COVID-19: possible role of vitamin D supplementation in preventing infection and reducing symptom severity

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ABSTRACT

The COVID-19 pandemic and its consequences have demonstrated that viral infections still pose great challenge to health systems worldwide. The immune system plays a primary role in fighting infections, and non-specific innate immunity, in particular, is a key defense mechanism engaged during the first phases of an infection. The subsequent triggered immune response, characterized by specific cytokine patterns, can be exaggerated and lead to a cytokine storm, causing multiorgan damage and intravascular disseminated coagulation. Calcitriol, the active form of vitamin D, a key player in mineral homeostasis, is able to control the immune response by favoring the production of antimicrobial peptides (e.g., cathelicidin), decreasing viral shedding, and modulating the inflammatory process. A poor vitamin D status has been demonstrated to be associated with increased risk of infections, especially in fragile subjects, as also recently shown in COVID-19 disease. Unfortunately, this does not demonstrate that supplementation with vitamin D is significantly linked to prevention or modulation of the course of viral infections. Large intervention trials are necessary to produce evidence that vitamin D supplementation is linked to decreased rates of infections and related complications. At present, cholecalciferol and calcidiol regimens commonly employed in clinical practice to prevent and correct musculoskeletal abnormalities are advisable, at least in fragile, vitamin D-deficient individuals, in order to reach a target of serum 25(OH)D of 30–50 ng/ml, avoiding large doses, which can be responsible for hypercalciuria-hypercalcemia or increased falls.

KEYWORDS

Cholecalciferol, calcidiol, immune system, coronavirus, respiratory tract infections.

Introduction

December 2019 brought the first reports of a novel coronavirus (CoV) disease caused by a newly identified β-coronavirus, designated SARS-CoV-2 by the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses. The World Health Organization (WHO) officially named this disease coronavirus disease 2019 (COVID-19). The first familial clusters of pneumonia associated with the infection were observed in Wuhan, China [1]. The world has previously seen two other CoV disease epidemics, namely the ones caused by the severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) that started, respectively, in China in 2003 [2] and in the Middle East in 2012 [3]. Initial animal-to-human infection and the development, in some cases, of severe atypical pneumonia [4,5] are features common to all three epidemics. In addition to respiratory failure, COVID-19 patients can manifest a cytokine storm and a hypercoagulable state responsible for disseminated intravascular coagulation (DIC) that induces multiple organ dysfunction and dramatically increases the risk of death [6,7].

SARS-CoV-2 is an enveloped non-segmented positive-sense RNA virus (belonging to the subgenus sarbecovirus, Orthocoronavirinae subfamily) [8]. Its sequence is 96.2% identical to that of the bat CoV RaTG13 virus, and 79.5% identical to that of SARS-CoV. Hence, the bat has been hypothesized to be the natural host of origin of the virus, the suggestion being that SARS-CoV-2 was transmitted from bats, possibly via intermediate hosts, to humans [8-11].

The COVID-19 epidemic spread very quickly all over the world, steadily growing through human-to-human transmission, and reaching pandemic proportions with unprecedented speed, causing lockdowns to be enforced worldwide. From the outset, Italy was particularly affected, seeing an exponentially growing number of infected patients, requiring critical and intensive care. This situation caused great concern within the country's National Health System, as intensive care units soon reached maximum capacity and saturation [12-13].

In the case of SARS-CoV-2, release of viral RNA into

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host cells occurs through binding of the spike (S) glycoproteins present on the surface of the virus to the human angiotensin-converting enzyme 2 (ACE2) receptors. ACE2 receptors are expressed in several human tissues including the upper and lower respiratory tract. The expression of these receptors is higher in current smokers and in COPD patients than in normal subjects [14-16]. The S-protein of SARS-CoV-2 has a binding affinity for ACE2 that is 10- to 20-fold higher than that of SARS-CoV17. The binding of viral S-glycoproteins to these receptors allows membrane fusion and release of the viral genome RNA into the cytoplasm [18].

An efficient immune response is essential for the control and resolution of CoV infections. The innate component of the human immune system is our body's first line of defense; its function is based on poorly-specific recognition and alert activity oriented against foreign molecules, which leads to a consequent and immediate startup of responses [19]. A key aspect of this nonspecific action is that it can be carried out not only by specialized cells such as neutrophils, macrophages and dendritic cells, but also by epithelial cells and fibroblasts. On the surface as well as internally, these cells express so-called pattern recognition receptors (PRRs), which are able to recognize and interact with the pathogen-associated molecular patterns (PAMPs) expressed by a wide number of bacterial and viral pathogens [20]. Viral RNAs are detected as PAMPs by three primary families of PRRs: toll-like receptors (TLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), and NOD-like receptors (NLRs). TLRs detect viral components in the endosome [21-23], while RLRs and NRLs do so in the cytoplasm [24,25]. Remarkably, RIG-I is a cytoplasmic RNA helicase that stimulates specific signaling pathways leading to activation of NF-κB, which in turn triggers an inflammatory cytokine cascade through the realizing of IFN-γ [25,26]. The RIG-I association with uncapped ssRNA [27] is crucial in the antiviral IFN response. In fact, upon detection of pathogen components, RIG-I induces both direct and indirect modulation of proinflammatory cytokines. Plasma cytokines and chemokines found to be increased in COVID-19 patients include interleukin-1 (IL-1), IL-2, IL-4, IL-7, IL-10, IL-12, IL-13, IL-17, granulocyte colony-stimulating factor (GCSF), macrophage colony-stimulating factor (MCSF), IP-10, MCP-1, MIP-1α, hepatocyte growth factor (HGF), interferon-gamma (IFN-γ) and TNF-α [28,29]. Overall, the complex virus-cell interaction produces various immune mediators, crucial in protecting against the invading virus. Precise regulation of the inflammatory reaction is needed to eliminate the virus without generating an uncontrolled cytokine storm 16, as a cytokine storm can induce a catastrophic attack, by the immune system, against vital organs, provoking death by multiple organ failure, DIC and/or severe acute respiratory syndrome (SARS). Based on this hypothesis, the administration of tolicizumab, a monoclonal antibody against the receptor of one of the more active cytokines, IL-6, has been proposed. After preliminary data demonstrating its efficacy, a phase III multicenter clinical trial in Covid-19 patients with high levels of IL-6 has been started.

Epidemiological data relating to the COVID-19 pandemic show that the proportion of infected patients who are hospitalized increases with age. Also, the mortality rate increases with age, reaching a maximum of 18.4% in patients aged over 80 years [30].

Low vitamin D levels could be a possible explanation for the increased risk of complications observed in elderly patients with COVID-19. The aging-related decrease in serum 25(OH) D levels is linked to lower concentrations of precursors in the skin, less opportunity to get sunlight, and increased use of drugs [31,32]. Vitamin D is known to have a broad spectrum of properties, beyond control of bone remodeling and skeletal homeostasis. In this respect, vitamin D could perform a protective action against COVID-19 infection, through both an immunomodulatory action and antiviral properties [33,34]. The wide pleiotropic effects of vitamin D, mediated by its biologically active form, i.e., 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], can be explained by the expression of its receptor (VDR) in multiple tissues and cell types, most of which also contain the enzyme CYP27B1 (cytochrome P450-associated 25(OH)D3-1α-hydroxylase), responsible for the local conversion of circulating 25-hydroxy-vitamin D (25 hydroxy-D) to the active metabolite.

The aim of this review is to examine, on the basis of data from previous and recent studies, the possibility that vitamin D level could possibly be directly correlated with a reduction of COVID-19 infection, and to suggest that vitamin D supplementation could be a beneficial contributor to preventing severe disease evolution.

Immunomodulatory action of vitamin D

Several research groups have extensively examined the immune-modulatory role of 1,25(OH)2D, also named calcitriol [35]. In recent years the potential role of vitamin D has been elicited from unbiased genomic screening analyses, with the identification of hundreds of 1,25(OH)$_2$D target genes in cells belonging to the immune system [36]. Monocytes, macrophages, dendritic cells (belonging to the innate immune system) and B and T cells (belonging to the adaptive immune system) all express CYP27B1 and VDR [37,38], while 1,25(OH)$_2$D can exert its biological effects via all the typical endocrine signaling pathways, including intracrine and paracrine ones. Moreover, in macrophages, CYP27B1 and VDR expression is upregulated in response to a pathogen challenge [39]. Both anabolic and catabolic pathways in vitamin D metabolism are strictly regulated by a feedback system acting on 1α-hydroxylase, with serum parathormone (PTH) and calcium being positive regulators, and fibroblast growth factor-23 (FGF-23), phosphate and 1,25(OH)$_2$D itself being negative regulators. Additionally, in cells of the immune system, CYP27B1 is stimulated by cytokines [35]. Active vitamin D inhibits the production of IFN-γ, IL-2 and IL-12, by downregulating the Th1 response, while it promotes a Th2 response by decreasing the production of IL-4, IL-5 and IL-10. Recently, 1,25(OH)$_2$D has also been shown to play a role in suppressing Th17 recruitment through the downregulation of IL-23 and IL-6 40. A study reported that the innate immune response to viral infections through IFN-γ production and the subsequent cytokine storm is responsible for the major lung epithelial cell injury observed following infection with coronaviruses [41]. This scenario has already been

demonstrated in the most severe cases of SARS-CoV [42] and MERS-CoV [43] infection, while in SARS-CoV2 infection DIC has also been found to be a direct cause of death [44].

Interestingly, it has been demonstrated *in vitro* that calcitriol induces increased expression of ACE2 by exerting profound modulation of the ACE2/Ang(1-7)MasR system [45]. Thus, calcitriol could play a role in reducing the viral shedding.

Innate immunity as the first line of defense and the antiviral role of vitamin D

The innate immune system is the crucial first line of defense against infectious agents, including viruses, although it exerts a nonspecific response, by initially detecting invading pathogens and subsequently activating adaptive immunity [20]. In this context, the PRRs expressed in monocytes, macrophages, polymorphonuclear cells and epithelial cells play an essential role in recognizing PAMPs: RLRs, TLRs and NLRs are able to recognize several antigens belonging to RNA species, present in respiratory viruses including coronavirus [46]. Once activated, they also increase expression of co-stimulatory molecules such as CD40, CD80 and CD86, essential for the activation of T cells and leading to acquired immune reactions. NLRs, instead, are involved in interleukin-1β (IL-1β) maturation and the subsequent cytokine cascade [47,48]. Antimicrobial peptides (AMPs) such as cathelicidins and β-defensins play a central role in innate immunity [49,50] and $1,25(OH)_2D$ is involved in their regulation. AMPs are a variable group of short, positively-charged oligopeptides with diverse structures and functions, rapidly activated following infections and widely expressed among a wide range of cells that typically come into close contact with pathogens: antigen-presenting cells, neutrophils, natural killer (NK) cells, and various epithelial cells, such as airway, urinary and gastrointestinal cells. All subgroups have been shown to exert a rapid response to infections with bacteria, viruses, fungi or protozoa [51]. Because of their inhibitory and immunomodulatory properties, AMPs have been thoroughly studied and proposed as alternatives to antibiotics in bacterial and viral infections [52].

In 2006 the first *in vitro* evidence of intracellular vitamin D-mediated production of a human antimicrobial peptide, i.e. cathelicidin, upon activation of TLRs by Mycobacterium tuberculosis was published [53].

Cathelicidins are linear peptides with a conserved 100-amino-acid cathelin domain, folded into amphipathic α-helical structures, frequently cleaved from the highly variable C-terminal antimicrobial domain [54,55]. In nature there are multiple cathelicidins, known to contribute to immune response by recruiting leukocytes, to induce chemotaxis of immune-competent cells to the infection site, and to inhibit lipopolysaccharide-dependent activation of the endothelium vasodilation. In humans, only one cathelicidin gene is known, named human cationic antimicrobial peptide 18 (hCAMP-18) or LL-37. In 1995, LL-37 was isolated from human neutrophils [56,57].

Cathelicidins are synthesized as pre-propeptides, then stored in neutrophil granules as inactive propeptides, after removal of the signal peptide. They can also be produced by epithelial cells: in skin cells they are further cleaved into smaller molecules and may exert a strong activity against various pathogens [58,59]. Moreover, monocytes, NK cells, mast cells, B cells, colon enterocytes and keratinocytes [57] can all produce LL-37, which can be detected in multiple tissues and biological fluids, including sweat, breast milk, tracheal aspirates of newborns, and seminal plasma [54]. Several endogenous factors regulate the expression of LL-37, including pro-inflammatory cytokines and active vitamin D [57]. In this scenario, LL-37 is rapidly released by epithelial cells and leukocytes following infection, and acts as a chemoattractant for neutrophils, monocytes, dendritic cells and T-cells [59,60]. During the immune response, LL-37 can also stimulate IL-6 production in human dendritic cells, possibly acting as both an anti- and a pro-inflammatory factor [60]. Very recently, intralesional injection of vitamin D has been demonstrated to be an effective and safe treatment for verruca vulgaris, acting by causing increased LL-37 expression [61].

Notably, cathelicidin-deficient individuals show an increased susceptibility to infections [58] and in the absence of 25(OH)D, VDR, or CYP27B1, cathelicidin expression in macrophages and keratinocytes is significantly impaired [53,62].

In this context, it has been demonstrated that 1,25(OH)2D upregulates the gene expression for gap, adherens and tight junction proteins in epithelial cells, enhancing their barrier function [33]. In particular, β-defensins are primarily expressed by epithelial cells and keratinocytes, but can also be produced by neutrophils, macrophages, mast cells, NK cells, dendritic cells and lymphocytes [49,50,58,63] as pre-propeptides. In humans, three β-defensins (HBD-1 to 3) have been fully characterized, while HBD-4 was only recently identified. Since 1986, when defensins were first reported to have antiviral activity [58], several studies have demonstrated how they can protect against different human viruses, such as influenza A virus (IAV), human immunodeficiency virus (HIV), human papillomavirus (HPV), human adenovirus (HAdV), herpes simplex virus (HSV), respiratory syncytial virus (RSV) and, significantly, severe acute respiratory syndrome coronavirus (SARSC) [44,52,58,64-67]. Although the multiple mechanisms associated with the antiviral activity of defensins need to be better elucidated, it seems clear that they can act directly on virus particles but also interfere indirectly at various stages of the viral life cycle [58,67]. Several studies suggest that defensins exert their antiviral activity particularly at the initial steps of viral entry; nevertheless, a role affecting viral tracking within infected cells has also been suggested [68]. The antiviral action of defensins is triggered by destruction of the envelope proteins and disruption of the membrane. Vitamin D is also involved in the regulation of defensins. In fact, vitamin D exerts antiviral effects by inducing human β-defensin 2, which attracts neutrophils and monocytes [34,69,70].

Vitamin D status and COVID-19

Since the early 1900s, hypovitaminosis D has been recognized as a global public health problem and vitamin D supplementation has been employed to prevent or treat diseases characterized by abnormal mineralization, i.e., rickets and osteomalacia.

The definition of hypovitaminosis D is not universally shared. Over the years, the "normal level" cut-off of 25-hydroxyvitamin D (the most reliable indicator of the storage of vitamin D and the major circulating vitamin D metabolite) was progressively increased from 12 to 20 and, finally, to 30 ng/mL (30, 50, 75 nmol/L, respectively) in fragile individuals, meaning those with impaired mineral metabolism [71] (Scientific Advisory Committee on Nutrition (SACN) Vitamin D and Health Report; Crown Copyright 2016. Available online at: https://www.gov.uk/government/groups/scientific-advisory-committee-on-nutrition). Although concentrations of around 20 ng/ml may be adequate for bone health in the majority of individuals, higher concentrations may be needed to obtain extraskeletal effects, such as the immunomodulatory action [72]. Nonetheless, a serum 25(OH)D target of between 20 and 50 ng/ml is indicated as desirable in order to avoid non-classic toxic effects observed at levels exceeding 50 ng/ml, such as an increased risk of falls, associated with levels higher than 50–60 ng/ml in older individuals. Several factors, such as season, latitude, race, nutrition and age, can impact a person's level of vitamin D. The age-related decline in serum 25(OH)D levels is linked to a lower concentration of precursors in the skin, a decreased capacity for vitamin D production, limited sun exposure, and increased use of drugs interfering with vitamin D metabolism [31,32]. Notably, the seasonal peak for acute respiratory tract infections (RTIs) is generally during the winter, when 25(OH)D concentrations are lowest, and there is clear evidence [73] that higher levels of 25(OH)D may be associated with a decreased risk of acute RTIs, including those caused by influenza [74]. A consistent, independent association between low serum concentrations of 25-hydroxyvitamin D and susceptibility to acute RTI is reported in many observational studies [75,76]. With regard to vitamin D supplementation, in a recent meta-analysis including 25 randomized controlled trials with more than 11,000 participants, Martineau *et al*. demonstrated that vitamin D supplementation reduced the risk of acute RTIs. In subgroup analyses, individuals with very low serum 25(OH)D levels [25(OH) D<25 nmol/l, i.e., 10 ng/ml] and those receiving oral or weekly vitamin D, and not the ones receiving one or more bolus doses, appeared to be, overall, more protected [77]. Consequently, a large proportion of the population is at risk of profound vitamin D deficiency during the wintertime, in particular older individuals, and might benefit from vitamin D supplementation administered at daily or weekly doses to prevent RTIs, while the administration of bolus doses is debated. Older individuals are at major risk of developing severe COVID-19-related disease, with higher mortality rates recorded in people older than 65 years, whether community-dwelling or living in residential homes.

Considering the huge impact of COVID-19 epidemics on health system costs, a low-cost way of reducing the risk and/or progression of the infection, widely and easily applicable to large at-risk populations, is certainly desirable. To date, given that the COVID-19 pandemic is still a recent phenomenon, no randomized controlled trials have been carried out in at-risk individuals with the aim of decreasing COVID-19 disease outbreaks and COVID-19-related morbidity and mortality.

Cross-sectional studies on the possible association between poor vitamin D status and SARS-CoV-2 infection have recently been published.

A recent retrospective study carried out in a cohort of Swiss patients found serum 25(OH)D levels to be lower in individuals positive and symptomatic for SARS-CoV-2 (11.1 ng/ml), as revealed by PCR in swabs, as compared with negative patients (24.6 ng/ml), independently of age [78]. Despite the small size of the study group, coming from a single hospital center, these results confirm other, as yet unpublished, observations of this kind (unpublished data, available at: https://www.medrxiv.org/content/10.1101/2020.05.01.20079376v2). Another cross-sectional analysis has found negative correlations between mean levels of serum 25(OH)D and both the number of COVID-19 cases and COVID-19 mortality per million individuals in different European countries. Although these observations do not prove a causal relationship between low 25(OH)D levels and COVID-19 infections, they lend support to the hypothesis that optimal vitamin D levels might protect against COVID-19 disease [79,80]. Nonetheless, a further study assessing the relationship between serum 25(OH)D concentration and COVID-19 infection in a UK biobank (over 300,000 individuals) did not find any association in a multivariable logistic regression analysis after adjustment for confounders [81]. It would be interesting, in the future, to assess the relationship between ACE2 expression in the lungs and serum 25(OH)D levels, since age- and gender-dependent decreases in ACE2 seem to be correlated with increased COVID-19 morbidity and mortality.

A recent paper, still unpublished, fully confirms in a large dataset the results of the studies listed above. In this work, the correlation of serum 25(OH)D data and severity of COVID-19-related disease was analyzed in COVID-19 patients from several countries with similar screening strategies (Germany, South Korea, China, Switzerland, Iran, UK, US, France, Spain and Italy). In this paper, CRP, a surrogate marker for the cytokine storm, was found to be inversely correlated with 25(OH)D levels (unpublished data, available at: https://www.medrxiv.org/content/10.1101/2020.04.08.20058578v4).

Correction of vitamin D status is advisable in older individuals at risk of musculoskeletal impairment related to low vitamin D levels. In the absence of stronger evidence from clinical trials, many countries' guidelines consider a serum level of 20 ng/ml (50 nmol/l) 25(OH)D to be acceptable and, given the worldwide levels of vitamin D deficiency, take this concentration as the primary treatment goal, even though some data suggest that a higher threshold may possibly be beneficial [82]. Another study indicates that a concentration of 20 ng/ml appears to be adequate to reduce the risk of skeletal problems and ARTIs, while concentrations above 30 ng/ml can be correlated with reduced risks of cancer, type 2 diabetes mellitus and adverse pregnancy and birth outcomes [83]. Daily doses of cholecalciferol up to 4000 IU (upper safety limit advised by the Institute of Medicine) can be administered as loading doses to quickly achieve the desired serum 25(OH)D target (30–50 ng/ml). Unfortunately, at this time there is no evidence that serum 25(OH)D levels above 50 ng/ml are safe and/or offer extraskeletal benefits [84]. Highly concentrated doses of vitamin D must be avoided, as they are linked to mineral metabolism disturbances and increased fall risk. Calcidiol, i.e., 25(OH)D3, can be administered daily or weekly in order to promptly and safely achieve an optimal vitamin D status. Interestingly, a pi-

lot study has shown promising results with this drug [85]. In a recently published study, 76 consecutive patients admitted to hospital for SARS-CoV-2-mediated acute respiratory infection were randomized 2:1 to receive calcidiol (0.532 mg at admission, then 0.266 mg on days 3 and 7, and then weekly until discharge) or placebo, in adjunct to standard treatment (a combination of hydroxychloroquine and azithromycin); the randomization groups were comparable in terms of baseline clinical and biochemical characteristics. In those receiving calcidiol the need for intensive care unit (ICU) admission was reduced to 2%, while in the placebo group it was 50%. This study underlined that increasing serum 25(OH)D levels in the short term, by administering a high dose of calcidiol (the main metabolite of the vitamin D endocrine system), significantly decreased the need for ICU admission of hospitalized patients with proven COVID-19 infection 86. These very promising findings need confirmation in larger trials.

Conclusion

The immunomodulatory action of calcitriol, favoring the induction of antimicrobial peptides, a possible decrease in viral shedding, and modulation of the inflammatory process, is unquestionable. In the field of RTIs and systemic diseases such as COVID-19-related SARS, intervention trials, especially in very high-risk individuals such as elderly people, are much needed. In the meantime, regimens of cholecalciferol and calcidiol commonly employed in clinical practice to prevent and correct musculoskeletal abnormalities are advisable, at least in fragile individuals. In the last two decades, although beneficial effects of high levels of 25(OH)D have been shown to be associated with decreased risks of many adverse health outcomes, including acute RTIs, evidence from large-scale trials is still lacking [73]. Given that the current outbreak of COVID-19-related infection is expected to last for the next two years, randomized controlled trials in the field or primary and secondary prevention of COVID-19-related disease could now be designed and carried out.

Several randomized clinical trials employing either oral cholecalciferol or oral calcifediol, alone or in combination with other supplements or drugs, to prevent or treat COVID-19 infection are currently ongoing [85] and will hopefully provide evidence supporting previous promising observations.

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