

## REVIEW

# Vitamin D supplementation and Covid-19 outcomes: A systematic review, meta-analysis and meta-regression

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## Summary

Vitamin D has many protective properties and potential role against acute lung injury. Low serum vitamin D is associated with high risk of pneumonia and development of acute respiratory distress syndrome. This study sought to analyse the efficacy of vitamin D in improving the outcomes of coronavirus disease 2019 (Covid-19) patients. Using specific keywords, we comprehensively searched the potential articles on PubMed, Europe PMC and ClinicalTrials.gov database until 8th May 2021. All published studies on Covid-19 and vitamin D were retrieved. Statistical analysis was conducted using Review Manager 5.4 software. A total of 11 studies with 22,265 Covid-19 patients were included in the meta-analysis. Our data suggested that vitamin D supplementation was associated with reduction in intensive care unit admission rate (OR 0.27; 95% CI: 0.09–0.76,  $p = 0.010$ ,  $I^2 = 70\%$ , random-effect modelling); reduction of the need for mechanical ventilation (OR 0.34; 95% CI: 0.16–0.72,  $p = 0.005$ ,  $I^2 = 61\%$ , random-effect modelling) and reduction of mortality from Covid-19 (OR 0.37; 95% CI: 0.21–0.66,  $p < 0.001$ ,  $I^2 = 50\%$ , random-effect modelling). Further analysis showed that the associations were influenced by age ( $p = 0.020$ ). Our study suggests that vitamin D supplementation may offer beneficial effects on Covid-19 outcomes. However, more randomized clinical trials are required to confirm this conclusion.

## KEYWORDS

coronavirus disease 2019, Covid-19, vitamin D

## 1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection continues to spread globally causing a pandemic and has become a major medical focus for the last couple of years. The

pandemic has involved over 164 million confirmed cases, with more than 3.4 million deaths as of 23 May 2021.<sup>1</sup> While some SARS-CoV-2 infections appear as mild upper respiratory symptoms and may be self-limiting, notable numbers of patients require hospitalizations and intensive treatment following progression into a more severe cases,

**Abbreviations:** ACE, angiotensin-converting enzyme; ARDS, acute respiratory distress syndrome; CI, confidence intervals; Covid-19, coronavirus disease 2019; ICU, Intensive Care Unit; IFN- $\gamma$ , interferon- $\gamma$ ; IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-6, interleukin 6; IU, International Unit; JBI, Joanna Briggs Institute; MOF, multi-organ failure; NF-kB, nuclear factor-kappa B; NK cells, natural killer cells; NKG2A, NK group 2 member A; NOS, Newcastle-Ottawa Scale; OR, odds ratio; RAS, renin angiotensin system; RT-PCR, Reverse Transcriptase-Polymerase Chain Reaction; SARS, severe acute respiratory syndrome; SARS-CoV-2, SARS-coronavirus-2; SNP, single nucleotide polymorphism; Th-1, T helper-1; TNF- $\alpha$ , tumour necrosis factor- $\alpha$ ; VDR, vitamin D receptor.

varying from simple lower respiratory tract infections to acute respiratory distress syndrome (ARDS) and eventually may turn into multi-organ failure.<sup>2,3</sup>

Individuals with comorbidities such as obesity, diabetes, chronic respiratory disease, cardiovascular disease and other immunocompromising conditions are facing higher risks in developing the severe form of SARS-CoV-2 infections.<sup>4-9</sup> For the past months, medical therapies to treat Covid-19 have been growing and evolving rapidly, ranging from supportive care, antivirals, anti-inflammatory agents and possible supplementations such as vitamin D.<sup>10-13</sup> Several studies have shown that vitamin D has antiviral properties and potential roles against acute lung injury or ARDS, making large interest on vitamin D has rapidly emanated even in the early beginning of pandemic.<sup>14,15</sup> Low vitamin D serum levels is associated with an increase in inflammatory cytokine levels and significant increase of risk to develop pneumonia and viral respiratory tract infections, which both contribute to the development of ARDS.<sup>16-18</sup> The goal of this study is to provide evidence whether or not vitamin D supplementation is associated with improved outcomes of Covid-19 based on the available studies.

## 2 | MATERIALS AND METHODS

### 2.1 | Eligibility criteria

The protocol of this systematic review and meta-analysis study of the observational and clinical trial studies was registered in PROSPERO (CRD42021256117). Included articles were selected as potentially fulfilling the entry criteria: comply the PICO framework (P: Covid-19 patients; I: vitamin D supplementation in any form; C: a group of patients who did not receive vitamin D, only receive standard of care therapy or any other medications as control/placebo; O: intensive care unit [ICU] admission, the need for mechanical ventilation and

mortality), cohort, case-control, cross-sectional and randomized or non-randomized clinical trial articles were included. All studies other than original research articles (review articles, letter to editor or correspondence), case-series or case report studies, studies reported other than in English language, studies focussing on populations below 18 years of age and pregnant women were excluded.

### 2.2 | Search strategy and study selection

The papers from three databases (PubMed, Europe PMC and ClinicalTrials.gov) were searched systemically. Search terms used include 'vitamin D' OR 'calcidiol' OR 'calciferol' OR 'calcifediol' OR 'cholecalciferol' OR 'calcitriol' AND 'SARS-CoV-2', OR 'coronavirus disease 2019' OR 'Covid-19' in a time range from 2019 until 8 May 2021 with English-language restriction. Our searching strategy details are listed in Table 1. Initial screening of titles and abstracts was conducted to identify eligible articles. Searches of potential articles were also done by analysing the list of references of eligible studies. The search strategy was presented in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram.

### 2.3 | Data extraction and quality assessment

Two authors (TIH and DI) performed the data extraction. An extraction form was developed to list the essential information about the study and its population characteristic (age, gender, hypertension, diabetes and corticosteroids usage/consumption), vitamin D dose, the number of patients receiving vitamin D and the control group, as well as the outcome of Covid-19 patients.

The outcomes of interest are the rate of ICU admission, the need for mechanical ventilation and the mortality. The ICU admission rate is defined by the number of patients who were subsequently

TABLE 1 Literature search strategy

Database	Keyword	Result
PubMed	('vitamin d'[MeSH Terms] OR 'vitamin d'[All Fields] OR 'ergocalciferols'[MeSH Terms] OR 'ergocalciferols'[All Fields]) OR ('calcifediol'[MeSH Terms] OR 'calcifediol'[All Fields] OR 'calcidiol'[All Fields]) OR ('ergocalciferols'[MeSH Terms] OR 'ergocalciferols'[All Fields] OR 'calciferol'[All Fields]) OR ('calcitriol'[MeSH Terms] OR 'calcitriol'[All Fields]) AND ('COVID-19'[All Fields] OR 'COVID-19'[MeSH Terms] OR 'COVID-19 Vaccines'[All Fields] OR 'COVID-19 Vaccines'[MeSH Terms] OR 'COVID-19 serotherapy'[All Fields] OR 'COVID-19 Nucleic Acid Testing'[All Fields] OR 'covid-19 nucleic acid testing'[MeSH Terms] OR 'COVID-19 Serological Testing'[All Fields] OR 'covid-19 serological testing'[MeSH Terms] OR 'COVID-19 Testing'[All Fields] OR 'covid-19 testing'[MeSH Terms] OR 'SARS-CoV-2'[All Fields] OR 'sars-cov-2'[MeSH Terms] OR 'Severe Acute Respiratory Syndrome Coronavirus 2'[All Fields] OR 'NCOV'[All Fields] OR '2019 NCOV'[All Fields] OR (('coronavirus'[MeSH Terms] OR 'coronavirus'[All Fields] OR 'COV'[All Fields]) AND 2019/11/01[PubDate]: 3000/12/31[PubDate]))	612
Europe PMC	'vitamin D' OR 'calcidiol' OR 'calciferol' OR 'calcifediol' OR 'cholecalciferol' OR 'calcitriol' AND 'SARS-CoV-2', OR 'coronavirus disease 2019' OR 'Covid-19'	5330
ClinicalTrials.gov	'vitamin D' OR 'calcidiol' OR 'calciferol' OR 'calcifediol' OR 'cholecalciferol' OR 'calcitriol' AND 'SARS-CoV-2', OR 'coronavirus disease 2019' OR 'Covid-19'	96

admitted into the ICU during the hospital stay. The need for mechanical ventilation is defined by the number of patients who need assisted ventilation. The total number of patients who were dead during the follow-up period with positive Covid-19 status was described as the mortality outcome.

Two authors (JEH and HH) assessed the quality of each study included in this study independently. The quality of clinical trials was assessed using the modified Jadad scale assessment where the random allocation, allocation concealment, blindness and withdrawals and drop-outs of each study were evaluated. The studies were scored from zero to seven and a study ranked as a high-quality study if the score was  $>4$ .<sup>19</sup> The quality of case-control and cohort studies were assessed using Newcastle–Ottawa Scale (NOS). The assessment reviews the selection, comparability and outcome of each study, then each study was assigned a total score from zero to nine. A study is graded as good quality if it scores  $\geq 7$ . Cross-sectional studies was assessed using the Joanna Briggs Institute (JBI) Critical Appraisal Tools for Analytical Cross Sectional Studies.

## 2.4 | Statistical analysis

The meta-analysis was performed using the Review Manager 5.4 (Cochrane Collaboration) software and Comprehensive

Meta-Analysis version 3. Mantel–Haenszel's formula was employed to calculate odds ratio (OR) and its 95% confidence interval (95%CI) for the ICU admission outcome and the need for mechanical ventilation outcome, while Inverse Variance method was used to obtain the OR and 95% CI for the mortality outcome. The heterogeneity was assessed by using the  $I^2$  statistic with a value of  $<25\%$ ,  $26\text{--}50\%$  and  $>50\%$  were considered as low, moderate and high degrees of heterogeneity, respectively. Random effects meta-regression was performed using a maximum likelihood for pre-specified variables including age, gender, hypertension, diabetes and the use of corticosteroids. The qualitative risk of publication bias was assessed with funnel plot analysis, while the quantitative risk of publication bias was assessed by using the Begg and Mazumdar rank correlation test.<sup>20</sup>

## 3 | RESULTS

### 3.1 | Study selection and characteristics

The searches on the databases yielded 6038 studies. A total of 5128 records remained following the elimination of duplicates. Screening the titles and abstracts and matching the inclusion and exclusion criteria, 5063 studies were removed. Among 65 evaluated full-text

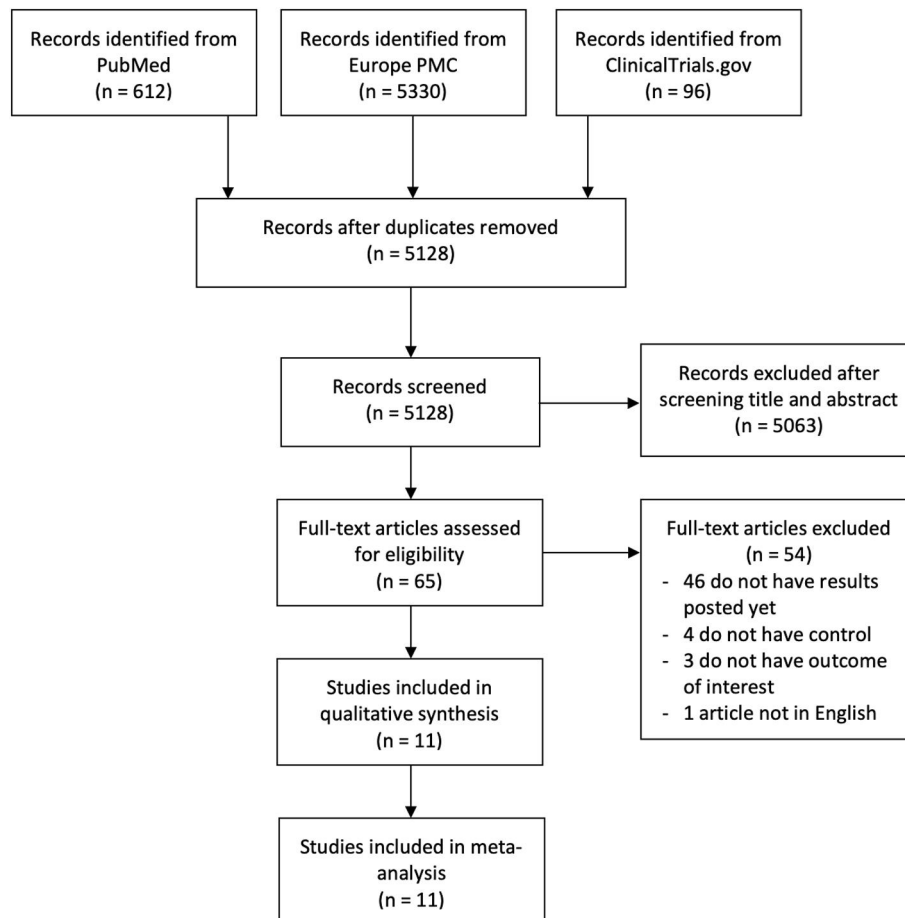


FIGURE 1 PRISMA diagram of the detailed process of selection of studies for inclusion in the systematic review and meta-analysis

TABLE 2 Characteristics of included studies

Study	Sample size	Design	Overall age mean $\pm$ SD	Male n (%)	Hypertension	Diabetes	Corticosteroid use	Vitamin D dose	Number of patients receiving Vitamin D versus Control
Annweiler C et al. <sup>21</sup> 2020	66	Retrospective cohort	87.7 $\pm$ 9	15 (22.7%)	N/A	N/A	4 (6.1%)	Cholecalciferol oral: 80,000 IU every 2–3 months	57 (86.3%) versus 9 (13.7%)
Annweiler G et al. <sup>22</sup> 2020	77	Retrospective cohort	88.3 $\pm$ 5.1	39 (50.6%)	49 (63.6%)	N/A	13 (16.9%)	Group 1: Cholecalciferol oral 50,000 IU per month, or the doses of 80,000 IU or 100,000 IU every 2–3 months. Group 2: Cholecalciferol oral 80,000 IU/months	45 (58.4%) versus 32 (41.6%)
Cangiano B et al. <sup>23</sup> 2020	98	Prospective cohort	89.6 $\pm$ 6.53	38 (24.2%)	48 (48.9%)	11 (11.2%)	8 (8.1%)	Cholecalciferol oral: 25,000 IU twice a months	20 (20.4%) versus 78 (79.6%)
Castillo ME et al. <sup>24</sup> 2020	76	Open-label, randomized clinical trial	53 $\pm$ 10	45 (59%)	26 (34.2%)	8 (10.5%)	N/A	Calcifediol oral: 0.532 mg on Day 1, then 0.266 mg on Day 3 and 7	50 (65.7%) versus 26 (34.3%)
Giannini S et al. <sup>25</sup> 2021	91	Retrospective cohort	74 $\pm$ 13	50 (55%)	N/A	30 (33%)	41 (45%)	Cholecalciferol oral: 200,000 IU daily for 2 consecutive days	36 (39.5%) versus 55 (60.5%)
Hernandez JL et al. <sup>26</sup> 2020	216	Retrospective case-control	59.5 $\pm$ 16.6	130 (60.1%)	88 (40.7%)	34 (15.7%)	47 (21.7%)	Cholecalciferol oral: 25,000 IU/monthly in 11 patients. Calcifediol oral: 0.266 mg/monthly in eight patients	19 (8.7%) versus 197 (91.3%)
Jevalikar G et al. <sup>27</sup> 2021	409	Cross-sectional	46.3 $\pm$ 15.4	134 (68%)	163 (39.8%)	188 (45.9%)	N/A	Cholecalciferol oral: 60,000 IU	197 (48.1%) versus 212 (51.9%)
Ling SF et al. <sup>28</sup> 2020	444	Cross-sectional	73.3 $\pm$ 14.8	245 (55.2%)	197 (44.4%)	129 (29.1%)	N/A	Cholecalciferol oral: 40,000 IU weekly (47.9%), 20,000 IU twice weekly (28.8%), 20,000 IU weekly (11%) for a maximum of 7 weeks	73 (16.4%) versus 371 (83.6%)

TABLE 2 (Continued)

Study	Sample size	Design	Overall age mean $\pm$ SD	Male n (%)	Hypertension	Diabetes	Corticosteroid use	Vitamin D dose	Number of patients receiving Vitamin D versus Control
Murai IH et al. <sup>29</sup> 2021	237	Double-blind, randomized clinical trial	56.5 $\pm$ 13.8	133 (56.1%)	126 (53.1%)	84 (35.4%)	150 (63.2%)	Cholecalciferol oral: 200,000 IU single dose	119 (50.2%) versus 118 (49.8%)
Tan CW et al. <sup>30</sup> 2020	43	Prospective cohort	61.7 $\pm$ 7.5	26 (60.4%)	24 (55.8%)	6 (13.9%)	N/A	Cholecalciferol oral: 1000 IU/day for up to 14 days	17 (39.5%) versus 26 (60.5%)
Vasheghani M et al. <sup>31</sup> 2021	508	Cross-sectional	56 $\pm$ 17	264 (52%)	35 (7%)	116 (23%)	27 (5.3%)	Cholecalciferol oral: at least 50,000 IU during the past months	88 (17%) versus 420 (83%)

TABLE 3 Quality appraisal of studies included in the meta-analysis using Jadad scale assessment

Study	Random allocation	Concealment schemes	Blinding	Withdrawals and drop-out	Total score	Interpretation
Castillo ME et al. <sup>24</sup> 2020	2	1	0	1	4	Moderate quality
Murai IH et al. <sup>29</sup> 2020	2	2	2	1	7	High quality

Note: Points were determined as follows: (1) random allocation: computer-generated random numbers, 2 points; not described, 1 point; inappropriate method, 0 point; (2) allocation concealment: central randomization, sealed envelopes or similar, 2 points; not described, 1 point; inappropriate or unused, 0 point; (3) blindness: identical placebo tablets or similar, 2 point; inadequate or not described, 1 point; inappropriate or no double blinding, 0 point; and (4) withdrawals and drop-outs: numbers and reasons are described, 1 point; not described, 0 point. The Jadad scale score ranges from 1 to 7; higher score indicates better RCT quality. If a study had a modified Jadad score  $>4$  points, it was considered to be of high quality; if the score was 3–4 points, it was moderate quality; and if the score was  $<3$  points, it was low quality.

articles for its eligibility, 46 articles were excluded due to unavailable of results (still recruiting or withdrawn), 4 articles had no control or comparison group, 3 articles did not mention the criteria of the outcome of interest and 1 article because the full-text was not in English. The meta-analysis included 11 studies<sup>21–31</sup> with a total of 2265 Covid-19 patients (Figure 1). Out of 11 studies, one study was double-blind randomized clinical trial, one study was open-label randomized clinical trial studies, four were retrospective cohort studies, two were prospective cohort studies and three were cross-sectional studies. All of the vitamin D doses were administered orally, although the dosage varied between each of the included studies, ranging from 25,000 IU/month up to 200,000 IU/day for two consecutive days. The baseline characteristics and severity between the vitamin D group and the control groups were already controlled in each of the included studies, meaning that there is no significant difference between two groups' characteristics. Table 2 presents the characteristics of the studies.

### 3.2 | Quality of study assessment

Jadad scale assessments suggested that one clinical trial study was graded high quality, while another study was graded moderate

quality (Table 3). Quality assessment of cohort and case-control studies using NOS scale and cross-sectional studies using JBI Critical Appraisal checklist indicated all included studies had a good quality (Table 4 and Table 5). Altogether, all studies were acceptable to be further analysed using meta-analysis.

### 3.3 | Vitamin D and ICU admission of Covid-19 patients

Five studies ( $n = 772$ ) reported the effect of vitamin D on the ICU admission of Covid-19 patients. Our pooled analysis showed that vitamin D supplementation was associated with reduction of ICU admission rate (OR 0.27; 95% CI: 0.09–0.76,  $p = 0.010$ ,  $I^2 = 70\%$ , random-effect modelling; Figure 2a).

### 3.4 | Vitamin D and the need for mechanical ventilation

Six studies ( $n = 1516$ ) reported the effect of vitamin D on the need for mechanical ventilation outcome. The pooled analysis suggested that vitamin D supplementation was associated with reduction of the

TABLE 4 Newcastle–Ottawa quality assessment of observational studies

First author, year	Study design	Selection	Comparability	Outcome	Total score	Result
Annweiler C et al. <sup>21</sup> 2020	Cohort	***	**	***	8	Good
Annweiler G et al. <sup>22</sup> 2020	Cohort	***	**	***	8	Good
Cangiano B et al. <sup>23</sup> 2020	Cohort	***	**	**	7	Good
Giannini S et al. <sup>25</sup> 2021	Cohort	***	**	***	8	Good
Hernandez JL et al. <sup>26</sup> 2020	Case-control	***	**	**	7	Good
Tan CW et al. <sup>30</sup> 2020	Cohort	***	**	**	7	Good

Note: \*\* means the score is 2, \*\*\* means the score is 3. All the scores were sum up to get the final score and to conclude the quality of included studies.

TABLE 5 Joanna Briggs Institute Critical Appraisal tool for cross-sectional study

	Jevalikar G et al. <sup>27</sup> 2021	Ling SF et al. <sup>28</sup> 2020	Vasheghani M et al. <sup>31</sup> 2021
1. Were the criteria for inclusion in the sample clearly defined?	Yes	Yes	Yes
2. Were the study subjects and the setting described in detail?	Yes	Yes	Yes
3. Was the exposure measured in a valid and reliable way?	Yes	Yes	Yes
4. Were objective, standard criteria used for measurement of the condition?	Yes	Yes	Yes
5. Were confounding factors identified?	Yes	Yes	Yes
6. Were strategies to deal with confounding factors stated?	Yes	Yes	Yes
7. Were the outcomes measured in a valid and reliable way?	Yes	Yes	Yes
8. Was appropriate statistical analysis used?	Yes	Yes	Yes
Overall appraisal	Include	Include	Include

need for mechanical ventilation (OR 0.34; 95% CI: 0.16–0.72,  $p = 0.005$ ,  $I^2 = 61\%$ , random-effect modelling; Figure 2b).

### 3.5 | Vitamin D and mortality of Covid-19 patients

Ten studies ( $n = 2223$ ) reported on mortality. The pooled estimate showed that vitamin D supplementation was associated with reduction of mortality from Covid-19 (OR 0.37; 95% CI: 0.21–0.66,  $p < 0.001$ ,  $I^2 = 50\%$ , random-effect modelling; Figure 2c).

### 3.6 | Meta regression

Our meta-regression suggested the association between vitamin D supplementation and mortality was affected by age ( $p = 0.027$ ; Figure 3a), meaning that older people will gain more protective measures towards mortality in comparison with younger people. The association between vitamin D supplementation and mortality was not affected by gender ( $p = 0.191$ ; Figure 3b), hypertension ( $p = 0.566$ ; Figure 3c), diabetes ( $p = 0.608$ ; Figure 3d), nor the use of corticosteroids ( $p = 0.070$ ; Figure 3e).

### 3.7 | Publication bias

Funnel plot analysis showed an asymmetrical inverted-plot for the ICU admission outcome (Figure 4a) and the need for mechanical ventilation outcome (Figure 4b), showing some indication of publication bias. Funnel plot analysis showed a relatively symmetrical inverted-plot for the mortality outcomes (Figure 4c). Begg and Mazumdar rank-correlation test were not statistically significant for ICU admission ( $p = 0.086$ ), the need for mechanical ventilation ( $p = 0.060$ ) and mortality outcome ( $p = 0.371$ ), showing no indication of publication bias. However, because the number of included studies in the ICU admission and mechanical ventilation outcomes are fewer than 10 studies, the funnel plots and statistical tests for detecting publication bias are not very reliable when compared with larger numbers of included studies in each outcome.<sup>32,33</sup>

## 4 | DISCUSSION

According to our pooled analysis, vitamin D supplementation had an association with a reduction of ICU admission rate, reduction in the need for ventilators and reduction of mortality from Covid-19. This

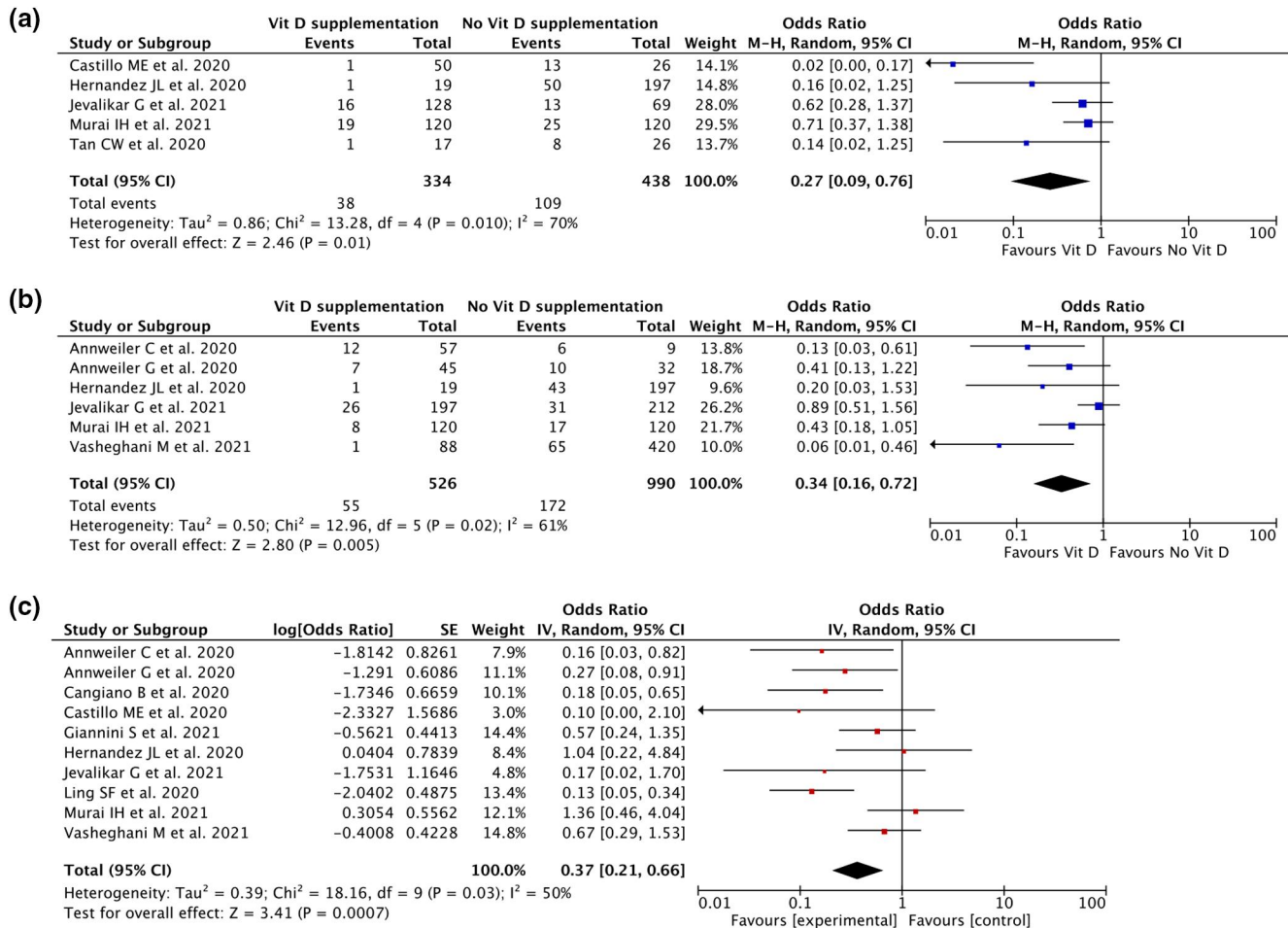


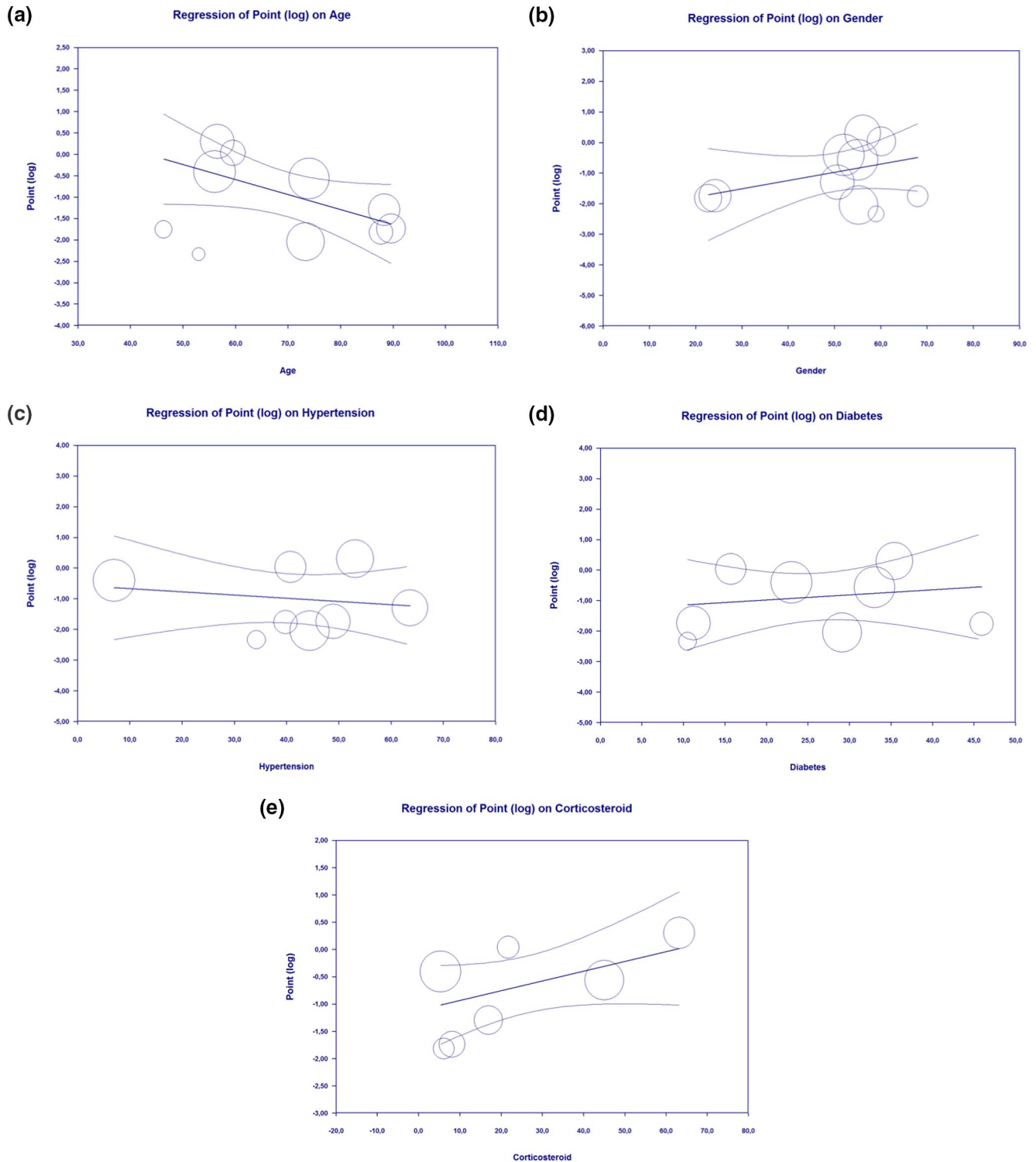
FIGURE 2 Forest plot that demonstrates the association of vitamin D supplementation with ICU admission rate (a), the need for mechanical ventilation (b) and mortality (c) outcomes

meta-regression reveals that age affects the association between vitamin D supplementation and Covid-19 mortality.

There are some explanations how vitamin D could affect the prognosis of Covid-19 patients. The interaction of SARS-CoV-2 infection and vitamin D are presented briefly in Figure 5. Both innate and adaptive arms of the immune system, triggered by SARS-CoV-2 infection, cause a destructive inflammatory reaction, resulting in local and systemic complications. The host immune or the inflammatory reaction is one of many severity-determining variables as proven by significant association between inflammatory proteins/indicators and disease severity.<sup>34,35</sup> Exhaustion markers (e.g., CD8+ T cells, NK cells, NKG2A) are increased during acute symptomatic period of Covid-19 and then return to normal in the convalescent period. Cytotoxic lymphocytes and natural killer cells aid control of viral infection, and both may serve as a predictor in determining the severity of the disease. Throughout the course of Covid-19 infection, Natural killer and cytotoxic lymphocytes will eventually reach functional exhaustion, as indicated by reduced total number.<sup>36</sup> Severe Covid-19 patients have high levels of various inflammatory proteins such as C-reactive protein, D-dimer and cytokines, including IL-6, IL-1 $\beta$ , TNF- $\alpha$ , also known as cytokine storm.<sup>37</sup> IL-6 can be used as a

good indicator of poor outcome in Covid-19 patients who suffer ARDS.<sup>38,39</sup> Cytokine storm leads to a severe pulmonary infiltration by neutrophils and macrophages that causes severe alveolar injury with hyaline membrane formation and alveolar wall thickening.<sup>38</sup> The cytokine storm increases inflammatory mediators and oxidative stress, while concomitantly reducing endothelial nitric oxide synthase. All these processes result in systemic inflammation and endothelial dysfunction, and ultimately cause hemodynamic instability, tissue injury and multiple organ failure.<sup>40</sup>

There are three main mechanisms through which vitamin D may reduce the risk of infection: enhance physical barriers, cellular innate immunity and adaptive immunity. Vitamin D strengthens cellular immunity by induction of antimicrobial peptides, including human cathelicidin LL-37, and through 1,25-dihydroxyvitamin D and defensins.<sup>41</sup> Cathelicidins are known to have direct antimicrobial effect towards a variety of pathogens comprising both Gram negative and positive bacteria, non-enveloped and enveloped viruses, and fungi.<sup>42</sup> Cathelicidins also promote the chemotaxis of cellular immunity to the site of infection and its expression was upregulated by vitamin D especially in respiratory epithelial cells, which play a major role in host defenses.<sup>42</sup> Furthermore, on the cellular level, vitamin D



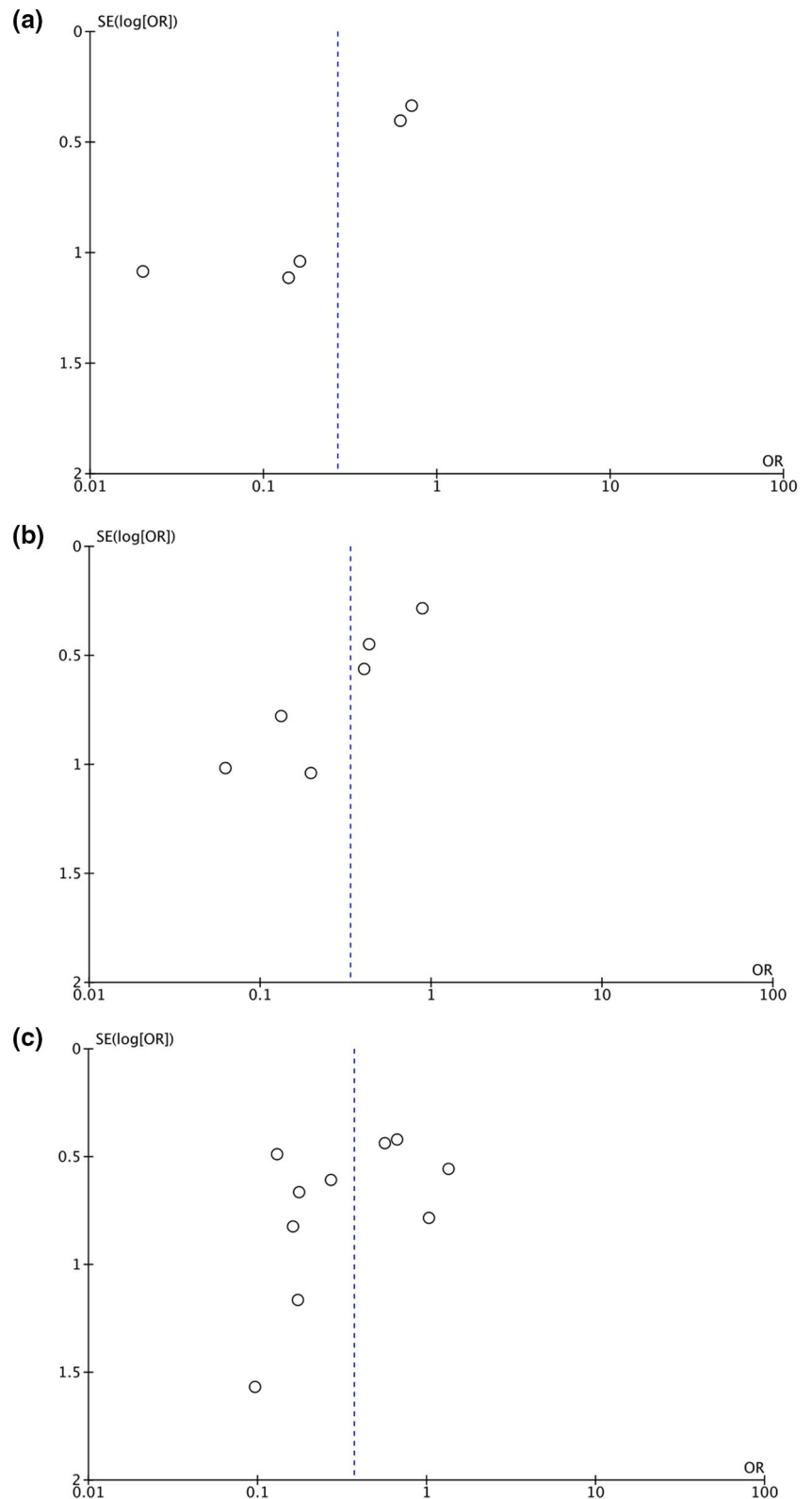
**FIGURE 3** Bubble-plot for meta-regression. Meta-regression analysis showed that the association between vitamin D supplementation and mortality outcome was affected by age (a), but not by gender (b), hypertension (c), diabetes (d) and the use of corticosteroids (e)

also stimulates gap junction genes, tight junction genes and adherens genes (e.g., E-cadherin) to strengthen cellular junction integrity and improve cell to cell communication, therefore maintaining the inter-cellular junctions to prevent further invasion of microorganisms, including viruses, which may lead to further inflammation and tissue

damage.<sup>43,44</sup> The vitamin D receptor (VDR) is possessed by the majority of immune cells including macrophages, B and T lymphocytes, neutrophils and dendritic cells. VDR activation leads to downstream cell signalling that produces immunomodulatory, anti-proliferative, and pro differentiative effects.<sup>42</sup> Vitamin D helps to reduce

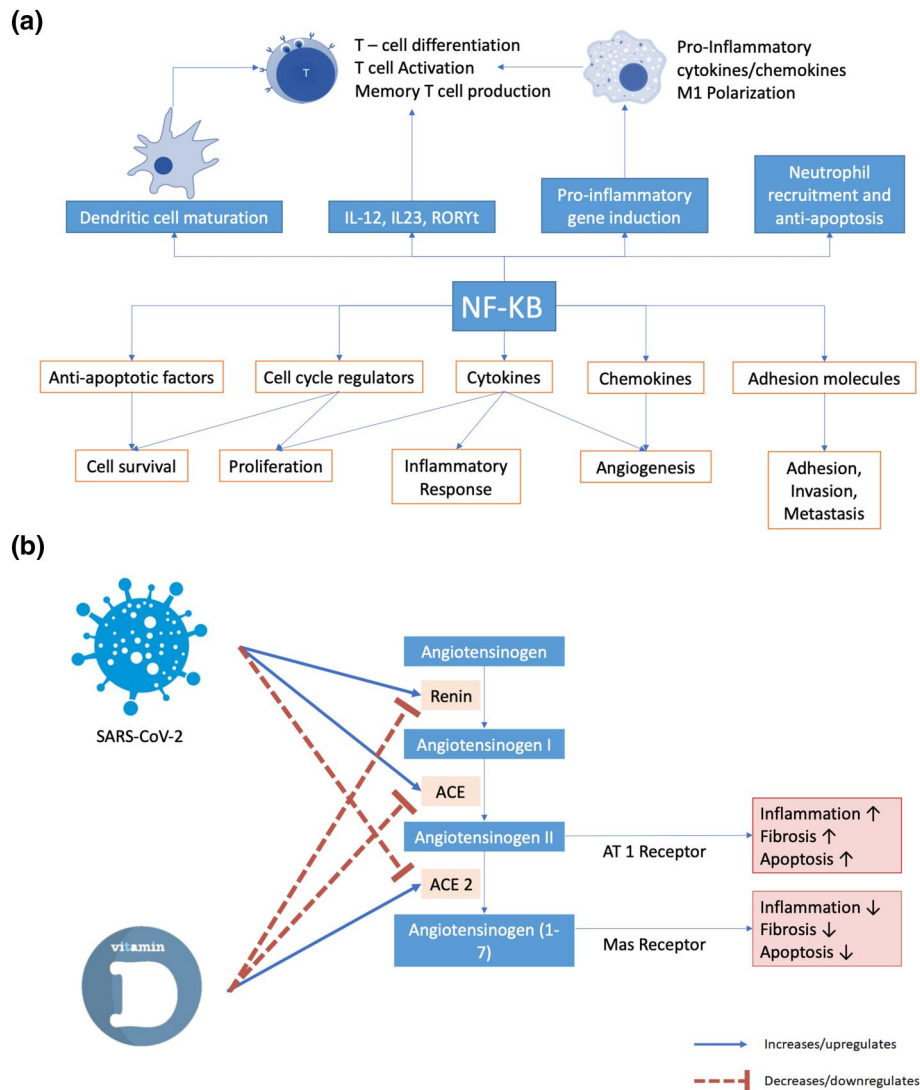


**FIGURE 4** Funnel plot analysis for the association of vitamin D supplementation with ICU admission (a), the need for mechanical ventilation (b) and mortality (c) outcomes



pro-inflammatory T helper 1 (Th1) cytokines, TNF- $\alpha$  and interferon (IFN)- $\gamma$  and helps the production of Th2 lymphocyte cytokines which indirectly suppress Th1 cells.<sup>41,45</sup> Additional evidence that supports immunoregulatory function of vitamin D is that 1,25(OH)<sub>2</sub>D<sub>3</sub> or calcitriol, the active form of vitamin D, is able to induce monocyte

differentiation into macrophage-like form.<sup>42</sup> VDR expressed by monocytes sensitizes them to the differentiating effects of calcitriol (autocrine mechanism for cell maturation). Vitamin D modulates macrophage response, and thus prevents overproduction of inflammatory cytokines and chemokines.<sup>42</sup> Calcitriol downregulates



**FIGURE 5** The role of vitamin D in suppressing the nuclear factor kappa B (NF- $\kappa$ B) signalling pathway (a) and the proposed potential therapeutic effect of vitamin D for COVID-19 and induced acute respiratory distress syndrome (b)

granulocyte-macrophage colony stimulating factor but stimulates the immunosuppressive prostaglandin E<sub>2</sub> production by the macrophages.<sup>41</sup> Importantly, vitamin D deficiency impairs macrophage maturation.<sup>41</sup> Several studies have shown that the risk of Covid-19 increases in people with vitamin D deficiencies; furthermore, lower concentration also contributes to the development of ARDS.<sup>46,47</sup> Aside from that, vitamin D has also been known to induce the production of type I IFNs, which able to suppress and keeping viral replication under control causing the prevention of further inflammatory response.<sup>48,49</sup> Several studies have reported that severe form of Covid-19 is frequently related with increased hypercoagulable state, which could be worsen by excessive inflammation and may accelerates upcoming thrombotic events. Vitamin D has the potential role to promote antithrombin and thrombomodulin gene expression to suppress that hypercoagulable state, causing significant protection during the course of severe form of Covid-19.<sup>49–51</sup> Vitamin D supplementation and serum levels above 50 ng/ml have

been observed and may help in reducing severity and the course of viral diseases including Covid-19.<sup>41</sup>

Another role of vitamin D in the pathogenesis of Covid-19 is through its ability to inhibit the RAS and nuclear factor kappa B (NF- $\kappa$ B) pathway. Because ACE2 has a protective role against lung injuries, interference with that receptor by SARS-CoV-2 might be linked with ARDS. Lung injury caused by ischaemia and reperfusion are promoted by the increased expression of ACE/Ang II/AT1R axis and reduced levels of ACE2/Ang-(1-7). ACE2 plays its protective function to the lung through Ang-(1-7)/MasR pathway and prevents activation of NF- $\kappa$ B pathway/extracellular signal-regulated kinase.<sup>52</sup> The 1 $\alpha$ ,25 (OH)<sub>2</sub>D<sub>3</sub>, the biological active form of vitamin D, contributes its protective role and reduces lung injury by regulating RAS biosynthesis (renin, ACE/Ang II/AT1R axis and ACE2/Ang-(1-7) axis stimulation).<sup>53</sup> The VDR and calcitriol also prevent lung, liver and kidney fibrosis through the downregulation of RAS and the inhibition of NF- $\kappa$ B and wnt/ $\beta$ -catenin.<sup>54,55</sup>

A cohort study that investigates the influence of genetic variation in vitamin D pathway found that three SNP in VDR (rs4334089, rs11568820 and rs7970314) are associated with increased risk of upper respiratory tract infection.<sup>56</sup> A systematic review and meta-analysis study which evaluates the association between VDR polymorphism and severe Respiratory Syncytial Virus (RSV)-bronchiolitis supports the association between FokI polymorphism and severe RSV infection. This study also examines the role of six VDR polymorphisms (Cdx, A1012G, FokI, BsmI, Apal and TaqI) on infection susceptibility to enveloped virus found that a polymorphism at locus rs2228570 (FokI) is associated with viral infections.<sup>57</sup> The TT genotype and T allele were reported to be risk factors for infections with enveloped viruses, including RSV.<sup>58,59</sup> Being an enveloped virus, it may be the same case for SARS-CoV-2. The mechanism that explains the association of FokI polymorphism with increased viral infection is that the FokI polymorphism creates a shorter VDR proteins that causes higher rate of transcription driven by the NF- $\kappa$ B, increased IL-12 and higher lymphocyte proliferation.<sup>56</sup> Therefore, supplementation with vitamin D may help in ameliorating those potentially negative effects from viral infection. All of these properties and roles might explain the benefit of vitamin D on the outcomes of Covid-19.

This study has some limitations. Significant heterogeneities were identified on most of the outcomes of interests included in this study. This was probably caused by the difference in the given vitamin D doses and co-administered medications with vitamin D as Covid-19 treatment. Importantly, we have made rigorous efforts to ensure that only sound studies were included, and several pre-print studies were included to minimize the risk of publication bias.

## 5 | CONCLUSION

Our meta-analysis indicates that vitamin D supplementation had an association with favourable outcomes of Covid-19, compromising reduction in the rate of ICU admission, reduction in mechanical ventilation usage and reduction of mortality rate from Covid-19. This study suggests that vitamin D might be a potential therapeutic agent for the management of Covid-19 to give better outcomes for the patients. However, more randomized clinical trial studies are still necessary and should be done to confirming the results of our study. Finally, vitamin D might be considered as an essential drug for future Covid-19 therapy models.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTION

Conceptualization, methodology, formal analysis, data curation, writing-original draft, visualization, writing-review and editing: Timotius Ivan Hariyanto. Conceptualization, methodology, formal

analysis, data curation, writing-original draft, writing-review and editing: Denny Intan. Conceptualization, validation, supervision, writing-review and editing: Joshua Edward Hananto. Conceptualization, validation, supervision, writing-review and editing: Harapan Harapan. Conceptualization, validation, supervision, writing-review and editing: Andree Kurniawan.

## DATA AVAILABILITY STATEMENT

Data analysed in this study were a re-analysis of existing data, which are openly available at locations cited in the reference section.

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