The Role of Vitamin D in Prevention and Treatment of Infection

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Abstract

Vitamin D is well known for its classic role in the maintenance of bone mineral density. However, vitamin D also has an important “non-classic” influence on the body’s immune system by modulating the innate and adaptive immune system, influencing the production of important endogenous antimicrobial peptides such as cathelicidin, and regulating the inflammatory cascade. Multiple epidemiological studies in adults and children have demonstrated that vitamin D deficiency is associated with increased risk and greater severity of infection, particularly of the respiratory tract. Although the exact mechanisms by which vitamin D may improve immune responses to infection continue to be evaluated, vitamin D supplementation trials of prevention and adjunct therapy for infection are underway. Given its influence on the immune system and inflammatory cascade, vitamin D may have an important future role in the prevention and treatment of infection.

Keywords

Antimicrobial; cathelicidin; immune system; infection; inflammation; vitamin D

INTRODUCTION

Vitamin D is best known for its influence on bone mineral density, and a daily intake up to 800 IU/day of vitamin D has been recommended by the Institute of Medicine (IOM) to maintain levels optimum for bone health [1]. The American Academy of Pediatrics (AAP) endorsed the IOM recommendations, which recommends that infants who are formula fed or breastfed meet a daily intake of 400 IU/day [2]. Most experts define frank vitamin D deficiency as 25-hydroxyvitamin D (25OHD) levels less than 20 ng/mL (50 nmol/L), based on bone health outcomes such as prevention of osteoporosis and rickets. However, mounting evidence suggests that higher levels may be needed for reported non-skeletal benefits of vitamin D, including optimal immune function. Although controversial, 25OHD levels greater than 30 ng/mL (75 nmol/L) may be required [3]. In addition, there are now clear data that the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25OHD) is a hormone that regulates gene expression in multiple signaling pathways apart from those impacting bone mineral density, specifically those affecting immune function and inflammation [4]. Indeed,
vitamin D has immunomodulatory effects on both the innate and adaptive immune system, modulates the expression of antimicrobial peptides such as cathelicidin, and influences the inflammatory cascade via NFκB. However, the optimum levels of 25OHD considered “sufficient” to optimize vitamin D’s actions on these other signaling pathways are not yet confirmed.

Given its increasingly recognized role as an immunomodulator, numerous studies have begun to explore the relationship between vitamin D deficiency and the incidence and severity of infection in adults and children. Many of these studies have focused on respiratory disease, although there is now evidence that vitamin D deficiency is associated with systemic infection (see separate article in this issue on vitamin D and sepsis) [5]. We will briefly review recent studies exploring the role of vitamin D as an immunomodulator, especially as it relates host susceptibility to infection, and identify important gaps in our understanding of these mechanistic pathways. Additionally, we will examine epidemiological studies linking relative vitamin D deficiency to risk of infection, as well as completed and ongoing clinical trials assessing the efficacy of vitamin D supplementation for prevention and treatment of infection. The growing body of evidence implicating vitamin D as a key component of immune regulation has led to further questions about its mechanisms of action and its therapeutic potential that will require further study.

**IMMUNOMODULATORY ACTIONS OF VITAMIN D**

Recent studies indicate that vitamin D has extensive influence on gene expression affecting the immune system and the inflammatory cascade. Multiple cell types of the innate immune system, including respiratory epithelial cells, macrophages, monocytes, and dendritic cells, possess both the enzyme (CYP27B1) required to convert inactive, circulating 25OHD to active 1,25OHD and its receptor, the vitamin D receptor (VDR) [6–8]. Perhaps the best known immunomodulatory pathway was described by Liu and colleagues, identifying a possible mechanism by which vitamin D could mediate the host response to tuberculosis infection. This group demonstrated that in the macrophage, activation of the toll-like receptor 2 (TLR2) by Mycobacterium Tuberculosis (M.Tb) upregulates both CYP27B1 and VDR. These events led to downstream production of the antimicrobial peptide cathelicidin, causing enhanced killing of M.Tb [9]. Subsequent experiments have confirmed this vitamin D-dependent induction of cathelicidin [10–11].

Cathelicidin is primarily an antimicrobial peptide that is able to directly kill pathogens or bind to endotoxin, by forming ion channels and creating greater membrane permeability [12–13]. In addition to its role against M. Th, cathelicidin has activity against viruses and other bacteria. In the presence of viral infection, lung epithelial cells are able to convert inactive vitamin D to active vitamin D which in turn produces an increased amount of cathelicidin [8]. In vivo studies have demonstrated that in mice infected with Influenza A, treatment with cathelicidin reduces disease severity and viral replication [14]. In murine models lacking cathelicidin, mice were more susceptible to Group A Streptococcus skin infections and Pseudomonas aeruginosa [13]. In cystic fibrosis patients, supplementation with vitamin D caused induction of cathelicidin and increased antimicrobial activity against Pseudomonas aeruginosa and Bordetella bronchiseptica [11]. Whether or not vitamin D supplementation to deficient individuals increases cathelicidin levels in response to a pathogen, and thus confers greater local or systemic immunity to viral and bacterial infections, is an area of ongoing investigation.

Vitamin D also regulates the adaptive immune system, particularly T cells (TH1 cells, TH2 cells, TH17 cells, and T regulatory cells). Antigen stimulation of T cells leads to differentiation into different subgroups of TH1 cytokine producing cells or TH2 cytokine
producing cells. The TH1 cytokine profile is more pro-inflammatory in nature and includes interleukin (IL)-2, IFNγ, and tumor necrosis factor (TNF)-α, while the TH2 cytokine profile is more anti-inflammatory and allergic in nature and includes IL-3, IL-4, IL-5, and IL-10 [15]. Importantly, by activating the T cell VDR, vitamin D appears to suppress TH1 proliferation and cytokine production while promoting TH2 cell proliferation and cytokine production, as well as T regulatory cell production [7, 15]. Given the potent inflammatory effects of TNF-α, IFNγ, and the pro-inflammatory profile of the TH1 cytokines, optimizing vitamin D status may help to regulate inflammation in infected individuals. Conversely, vitamin D deficiency, then, may lead to a “dysregulated” and more pro-inflammatory state in infected individuals. In addition to their role in regulating inflammation, there is evidence that T-cell cytokines play a role in vitamin D-mediated cathelicidin production [16], which may influence susceptibility to bacteria and viruses. This hypothesis requires further investigation.

Finally, vitamin D also regulates the inflammatory cascade by modulating the nuclear factor kappa B (NFκB) pathway. Pathogen associated molecular patterns (PAMPs) are derived from bacteria, viruses, fungi, and protozoa and include lipopolysaccharide (LPS), lipoproteins, flagellin, bacterial DNA, and viral RNA. These PAMPs activate specific toll-like receptors (TLRs) present on various immune cell types. When activated by PAMPs, various TLR signaling pathways induce the NFκB pathway, which upregulates expression of pro-inflammatory cytokines. NFκB is regulated by interacting with inhibitor proteins known as IκB proteins [17]. In the airway epithelium during viral infection, vitamin D upregulates IκBα, which decreases NFκB signaling by binding to NFκB subunits serving to decrease multiple pro-inflammatory cytokines [18]. The upregulation of IκBα by vitamin D has also been demonstrated in cystic fibrosis respiratory epithelial cells treated with *Pseudomonas* lipopolysaccharide. In cells that were exposed to vitamin D, total cellular IκBα increased leading to reductions in IL-6 and IL-8, downstream pro-inflammatory cytokines produced by the NFκB activation [19].

Based on these findings, Vitamin D appears to influence susceptibility to and severity of infection via multiple mechanisms. It has direct influence on production of the antimicrobial peptide cathelicidin, which may lead to increased susceptibility to viruses and bacteria, and it influences cytokine profiles during infection via the innate and adaptive immune system, as well as via the NFκB pathway. Therefore, vitamin D deficiency may lead to a pro-inflammatory phenotype, which may augment disease severity. More research is needed to further define the role that vitamin D may have in modulating these and other key pathways during infection.

**Epidemiologic Studies of Vitamin D and Infection in Adults**

The known actions on the immune system and inflammatory cascade described above provide plausible mechanism for the role of vitamin D in prevention and adjunct treatment of infection. In particular, there have been multiple studies demonstrating an association between vitamin D deficiency and increased risk and severity of respiratory infection. For example, in the largest such epidemiological study to date (n=18,883), Ginde and colleagues analyzed data from the Third National Health and Nutrition Examination Survey and demonstrated an increased prevalence of upper respiratory tract infection among subgroups with 25OHD levels less than 10 ng/mL and 10–30 ng/mL, when compared to those with levels 30 ng/mL or higher (24% vs 20% vs 17% respectively; p<.001) and was independent of potential confounders such as season, body mass index, smoking history, asthma, and chronic obstructive pulmonary disease [20]. In a study of Finnish soldiers, those with 25OHD levels less than 16 ng/ml (40 nmol/L) had more days absent from duty due to physician diagnosed respiratory infection than those who had levels 16 ng/ml or higher [21].
Another study measuring the association between vitamin D deficiency and the risk of acute viral respiratory tract infection showed that those with 25OHD level of 38 ng/mL or more had a significant reduction in the development of acute respiratory tract infection [22]. However, a recent study assessing human rhinovirus (HRV) exacerbations in patients with chronic obstructive pulmonary disease (COPD) showed no relationship between 25OHD level and HRV exacerbation [23]. Overall, although these studies were limited due to their observational nature, they seem to indicate that vitamin D deficiency is a modifiable risk factor for respiratory infection. However, the potential for reverse causation (that sicker patients are more likely to stay indoors and thus have greater vitamin D deficiency) and unmeasured confounding could also explain these associations. In addition, these observational studies did not specifically explore potential mechanisms for reported associations. Thus, causation could not be inferred and clinical trials of vitamin D supplementation are required to explore whether these associations are causal and reversible.

In addition to its association with respiratory infections, several observational studies have found an association between vitamin D deficiency and HIV infection [24, 25]. In addition, in-vitro studies have demonstrated that pre-treatment of monocytes with active vitamin D (1,25OHD) decreased HIV infectivity of these monocytes [26]. Furthermore, vitamin D deficiency may lead to increased proliferation of TH1 cells, which are particularly susceptible to HIV infection [25]. Accordingly, a recent observational study demonstrated that vitamin D deficiency is associated with greater levels of IL-6 in HIV infected individuals, suggesting increased levels of inflammation in these individuals [27]. These preliminary studies collectively demonstrate that vitamin D deficiency may be an important, modifiable risk factor for HIV infection.

In addition to prevention of infection, recent studies have also demonstrated that vitamin D deficiency is related to infection severity, including increased length of stay, increased cost, and increased mortality in those admitted to intensive care units [28, 29]. In a study assessing 25OHD levels in 112 patients admitted with community acquired pneumonia, those with severe deficiency (25OHD levels <12 ng/ml [30 nmol/L]) had higher 30 day mortality [30]. Furthermore, not only are 25OHD levels low in many patients who enter the ICU, but they drop during the initial days of their ICU course, possibly due to hemodilution or changes in the vitamin D binding protein [31].

Although vitamin D deficiency may be associated with greater prevalence and severity of respiratory infection, the mechanism responsible for this association remains speculative. Given the relationship that vitamin D appears to have with cathelicidin, the vitamin D-dependent production of cathelicidin during clinical infection continues to be explored in respiratory infection. In an in vitro study performed in respiratory epithelial cells from patients with cystic fibrosis (CF), epithelial cells were exposed to Pseudomonas aeruginosa in the presence or absence of vitamin D or vitamin D agonists. In the cells treated with vitamin D, cathelicidin mRNA levels increased [19]. Furthermore, after treating vitamin D insufficient serum obtained from African American individuals, Liu and colleagues observed a significant rise in cathelicidin mRNA levels with vitamin D supplementation in cell culture [9]. Animal models have demonstrated that mice infected with Influenza A who are treated with cathelicidin have reduced disease severity and viral replication [14].

However, observational studies that have been performed in several disease states have been inconclusive when attempting to correlate circulating 25OHD and cathelicidin levels. For example, in patients with community acquired pneumonia, serum 25OHD levels were not associated with serum cathelicidin levels, nor were cathelicidin levels associated with greater mortality [30]. In a study assessing 25OHD levels in those with active pulmonary tuberculosis, the prevalence of vitamin D deficiency was 86% with a mean baseline 25OHD
level of 20.4 ng/mL, but serum 25OHD levels did not correlate with serum cathelicidin concentrations [32]. In patients with hepatitis B or hepatitis C, vitamin D deficiency was detected in all patients but had no correlation to plasma cathelicidin levels [33]. It is possible, then, that other mechanisms may be responsible for the association between vitamin D deficiency and infection susceptibility and severity, or that local cathelicidin production is clinically relevant but not reflected by systemic measurements.

Another explanation for the inconsistent correlation between vitamin D status and cathelicidin may be that cathelicidin values vary according to the 25OHD levels at a specific threshold. In a study of healthy adults, plasma cathelicidin levels correlated with 25OHD only when levels were 32 ng/mL or less [4]. However, this study was limited because it had a small enrollment of only 19 patients. Because cathelicidin is produced in respiratory epithelial cells (among other local cells and tissue), perhaps cathelicidin measured from respiratory secretions in patients with respiratory infection would be a more accurate measurement of cathelicidin production, rather than plasma cathelicidin. Data from a study assessing 25OHD levels and cathelicidin levels from nasopharyngeal secretions in children with bronchiolitis showed no significant correlation between these factors. However, in this study, the majority of children had 25OHD of 30 ng/mL or more [34], and therefore were mainly higher than the aforementioned threshold of less than 32 ng/mL shown to correlate with cathelicidin.

**Epidemiologic Studies of Vitamin D and Infection in Children**

A strong association between vitamin D deficiency and respiratory infections has also been reported in infants and children. Vitamin D status at birth has been associated with development of RSV bronchiolitis later in life and those neonates who were born with cord blood levels of 25OHD less than 20 ng/ml (50 nmol/L) had greater risk of developing RSV lower respiratory tract infection in the first year of life compared to those who had levels greater than 30 ng/ml (75 nmol/L) [35]. Another study found that higher cord blood 25OHD levels in 922 newborns were associated with decreased risk of respiratory infection by 3 months of age and decreased risk of wheezing by ages 15 months, 3 years, and 5 years of age [36]. Additionally, in a group of newborns admitted for acute lower respiratory infection, the 25OHD level was significantly lower (9 ng/mL) when compared to controls (16 ng/mL) [37]. Even though sample size was small in this study (n=25), the 25OHD level in the mothers of those with acute lower respiratory infection (ALRI) was lower (13 ng/mL) when compared to mothers of the control group (23 ng/mL).

Given the early susceptibility that these vitamin D deficient infants have in the newborn period, it is possible that vitamin D may play a role in in utero lung development. To further explore this question, Zosky and colleagues used a mouse model to study the relationship between vitamin D deficiency and somatic growth, lung function, and lung structure at 2 weeks of age. In the offspring of vitamin D deficient mice, they found that lung volume was significantly reduced [38]. Given this potential in utero influence that vitamin D has on lung development, it is possible that vitamin D deficiency may augment susceptibility to infection by decreasing the respiratory reserve of infants in addition to the previously described effect on infection risk. More studies assessing vitamin D’s effects on lung development need to be performed to further understand the mechanisms responsible for these findings and the potential implications on infection.

Other observational studies also demonstrate increased prevalence of respiratory infection in vitamin D deficient children. In a case-control study assessing children admitted for pneumonia, greater proportion of children with pneumonia had rickets (and therefore were likely vitamin D deficient) than in those who did not have pneumonia (38% versus 4%) [39].
Another study showed that children with serum 25OHD levels greater than 9 ng/ml (22.5 nmol/L) had significantly less risk for developing ALRI [40]. These children were diagnosed with severe ALRI in the inpatient or outpatient setting and compared to controls who were being seen for immunization administration. In addition, 25OHD levels in children 1–18 months old hospitalized with ALRI were significantly lower when compared to controls (11.6 ng/mL vs 15.6 ng/mL) [41]. In addition to vitamin D deficiency, it appears that vitamin D receptor (VDR) polymorphisms may increase susceptibility to ALRI. In a study comparing children hospitalized for ALRI and those without a history of ALRI, 2 single-nucleotide polymorphisms (SNPs) that encode the VDR were examined (TaqI and FokI). The FokI ff genotype translates for a longer VDR protein and has decreased rates of transcription of VDR RNA. Indeed, this group found that the FokI ff genotype was associated with a relative odds of ALRI seven times more than FokI FF [42]. The importance of VDR polymorphisms have also been described in interventional trials of vitamin D supplementation (discussed later in this review).

Furthermore, the degree of vitamin deficiency in children has been associated with severity of respiratory disease, as those with more severe deficiency were more likely to require pediatric ICU admission in children with acute respiratory tract infection [43]. In children admitted with ALRI secondary to bronchiolitis, those who had 25OHD levels of 15 ng/mL or less had increased need for supplemental oxygen and ventilator management [44]. Other studies have assessed vitamin D status in children admitted to the pediatric intensive care unit (PICU) for all disease states. For example, a study of 511 children admitted to the PICU, the overall median 25OHD level was 22.5 ng/mL, but in those admitted for septic shock the median level was 19.2 ng/mL [45]. In another study that enrolled 326 children admitted to the PICU, the prevalence of 25OHD levels less than 20 ng/ml (50 nmol/L) was high (69%), and vitamin D deficiency was associated with a longer length of stay [46]. Although there appears to be a strong association with severity of disease, none of these epidemiologic studies investigated possible mechanisms by which vitamin D deficiency may confer increased susceptibility to or severity of infection, nor can causation be inferred from these studies. They do, however, provide additional rationale to support clinical trials of vitamin D supplementation to explore the potential efficacy and underlying mechanisms.

**CLINICAL TRIALS WITH VITAMIN D SUPPLEMENTATION**

The earliest therapeutic application of vitamin D for infection may have been for the treatment of tuberculosis, which utilized sunlight and presumably raised the 25OHD levels in these individuals [47]. There were anecdotal benefits, but until recently, no clinical trials evaluated the ability of vitamin D to reduce the incidence and severity of infection. To date, there have been several trials assessing the effects of vitamin D supplementation on infection, and results have been mixed. Li-Ng and colleagues administered 2000 IU of daily vitamin D₃ or placebo to adults and measured the incidence and severity of upper respiratory infection (URI) in each group. They found that there was no difference between the incidence or severity of respiratory infection even though the 25OHD levels increased from 26 ng/mL to 35 ng/mL in the vitamin D supplementation group [48]. However, the duration of supplementation was relatively short (12 weeks), starting 25OHD levels were relatively high, and the supplementation started after the start of the winter respiratory season. More recently, the Vitamin D and Acute Respiratory Infection Study (VIDARIS) trial assessed the effect of vitamin D supplementation on incidence and severity of URI in adults. This was a randomized controlled, double blind study where participants received initial doses of 200,000 IU of vitamin D₃ and then monthly doses of 100,000 IU of vitamin D₃ or placebo for 18 months. At the end of the trial, there were no differences between the groups in number of URIs even though the group who received supplementation had an increase in 25OHD level from 29 ng/ml to 48 ng/ml [49]. However, the average 25OHD level prior to
supplementation in this population was high (29 ng/ml), indicating that may be limited benefit to additional supplementation with relatively high 25OHD levels at baseline.

Other trials have demonstrated that vitamin D supplementation may indeed decrease respiratory tract infections. Based on promising observational data in this population, a randomized controlled trial of 400 IU per day vitamin D3 supplementation versus placebo for 6 months found no difference in the primary outcome of number of days absent from duty secondary to acute respiratory infection. However, the intervention group was more likely to report no days absent from duty due to respiratory infection [50]. Another study tested the ability of vitamin D supplementation to prevent influenza in children. In this study, children were supplemented with 1,200 IU vitamin D3 daily or given placebo and the incidence of influenza A was measured. This study suggested that those who received supplementation had lower incidence of influenza A (10.8% vs 18.6%, respectively) than in those who received placebo [51]. Interestingly, the effect was remarkably strong in children with asthma (odds ratio 0.17), indicating that high risk subgroups, such as those with underlying lung diseases, may benefit more from the intervention. In a randomized double blind study of 744 Mongolian children, children received either unfortified milk or milk fortified with 300 IU of vitamin D for 7 weeks. The children who received fortified milk had higher 25OHD levels at the end of the study (7 ng/ml vs 19 ng/ml) and reported having fewer acute respiratory infections [52]. In a meta-analysis performed by Charan, five clinical trials of vitamin D supplementation were included and found that those in the vitamin D supplementation group had fewer respiratory tract infections (odds ratio = 0.58 (95% CI, 0.42 – 0.81) [53]. However, the dose, timing, and populations were variable, and the optimal balance of these factors for vitamin D supplementation in prevention of infection remains unclear.

Genetic polymorphisms provide an additional layer of complexity. In a recent study assessing vitamin D supplementation in patients treated for tuberculosis, Martineau and colleagues reported that four doses of 100,000 IU vitamin D over six weeks during the active treatment phase of tuberculosis did not decrease time to sputum conversion overall, but did significantly shorten time to sputum conversion in participants with the tt genotype of the TaqI VDR polymorphism [54]. This finding opens new doors for understanding the different effects of vitamin D supplementation based on genetic polymorphisms of VDR or key elements of vitamin D metabolism. In another study of adjunct treatment of tuberculosis, Coussens and colleagues measured the inflammatory response in those treated with four doses of 100,000 IU vitamin D3 versus those treated with placebo over six weeks. They found that those who were treated with vitamin D had suppression of the proinflammatory cytokine response [55]. Therefore, in addition to the antimicrobial role of vitamin D in enhancing cathelicidin in response to pathogens, vitamin D may decrease the inflammatory phenotype seen in patients with respiratory infection. These studies suggest new avenues for future interventional studies.

**FUTURE DIRECTIONS**

Given the complexity of vitamin D’s interaction with the immune system and inflammatory cascade, much work is needed to further define the role of vitamin D in optimizing immune function and reducing the incidence and severity of infections. Vitamin D status is known to be influenced by variants at gene loci affecting cholesterol synthesis, hydroxylation, and vitamin D transport [56], and recently, VDR polymorphisms have been implicated in variable phenotypes in response to vitamin D supplementation [54].

There are multiple interventional trials with vitamin D supplementation that are ongoing attempting to answer the question of whether or not vitamin D supplementation decreases
respiratory tract infections (www.clinicaltrials.gov), including special populations such as older adults (NCT01102374), COPD (NCT00977873), asthma (NCT01248065), dialysis (NCT01312714), and HIV patients (NCT01375010). These also include an ancillary study to the large (n=20,000) vitamin D and omega-3 (VITAL) trial, which will evaluate the role of 2,000 IU/day vs 800 IU/day of vitamin D supplementation in respiratory and non-respiratory infections, as well as serum cathelicidin levels (NCT01758081).

In addition, more research needs to be completed to further define the mechanisms by which vitamin D may regulate immune responses to potentially prevent or reduce the severity of infection. For example, a trial assessing vitamin D supplementation in patients with cystic fibrosis (CF) and assessing inflammatory cytokine levels in these patients is planned (NCT01426256). And, a study assessing sputum cathelicidin in patients with Tb who are treated with vitamin D is also planned (NCT00918086). Another study assessing vitamin D supplementation on anti-bacterial peptides, proinflammatory and regulatory cytokines, and T and B cell response is underway (NCT01399151).

While there is still much progress to be made, the balance of evidence continues to support vitamin D supplementation as a promising intervention for infection. Ongoing clinical trials should continue to clarify the impact of vitamin D supplementation on the incidence and severity of infection. In addition, mechanistic studies will continue to define vitamin D dependent immune pathways and help to inform the optimal intervention, patients, and target pathogens for this intervention.

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References


