

Vitamin D as a Preventive or Therapeutic Nutrient in COVID-19 Infection

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Abstract

SARS-CoV-2 has caused a global pandemic with limited treatment options available. Some studies have proposed that vitamin D may be associated with Covid-19 infection outcomes. The aim of this literature review is to analyze the evidence currently available from Randomized Controlled Trials (RCTs) and quasi-experimental studies regarding the impact of vitamin D used as a preventative or adjunctive therapeutic agent on the disease severity and mortality rate of Covid-19 patients. A literature search was performed using PubMed, Science Direct, and ProQuest. Sources listed in the citations of systematic reviews and meta-analyses were also evaluated. Articles meeting the inclusion criteria were selected after title, abstract, and article content were reviewed. Five studies, including two RCTs, met inclusion criteria. One RCT did not find significant benefit with vitamin D3 supplementation. The remaining studies, including mostly elderly participants using various vitamin D supplementation forms and doses, reported a reduced need for ICU treatment, faster recovery, decreased severity, and an improved survival rate for Covid-19 patients. An initial loading dose (21,280 IU Calcifediol) and regular supplementation of vitamin D (10,000-60,000 IU/day when ill to monthly doses of 50,000 IU or 80,000-100,000 IU/2-3 months) resulted in the greatest effectiveness. The results of this review suggest that regular vitamin D supplementation may be associated with reduced Covid-19 infection severity and mortality rate. However, more RCTs are needed to provide further robust evidence for more participants of different demographics and give insight into the most appropriate doses, forms, and timeline of vitamin D supplementation for patients with SARS-CoV-2.

Keywords: Vitamin D3; Calcifediol; Randomized controlled trials; Recovery rate; Survival rate

Introduction

The Covid-19 pandemic has caused a significant impact on a global scale. In late 2019, cases of an unknown type of pneumonia were first reported in Wuhan, China. Later identified as a novel type of coronavirus, this unknown virus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), or more commonly given the term Covid-19 [1]. Since then, researchers have sought potential treatment options for this dangerous virus with urgency, as there have been more than 44 million cases in the United States alone, causing more than 708,200 deaths as of September 2021 [2]. Globally, this virus is thought to have caused more than 4.8 million deaths [2]. SARS-CoV-2 infection occurs mainly through exposure to respiratory droplets from coughing, sneezing, speaking, singing, etc., when an infected person is in close contact with others [1]. The median incubation period is 4-5 days from the time of exposure, but it may take up to 14 days for symptoms to appear [1]. As an RNA virus, SARS-CoV-2 uses its spike "S" glycoproteins, which give it the "corona/crown" distinction, to bind to Angiotensin-Converting Enzyme 2 (ACE2) receptors once activated by the TMPRSS2, a serine protease [3]. The viral RNA then enters the cell via endocytosis, and viral replication initiates and infects more cells [4].

During viral replication, a person may remain asymptomatic [4]. In mild cases, estimated to occur in 81% of patients based

on one study in China, common symptoms include fever, dry cough, fatigue, myalgia, shortness of breath, headache, anosmia or ageusia, nausea, vomiting, or diarrhea [1]. Severe cases may include pneumonia and severe dyspnea. In critical situations, often developing around 10-12 days after the onset of symptoms (and estimated to occur in approximately 5% of cases), SARS-CoV-2 patients may develop respiratory failure, Acute Respiratory Distress Syndrome (ARDS), elevation in multiple inflammatory cytokines, sometimes termed as a cytokine storm, thromboembolic disease, septic shock, acute cardiac injury, acute kidney injury, and multi-organ/system failure or death [1,4]. Although most symptoms point to pulmonary disease, SARS-CoV-2 can also lead to cardiac, renal, hepatic, dermatological, hematological, and neurological complications [1]. In children, the multisystem inflammatory syndrome has also been a complication. Symptoms in these cases include severe abdominal pain, multisystem inflammation, shock, cardiac dysfunction, and, rarely, coronary artery aneurysm [1]. Certain characteristics and comorbid conditions have been found to contribute to the increased risk of severe or critical illness. These factors include older age, cardiovascular disease, respiratory disease, diabetes, hypertension, cancer, renal disease, obesity, sickle cell disease, pregnancy, other immunocompromising conditions, and organ transplantation recipients. The mortality rate is highest in individuals aged >70 yrs., regardless of the presence of other

conditions [1].

One step that must be taken when considering possible treatment options is analyzing the pathology of SARS-CoV-2. It is understood that the early course of the infection is driven by viral replication. The main driver of illness in the latter part of infection is an exaggerated immune and inflammatory response, which may involve tissue damage [5]. Therefore, antiviral and anti-inflammatory/immunosuppressant therapies are most often considered. Currently, remdesivir, an antiviral agent, is the only drug approved by the FDA for the treatment of Covid-19 in hospitalized patients [5]. It aids by inhibiting viral entry, the activity of enzymes associated with facilitating viral entry, membrane fusion, or endocytosis [4]. However, some studies point to its lack of benefit in advanced stages of the disease, especially in people receiving mechanical ventilation [5]. The National Institutes of Health (NIH) treatment guidelines highlight dexamethasone, a corticosteroid, to improve the survival of hospitalized patients who are receiving mechanical ventilation [5].

Furthermore, thanks to Operation Warp Speed, whose aim has been to produce vaccines to immunize the American public against Covid-19, vaccines have been developed and are currently being administered across the country. The mRNA vaccines developed by Moderna and Pfizer have since reported 95% efficacy and are found to be effective in preventing severe disease [6]. More recently, Johnson & Johnson has developed a vaccine based on an adenovirus carrying DNA for coronavirus spike protein, which has been reported to have reduced severe disease alone by 85% and prevented 100% Covid-related hospitalization or death [7]. In addition, AstraZeneca and the University of Oxford developed a vaccine based on adenovirus-based DNA, which is mainly authorized to be used in European countries. This vaccine is effective at preventing Covid-19, with no hospitalizations or severe cases in people receiving it. A combined analysis of two trials showed an average efficacy of AstraZeneca is 70% [8]. Russia's Sputnik V vaccine is also approved by Russia's National Research Center for Epidemiology and Microbiology, which claimed an efficacy rate of 92% after the second dose [9]. The vaccine shares one of its two human adenoviral vectors with AstraZeneca to increase the AstraZeneca vaccine's efficacy [9]. China-based Sinovac Biotech developed a vaccine based on inactivated SARS-CoV-2 virus and reported a 50.38% -90% varying efficacy in clinical trials in Brazil, Indonesia, and Turkey [10]. Novavax developed a full-length, perfusion Covid spike protein-based vaccine [NVX-CoV2373], which showed an efficacy of 89.3% in its Phase III trial in the U.K [10].

Although these medications and strategies for preventing Covid-19 are expected to have a major impact in addressing the Covid-19 pandemic, other methods and possible interventions continue to be sought. One of these strategies' stems from the apparent suggestions from epidemiological reviews in Europe that found an association between vitamin D deficiency in the population and increased Covid-19 mortality [11]. When

examining the possible mechanisms of vitamin D's therapeutic use in Covid-19, researchers found that vitamin D, through its modulatory effect on the body's inflammatory functions, may have both antiviral effects and mechanisms that help in reducing the severity of Acute Respiratory Distress Syndrome (ARDS) [12]. A previous meta-analysis had found vitamin D supplementation to reduce the risk of acute respiratory infections when the regimen consisted of daily Vitamin D \geq 2000 IU following a loading dose of $<60,000$ IU [12]. Thanks to this and other preliminary data pointing to these associations, numerous retrospective studies and a few randomized controlled trials have been performed in regard to vitamin D and Covid-19. Several systematic reviews have examined the current evidence available and have found that serum vitamin D status may determine the risk of Covid-19 infection, severity, and mortality [12]. In one such review, vitamin D deficiency was found to be associated with Covid-19 severity, hospitalization, and odds of ICU admission [13]. Another recent study found that the risk of having a positive Covid-19 test for black individuals was 2.64-fold greater with a vitamin D level less than 40 ng/mL than it was with a level greater than 40 ng/mL [14]. Another meta-analysis that examined retrospective studies found that Covid-19 patients with a poor prognosis had significantly lower levels of vitamin D when compared to patients with a good prognosis [15]. In contrast, another systematic review did not find a significant association between vitamin D deficiency and infection but found that severe cases of Covid-19 presented with 64% more vitamin D deficiency compared to mild cases [16]. However, the most compelling evidence regarding the use of vitamin D as an adjunctive therapy agent comes from randomized controlled trials and clinical trials as they can determine whether there was a direct association between vitamin D supplementation and Covid-19 clinical outcome while controlling confounding factors. Therefore, using data from preliminary randomized controlled trials and quasi-experimental studies, this review sought to analyze what evidence currently exists from randomized controlled trials and clinical trials regarding the impact of vitamin D used as a preventative and adjunctive therapy agent on the disease severity and mortality

Materials and Methods

The standard literature review pertaining to the topic of interest was conducted from January to February 2021. Eligible articles were sourced from the PubMed, Science Direct, and ProQuest databases. The aim was to include quasi-experimental and randomized-controlled articles that examined the impact of vitamin D use on disease severity and mortality of Covid-19 patients. Search terms regarding vitamin D and Covid-19 were used as described in detail below. Initially, current review articles and meta-analyses regarding Covid-19 and vitamin D were reviewed, and relevant articles referenced in these reviews were further studied. Two of these referenced articles were included in the review as they met the inclusion criteria. Then, additional articles were chosen for review that directly addressed the review question and met the inclusion criteria. The inclusion criteria were checked for several items: that the piece was

published within the last five years, that it was peer-reviewed or stated as pre-print, that it described a randomized controlled trial or clinical trial or quasi-experimental study with vitamin D supplement administration and that it addressed the outcomes of the review question. The review question asked, what evidence currently exists from randomized controlled, quasi-experimental, and clinical trials regarding the impact of vitamin D used as a preventative and adjunctive therapy agent on the disease severity and mortality rate of Covid-19 patients?

Search Strategies and Data Collection

Terms used in the search included the following combinations: "Covid-19" or "SARS-CoV-2" and "Vitamin D" or "Cholecalciferol" or "Calciferol" or "Calcifediol" and "Randomized-Controlled Trial" or "Quasi-experimental" or "Systematic Review." All results were reviewed on PubMed, Science Direct, and ProQuest, and then article titles were screened to filter out irrelevant articles. If studies appeared relevant to the topic of interest, the abstracts were read to further screen for relevance, and if the study met the criteria for review, it was reviewed. On PubMed, results were additionally filtered as "randomized-controlled trials" or "clinical trials." On PubMed, this filtered search resulted in five studies, only three of which were relevant as one was a study protocol without results, and the other did not address the objectives of the review question. These three articles, along with those obtained from other databases, brought the total number of articles relevant to the parameters of this review to five. These include two randomized-controlled trials [17,18], one randomized, open-label double-masked clinical trial [19], and two quasi-experimental studies that will be reviewed in continuation [20,21].

Results

The review included a total of 499 adult individuals. Individuals in each of these studies either received a vitamin D supplement of various dosage rates and durations or were in the placebo or no-intervention group. In the quasi-experimental studies, some of the vitamin D supplementations were taken before the Covid-19 diagnoses in some participants [20, 21]. Table 1 provides a summary of the studies reviewed.

The first study, published in August 2020, was conducted at Reina Sofia University Hospital in Cordoba, Spain [19]. This pilot study, part of a larger study COVIDIOL, sought to assess the clinical effectiveness of treatment of hospitalized Covid-19 patients with *calcifediol* (25(OH)D₃) in the early stages of Covid-19. The objective of this study was to evaluate whether this treatment may reduce ICU admissions and death. This was a parallel pilot randomized open-label, double-masked clinical study. It included 76 patients hospitalized with a Covid-19 acute respiratory infection (exhibited by a positive PCR exam and radiographic evidence of viral pneumonia), excluding pregnant women and patients under 18 years of age. The mean age of the participants was 53 ± 10 years, and it included 45 men and 31 women. All patients received the best available therapy

(hydroxychloroquine-400 mg every 12 hours on Day 1 + 200 mg every 12 hours on the following days; and azithromycin-500 mg orally for 5 days). Patients were separated into two groups at a 2:1 ratio, with 50 patients receiving the best available therapy and high-dose oral calcifediol supplements (0.532 mg/21,280 IU on the 1st day + 0.266 mg/10,640 IU on Days 3 and 7 and then weekly until discharge or ICU admission) and 26 patients received only the best available therapy [19].

As far as the results of the patients treated with calcifediol, one patient (2%) was admitted to the ICU, none died, and all were discharged without complications. Among the patients not treated with calcifediol, 13 patients (50%) were admitted to the ICU, and two died, with the remaining patients being discharged without complications ($p < 0.001$) [19]. Multivariate Risk Estimate Odds Ratio assessment for ICU inpatients with calcifediol treatment vs. without calcifediol treatment showed that ICU was 0.02 (95% CI 0.002– 0.17) and when adjusted by hypertension (HTN) and Diabetes Mellitus Type 2 (DM2), it was 0.03 (95% CI: 0.003-0.25). This study demonstrated that the use of high-dose calcifediol significantly reduced ICU admission of Covid-19 patients requiring hospitalization and that calcifediol seems to be effective in reducing the severity of the disease. It should be noted that the NIH no longer recommends the standard therapy used in this study and that some weaknesses of the study include the study design, the lack of baseline vitamin D serum concentration, lack of BMI, and race collection data (other risk factors pertaining to Covid-19). This study raised several questions: What would happen if calcifediol use is compared to cholecalciferol use, as calcifediol is more readily absorbed? What if calcifediol was administered in earlier or later stages of Covid-19? Would the results differ if the patients were treated with only high-dose calcifediol without the standard therapy of hydroxychloroquine, azithromycin, or ceftriaxone medications? While this study showed promise for the use of calcifediol supplementation for Covid-19 patients, it also exposed research gaps and highlighted the need for more extensive randomized controlled trials that examine the use of vitamin D as an adjunctive therapy for Covid-19 patients.

A randomized, placebo-controlled study known as the SHADE study was conducted by Rastogi et al. [17]. Contrary to the previous study reviewed, this study included participants who were only mildly symptomatic or asymptomatic but SARS-CoV-2 positive and vitamin D deficient (25(OH)D <20 ng/ml). The patients who required invasive ventilation or who had significant comorbidities were excluded. The aim of the study was to examine the effect of high-dose oral cholecalciferol supplementation on viral clearance, which would be measured as participants who would be SARS-CoV-2 negative before day 21 [17]. Furthermore, a change in inflammatory markers was a secondary outcome and would be measured with levels of fibrinogen, D-dimer, procalcitonin and (CRP), and ferritin. The study included 40 participants who received standard care for the SARS-CoV-2 infection and pre-existing comorbidities as per the institute protocol (not described). The intervention group ($n = 16$, 6

Table 1: Randomized Trails and Quasi-Experimental Studies on Vitamin D in COVID

Name of Trial	Design	Sample Size	Age	Intervention	Vitamin D Dose	Placebo	Outcome	Results	Conclusion	References
Effect of Calcifediol Treatment and Best Available Therapy versus Best Available Therapy on Intensive Care Unit Admission and Mortality Among Patients Hospitalized with Covid-19: A Pilot Randomized Clinical Trial	Parallel pilot randomized open-label, double-masked clinical trial.	76; 50 patients with intervention and 26 patients without	53 +/- 10 yrs.	Calcifediol Treatment: Day of Admission: 0.532 mg/21,280 IU Calcifediol, Day 3 and 7: 0.266 mg/10,640 IU Calcifediol; then weekly until discharged + Standard Care	Loading dose of 21,280 IU then 10,640 IU on Day 3 and 7 and then weekly	Standard Care	Effects of Calcifediol on Intensive Care Unit Admission Rates and Mortality	2% (n = 1) of Patients in Calcifediol Group required ICU admission vs. 50% (n = 13) of Untreated Patients, P < 0.001; No deaths with Calcifediol Group vs. 2 Deaths with Untreated Group	Administration of High-Dose Calcifediol or 25-Hydroxy vitamin D significantly reduced the need for ICU treatment of patients requiring hospitalization due to Covid-19. Calcifediol may reduce the severity of the disease.	[19]
Short-term, High-dose Vit. D Supplementation for Covid-19 Disease: A Randomized, Placebo-Controlled Study (SHADE study)	Randomized, placebo-controlled study	40 participants, 16 with intervention and 24 in control	NA	Daily high-dose cholecalciferol for 7 days with a therapeutic target of 25(OH)D > 50 ng/mL; supplementation continued after 7 days until target reached	60,000 IU cholecalciferol/day	Received no supplement, only standard care	The proportion of Patients SARS-CoV-2 negative before day 21 and change in inflammation markers.	Intervention Group: 10/16 patients achieved >50 ng/mL by day 7 and additional 2 patients achieved >50 ng/mL by day 14; 62.5% of patients became Covid-19 negative (n = 10); Fibrinogen levels decreased significantly p = 0.007 (intergroup difference of 0.70 ng/mL); Control Group: 20.8% (n = 5) became Covid-19 negative	A greater proportion of vitamin D-deficient individuals with Covid-19 infection turned Covid-19 negative with a significant decrease in fibrinogen on high-dose cholecalciferol supplementation.	[17]
Effect of Single High dose of vitamin D3 on Hospital Length of Stay in Patients with Moderate to Severe Covid-19: A Randomized-Clinical Trial	Multicenter, Double-Blind, Randomized Controlled Trial	240 Patients (232 Patients analyzed)	56 years	A single oral dose of 200,000 IU vitamin D3	Single-dose of 200,000 IU vitamin D3	Received no supplement	Hospital Length of Stay, Mortality, Admission to ICU, Mechanical Ventilation Requirement, Serum Levels of 25(OH)D, Creatinine, Calcium, C-reactive Protein, D-dimer levels	Of 232 analyzed Patients: Hospital Length of Stay was comparable (7 days for both groups) Rate of Mortality (7.0% vs. 5.1%, p = 0.59), Admission to ICU (7.0% vs. 14.4% p = 0.90) didn't significantly differ between groups Vitamin D3 Supplementation significantly increased 25(OH)D levels compared to placebo (difference of 24 ng/ml p = 0.001)	Supplementation was safe and increased 25(OH)D levels but didn't significantly reduce the length of stay or other relevant outcomes. This trial does not support the use of Vitamin D3 supplements as adjuvant therapy.	[18]
Vitamin D and Survival in Covid-19 Patients: A Quasi-Experimental Study	Quasi-Experimental Study	66	87.7 +/- 9 years	Received Bolus vitamin D3 during Covid-19 or in the Preceding Month	Single Dose 80,000 IU Vitamin D3 every 2-3 months given without serum D-level knowledge	Received no vitamin D3 supplementation	Covid-19 mortality and OSCI score in the acute phase	82.5 % of participants in the Intervention group survived Covid-19, compared to only 44.4 % in the Comparator group (p = 0.023). Kaplan-Meier distributions showed that the Intervention group had a longer survival time than the Comparator group (log-rank p = 0.002). Vitamin D3 supplementation was inversely associated with OSCI score for Covid-19 (β = -0.84 [95 %CI: -6.07; -1.62], p = 0.001).	Bolus vitamin D3 supplementation during or just before Covid-19 was associated in frail elderly with less severe Covid-19 and better survival rate.	[20]
Vitamin D Supplementation Associated to Better Survival in Hospitalized Frail Elderly Covid-19 Patients: GERIA-COVID Quasi-Experimental Study	Quasi-Experimental Study	77 Patients	88 +/- 5 years	Group 1: Regularly supplied with Vit. D over the year preceding Covid-19 Group 2: Supplied with Vit. D after Covid-19 diagnosis	Group 1: 50,000 IU vitamin D3 every month or 80,000 IU or 100,000 IU vitamin D3 every 2-3 months	No vitamin D Supplementation	14-day mortality and the highest score on OSCI measured in the acute phase	In Group 1 (n = 29), 93.1% of Covid-19 participants survived at day 14, compared to 81.2% survivors in Group 2 (n = 16) (p = 0.33) and 68.7% survivors in Group 3 (n = 32) (p = 0.02). Group 1 had longer survival time than Group 3 (log-rank p = 0.015), although there was no difference between Groups 2 and 3 (log-rank p = 0.32). Group 1, but not Group 2 (p = 0.40), was associated with lower risk of OSCI score ≥5 compared to Group 3 (odds ratio = 0.08, p = 0.03).	Regular bolus vitamin D supplementation was associated with less severe Covid-19 and better survival in frail elderly.	[21]

males and 10 females with average age 50 years) received high-dose vitamin D supplementation in the form of oral nano-liquid droplets (60,000 IU cholecalciferol/day) with standard care and the control group (n = 24, 14 males and 10 females with average age 47.5 years) only received standard care but no vitamin D

supplementation. The daily supplementation occurred for seven days with a therapeutic target of 25(OH)D > 50 ng/mL [17]. On day 7, 25(OH)D levels were assessed, and supplementation was continued if the therapeutic target was not reached. The serum inflammatory markers were also measured periodically.

Baseline 25(OH)D level for the intervention group was 8.6 (7.1 to 13.1) ng/mL and 9.54 (8.1 to 12.5) ng/mL for the control group ($p = 0.730$). The results showed that for the intervention group, 10 out of 16 participants achieved >50 ng/mL of 25(OH)D by day seven, and an additional two patients achieved >50 ng/mL of 25(OH)D by day 14. In total, 75% of the intervention participants achieved the desired 25(OH)D levels by day 14. Ten (62.5%) participants from the intervention became SARS-CoV-2 negative, while five (20.8%) participants of the control group became SARS-CoV-2 negative within 21 days [17]. Furthermore, fibrinogen levels significantly decreased in the intervention group compared to the control (intergroup difference 0.70 ng/mL; $p = 0.007$). This study indicated that therapeutic high-dose cholecalciferol supplementation led to a greater proportion of vitamin D-deficient individuals who were Covid-19 positive to become Covid-19 negative, indicating that this supplementation was useful for viral SARSCoV-2 RNA clearance. Supplementation with 25(OH)D also led to a significant decrease in fibrinogen levels. This outcome raises some questions to consider for further study: Would high-dose cholecalciferol use also shorten the illness length for individuals with severe symptoms? What level of 25(OH)D use is the most effective in providing immunomodulatory benefits? Would cholecalciferol supplementation influence inflammatory cytokine levels? It is furthermore of interest to note that according to the NIH health professionals' fact sheet, the recommendation for adequate vitamin D serum levels for adequate bone and overall health for healthy individuals is ≥ 20 ng/mL and that >50 ng/mL is linked to potential adverse effects (Vitamin D Fact Sheet for Health Professionals, 2020). This study aimed for a therapeutic target of 25(OH)D >50 ng/mL as an arbitrary cutoff point as the appropriate level for 25(OH)D for immunomodulatory effects is unknown [17].

The third randomized-controlled trial examined in this review was published in February 2021 [18]. This multicenter, double-blind, randomized controlled trial was conducted at two sites in Sao Paulo, Brazil, to examine whether a single high dose of vitamin D3 would significantly affect hospital length of stay in patients with Covid-19. Additional secondary outcomes included mortality during hospitalization, the number of patients admitted to the intensive care unit, the number of patients who required mechanical ventilation, the duration of mechanical ventilation, and serum levels of 25-hydroxyvitamin D, total calcium, creatinine, and C-reactive protein. Out of 240 patients who participated in this study, 120 patients were randomly assigned to receive the intervention of 200,000 IU of vitamin D3, and the other 120 patients were in the placebo group. Analysis was performed on 237 patients who completed the study (104 females and 133 males; $n = 119$ intervention group, $n = 118$ placebo group).

Analysis of data showed that the mean age of the participants was 56 years, and the baseline 25(OH)D level for the intervention group was 21.0 ng/mL (10.2), while it was 20.6 ng/mL (8.1) ($p = 0.747$) for the placebo group. The results of this study showed that hospital length of stay was comparable (7 days for both

groups), the rate of mortality was not significantly different (7.0% vs. 5.1%, $p = 0.59$), and admission to the intensive care unit was not significantly different either (7.0% vs. 14.4%, $p = 0.90$). The only significant difference between the two groups was the increase in 25(OH)D levels in the intervention group compared to the placebo group (difference of 24 ng/mL, $p = 0.001$). This study concluded that the data did not support the use of vitamin D3 for moderate to severe Covid-19 patients [18]. However, some limitations mentioned in this study should also be noted: The sample analyzed had heterogeneity of treatment due to diverse medication regimens, the percentage of patients who participated with vitamin D deficiency was relatively low, only one dose of vitamin D was administered, and the patients were given vitamin D3 relatively late during symptom onset (mean of 10.3 days after symptom onset). Other studies reviewed in this article that showed a positive outcome included vitamin D supplementation earlier during the disease and provided regular vitamin D supplementation [18,19]. Furthermore, it is notable to mention that the individuals were not severely deficient in vitamin D at baseline as the adequate serum 25(OH)D level for bone and overall health is ≥ 20 ng/mL [22].

Aside from the three aforementioned randomized controlled trials, two quasi-experimental studies conducted in France provided interesting data [20,21]. In France, it was recommended that all nursing homes regularly and systematically supplement all residents with a single oral dose of 80,000 IU vitamin D3 every 2-3 months due to the very high prevalence (90-100 %) of hypovitaminosis D in the nursing-homes population [21,22]. This practice has provided interesting data as this population was regularly supplemented with vitamin D3. Furthermore, supplementation was often provided without knowledge of baseline serum vitamin D levels, as vitamin D deficiency is frequent in healthy adults and is often severe in the French general population [23].

The first of these two studies occurred with 77 frail elderly hospitalized Covid-19 patients who were 88 \pm 5 years old [20]. The objective of this study was to determine whether vitamin D supplementation is taken regularly in the preceding year or after diagnosis with Covid-19 was able to improve 14-day mortality and the highest (worst) score on the Ordinal Scale for Clinical Improvement (OSCI) measured during the acute phase of Covid-19. The participants were divided into three groups: Group 1 had 29 participants who were supplemented with 50,000 IU Vitamin D3 every month or 80,000 - 100,000 IU vitamin D3 every 2-3 months, Group 2 was supplemented with 80,000 IU vitamin D3 within hours of Covid-19 diagnosis, Group 3 was not supplemented with vitamin D3 [20].

The results of this study showed that Group 1 had the highest survival percentage at day 14 (93.1%) compared with Group 2 (81.2%, $p = 0.33$), and Group 3 had the lowest survival rate at Day 14 (68.7%, $p = 0.02$). The Kaplan-Meier distributions showed that Covid-19 participants in Group 3 had shorter survival time (log-rank $mboxemph = 0.015$) and 14-day mortality rates (6.9%

in Group 1 versus 31.3% in Group 3, $p = 0.02$) than those in Group 1. Interestingly, there was no difference between Groups 2 and 3 (log-rank $p = 0.32$) and between Groups 1 and 2 (log-rank $p = 0.22$). Furthermore, Group 1 was associated with a lower risk of World Health Organization's Ordinal Scale for Clinical Improvement score (OSCI score) ≥ 5 compared to Group 3 ($p = 0.03$). This study demonstrated that for the frail elderly, regular bolus vitamin D3 supplementation (as seen in Group 1) was associated with less severe Covid-19 and better survival. However, one-time supplementation of vitamin D3 after the diagnosis of Covid-19 was not associated with significant improvements in Covid-19 outcomes. Based on this observation, the researchers of this study are conducting the COVID-TRIAL study, which aims to analyze the effects of high-dose versus standard-dose vitamin D3 on the 14-day mortality rate of elderly patients [21]. One downside to this study is that baseline serum 25(OH)D levels are not available, which could be a potential confounder, and that the participant population was elderly and did not reflect the general population. Furthermore, the study design could be stronger if patients were randomized to vitamin D treatment. The study reported that the participants who regularly received vitamin D supplementation (Group 1) might be treated better by their family physicians than the others, thereby exhibiting more stable chronic diseases such as cardiovascular comorbidities [20]. Nevertheless, this study suggests that regular vitamin D3 supplementation may be an effective adjunctive treatment for Covid-19, especially in the elderly.

The second quasi-experimental study examined 66 residents in a French nursing home aged 87.7 \pm 9 years. It sought to consider whether bolus vitamin D3 supplementation affected Covid-19 mortality and OSCI in the acute phase [21]. The intervention group received bolus vitamin D3 during Covid-19 illness or in the preceding month. The bolus vitamin D3 supplementation consisted of a single dose of 80,000 IU cholecalciferol, which was usually given every 2-3 months to nursing home residents without prior knowledge of their serum vitamin D levels. After adjusting for potential confounders, the results showed that 82.5% of the intervention participants survived vs. 44.4% of the comparator group who had not been supplemented with vitamin D3 ($p = 0.023$). The Kaplan-Meier distributions showed that the residents who had not recently received vitamin D3 supplements had shorter survival times than those having received vitamin D3 supplementation during or just before Covid-19 (log-rank $p = 0.002$). Furthermore, the bolus vitamin D3 supplementation during or just before Covid-19 was inversely associated with the OSCI score ($p = 0.001$) for Covid-19 in the acute phase. This study showed that bolus vitamin D3 supplementation during or just before Covid-19 was associated with less severe Covid-19 and a better survival rate for frail elderly. Similar limitations are present in this study in comparison with the other quasi-experimental study. This one also only analyzed the frail elderly and does not represent a greater variety of age groups. It also did not measure serum concentrations of 25(OH)D at baseline, which may be a confounder, and the study design did not contribute to

randomization. However, this study provided data consistent with many of the other studies mentioned above that point to a possible association between vitamin D use and Covid-19 severity and mortality.

Discussion

The most relevant data currently available regarding experimental trials on the use of vitamin D in the management of Covid-19 includes three randomized controlled trials (one of which is a pilot study) and two quasi-experimental studies that are partially retrospective. Of the randomized controlled trials, two studies associated vitamin D use with the severity and duration of the disease [17,19], while the third did not find any significant association with positive outcomes of vitamin D use in Covid-19 patients [18]. Some questions and concerns arose regarding the methodology of this study, including differing baseline 25(OH)D levels or lack of knowledge thereof and a single vitamin D intervention later in the disease stage and at differing dosage levels. This warrants studies addressing when vitamin D intervention may be optimal and positively affects Covid-19 outcome. The current RDA for vitamin D is 600-800 IU/day for most adults. The tolerable upper intake level is 4,000 IU for adults, but the levels supplemented in most of these studies were mega-doses [22]. As a fat-soluble vitamin, monitoring the toxicity of this supplement is also important. However, these studies did not report any adverse side effects. Both quasi-experimental studies found vitamin D use was associated with less severe Covid-19 symptoms and better survival rates [20,21] despite a small sample size ($n = 499$) and limited to elderly populations. Additional randomized-controlled trials investigating the use of various doses of vitamin D at various stages of the disease, with a larger sample size, would be very beneficial in helping to substantiate and provide more robust evidence on the benefits of vitamin D supplementation for Covid-19 patients.

Interestingly in a recent multicenter randomized clinical trial, a low dose of 5000 IU vitamin D treatment daily for 2 weeks to Covid-19 patients with mild to moderate symptoms significantly shortened the time to recovery in resolving cough (6.2 ± 0.8 days versus 9.1 ± 0.8 days; $p = 0.039$), and ageusia (11.4 ± 1.0 days versus 16.9 ± 1.7 days; $p = 0.035$) [24]. The data suggest that a substantially low dose of vitamin D can effectively reduce the Covid-19 symptoms. However, this study did not include Covid-19 patients with severe symptoms. It is not clear if low doses of vitamin D will be equally effective in Covid-19 patients with severe symptoms having the risk of mortality as reported in other trials where high doses of vitamin D were used.

Vitamin D can provide potential benefits to Covid-19 patients by having antiviral effects, regulating the renin-angiotensin system [25], stimulating innate and modulating adaptive cellular immunity processes [26], decreasing the cytokine and chemokine storm [27], and maintaining the integrity of the pulmonary epithelial barriers [28]. Vitamin D may induce antimicrobial peptides with direct antiviral activity against enveloped and non-enveloped viruses, including the coronavirus family [29].

One mechanism of vitamin D, specifically the active form of calcitriol, involves the renin-angiotensin system, which regulates hemodynamics and functions as a pro-inflammatory system [30]. SARS-CoV-2 uses the cell's ACE2 as a receptor for viral cell entry [31]. This down-regulates ACE2 expression, which results in more angiotensin II available to cause inflammatory damage, as angiotensin II can increase vasoconstriction, inflammation, and oxidative stress [32]. The problem with the down-regulation of ACE2 is that it has a protective effect against inflammation, especially in endothelial and pulmonary alveolar cells [33,34]. This can lead to increased inflammation, which can exacerbate into a cytokine storm and lead to ARD [35]. Vitamin D has been shown to prevent ARD symptoms in animal experiments by reducing pro-inflammatory cytokine production [29,36]. In addition, vitamin D has the ability to negatively regulate the renin-angiotensin system, reducing the activity of ACE and increasing the activity of ACE2 while lowering angiotensin II levels [26]. Vitamin D deficiency has also been shown to increase renin production and increase ACE and Angiotensin II production [32,37]. These observations suggest that the use of vitamin D has the ability to disrupt ACE2-mediated cytokine storm, a hallmark of Covid-19 infection.

Vitamin D also has the ability to reduce the production of pro-inflammatory Th1 cytokines, including TNF-alpha and INF-gamma. At the same time, it increases the expression of anti-inflammatory cytokines by macrophages [29]. It also helps to regulate T-cell induction and promotes the production of anti-inflammatory Th2 cells [38]. Furthermore, vitamin D has been shown to induce the expression of antimicrobial peptides, such as beta-defensin-2 and cathelicidins, inactivated monocytes, and macrophages [39]. This effect is directly mediated by vitamin D binding to vitamin D receptors (VDRs), resulting in increased production of defensin and cathelicidin [26]. These peptides exhibit antiviral activities by penetrating the viral membrane, and this increase in viral membrane permeability was correlated with 25(OH)D levels [29,39,40]. Cathelicidins can help reduce the cytokine storm, and it has been found that a minimum of 30 ng/mL vitamin D is required for optimum production of cathelicidins [41]. Another consequence of activated macrophages or damaged parenchymal cells in Covid-19 infection is the induction of fibrosis, which is initiated from the release of TGF-b. TGF-b signaling mediates phosphorylation of SMAD2 and SMAD3, which then translocates as a complex (SMAD2/3/4) into the nucleus and drives the expression of pro-fibrotic genes [42]. The VDR directly interacts with SMAD3 and inhibits TGF-b-SMAD mediated upregulation of pro-fibrotic genes [42,43]. Vitamin D inhibition of the TGF-b-SMAD signaling pathway may offer a useful therapeutic approach in Covid-19 patients [44]. Finally, vitamin D helps to maintain stable physical barriers by stimulating genes that code for cell integrity. Vitamin D may stimulate the production of several epithelial proteins: occludin, located at tight junctions; connexin 43, located at gap junctions; and E-cadherin, located at adherens junctions, keeping cellular tight junctions via E-Cadherin [45]. Furthermore, vitamin D has

been found to alter 25% of the genes encoding proteins that serve as a target for SARS-CoV-2, which shows potential in its use as an adjunctive therapy agent for Covid-19 treatment [46]. Therefore, the use of vitamin D could reduce severity in Covid-19 symptoms by multiple mechanisms and can be beneficial in preventing death from Covid-19.

Conclusion

Based on the results of randomized-controlled trials and quasi-experimental studies published until February 2021 using vitamin D supplementation in Covid-19 situations, regular vitamin D supplementation may reduce Covid-19 severity and mortality rate. More specifically, an initial loading dose (21,280 IU Calcifediol) and regular supplementation of vitamin D (10,000-60,000 IU/day when ill to monthly doses of 50,000 IU or 80,000-100,000 IU/2-3 months) resulted in the most effectiveness in the reviewed studies. However, more randomized controlled trials are needed to provide more robust evidence for a variety of participants and to give insight into the most appropriate dose, form, and timeline of vitamin D supplementation for patients with SARS-CoV-2.

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