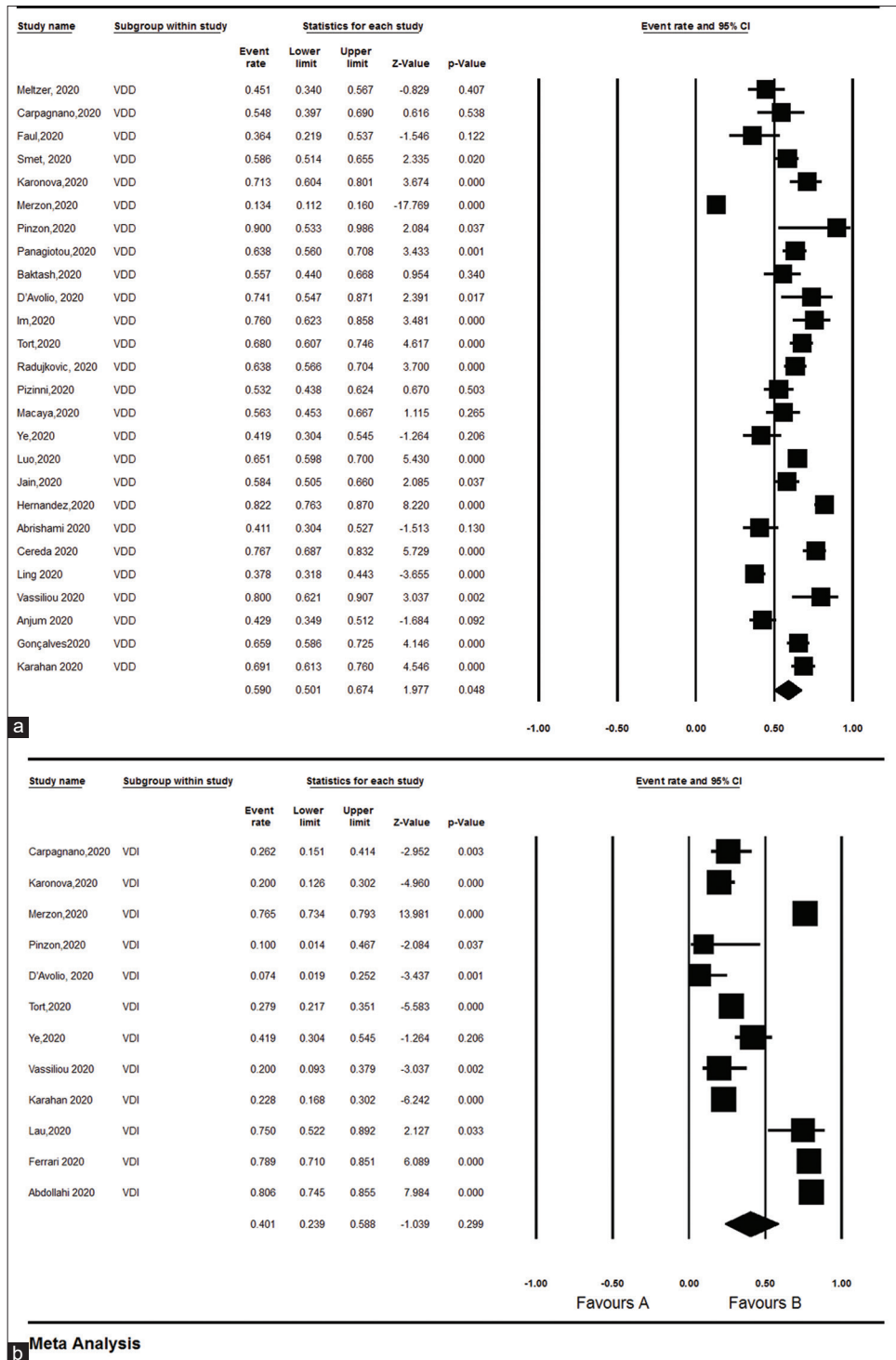


Figure 2 : Forest plot of event rates of: (a) vitamin D deficiency (VDD), and (b) vitamin D insufficiency (VDI) in patients with COVID-19

overall validity of the results. Therefore, we decided to exclude this study from our analysis. The pooled analysis revealed a significant association between VDD/VDI and the severity of symptoms in patients with COVID-19 (OR = 3.38, 95% CI: 1.94–5.87, $P < 0.0001$). Figure 3a shows the forest plot of VDD/VDI versus normal vitamin D and severity of symptoms of COVID-19 (composite outcomes).

Moderate heterogeneity was noted ($I^2 = 67%$). On sensitivity analysis, three studies^[43,44,58] were the main reasons for heterogeneity, and the heterogeneity test (I^2 score) dropped to 12% (low level of heterogeneity) when these three studies were omitted from the analysis and the effect size was still significant (OR: 2.51, CI: 1.79–3.52, P value < 0.0001). Another pooled analysis revealed significantly lower mean

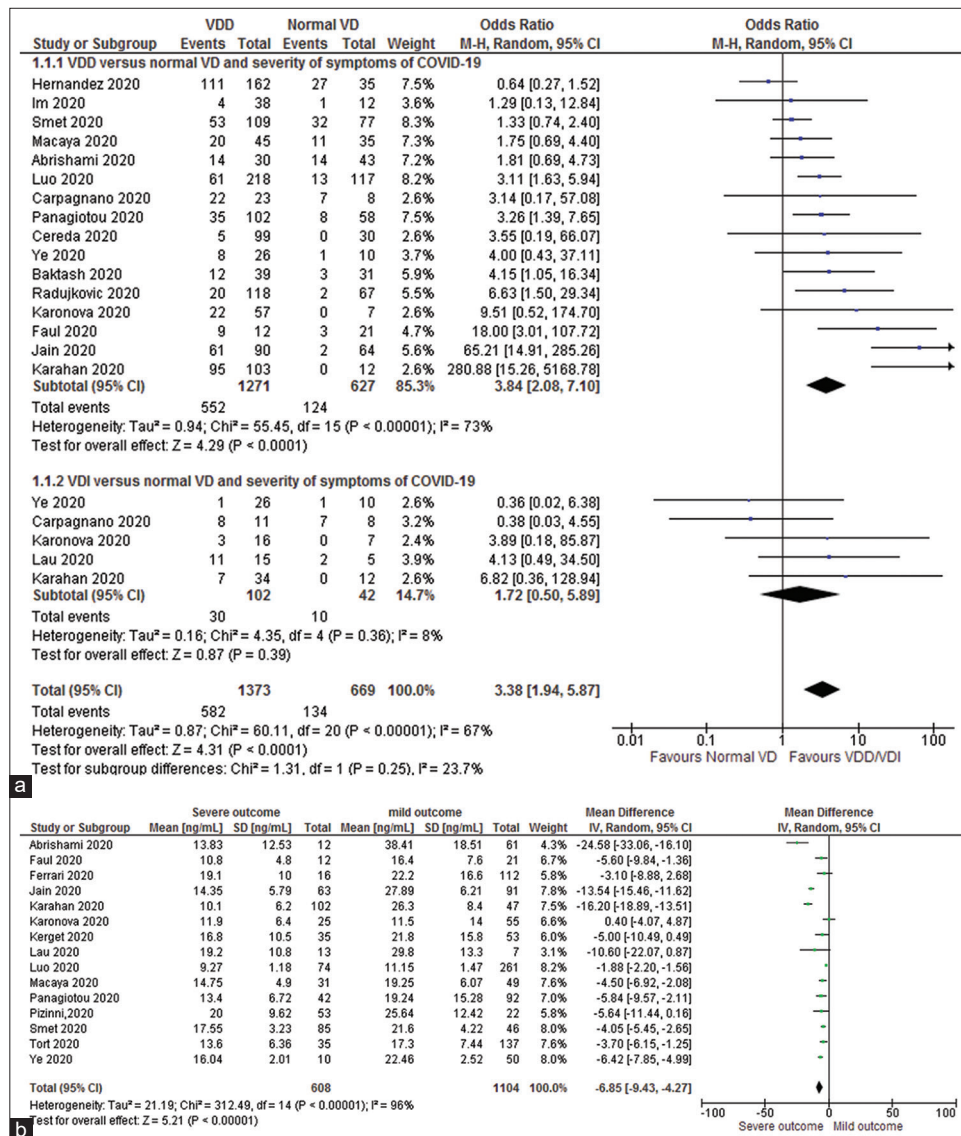


Figure 3: (a) Forest plot: Vitamin D deficiency/insufficiency versus normal vitamin D and severity of symptoms of COVID-19 (composite outcomes). (b) Pooled analysis of mean vitamin D level (ng/mL) and severity of symptoms of COVID-19

levels of vitamin D in patients with COVID-19 who had severe symptoms when compared to those with mild/ordinary outcomes (standardized mean difference: -6.85, CI: -9.43– -4.27, P value <0.00001, I² = 96%). Figure 3b illustrates the pooled analysis of mean vitamin D level (ng/mL) and severity of symptoms of COVID-19.

Additional sub-analysis between VDD and individual outcomes revealed a significant association between VDD and mechanical ventilation (OR: 2.73, CI: 1.55–4.82, P value <0.001, I² = 0%), radiological worsening (OR: 1.60, CI: 1.03–2.48, P value = 0.04, I² = 0%), ICU admission (OR: 5.73, CI: 3.48–9.45, P value ≤ 0.001, I² = 72%) and ARDS development (OR: 11.27, CI: 2.63–48.26, P value = 0.001, I² = 1%). The forest plot of VDD/VDI versus normal vitamin D and severity of symptoms of COVID-19 (individual outcomes) can be found in the Supplementary Figure 1.

VDD/VDI and the case fatality rate in patients with COVID-19

Seventeen studies assessed the association between VDD/VDI and case fatality rate.^[13,34,35,37-44,46,48,51,54,56,58] A significant association was observed between the pooled effects of VDD/VDI and case fatality rate (OR = 2.30, 95% CI: 1.47–3.59, P value <0.00001). Pooled analysis of VDD/VDI and case fatality rate in patients with COVID-19 is depicted in Figure 4. A moderate level of heterogeneity, although not significant, was noted (I² = 36%, P value = 0.06). Notably, sensitivity analysis showed a persistently significant association between low vitamin D level and case fatality rate when two studies conducted by Jain *et al.* (2020)^[43] and Karahan *et al.* (2020)^[58] were omitted from the analysis (OR = 1.87, 95% CI: 1.27–2.76, P value = 0.002) and the heterogeneity test (I²) dropped from 36% to 18%.

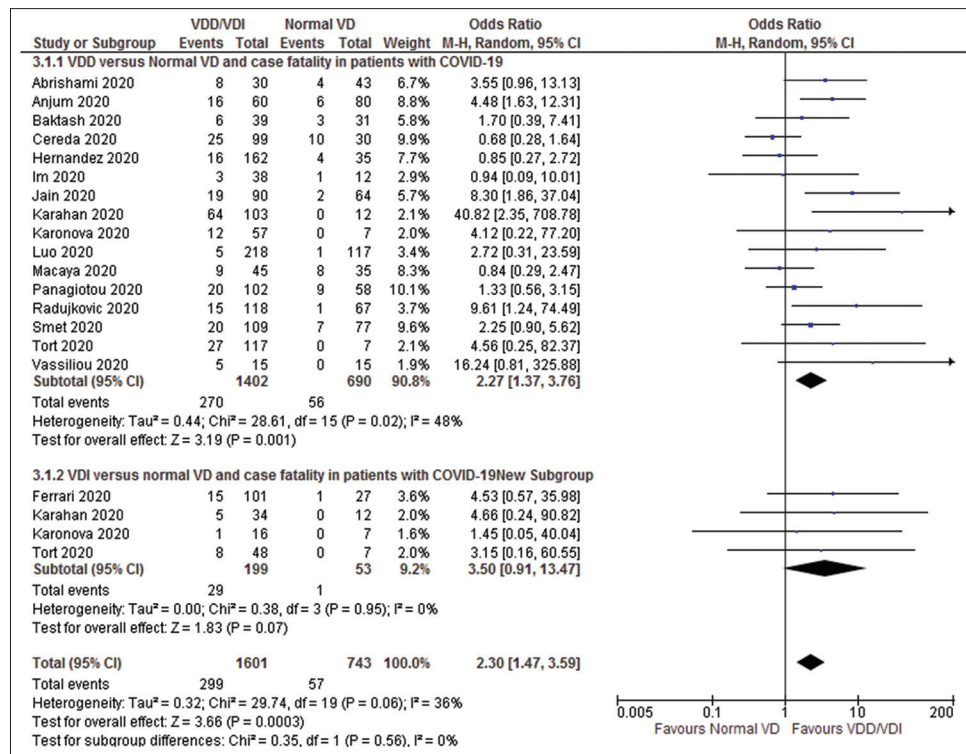


Figure 4: Pooled analysis of vitamin D deficiency/insufficiency and case fatality rate in patients with COVID-19

Pooled analysis of adjusted Odds ratio

Three,^[15,28,40] five,^[37,41,44,46,59] and four studies^[35,37,53,59] studies that adjusted for confounders were included in the pooled analysis of VDD and risk, the severity of symptoms, and mortality rate of patients with COVID-19, respectively. Pooled analysis of the adjusted OR can be found in Supplementary Figure 2a-c and revealed a significant association between VDD and COVID-19 risk (adjusted OR: 1.971, CI: 1.68–2.3, P value = 0.000), the severity of symptoms (adjusted OR: 2.673, CI: 1.62–4.41, P value = 0.000), and mortality rate (adjusted OR: 1.555, CI: 1.09–2.21, P value = 0.014). Notably, using a fixed-effect model in view of the small number of studies that adjusted for confounders makes inferences about the general population challenging.^[22]

Quality of studies

The quality of the included studies was limited by the following factors: i) with the exception of the studies conducted by Im *et al.* (2020),^[34] Jain *et al.* (2020),^[43] Cereda *et al.* (2020),^[53] Vassiliou *et al.* (2020),^[54] Bakshat *et al.* (2020),^[48] Radujkovic *et al.* (2020),^[39] Anjum *et al.* (2020),^[56] Pizzini *et al.* (2020),^[32] and Kerget *et al.* (2020),^[52] all the included studies were of retrospective design; ii) in the majority of studies, it was not clear how subjects were invited and recruited, which therefore predisposed the findings to selection bias; iii) with the exception of studies conducted by Smet *et al.* (2020),^[13] Kaufman *et al.* (2020),^[14] Hastie *et al.* (2020),^[15,16] Darling *et al.* (2020),^[23] Li *et al.* (2020),^[24] Raisi-Estabragh *et al.* (2020),^[27] Katz *et al.* (2020),^[28] Israel *et al.* (2020),^[29] Meltzer *et al.* (2020),^[31] Macaya *et al.* (2020),^[41] Ye *et al.* (2020),^[47] Hernández

et al. (2020),^[44] Cereda *et al.* (2020),^[53] Radujkovic *et al.* (2020),^[39] Luo *et al.* (2020),^[46] Abrishami *et al.* (2020),^[35] Merzon *et al.* (2020),^[40] Karonova *et al.* (2020),^[37] and Ling *et al.* (2020),^[59] no adequate adjustment for confounding factors such as body mass index (BMI), comorbidities, and socioeconomic status was performed. A detailed quality assessment of the included studies is depicted in Table 1.

Publication bias

Egger’s regression intercept analysis was carried out for the two meta-analyses (severity of symptoms and case fatality rate). It revealed a non-significant risk of publication bias for the association between low vitamin D level and severity of symptoms (P-value: 0.071). However, the risk of publication bias was significant for the association between low vitamin D level and mortality rate (P-value: 0.023). Figure 5 illustrates the funnel plots and Egger’s regression intercept.

DISCUSSION

The results of our meta-analysis support a high prevalence of VDD/VDI in patients with COVID-19. Besides, a significant association was illustrated between VDD/VDI and severity of symptoms and case fatality rate among patients with COVID-19.

The results of a meta-analysis by Zhou *et al.* (2019)^[62] suggest an inverse correlation between VDD and the risk of development of community-acquired pneumonia (CAP),^[62] although one can argue that these findings cannot be generalized for infection caused by this novel coronavirus (SARS-CoV-2). Preliminary

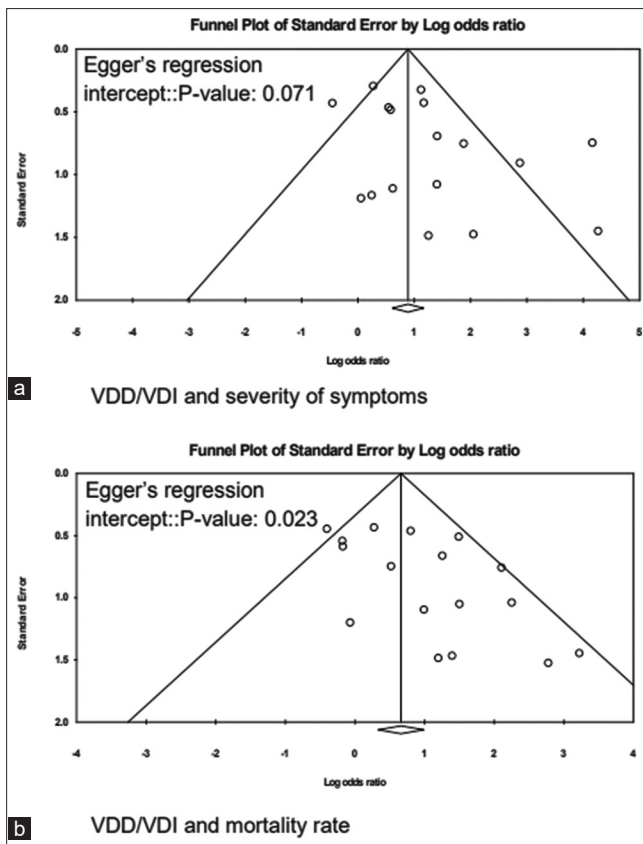


Figure 5: Funnel plots and Egger's regression intercept: A. VDD/VDI and severity of symptoms. B. VDD/VDI and mortality rate, in patients with COVID-19

evidence from epidemiological studies published so far suggests an inverse correlation between vitamin D levels and risk of symptomatic COVID-19 infection. Another ecological analysis underlined the higher vulnerability to COVID-19 infection in populations with lower vitamin D levels.^[63] Low vitamin D levels were also observed to be an independent predictor of COVID-19 risk in multivariate analyses conducted by Meltzer *et al.* (2020)^[31] (relative risk [RR] = 1.77, $P < 0.02$) and Merzon *et al.* (2020)^[40] (OR: 1.50, $P = 0.001$). The possible mechanisms of such an association could be ascribed to the immunomodulatory role of vitamin D. Notably, individuals with low vitamin D levels have a reduced concentration of vitamin D receptors (VDRs) and antimicrobial peptides, which increases their susceptibility to bacterial and viral infections.^[64]

Our analysis identified a significant association between low vitamin D levels (VDD/VDI) and the severity of COVID-19 symptoms. Emerging evidence points toward the cytokine storm as the main drive for ARDS and a severe course of COVID-19.^[65,66] The cytokine storm involves the release of increased amounts of pro-inflammatory cytokines (interleukin IL-1B, IL-6, IL-12, interferon- γ) and chemokines (CXCL10 and CCL2), which trigger the systemic inflammatory response. Besides, being a potent immune modulator of different immune cells, vitamin D is associated with the down-regulation of some important cytokines, such as IL-6 and TNF- α , which play

an essential role in the development of cytokine storm.^[4,5,66] Therefore, low vitamin D levels impair the down-regulation of these inflammatory cytokines and potentially contribute to the development of ARDS. Vitamin D receptors are richly expressed in the lungs and shown to protect against sepsis-induced lung injury by inhibiting the angiotensin-2-TEK receptor tyrosine kinase-myosin light-chain kinase pathway. In animal models, 1,25 hydroxyvitamin D has been shown to attenuate lipopolysaccharide (LPS)-induced lung damage by modulating the renin-angiotensin system (RAS).^[67] Vitamin D has been shown to induce ACE2/angiotensin axis activity and increase the level of ACE2 in comparison to ACE1, which thereby attenuates angiotensin II-mediated vasoconstriction and lung injury.^[68]

Also, vitamin D promotes autophagy, which is an essential biological process in maintaining cellular hemostasis and enhancing the antiviral environment.^[69] Besides, some evidence suggests that vitamin D enhances the production of lung surfactants, which are essential in maintaining the integrity of alveolar-air interface and thereby gas exchange in the lungs.^[70] A summary of the postulated mechanisms of action of vitamin D on COVID 19 is depicted in Supplementary Figure 3.

The increased case-fatality in COVID-19 has been attributed to the development of ARSD due to a cytokine storm apart from a pro-thrombotic state. The mechanistic basis of the contribution of lower vitamin D levels to a pro-inflammatory and acute lung injury remains a plausible hypothesis. The results of our meta-analysis suggest a significant association between VDD/VDI and the case fatality rate in COVID-19 patients, which remains significant even after adjusting for some confounders. However, given the small sample sizes of the included observational studies that addressed the case-fatality rate and vitamin D status in patients with COVID-19 and the significant risk of publication bias, it is prudent to interpret these findings cautiously. Nonetheless, Ilie *et al.* (2020)^[71] found a negative correlation between the mean vitamin D level and mortality rate from COVID-19 in 20 European countries. Although most of the evidence related to VDD/VDI and case fatality rate in patients with COVID-19 is driven from ecological studies, these results are subject to the ecological fallacy and cannot, therefore, be applied on an individual level.

Implications

This systematic review will add supplementary evidence to the existing dataset about the relationship between vitamin D levels and viral respiratory illness. We believe that our meta-analysis comprehensively summarizes the available data on the relationship between VDD/VDI and the severity of COVID-19 symptoms and mortality rate. Considering the availability and safety of vitamin D supplements at the required doses, local guidelines need to be followed to prevent VDD. For example, the UK government advises all people to consider taking 10 micrograms (400 IU) of vitamin D supplements daily during the COVID-19 pandemic to maintain their musculoskeletal

health, especially if they spend most of their time indoors.^[72] However, it is debatable if such an approach will mitigate the severity of symptoms in patients with COVID-19 as perhaps a higher prophylactic dose of vitamin D is needed to provide immunomodulatory benefits.

Limitations

Our study is subject to several limitations. First, most of the studies included were retrospective observational designs that may impede the inference of a causal relationship. It was not clear whether low vitamin D level predisposes patients to an increased risk of severe COVID-19 or the low vitamin level is a consequence of COVID-19. However, applying Hill's criteria^[73] to the causality reveals a consistent and strong association between VDD and COVID-19 severity.^[13,35-37,39,42,43,46-48] Also, the temporal precedence of vitamin D testing in several studies^[14,31,40,41] with the exception of one study^[32] in which testing was done at 8 weeks post-infection, increases the probability of the low vitamin D level being a factor in increasing the severity of COVID-19. Second, the SARS-CoV-2 positivity based on PCR testing in the large population-based studies is only considered as a precursor for COVID-19 disease and many people with a positive test do not develop the disease.^[14,40] Second, moderate heterogeneity was noted, which implies a cautious interpretation of our findings. However, the heterogeneity test (I^2) dropped from 67% to 12% and the results were still statistically significant, when sensitivity analysis of the association between VDD/VDI and COVID-19 severity of symptoms was performed. Third, different cut-off values were used to define VDD, VDI, and normal vitamin D levels. However, we believe that the cut-off values used in this study had correctly combined the majority of studies. Fourth, a considerable number of studies lacked any adjustment for confounding factors, which might have influenced the overall results. Besides, the lack of the seasonality adjustment of vitamin D level in the majority of studies is an important factor that might have impacted our findings. However, a large population-based study substantiated a strong inverse association between vitamin D level and positivity for SARS-CoV-2 testing even after adjustment for seasonality effect on vitamin D level.^[14] Fifth, different criteria for the severity of symptoms were used, which might also affect our findings. However, a separate analysis of each severity outcome was done and revealed similar results [Supplementary Figure 1]. Sixth, the relatively small sample size of some studies might have overestimated the true association between vitamin D levels and COVID-19. Seventh, we acknowledge that the methods used for vitamin D testing might affect the accuracy of vitamin D levels across different studies. Eighth, only a few studies in this systematic review were population-based^[14,16,23,24,27,29,40] and the majority were conducted on hospitalized patients, which could inevitably affect the generalizability of our findings.

Future directions

Ongoing clinical trials should provide further clarity regarding these preliminary results, which suggest an inverse correlation between vitamin D levels and COVID-19 disease severity.

CONCLUSIONS

Vitamin D deficiency and/or insufficiency are relatively common in patients with COVID-19. A considerable amount of evidence indicates an inverse correlation between vitamin D levels and COVID-19 related clinical outcomes, including the case-fatality rate although most of the data so far have been derived from medium-quality observational studies. The ongoing, well-structured prospective clinical trials with a larger sample size should provide further evidence if prophylactic or therapeutic intervention with vitamin D improves COVID-19-related clinical outcomes.

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Nil.

Conflicts of interest

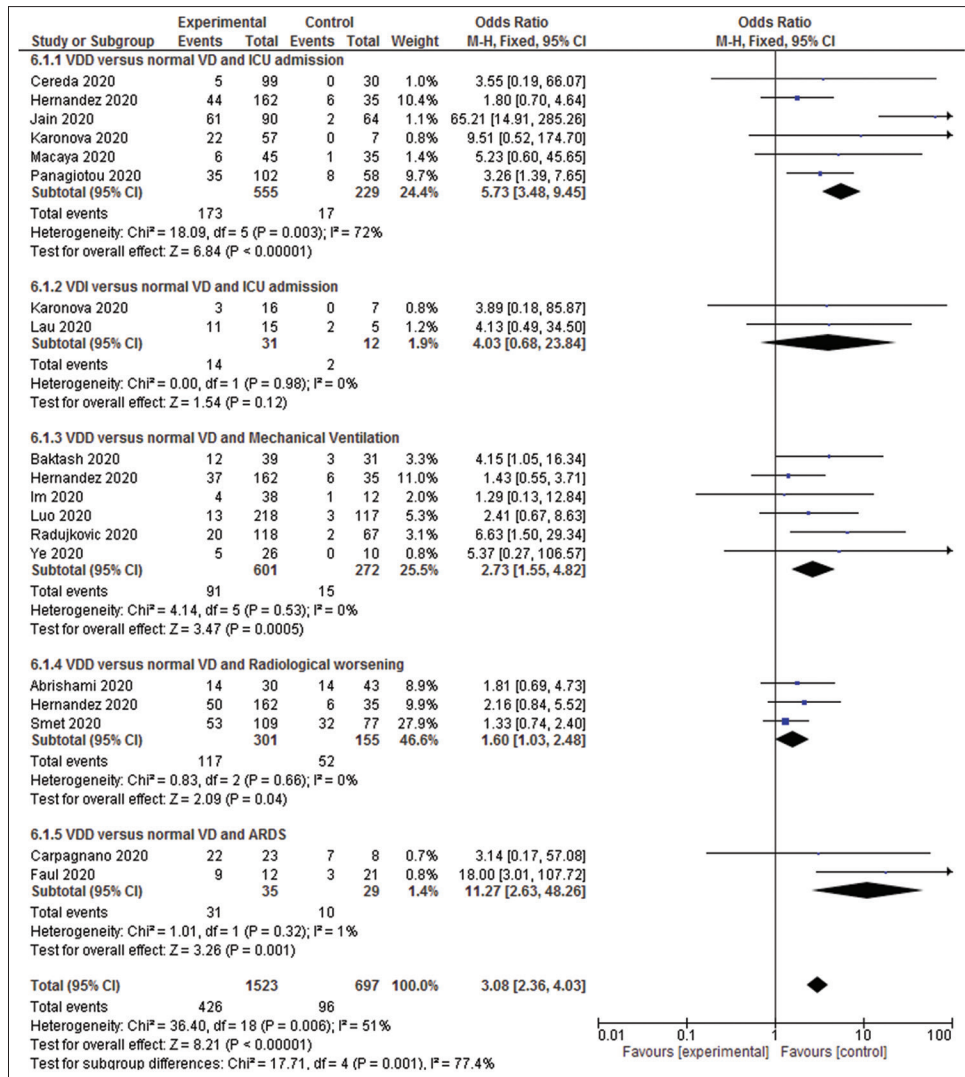
There are no conflicts of interest.

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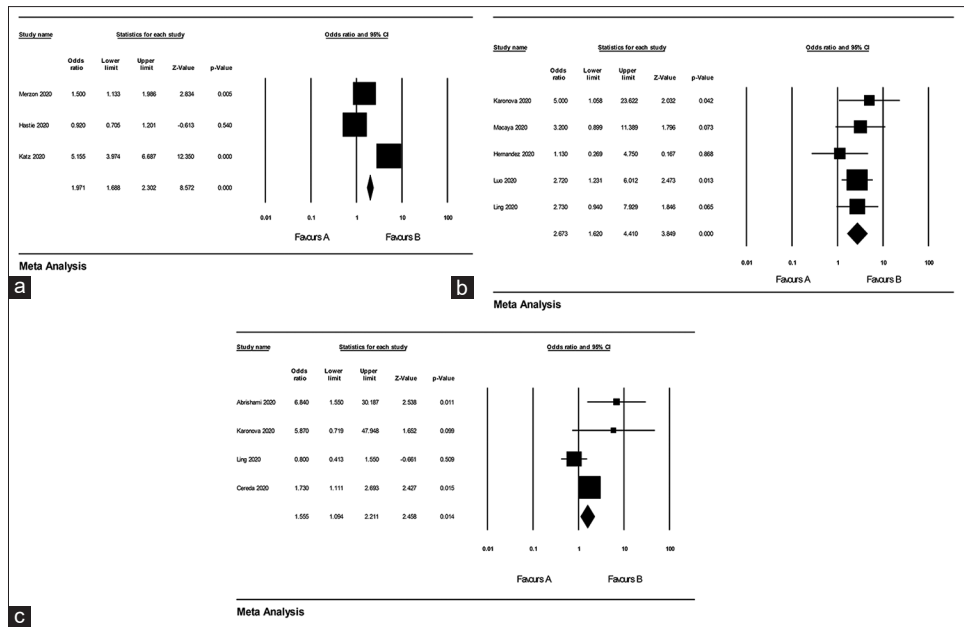
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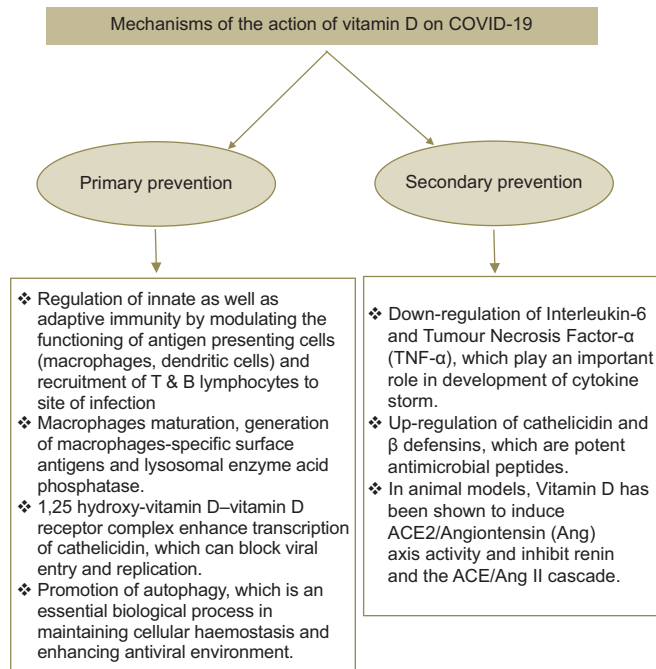
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Supplementary Figure 1: Forest plot: Vitamin D deficiency/insufficiency versus normal vitamin D and severity of symptoms of COVID-19 (individual outcomes)



Supplementary Figure 2: Forest plot: Adjusted odds ratio: (a) VDD and COVID-19 risk. (b) VDD and severity of COVID-19. (c) VDD and mortality rate in patients with COVID-19



Supplementary Figure 3: Summary of the postulated mechanisms of the action of vitamin D on COVID-19