VIA MEDICA

REVIEW ARTICLE

Disaster and Emergency Medicine Journal
2022, Vol. 7, No. 2, 124–131
DOI: DEMJ.a2022.0016

ISSN 2451–4691, e-ISSN 2543–5957

ASSOCIATION BETWEEN LOW SERUM VITAMIN D AND INCREASED MORTALITY AND SEVERITY DUE TO COVID-19: REVERSE CAUSALITY?

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ABSTRACT

We are very close to completing two years since the start of the COVID-19 pandemic. Even though vaccines have been developed and applied to more than 4 billion people in the world, SARS-CoV-2 continues to be a challenge for humanity. Therefore, it is important to study modifiable risk factors that may increase the severity of COVID-19, and one of the most discussed has been vitamin D. Currently, there is some evidence of association between low serum 25-hydroxyvitamin D [25(OH)D3] and increased mortality and severity due to SARS-CoV-2 infection. Before the pandemic, experimental evidence in animal and human studies had reported that an acute inflammatory process can cause a secondary decrease in 25(OH)D3. COVID-19 can be associated with a severe inflammatory process with an elevation of inflammatory markers; in this light, the reported association between low 25(OH)D3 and COVID-19 severity and/or mortality may be an epiphenomenon of the inflammatory process induced by SARS-CoV-2 and be an example of reverse causality.

KEY WORDS: SARS-CoV-2; COVID-19; Vitamin D; 25-hydroxyvitamin D; severity; mortality

Disaster Emerg Med J 2022; 7(2): 124–131

INTRODUCTION

Until December 31 2021, the COVID-19 pandemic has caused more than 5.4 million deaths worldwide, although mortality has decreased significantly due to the vaccination of more than 4.4 billion people [1]. Given the appearance of 8 new variants whose virulence and coverage by current vaccines are still under study [2, 3], it is important to study modifiable risk factors that may reduce the risk of developing severe or fatal forms of SARS-CoV-2 infection. One of the most studied modifiable risk factors has been vitamin D. So far, several systematic and meta-analytic studies have been published, which have concluded that there may be a cause-effect relationship between low serum 25-hydroxyvitamin D (25(OH)D3) and increased mortality and severity due to SARS-CoV-2 infection [4–9]. However, the robustness of this cause-and-effect relationship is currently being questioned and it has been suggested that this premature conclusion should be taken with caution for several rea-

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Received: 18.01.2022 Accepted: 23.05.2022 Early publication date: 22.06.2022

sons [10]. First, to make the causality inference, ecological and observational studies were used; most of these studies did not have baseline levels of 25(OH)D3 (before SARS-CoV-2 infection). Second, there is experimental and clinical evidence before the COVID-19 pandemic that acute inflammatory diseases can cause a decrease in 25(OH)D3 concentration and not the opposite. Third, intervention studies with vitamin D in patients with severe COVID-19 and hypovitaminosis have not had effective results in reducing mortality from COVID-19 [11, 12]. In this light, we argue that the low levels of 25(OH)D3 seen in severe COVID-19 are probably an epiphenomenon of the severe inflammatory process of COVID-19 and not its cause. That is, the association of low serum 25(OH)D3 and increased mortality and severity due to SARS-CoV-2 infection may be a phenomenon of reverse causality.

SERUM 25(OH)D3 AS A NEGATIVE MARKER OF INFLAMMATION IN THE PRE-PANDEMIC ERA

Vitamin D has been studied extensively before the pandemic as a negative marker of inflammation; that is, in patients who previously had normal 25(OH)D3 levels, an inflammatory process can make vitamin D levels decrease [13, 14].

Studies in animals

Studies in dogs on vitamin D status before and after surgery have found a significant decrease in total 25(OH)D3 concentration, which increased after the operation and then normalized on day 60 [15]. C-reactive protein (CRP) increased significantly and albumin also decreased significantly [15].

Studies in humans

In a systematic study published by Silva et al. [16] in 2014, 6 of 8 included studies found evidence that the concentration of serum 25(OH)D3 decreases during the acute-phase response in humans. This has been observed during knee/hip arthroplasty [17–19], acute myocardial infarction [20], acute pancreatitis [21], the first dose of IV bisphosphonate [22], and cardiopulmonary bypass (23). In all the studies, CRP was elevated. Recovery to baseline 25(OH)D3 concentrations took 2 weeks, although in 2 studies it took up to 90 days. In two studies, the level of total 25(OH)D was not modified, but the baseline sample was taken on the second day of the event [16]. None of the 6 studies mentioned above measured free 25(OH)D3. Recently, Binkley et al. found that on the first day after surgery (total hip arthroplasty), total and free 25(OH)D (reduction from 21 to 34%), as well as DBP (vitamin D-binding protein), calcium, creatinine, alkaline phosphatase, and plasma hemoglobin declined 8–22% ($p < 0.0001$) with respect to measurements before surgery [24]. On the other hand, the urinary DBP/creatinine ratio (UDBP/Cr) increased significantly and the levels of total and free 25(OH)D returned to baseline levels 6 weeks after surgery.

Association between low serum 25-hydroxyvitamin D and infections

Smolders et al. investigated whether systemic inflammation lowers circulating 25(OH)D levels using the experimental human endotoxemia model (bolus of E. Coli-derived lipopolysaccharide, LPS) [25]. They found a significant reduction in 25(OH)D levels 2–3 hours after infusion, compared to baseline levels; the fall in the levels of 25(OH)D coincided with the elevation of the levels of proinflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-8; in turn, 25(OH)D levels recovered to baseline 6 hours following cessation of LPS infusion.

Postulated mechanisms of 25(OH)D reduction induced by acute inflammation

Several main mechanisms have been postulated that could explain the reduction in 25(OH)D associated with surgical interventions [24]. A surgical intervention, especially interventions in traumatology (e.g., knee/hip arthroplasty) would cause an increase in uptake and/or consumption of 25(OH)D in the immediate post-operative interval when demands for tissue regeneration are high. Part of the reduction in 25(OH)D could be due to the increased volume of distribution, produced using water solutions during operations. Another possible explanation is an increase in the catabolism and elimination of 25(OH)D from the intravascular compartment or increased urinary loss. Concordant with this last hypothesis, reduced serum DBP and increased urinary DBP/creatinine ratio have been found immediately after surgery [24].

There is evidence that serum 25(OH)D is a negative acute-phase reactant. The rationale for this hypothesis is the inverse relationship between the reduction in serum concentration of 25(OH)D and increased circulating inflammatory marker levels

[17, 18, 26]. An inverse relationship of CRP with low levels of 25(OH)D has been found during total knee arthroplasty (TKA) and other orthopedic surgeries [16]. It has also been observed that an increase in Interferon-gamma (IFN-gamma) levels occurs with a simultaneous decrease in 1.25(OH) D in the circulation after anterior cruciate ligament reconstruction [27]. Interestingly, IFN-gamma has been found to mediate the enzymatic conversion of 25(OH)D to 1,25(OH)D in circulating immune cells [28–30]. The Th1 cytokine IFN-gamma up-regulated TLR2/1 induction of 25-hydroxyvitamin D-1α-hydroxylase (*i.e.*, CYP27B1), leading to enhanced bioconversion of 25-hydroxyvitamin D3 to its active metabolite 1,25(OH)D3. In contrast, the Th2 cytokine IL-4, by itself and in combination with the TLR2/1 ligand (TLR, Toll-like receptor), induced catabolism of 25(OH)D3 to the inactive metabolite 24.25(OH)D3, and was dependent on expression of vitamin D-24-hydroxylase (*i.e.*, CYP24A1) [28]. Another important finding is that tumor necrosis factor-alpha induces vitamin D-1-hydroxylase activity in normal human alveolar macrophages [31]. Henriksen et al. reported a reduction in serum 25(OH)D concentrations after total knee arthroplasty, at the beginning mildly (12%) from pre-surgery to 2 days post-surgery and the more pronounced decrease (74%) from 3 to 8 weeks post-surgery; simultaneously, they observed an increase in serum pro-inflammatory cytokine concentrations (*i.e.*, TNF-a, IFN-gamma, IL-1b, GM-CSF, and IL-6) [26].

REVERSE CAUSALITY IN MEDICINE

Sir Austin Bradford Hill, 56 years ago published his famous article on the 9 criteria to take into account to establish an association between two variables, and among all these criteria one of the most important is the fourth, which he called temporality [32]. Temporality is defined as the necessity for exposure, or a hypothetical cause, to precede an outcome, or an effect, in time [33]. Sir Austin Bradford Hill explained the concept of temporality in a very simple way, resorting to the analogy of analyzing and answering the question in an association study between two variables: "which is the cart and which the horse?"; that is, avoid confusing cause and effect [34].

Reverse causality (also called reverse causation, retrocausality, or backward causation) is defined as a phenomenon in which the outcome precedes and causes the exposure [35–37]. Reverse causality is one of the most important biases that can be committed in observational studies, especially in those that seek to associate a risk factor with a disease. In medicine, several examples of reverse causality have been described, especially in studies of cardiovascular risk factors [38], such as low body mass index in many observational studies of chronic disease (*e.g.*, heart failure, renal disease, rheumatoid arthritis) associated with greater mortality risk [39], low cholesterol associated with higher cancer risk [40] and low hemoglobin A1c associated with higher mortality risk in diabetes [41], among others.

ASSOCIATION BETWEEN LOW SERUM 25-HYDROXYVITAMIN D AND INCREASED MORTALITY AND SEVERITY DUE TO SARS-COV-2 INFECTION: REVERSE CAUSALITY?

Here, we summarize evidence to support that the association between low serum 25(OH)D and increased mortality and severity due to SARS-CoV-2 infection is probably a phenomenon of reverse causality (Fig. 1).

Ecological studies

Ecological studies consist of taking the information contained in databases of 25(OH)D concentrations of populations and countries and associating it with mortality, recovery, severity, or susceptibility to SARS-CoV-2 infection, based on geographic altitude [42]. It should be noted that the inference is indirect, that is, the concentration of vitamin D in the patients is not taken directly. In an ecological study carried out with data from 46 countries, Mariani et al. [43] found an association between vitamin D deficiency and COVID-19 incidence, complications, and mortality. In another ecological study with mortality data from 117 countries taken on May 17, 2020, Rhodes et al. [42] also found an association between mortality from COVID-19 and geographic latitude, inferring that some countries do not have sufficient exposure to ultraviolet B to maintain normal vitamin D blood levels throughout winter. The most important limitation of ecological studies is the possibility that an ecological fallacy is being observed, that is, a conclusion about individuals based only on analyses of group data [43]. Another limitation of this type of study applied to COVID-19 is that mortality is very dynamic and changing, a characteristic that makes them less powerful, which differentiates it when

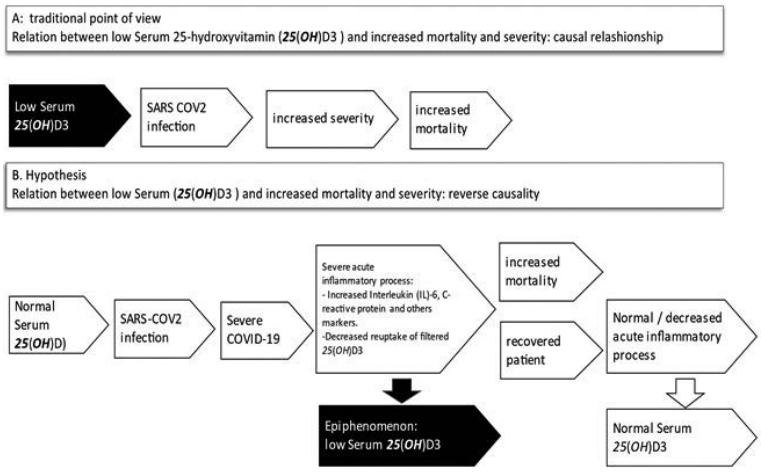

FIGURE 1. **A.** The increase in severity and mortality from COVID-19 would occur because, in addition to other factors, the patient would have a low concentration of 25(OH)D3. However, there is a lack of studies showing baseline vitamin D levels before infection; **B.** The reverse causality hypothesis postulates that patients before infection could have normal levels of 25(OH)D3, but later it decreases as an epiphenomenon of the acute inflammatory process associated with severe COVID-19. If the patient manages to recover from the disease and the acute inflammatory process decreases, 25(OH)D3 levels could potentially increase or normalize

studies are carried out on chronic diseases and vitamin D. Another limitation is that the concentration of vitamin D can change in the individual, in a pandemic for example due to isolation and because the individual begins to take vitamin D due to advertisements.

Mendelian randomization studies

Mendelian randomization studies use the genetic variant as a surrogate variable for 25(OH)D deficiency, to infer the causal effect of exposure [25(OH)D concentration] to an outcome, for example, susceptibility to infection by SARS-CoV-2, severity or mortality [44]. Butler-Laporte et al. [44] conducted a Mendelian randomization study with genetic variants associated with 25(OH)D levels in a genome-wide association study (GWAS) with 443,734 participants of European ancestry, where they found no association between 25(OH)D levels and COVID-19 susceptibility, severity, or hospitalization. Patchen performed another two-sample Mendelian randomization study in the population-based UK Biobank and SUNLIGHT Consortium, applied to meta-analyzed results of genome-wide analyses in the COVID-19 Host Genetics Initiative, and they also found no evidence of causality association between serum vitamin D concentrations and susceptibility to and severity of COVID-19 infection, including severe respiratory infection and hospitalization [45]. Cui et al. [46] performed a two-sample Mendelian randomization using summary-level GWAS data, and they found no evidence to support the causal associations between the genetically lowered serum 25(OH)D concentrations and the risk of COVID-19 susceptibility.

Observational studies

Most of the observational studies and their respective meta-analytic studies are based on the determination of 25(OH)D levels when the patient is hospitalized or in the ICU, and for obvious reasons, they do not have baseline determinations. Therefore, it is strictly difficult to affirm or deny the inference of causality with these data only. However, recent studies are showing evidence that is in favor of a reverse causality explanation. Evidence is of two types: studies showing an increase in inflammatory markers with a simultaneous decrease in 25(OH)D; and studies where 25(OH)D was measured during the course of the disease. A recent systematic study found that mean values of inflammatory markers [C-reactive protein (CRP), interleukin 6 (IL-6), and tumor necrosis factor a (TNF-a)] were higher in the low 25(OH) D groups [47, 48]. Other recently published studies also found an increase in inflammatory markers with a simultaneous reduction in 25(OH)D levels [48–51]. Balzanelli et al. have described patients with severely low levels of vitamin D, and who also have extremely high levels of IL-6 and low glomerular filtration rate (eGFR), and they have postulated that there would be a reduction in the reuptake of filtered 25-hydroxyvitamin D in proximal kidney tubules. Some obser-

vational studies have found evidence of an inverse relationship evolution between the evolution over time of a patient with COVID-19 and the markers of acute inflammation. In a case-control study, Gameil et al. found that patients who had elevated CRP 3 months after COVID-19 also had lower levels of serum 25(OH)D compared to controls [52].

In a case-control study, Gupta et al. [53] compared 25(OH)D levels taken up to 6 months before, with those taken after SARS-CoV-2 infection, and they found that COVID-19 positive individuals had lower serum 25(OH)D measurements compared to controls; they postulated the possibility that reduced serum 25(OH)D may be a consequence and not a cause of COVID-19 infection. Gallelli et al. [54] studied 25(OH)D levels in patients during COVID-19 disease, and found that during the acute phase they were reduced, but increased significantly after recovery; with these findings, it could be interpreted that once the inflammatory process ceases, 25(OH)D levels tend to normalize.

Clinical trials and metanalytic studies

The best evidence in favor of a causal association between vitamin D concentrations and clinical outcomes in patients with COVID-19 could be that supplementation in patients with low levels should improve patient survival; however, the studies published to date do not support this hypothesis. Chen et al. recently published a meta-analysis that included 11 observational studies and two clinical trials, and they found no relationship between vitamin D levels and severity and mortality from COVID-19; on the other hand, another important finding was that vitamin D supplements did not significantly improve clinical outcomes in patients with COVID-19 [55]. In a meta-analysis with 13 studies and 3 clinical trials, Pal et al. found that vitamin D supplementation was associated with improved clinical outcomes only in patients receiving the drug post-COVID-19 diagnosis and not in those who had received vitamin D before diagnosis [56]. The authors of the latter study found several limitations of the included studies, among which were that the included studies did not have baseline vitamin D levels (before COVID-19 infection); vitamin D supplementation was administered irrespective of the baseline serum 25(OH)D levels of the patients; and another limitation was that, except for two randomized controlled trials [57, 58], none of the studies mention the degree of rising in serum 25(OH)D levels post-vitamin D supplementation; hence, one can only speculate if adequate vitamin D levels had been achieved to exert immunomodulatory effects [56]. Stroehlein et al. [11] also found no evidence to determine the benefits and harms of vitamin D supplementation as a treatment of COVID-19. In a meta-analytic study, Rawat et al. [12] also found no significant difference between vitamin-D supplementation on major health-related outcomes in COVID-19.

WHY IS IT IMPORTANT TO DETERMINE THE NATURE OF THE ASSOCIATION BETWEEN LOW SERUM VITAMIN D AND INCREASED MORTALITY AND SEVERITY DUE TO SARS-COV-2 INFECTION?

It is important to elucidate this issue for two reasons, first to avoid requests for tests of serum 25(OH)D in patients indiscriminately [59]; and secondly, to prevent vitamin D toxicity reactions from overtreatment [60–62]. Even though vitamin D toxicity is not likely when oral supplements are taken below 2000 international units/day, before the COVID-19 pandemic, acute kidney injury due to overcorrection of hypovitaminosis D has been reported [63, 64]. On the other hand, before the pandemic, an increase in requests for potentially inappropriate testing for vitamin D deficiency had been observed [65, 66], which has led to the possibility of overtreatment [67, 68]. During the pandemic, Arroyo-Díaz et al. [69] found in a cross-sectional study higher in-hospital mortality and/or invasive mechanical ventilation among subjects treated with vitamin D prior to hospital admission in the crude analysis, which was not confirmed in the fully adjusted model. Therefore, more studies are necessary on the safety of vitamin D supplementation in patients with severe forms of COVID-19.

In conclusion, there is currently evidence to postulate that the association between low serum 25(OH)D and increased mortality and severity due to SARS-CoV-2 infection is probably an example of reverse causality. This evidence comes from the negative results of this association in Mendelian randomization studies and systematic and meta-analytic studies that do not observe a reduction in severity or mortality from vitamin D therapy in patients with severe COVID-19. Additionally, this hypothesis is reinforced by the antecedents of studies before the pandemic in relation to the fact that inflammatory states decreased the concentration of serum 25(OH)D.

Contributors and sources

TJO (Physician), AC (Clinical pharmacology), and RRO (Geriatrician) conceptualized and wrote the first draft of the manuscript. RAG contributed his experience in studies on vitamin D and factors that affect serum levels. All authors reviewed, edited, and approved the final version of the manuscript. TJO and RRO are guarantors.

Conflict of interest

We have read and understood BMJ policy on the declaration of interests and have no relevant interests to declare.

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