The role of vitamin D in outcomes of critical care in COVID-19 patients: Evidence from an umbrella meta-analysis of interventional and observational studies

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Abstract:

Objectives: Several meta-analyses have suggested the beneficial effect of vitamin D on patients infected with SARS-CoV-2. This umbrella meta-analysis aims to evaluate influence of vitamin D supplementation on clinical outcomes and the mortality rate of COVID-19 patients.

Design: Present study was designed as an umbrella meta-analysis. The following international databases were systematically searched till March 2023: Web of Science, PubMed, Scopus, and Embase.

Settings: Random-effects model was employed to perform meta-analysis. Using AMSTAR critical evaluation tools, the methodological quality of the included meta-analyses was evaluated.

Participants: Adult patients suffering from COVID-19 were studied.

Results: Overall, 13 meta-analyses summarizing data from 4 RCTs and 9 observational studies were identified in this umbrella review. Our findings revealed that vitamin D supplementation and status significantly reduced mortality of COVID-19 [Interventional studies: (ES= 0.42; 95% CI: 0.10, 0.75, p <0.001; I^2 = 20.4%, p=0.285) and observational studies (ES= 1.99; 95% CI: 1.37, 2.62, p <0.001; I^2 = 00.0%, p=0.944). Also, vitamin D deficiency increased risk of infection and disease severity among patients.

Conclusion: Overall, vitamin D status is a critical factor influencing the mortality rate, disease severity, admission to ICU and being detached from mechanical ventilation. It is vital to monitor the vitamin D status in all patients with critical conditions including COVID patients.

Keywords: Vitamin D; Mortality; Intensive care unit; Critical illness; COVID-19; Umbrella meta-analysis.

Introduction:

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), caused a novel pandemic named coronavirus disease 19 (COVID19). SARS-CoV-2 generates an inflammatory status and induces the production of c-reactive protein (CRP), d-dimer, interleukin-6 (IL-6), etc. which could lead to acute distress syndrome (ARDS) especially in the second week due to cytokine storm [(1)]. Besides auxiliary drugs to treat and reduce the complications of COVID-19 such as corticosteroids, no proven drugs have been generated yet and the search for current available medications has been prioritized.

Vitamin D is a vital component in modulation of the immunological response in both infectious and autoimmune diseases in different ways [(2)]. A substantial body of evidence indicates that active form of vitamin D (1,25 dihydroxy vitamin D) is essential for the modulation of innate and adaptive immunity (T lymphocytes activation and B lymphocytes proliferation) [(3)], reduce the risk of cytokine storm and proinflammatory markers [(4)] and maintenance of pulmonary barrier integrity [(5)]. In case of vitamin D deficiency these mechanisms will fail and make host vulnerable to different types of infections such as respiratory diseases. Several studies now support that vitamin D sufficiency has a beneficial effect on acute respiratory tract infections [(6-8)] and attenuates the risk of respiratory tract infections. Initially, it was indicated that vitamin D deficiency could lead to higher mortality rates, longer stay in intensive care unit (ICU), higher mechanical ventilation rate and its severity. Hence, during the pandemic, vitamin D attracts an attention on COVID-19 treatment and its complications.

Relationship between vitamin D in COVID-19 outcomes is not based on solid evidence. High heterogeneity among the meta-analysis studies lead to dubious results on the effects of vitamin D on COVID-19 severity and its complications and majority of the reviews remained inconclusive. Several meta-analyses have shown that vitamin D sufficiency and supplementation has a positive impact on COVID-19 outcomes [(9-12)]. While, others did not support these results [(13-15)]. Therefore, present umbrella meta-analysis aimed to assess the role of vitamin D on clinical outcomes such as ICU admission, mechanical ventilation rate, severity and mortality in COVID-19 positive patients to provide valid and authentic evidence.

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2. Method and materials:

Present umbrella meta-analysis has been developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines. The question of this study was based on PICO criteria: Participants (Patients suffering from COVID-19), Intervention (Vitamin D supplementation or status), Comparison (Control), Outcome (risk of infection, ICU admission, mechanical ventilation rate, severity and mortality).

2.1. Search strategy and study selection

The scientific international databases including Web of Science, PubMed, Scopus and EMBASE were searched up to March 2023 to identify relevant studies. The search strategy was developed using the following MeSH and title/abstract keywords. The full search strategy for all databases is presented in **Supplementary Table 1**. The wild-card term"*" was utilized to enhance the sensitivity of the search method. Also, the articles were confined to English language.

2.2. Inclusion and exclusion criteria

Systematic reviews and meta-analysis studies of investigating the effects of vitamin D were included in the current umbrella meta-analysis if they reported the effect of vitamin D on COVID-19 positivity status, severity, infection status, ICU admission, mechanical ventilation, and prognosis including effect sizes (ES) and corresponding confidence intervals (CI). In vitro, in vivo, and ex-vivo studies were excluded from this meta-analysis of meta-analyses.

2.3. Quality assessment

The quality evaluation of the methodology of the included studies was examined by two reviewers (VM, and FHK), using the AMSTAR [(16)] independently. The AMSTAR questionnaire consists of 11 questions in which reviewers must respond with “yes”, “no”, “not applicable” or “can't answer”. Eleven is the highest score. Articles with a score of 7 or higher are regarded to be of good quality.

2.4. Data extraction

Two independent reviewers (FHK, and VM) screened the studies based on the eligibility criteria. In the first stage, the title and abstract were evaluated. Second, the full text of relevant papers was reviewed to determine whether the study could be included in the umbrella meta-analysis. All discrepancies were resolved by senior author's decision (MZ).

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The year of publication, sample size, study location, study types, vitamin D deficiency definition, ESs [(weighted mean difference (WMD), standardized mean difference (SMD), OR, RR and HR] and CIs for COVID-19 positivity status, severity, infection status, ICU admission and mortality, mechanical ventilation, and mortality.

2.5. Data synthesis and statistical analysis

The overall effect size was calculated by combining the ES and CI for each included meta-analysis. A random-effects model was employed to perform the analysis. I^2 statistics and Cochrane's Q test were used to determine between-study heterogeneity; in the matter of I^2 value >50% or P<0.1 for the Q-test, it was regarded as significant heterogeneity. Sensitivity analysis was conducted to determine whether the overall effect size was associated with the removal of one specific study from overall analysis. Begg's test was used to assess publication bias. If the p-value for Begg's test was <0.05, trim and fill analysis was carried out to adjust the publication bias. Stata software version 17.0 (Stata Corporation, College Station, TX, US) was used for all of the statistical analyses. P< 0.05 was considered as significant level.

3. Results

3.1. Systematic review

In initial search, a total of 1,432 citations were identified. After discarding duplicates and screening of the remaining studies, of the 19 full-texts, 13 meta-analyses summarizing data from 4 RCTs and 9 of observational studies were included in the present analysis. The PRISMA flow chart of the screening process is presented in in **Fig. 1**. All included studies were published from 2019 to 2021. About 712,354 participants in observational studies and 4,191 participants in experimental studies were included in this review. Observational studies were conducted in Iran [(17)], Turkey [(4)], China [(11)], Brazil [(9)], Ethiopia [(18)], Ireland [(19)], Lebanon [(15)], Poland [(2)], and USA [(20)]. Three of four experimental studies were conducted in India [(5, 12, 14)] and one in Iran [(21)]. Calcifediol, cholecalciferol, and calcitriol were types of vitamin D supplementation which used in experimental studies. **Table 1** provides the details of characteristics of included observational and experimental studies reviewed.

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3.2. Risk of bias assessment

Based on AMSTAR questionnaire, all included meta-analysis studies evaluated as good quality. The quality score of six out of 13 studies was 10 and 11, and the remaining studies scored 8 and 9. The results are presented in **Table 2**.

3.3. Meta-analyses on vitamin D and COVID-19 mortality

3.3.1. Interventional studies

The pooled results of the 3 meta-analyses [5, 14, 21] indicated that vitamin D supplementation significantly decreased mortality (ES= 0.42; 95% CI: 0.10, 0.75, p <0.001; I^2 = 20.4%, p=0.285). Sensitivity analysis showed that the removal of 1 study (Rawat et al.) affected the overall effect size (ES= 0.47; 95% CI: -0.13, 1.08) **Fig. 2A.**

3.3.2. Observational studies

The results of the present umbrella meta-analysis from 5 studies indicated that vitamin D deficiency significantly increased mortality (ES= 1.99; 95% CI: 1.37, 2.62, p <0.001; I^2 = 00.0%, p=0.944) **Fig. 2B.**

3.4. Meta-analyses on serum vitamin D and COVID-19 positivity status

The pooled results of the 3 meta-analyses did not show any significant relation between serum vitamin D and positive cases of COVID-19 (ES= 2.12; 95% CI: 0.96, 3.27, p=0.063; I^2 = 89.4%, p <0.001) **(Fig. 3A).**

3.5. Meta-analyses on serum vitamin D deficiency and risk of infection in COVID-19 patients

Four meta-analyses were included in the analysis of the relation between vitamin D deficiency and risk of infection. Vitamin D deficiency significantly increased the risk of infection among COVID-19 patients (ES= 1.64; 95% CI: 1.40, 1.88, p <0.001; I^2 = 67.3%, p =0.027) **(Fig. 3B).**

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3.6. Meta-analyses on serum vitamin D and COVID-19 severity

The pooled results of the 3 meta-analyses indicated a significant association between vitamin D deficiency and COVID-19 severity. Vitamin D deficiency increased severity of COVID-19 (ES= 1.77; 95% CI: 1.45, 2.10, p <0.001; I^2 = 00.0%, p =0.463). Asma Kazemi et al. study was excluded from the analysis due to the wide CI and insignificant weight (weight= 0.02) **(Fig. 4).**

3.7. Systematic Reviews on vitamin D and other major health related outcomes in COVID-19:

Associations between vitamin D and ICU admission, mechanical ventilation, and prognosis as the other health related outcomes in COVID-19 have been reviewed in studies.

3.8. ICU admission

Two review studies have assessed the impact of serum vitamin D status on ICU admission and severity of COVID-19. One study reported a positive but insignificant trend between vitamin D deficiency and increased risk of ICU admission [15]. The second study reported high prevalence of vitamin D deficiency among severe COVID-19 cases compared to mild cases [9]. In another study Pooled analysis of unadjusted data from observational and RCT studies showed that vitamin D supplementation in COVID-19 was significantly associated with reduced ICU admission [12]. The results regarding ICU admission and vitamin D were contradictory in two systematic review of experimental studies: Rawat et al. found that vitamin D didn't reduce ICU admission rates [5], while Shah et al. reported lower ICU admission rate in patients supplemented with vitamin D compared to patients without supplementation [14].

3.9. Mechanical ventilation

Results regarding vitamin D and mechanical ventilation from two systematic review studies did not show any significant positive effect of vitamin D serum status or vitamin D supplementation on reducing risk of invasive, and non-invasive mechanical ventilation [5, 15].

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3.10. Poor prognosis

Finally, review of five studies revealed that patients with poor prognosis had significantly lower serum levels of vitamin D compared to those with good prognosis [20].

Discussion

The current umbrella meta-analysis summarizes 13 meta-analyses, 57 observational studies and 23 randomized controlled trials (RCT). According to results, vitamin D supplementation was efficient in reducing mortality, and vitamin D deficiency significantly increased mortality, severity of COVID-19, and risk of infection among patients. In addition, lower serum levels of vitamin D was significantly associated with poor prognosis. However, there was no significant relationship between serum vitamin D and positive cases of COVID-19, and the results regarding ICU admission and vitamin D were contradictory. Furthermore, results didn't show any significant positive effect of vitamin D serum status or vitamin D supplementation on reducing risk of invasive or/and non-invasive mechanical ventilation. Due to limited number of studies for each variable, sub-group analyses was not possible.

In this umbrella meta-analysis, we discussed the multiple aspects of vitamin D deficiency and risk of mortality and COVID-19 health status outcomes. Vitamin D is a fat-soluble vitamin with anti-inflammatory, antioxidant, and antiviral features [21]. The regulatory role of vitamin D on acquired and innate immunity, explains its possible role in infectious diseases such as COVID-19 [18]. Based on the findings of clinical trials, vitamin D supplementation is efficient in reducing mortality. The beneficial effects of vitamin D in treating COVID-19 is by preventing "cytokine storm" and subsequent ARDS, known as the main cause of mortality [22]. After activation of the angiotensin-converting enzyme 2 (ACE2) receptor by the coronavirus, vitamin D provides its protective role via activating the renin–angiotensin–aldosterone system (RAAS), modulating the cytokine storm and neutrophil activity, maintaining the pulmonary epithelial barrier, stimulating epithelial repair, and reducing the damage caused by pro-inflammatory cytokines. Moreover, vitamin D augments the activity of the ACE2/Ang (1–7) axis, which has anti-inflammatory and antioxidant functions and also suppresses renin and the ACE/Ang II/AT1R axis, thereby enhancing the expression and concentration of ACE2, MasR and Ang-[1–7] [5, 15, 23].

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Vitamin D increases cathelicidin (LL-37)/defensin expression and displays antimicrobial and antiviral activities. Cathelicidin and defensin, furthermore, stimulate the expression of antiviral cytokines and chemokines involved in the recruitment of monocytes/macrophages, natural killer cells, neutrophils, and T cells and eventually enhance the host defense. The vitamin D receptor and CYP27B1 dignify the expression and cellular production of cathelcidin and defensin, which is effected by the interactions of pathogens and membrane pattern recognition receptors, including toll-like receptor and toll-like receptor 2 [(4)]. Additionally, vitamin D indorses the upregulation of Interleukin (IL-10) (anti-inflammatory cytokine), and downregulation of IL-1, IL-6 (proinflammatory cytokines), and tumor-necrosis factor-alpha (TNF-α) [(12)]. Vitamin D also increases the expression of genes involved in the antioxidant system, such as the glutathione reductase gene [(17)].

Although the majority of studies confirmed the efficiency of vitamin D supplementation in declining mortality; accurate evidence-based recommendations on circumstances of vitamin D administration in clinical practice, can be confirmed by well-designed randomized controlled trials on health outcomes of COVID-19 [(15)]. In this regard, different aspects of vitamin D supplementation in COVID-19 in RCTs must be discussed thoroughly. For example, some studies were accomplished on aged individuals which already have several comorbidities, are less exposed to sunlight, display lower 7-dehyrocholesterol values in the skin, have enhanced markers of cytokine release syndrome, and are at high risk of respiratory failure [(2, 9, 21)]. Also, study population was not stratified based on serum vitamin D status at baseline, since vitamin D-deficient patients benefit more from supplementation. Differences in the dose of supplementation, frequency of supplementation, route of prescription, and duration are other limiting factors [(21, 23)]. Heterogeneity in the study design, population characteristics, methodology, baseline characteristics, and small sample size of the population enrolled have also been mentioned in a number of studies [(5, 14, 21)]. Differences in the type and timing of vitamin D supplementation is another confounding factor. In regards to source of vitamin D, it has been mentioned that cholecalciferol supplementation may lead to faster recovery from COVID-19 [(15)]. Most studies administered 1,25-hydroxy cholecalciferol (DHCC), as the active form of vitamin D and few studies used calcefediol [(5)]. Moreover, one study indicated that patients supplemented with vitamin D after COVID-19 diagnosis, benefited more than those supplemented with the drug prior to the diagnosis [(12)].

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According to observational studies, there was an inverse relationship between vitamin D deficiency and mortality. Vitamin D deficiency is related with reduced innate cellular immunity and cytokine storm stimulation [11]. The mechanism of action of vitamin D and ACE has been discussed earlier. High levels of ACE have been observed in patients with severe COVID-19 with low vitamin D level [23]. Vitamin D receptors are present on the nuclei membrane and are responsible for regulating different defensive proteins and receptors. Receptors recognize pathogens and their interaction affect the expression of pathogenic genes. Vitamin D inhibits T helper type 1 (TH1) proliferation and shifts towards TH2 proliferation, leading to decline in oxidative compounds synthesized via TH1, affecting T-cells maturation, and producing anti-inflammatory subtypes [21]. McGregor et al., claimed that CD4+ T cells present in the bronchoalveolar lavage fluid (BALF) of patients diagnosed with COVID-19 are Th1-skewed and the genes induced by SARS-CoV-2 are regulated by VDR [24]. Furthermore, vitamin D induces transcription factors including STAT3 (signal transducer and activator of transcription 3), c-JUN and BACH2 (BTB Domain And CNC Homolog 2) that cooperatively suppress Th1 and Th17, and eventually induce IL-10 via IL-6-STAT3 signaling [23]. Jain et al., reported that inflammatory markers such as IL-6, TNF-α and serum ferritin levels were shown low in COVID-19 patients with serum vitamin D level below 50 nmol/L [25]. Additionally, high concentrations of transforming growth factor β have been observed in the acute phases of COVID-19 and are relatively suppressed by VDR [17]. Mechanistic pathways are comprehensively and schematically demonstrated in **Figure 5**.

The association between vitamin D deficiency and COVID-19 mortality must be discussed from other perspectives as well; for example, it is not clear whether low vitamin D is the cause or consequence of COVID-19. Multiple factors may affect the reduced vitamin D level in patients diagnosed with COVID-19, including age, sex, region, season, sun exposure, body mass index, comorbidities, and race. In favor to age, in the majority of studies, patients were over 50 years old with basic low vitamin D level [11, 15, 21]. Obesity alone is an independent risk factor for severe sequences of the disease [2]. COVID-19 broke out at winter when in the northern hemisphere, sunlight was low and individuals in that region had low 25-hydroxyvitamin D level [4, 11]. Moreover, patients were enforced to be isolated or hospitalized, which prevented them from obtaining sunlight and a balanced diet [11]. Ecological studies have revealed that people

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living in higher latitude with decreased vitamin D level are prone to infection, related complications, and mortality [21].

In regards to studies, Liu et al., claimed inconsistency in the number and sample size of included studies, significant heterogeneity, publication bias, and variations in effect size estimates as reasons for the inconsistent results observed [11]. Bassatne et al., reported low quality and inevitability of evidence, as well as variation in the definition of vitamin D deficiency, serum 25(OH)D cutoffs and the timing of blood sampling and COVID-19 diagnosis and related outcomes in the included studies. Also, decline in the synthesis of vitamin D binding protein and increase in 25(OH)D renal excretion which significantly regulate vitamin D level in critical illnesses [15].

Vitamin D deficiency also significantly enhanced the risk of COVID-19 infection and severity of COVID-19. According to the D-CIMA meta-analysis, patients with serum 25(OH)D< 20 ng/mL or 50 nmol/L were 1.64 times more likely to be infected with COVID-19 and also individuals with serum 25(OH)D< 20 ng/mL or 50 nmol/L were 2.42 times more likely to have severe COVID-19 [4]. One study claimed that vitamin D supplementation declined the frequency of infection and was beneficial in patients receiving daily or weekly doses of 25(OH)D, protective effects were stronger in patients with baseline 25(OH)D less than 25nmol/L, and that this relationship was not significant in those receiving bolus doses [19]. The mechanism of action is related to the disruption of the parathyroid-vitamin D-axis [26]. Moreover, vitamin D acts by stimulating the chemotaxis of T-lymphocytes and eliminating respiratory pathogens by inducing apoptosis and autophagy in the infected epithelium [4]. Hence, vitamin D declines the risk of microbial infection by modulating the innate and adaptive immunity, inhibiting cytokine storm, and declining pro-inflammatory cytokine production, due to its antiviral and anti-inflammatory properties [17, 18]. Several aspects of this association must be further discussed. It is not clear whether the low concentrations of 25(OH)D in patients with severe COVID-19 infection is a cause or consequence of severe COVID-19 infection. Three perspectives have been mentioned: First, absence of baseline 25(OH)D measurement prior to infection. Second, the concentration of C-reactive protein (CRP) was not measured for patients with severe COVID-19 infection. Third, 25(OH) D concentration decrease, as a consequence of inflammation, is considered solely as a negative acute phase reactant. Furthermore, a majority of studies did not report whether 25(OH)D concentrations was measured before or during COVID-19 infection [19].

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Patients with poor prognosis had significantly lower serum levels of vitamin D compared to those with good prognosis. One study claimed 25(OH)D concentration may be considered as a negative acute phase reactant and a poor prognosis in COVID-19 infection [19]. In Sun et al.'s study, 74% of patients with severe COVID-19 had low calcium and 25(OH)D level and hypoproteinemia. They reported hypocalcemia as a biomarker of clinical severity and prognosis [27]. As mentioned earlier, calcitriol as the active form of vitamin D is the regulator of renin-angiotensin system (RAS) and this overactivation is related to poor prognosis [2].

According to the results of the present study, there was no significant relationship between serum vitamin D and positive cases of COVID-19. Bassatne et al., reported uncertain evidence regarding the association between positive cases of COVID-19 and serum 25(OH)D levels <20 ng/ml; however, increasing the cutoff of low 25(OH)D levels to 30 ng/ml showed significant results [15]. Other studies showed that COVID-19 positive cases had lower vitamin D level compared to negative cases. However, significant heterogeneity and publication bias was reported in these studies [2, 11].

The results regarding ICU admission and vitamin D were contradictory. Bassatne et al., claimed an increased risk of ICU admission in COVID-19 patients with 25(OH)D levels<20 ng/ml, also indicated that calcifediol supplementation may have a protective effect on COVID-19 related ICU admissions [15]. Similarly, a pilot trial showed that only 1 out of 50 patients receiving calcifediol needed ICU admission, while 50% of patients not receiving vitamin D were admitted to ICU (odds ratio (OR)=0.03). However, the reported OR was unreliable mainly due to indeterminate allocation concealment and patient blinding [28]. One study [21] observed decline in ICU admission rate after vitamin D administration. However, this study did not include a RCT that had major influence on the findings of other studies which showed no association between ICU admission and vitamin D supplementation [5]. The main reason for the contradictory findings observed were the limited number of studies assessing the relationship between ICU admission and vitamin D.

The current study also didn't show any significant positive effect of vitamin D serum status or vitamin D supplementation on reducing risk of invasive, and non-invasive mechanical ventilation. One study showed that COVID-19 patients who required mechanical ventilation had at least on nutrient deficiency [2]. Hence, a clear association between vitamin D serum status and mechanical ventilation cannot be obtained. The main reason for the inconsistent results observed

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is the small number of studies assessing this association. The majority of studies didn't observe any significant results, and the few ones lacked important methodological qualifications [2, 5, 9, 17].

Strengths and limitations

The present study summarized the current evidences on the effects of vitamin D supplementation and deficiency in COVID-19 as the first umbrella meta-analysis. The current study was registered in PROSPERO or Cochrane library and several aspects of COVID-19 health status outcomes were assessed. Based on the AMSTAR questionnaire, all included meta-analyses were evaluated as high quality. The limitations were the significant heterogeneity observed in few outcomes and also, due to the limited number of studies, sub-group analysis was not possible. The novelty of the subject was in favor for the small number of studies included, especially RCTs.

Conclusion

The present umbrella of meta-analyses confirms the efficiency of vitamin D supplementation in reducing COVID-19 mortality. This review also indorses an inverse association between vitamin D deficiency and risk of mortality and infection among COVID-19 patients, and the severity of COVID-19. In addition, lower serum levels of vitamin D was significantly associated with poor prognosis in patients. Hence, vitamin D supplementation is supported for preventing catastrophic outcomes of COVID-19.

Conflict of interest

None.

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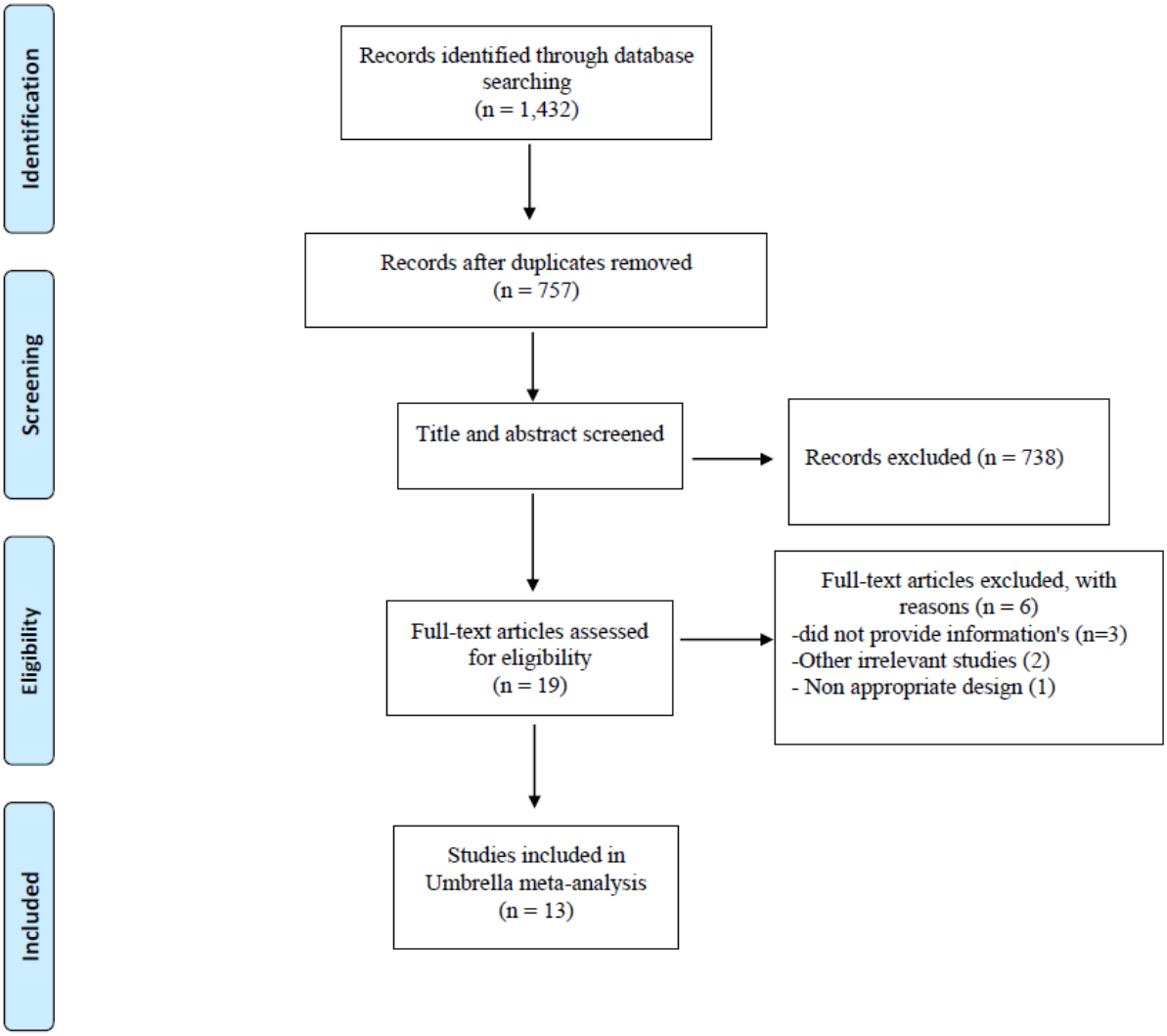

Figure 1. PRISMA flow chart of the study

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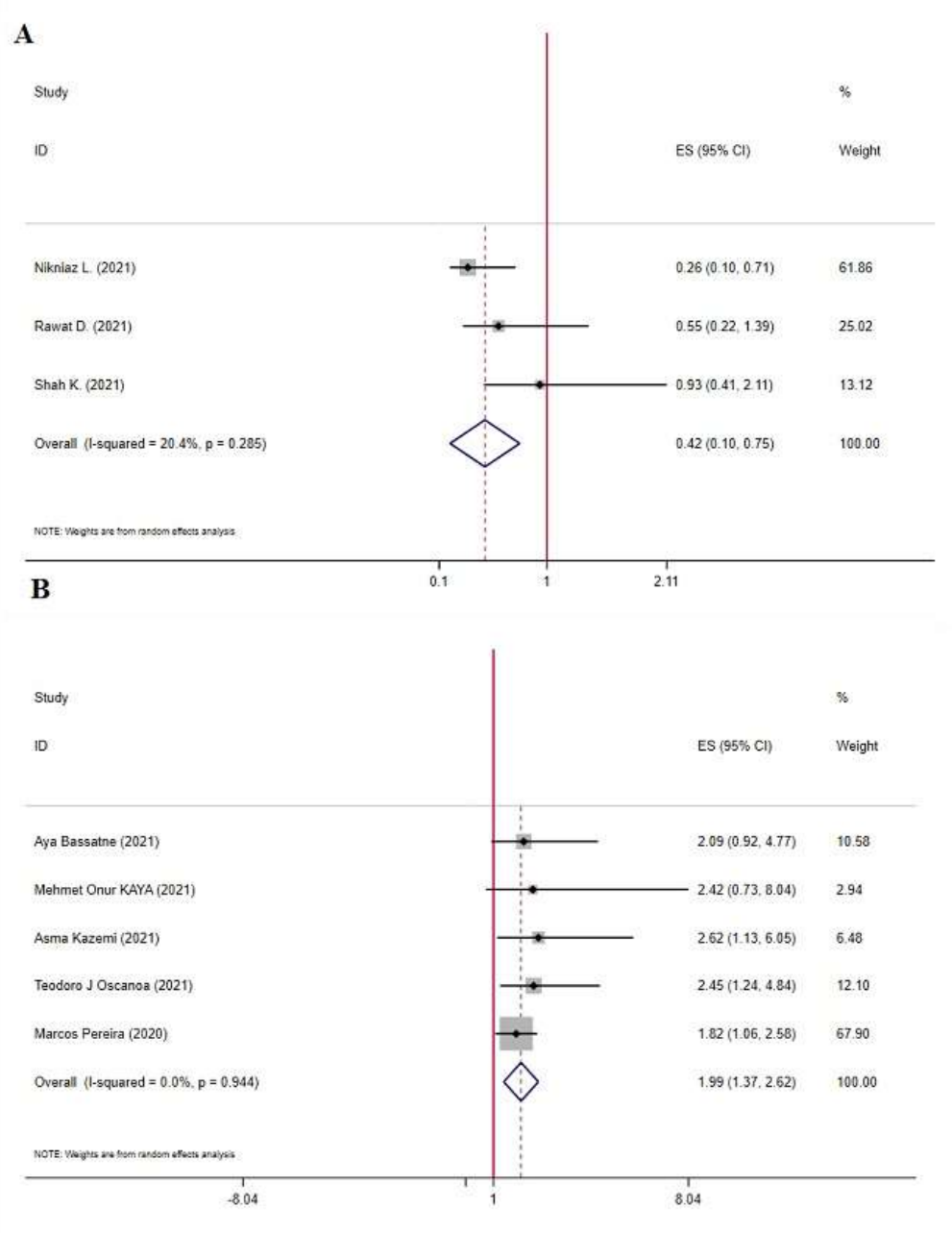

Figure 2. The Forest plot of umbrella meta-analysis on effect of vitamin D supplementation on mortality according to interventional studies (A) and observational studies (B).

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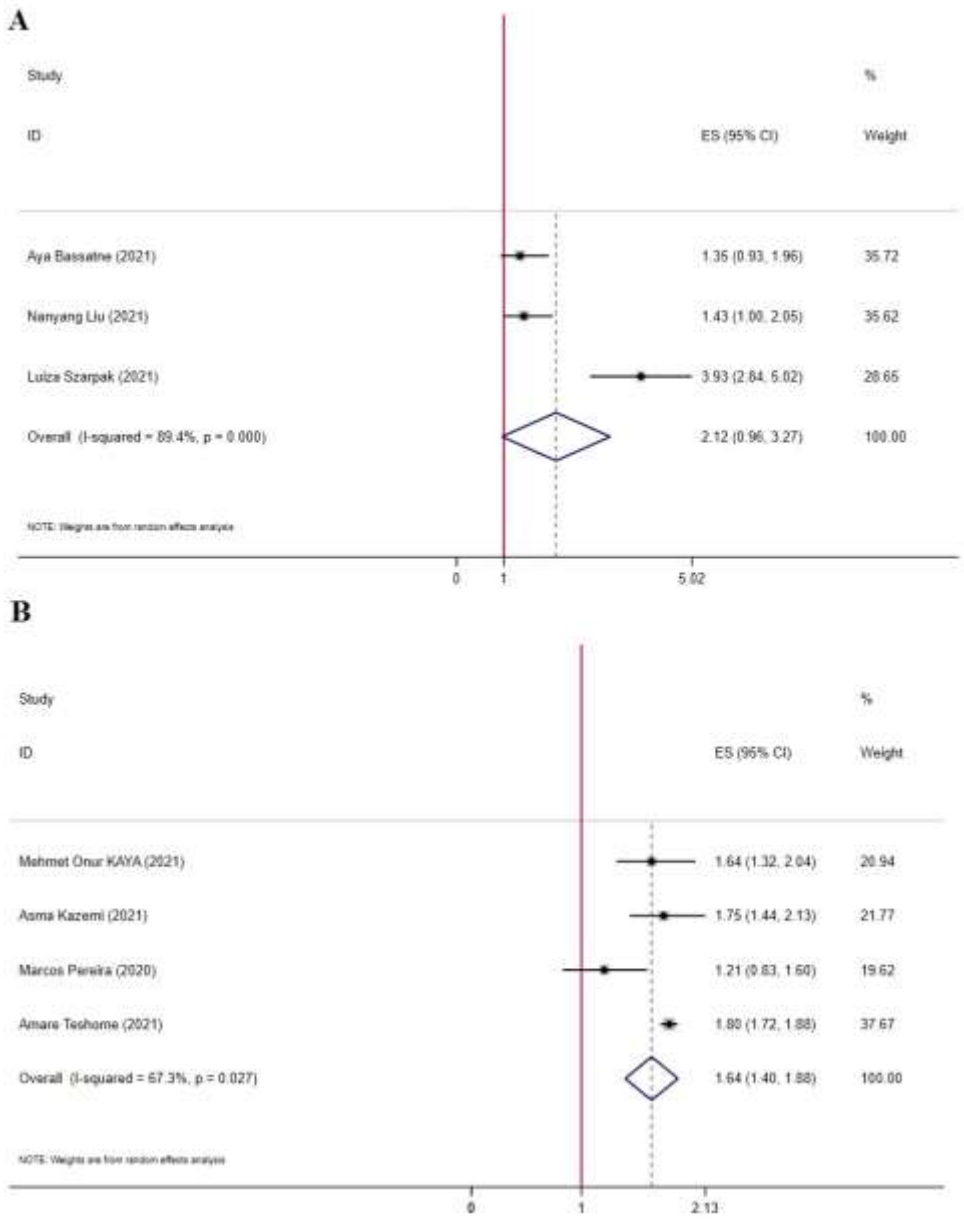

Figure 3. The Forest plot of umbrella meta-analysis on association of serum vitamin D with COVID-19 positivity status (A) and association of vitamin D deficiency with risk of infection in COVID-19 patients.

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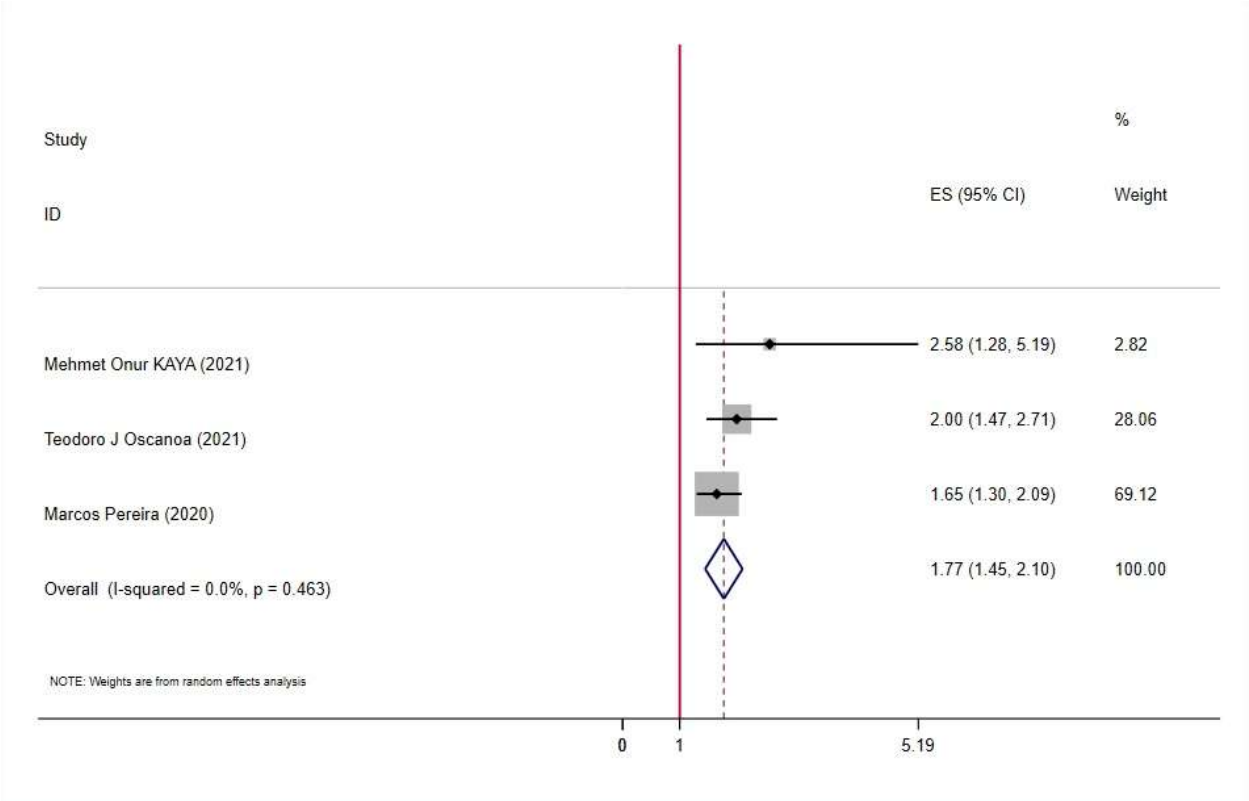

Figure 4. The Forest plot of umbrella meta-analysis on association of vitamin D deficiency with COVID-19 severity.

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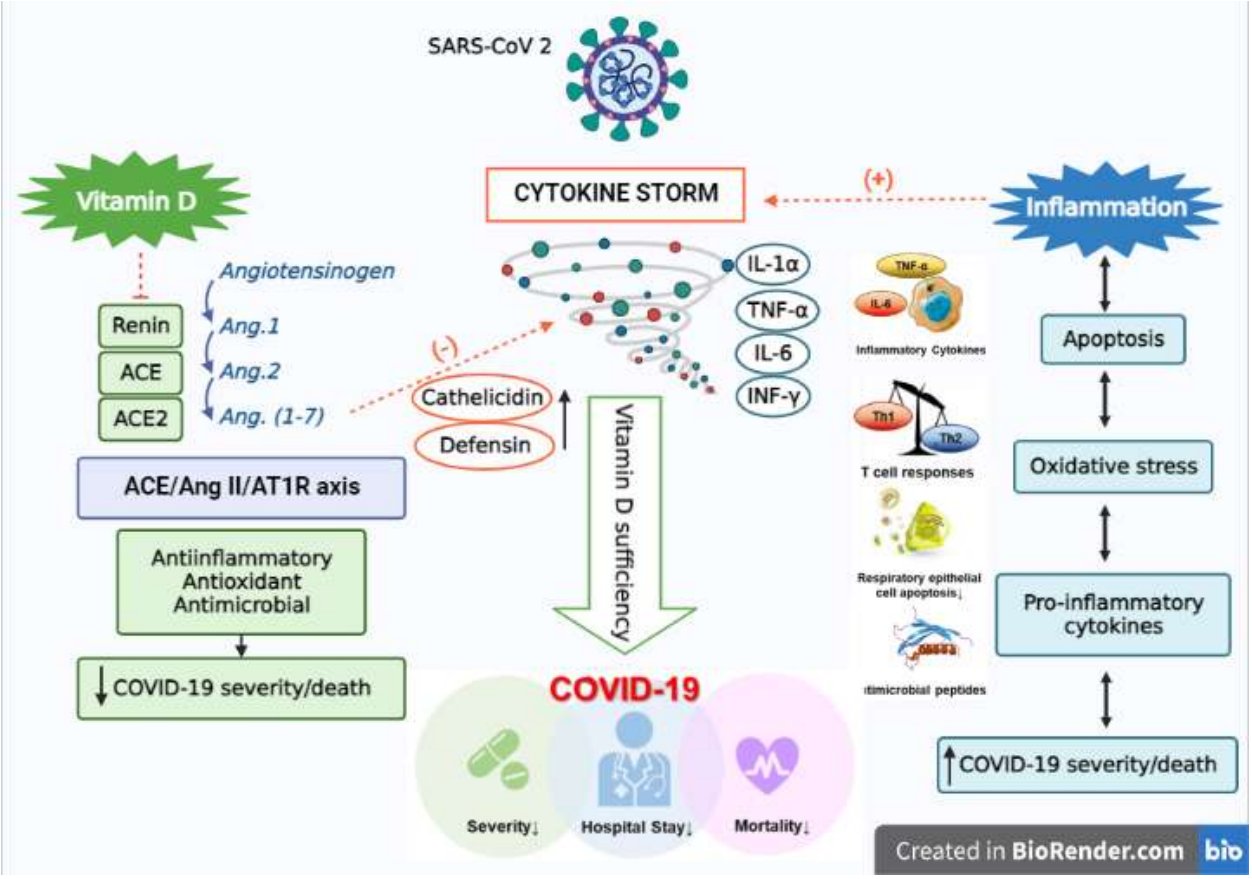

Figure 5. Mechanistic pathways demonstrating how vitamin D is affective on COVID-19 patients.

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Table 1. The characteristics of included meta-analyses

Citation (First author et al., year)	Country	NO. of participants	No. of Studies in Meta-analysis	Age (year)	Primary outecome / intervention	Study types	Quality Assessment Scale
Obsrvational study							
Kaya et al. 2021	Turkey	205,869	23	18-85	Occurrence of the risk of Covid-19 infection, severity and mortality	case-control, cohort, cross-sectional	Yes (NOS)
Kazemi et al. 2021	Iran	9110	31	7-81	Association of vitamin D status with COVID-19 severity	Case-control, cohort, cross-sectional	Yes (NOS)
Pereira et al. 2020	Brazil	8176,	26	35-81	Vitamin D deficiency and COVID-19 severity	Restrospective,Cohort, crosssectional	Yes (RTI–Item Bank)
Teshome et al. 2021	Ethiopia	91,120	14	NR	COVID-19 infection	Case-control, cohort, cross-sectional	Yes (JBI tools)
Oscanoa et al. 2021	Ireland	2692	23	30-60	Association between 25-hydroxyvitamin D concentration and SARS-CoV-2 infection severity or mortality	Case-control, cohort, cross-sectional	Yes (NOS)
Bassatne et al. 2021	Lebanon	18601	31	42-88	Mortality rate from COVID-19 infection	Case-control, cohort, cross-sectional	Yes (NOS)
Liu et al. 2021	China	361,934	10	18-81	Incident COVID-19	Case-control	Yes (NOS)
Szarpak et al. 2021	Poland	14,485	13	40-83	Incident COVID-19	Case-control, cohort	Yes (RoB 2 tool)
Munshi et al. 2020	USA	376	6	49-72	Association of vitamin D serum levels with COVID-19 severity and prognosis	Case-control, cohort	NR
Interventional study							
Nikniaz et al..2021	Iran	259	4	47-88	Impact of Vitamin D Supplementation on Mortality Rate / Calcifediol, Cholecalciferol, calcitriol	Clinical Trials, Quasi-Experimental, Interventional Pilot	Yes (JBI Critical Appraisal Tools)

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						Studies	
Rawat et al.2019	India	467	5	53-87	Impact of Vitamin D Supplementation on Mortality Rate and ICU addmission / Calcifediol, Cholecalciferol, calcitriol	Clinical Trials, Quasi-Experimental, Interventional Pilot Studies	Yes (Cochrane)
Shah et al.2021	India	532	3	NR	Impact of Vitamin D Supplementation on Mortality Rate and ICU addmission / Cholecalciferol, calcitriol	Retrospective case–control study, Clinical Trials	Yes (Cochrane)
Pal et al.2021	India	2933	13	47-74	Impact of Vitamin D Supplementation on ICU addmission / Calcifediol, Cholecalciferol	Quasi-experimental Study, Retrospective, Observational study, Prospective, Observational study	Yes (Cochrane)

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Table 2. Results of assessment of methodological quality of meta-analysis using AMSTAR questionnaire.

Study	A priori design	selection and data extraction	literature search	publication type	list of studies	characteristics of the included studies	assessed scientific quality	scientific quality formulating conclusions	methods used to combine the findings	assessed publication bias	conflict of interest stated	Quality score
Kaya et al. 2021	+	+	+	+	-	+	+	+	+	+	+	10
Kazemi et al. 2021	+	+	+	+	+	+	+	+	+	+	+	11
Pereira et al. 2020	+	+	+	-	-	+	+	+	+	+	-	8
Teshome et al. 2021	+	+	+	+	-	+	+	+	+	+	+	10
Oscanoa et al. 2021	+	+	+	-	-	+	+	+	+	+	-	8
Bassatne et al. 2021	+	+	+	+	-	+	+	+	+	+	+	10
Liu et al. 2021	+	+	+	+	-	+	+	+	+	+	+	10
Szarpak et al. 2021	+	+	+	-	-	+	+	+	+	+	-	8
Munshi et al. 2020	+	+	+	-	-	+	+	+	+	+	-	8
Nikniaz et al..2021	+	+	+	+	-	+	+	+	+	+	+	10
Rawat et al.2019	+	+	+	-	-	+	+	+	+	+	+	9
Shah et al.2021	+	+	+	-	-	+	+	+	+	+	-	8
Pal et al.2021	+	+	+	-	+	+	+	+	+	+	-	9

The result of assess the methodological quality using AMSTAR: each item for included studies (? : can't answer; - : means no; + : means yes).

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