Vitamin D and the Respiratory Tract – Emerging Roles in Immune Regulation

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Introduction

In 1903, Niels Finsen was awarded the second Nobel Prize in Physiology and Medicine for treating tuberculosis of the skin successfully with ultraviolet light.[1] By the 1920s, pulmonary tuberculosis was being treated with graded sun exposure.[1] With the advent of sulfanamides after the First World War and the subsequent development of more effective antibiotics, these observations were neglected. However during the last decade, vitamin D research has provided new insights into these historical interventions against tuberculosis and other infectious diseases.[2]

Vitamin D

Vitamin D is made largely from 7-dehydrocholesterol in the skin by ultraviolet-B (UVB) radiation, with lesser amounts coming from the diet.[3] Vitamin D is also found naturally in cod liver oil and oily fish and is added to some foods. It then circulates in the blood, where the liver converts it to 25-hydroxyvitamin D [25(OH)D]. 25(OH)D is measured in the blood to assess overall vitamin D status.[3] Until recently the kidneys were thought to be the main organ that converted 25(OH)D to its active form - 1,25 -dihydroxyvitamin D [$1,25(OH)_2D$], however it is now recognized that most organs are able to do this at a cellular level.[3]

Most authorities consider that a vitamin D level <50 nmol/L (to convert to ng/ml, divide by 2.496) represents a vitamin D deficiency.[3] Using this criteria, vitamin D deficiency is common in many countries around the world.[3] In a small survey of patients attending a general ENT clinic in South Auckland, New Zealand 2% had 25(OH)D levels <17.5 nmol/L (a level associated with osteomalacia) and 58% had 25(OH)D levels <50 nmol/L.[4] Increasing evidence suggests that **Vitamin D deficiency might be a factor in acute respiratory tract infection and related diseases, such as asthma.**

Vitamin D and innate immunity

Vitamin D has an important role in innate immunity through the production of antimicrobial peptides (AMPs), particularly LL-37 from cathelicidin. AMPs are synthesized and released largely by epithelial cells and neutrophils. AMPs have a broad spectrum of antimicrobial activity against viruses, bacteria and fungi.[5] Activation of Toll like receptors on macrophages by mycobacterial 19 kDa lipoprotein increases the expression of vitamin D receptors (VDRs) and the enzyme CYP27B1 that converts circulating 25(OH)D into the biologically active $1,25(OH)_2D$, which increases cathelicidin expression in cells.[2]

Bacteria can exist in sophisticated communities called biofilms. In a biofilm state, bacteria produce an extracellular matrix (often referred to as “slime”), which

protects its inhabitants against environmental threats. The biofilm matrix might also protect bacteria from AMPs.[6] Bacteria in biofilms can make proteinases that can degrade AMPs [6,7] and the exopolysaccaride made by *S. epidermidis* and *S. aureus* is positively charged; it thus repels positively charged AMPs.[6] AMPs are also inactivated by the products of inflammation such as lipopolysaccaride from Gram-negative bacteria.[8] Innate immune mechanisms through AMP production may prevent infection, but may be less effective once biofilms are established.

Vitamin D and adaptive immunity

VDRs are present in dendritic cells, T and B lymphocytes, neutrophils, and macrophages. Vitamin D is able to influence the adaptive response of all these cells.[2] Dendritic cell differentiation, maturation and activation is inhibited by $1,25(OH)_2D$. $1,25(OH)_2D$ downregulates the T_H1 response by inhibiting T_H1 cell development and T_H1 cytokines production.[2] In a large British population cohort, deficient (<25 nmol/L) and excessively high (>135 nmol/L) 25(OH)D levels were significantly associated with elevated serum IgE levels.[9] $1,25(OH)_2D$ also influences T_H2 cell development and thus the allergic response, but the production of T_H2 cytokines may be more U-shaped and not linear.[9]

Vitamin D may influence other T_H lymphocytes such as IL-17 secreting T lymphocytes (T_H17 lymphocytes),[2] which may be beneficial in asthma.[10] Vitamin D may be able to reverse a lack of IL-10 induction which has also been implicated in steroid-resistant asthma.[11] $1,25(OH)_2D$ also influences the generation of another class of T lymphocytes called FoxP3+ CTLA-4+ T_{REG} lymphocytes, which cause an overall potent immune suppression.[2] The influence of vitamin D on the adaptive immune system is complex. $1,25(OH)_2D$ modulates T cell and B cell proliferation as well as cytokine production. Through a number of other mechanisms ranging from dendritic cell regulation to the production of FoxP3+ CTLA-4+ T_{REG} lymphocytes, vitamin D has significant anti-inflammatory effects.[2]

Vitamin D and upper respiratory infection

Low vitamin D status has been linked to an increased incidence of upper respiratory infection (URI).[1] Low 25(OH)D levels are seen in children with otitis media with effusion[12] and tonsil disease,[13] however in neither of these small studies was there a control group. Pinto and colleagues found **low 25(OH) D levels in urban African American,** but not in white subjects, **with chronic rhinosinusitis**.[14] Ginde and colleagues have shown an inverse linear relationship between 25(OH)D concentration **and recent URI**.[15] Sabetta and colleagues also found that 25(OH)D concentrations >95 nmol/L were associated with a two-fold reduction in the risk of developing acute viral respiratory tract infection as well as a marked reduction in the number of days ill.[16]

A growing number of interventional studies suggest that vitamin D supplementation protects against respiratory tract infection (**Table 1**).[1] In one study by Aloia and Li-Ng where 25(OH)D was given for skeletal health, a reduction in infection risk was noted.[17,18] By contrast, a recent study by Li-Ng and colleagues randomised 162 adults to receive vitamin D_3 (2000 IU) daily or matching placebo for 12 weeks.[19] No difference in the duration or severity of URI symptoms was found.

Table 1: Randomized controlled trials (RCT) testing the effect of vitamin D supplementation on infection and/or asthma

Study authors and sample size	Study details	Results	Comment
Aloia and Li-Ng[17] n=204	3-year randomized placebo control study using 2000 IU D_3/day in African American women	Number of flu or cold episodes in the treated group were 1/3 of the placebo group - 8 vs 26	Significant reduction of reported flu episodes - small sample
Avenell et al[18] n=3444	Randomized placebo controlled study using 800 IU D_3/day for 24-62 months for osteoporosis	No difference in infection or antibiotic usage in previous week when surveyed	Low vitamin D_3 dose
Li-Ng et al[19] n=162	12-wk randomized placebo control trial using 2000 IU D_3/day	No significant difference in incidence of flu or cold symptoms	3 month trial - with this dose regimen it may take more than 3 months to achieve adequate 25(OH)D levels
Urashima et al[20] n=334	4-month randomized placebo control trial using 1200 IU D_3/day in school children	RR of 0.58 for influenza A compared with control group (p=0.04). Asthma attacks significantly reduced in treatment group (p=0.006)	Significant reduction of influenza A but not of influenza B. Significant reduction in asthma attacks.
Laaksi et al[21] n=164	6-month randomized placebo controlled trial 400 IU D_3/day	Marginally significant difference in days off in first 6 weeks of study (p=0.06); supplemented group reported as healthier (p=0.045)	Despite the low level of D_3 supplementation 51.3% of people in treatment group remained healthy vs 35.7% in the placebo group
Manaseki-Holland et al[26] n=453	Randomized controlled trial of a single oral dose of 100,000 IU D_3	No difference in days to recovery, but time to repeat pneumonia lower in the intervention group (72 days vs 59 days)	
Makak et al[31] n=48	6-month randomized placebo controlled study of 500 IU D_3/ day in children	A significant reduction in asthma exacerbations in the treated group [17%vs 46%] (p=0.029).	Decreased 25(OH)D levels compared with a stable or increased 25(OH)D level were eight times more likely to have an asthma exacerbation
Martineau et al[27] n= 126	Randomized controlled trial of 100,000 IU D_3 at baseline, 12,28 and 42 days	No significant difference in time to sputum culture conversion [36 days vs 43 days] (p=0.14)	Sputum culture conversion hastened in *tt* genotype of the *Taql* VDR polymorphism (p=0.02)

The authors attributed their null finding to a number of reasons. Firstly, the subjects started vitamin D supplementation during winter, rather than in the fall season or even earlier. It takes up to three months for blood 25(OH)D levels to plateau with vitamin D supplementation if a loading dose is not given. This meant that the subjects were reaching optimum 25(OH)D levels at the end of winter and the end of the trial. Secondly, the vitamin D dosage may have been inadequate and thirdly, the baseline 25(OH)D levels were higher than in previous studies meaning that vitamin D supplementation may have been less effective in these healthy volunteers.

In a second randomized controlled trial (RCT), Urashima and colleagues gave 334 Japanese school children Vitamin D_3 1,200 IU/ daily.[20] There was a 50% reduction in children who were diagnosed with influenza A, the primary outcome. However if one combines the number of cases of influenza A and influenza B, there was no reduction in infections between the vitamin D treated and the control group. This would suggest that vitamin D supplementation was ineffective in reducing the overall incidence of respiratory tract infection.

In a third RCT, Laaksi and colleagues supplemented **164 young Finnish men with 400 IU/day of vitamin D_3.[21] Absence from duty due to respiratory tract infection and number of days absent was lower in the treated group** 2.2±3.2 days when compared to the placebo group 3.0±4.0 days. The proportion of subjects without any days absent was slightly higher in the vitamin D supplementation group (51.3%) when compared to the placebo group (35.7%).

Vitamin D and lower respiratory infection

Rickets is associated with an increased risk of acute respiratory tract infection, particularly pneumonia.[1] In Turkey, newborns with subclinical vitamin D deficiency have an increased risk of developing acute lower respiratory tract infection.[22] In Canada, significantly more children who were vitamin D deficient were admitted to a pediatric intensive care unit with acute lower respiratory tract infection.[23] A case-control study has also reported an association between serum 25(OH)D levels <50 nmol/L and acute lower respiratory tract infection in children.[24] This study led to recognition of a link between sub-clinical vitamin D deficiency, nonexclusive breastfeeding and increased risk for severe acute lower respiratory tract infections. One Canadian study has shown that 25(OH)D status was not associated with an increased risk of hospitalisation for acute lower respiratory tract infection, however the average 25(OH)D level of these infants was 77 nmol/L indicating that their vitamin D status was not going to be an important factor in their risk of developing infection.[25]

In a recent RCT, 453 Afghani children aged 1-36 months with pneumonia were given either a single high-dose of oral vitamin D_3 (100,00 IU) or placebo drops, as well as routine pneumonia treatment.[26] While the recovery times were similar, 45% of children had a further episode of pneumonia within 90 days of supplementation compared to 58% in the placebo group. Children in the supplemented group took longer to have a repeat episode of pneumonia (72 days vs 59 days).

Recently, Martineau and colleagues have shown that the administration of four doses of 100,000 IU of vitamin D_3 did not significantly influence time to sputum culture conversion in the whole pulmonary tuberculosis study population (median time to sputum culture conversion was 36 days in the intervention group and 43 days in the placebo group), but it did significantly hasten sputum culture conversion in participants with the *tt* genotype of the *TaqI* vitamin D receptor polymorphism.[27]

Vitamin D and asthma

Childhood wheezing and asthma exacerbations are usually due to uncomplicated acute respiratory infections. Asthma prevalence is highest in industrialised countries that are furthest from the equator and lower at high altitudes.[28] Camargo and colleagues found an inverse relationship between cord blood 25(OH)D level and cumulative risk of wheezing at age 15 months, 3 years, and 5 years, but no association with doctor-diagnosed asthma.[29] Brehm and colleagues examined the association in **1,022 children between serum 25(OH) D and risk of a severe asthma exacerbation** (defined as an asthma-related emergency department visit or hospitalization) in North America.[30] **Children** with 25(OH)D levels <75 nmol/L **were more likely to have a severe exacerbation over the following 4 years**. In the RCT where **Japanese children were given 1,200 IU/ daily, children with a previous diagnosis of asthma had a significant reduction in number of asthma attacks**.[20] Only 2 asthmatic children taking vitamin D while 12 taking placebo had "asthma attacks." Majak and colleagues have investigated the role of **vitamin D supplementation (Vitamin D_3 500 IU daily) for 6 months in Polish children newly diagnosed with asthma.[31] A significant reduction in asthma exacerbations due to acute upper respiratory tract infections was observed.** Children whose 25(OH)D levels decreased over the study were eight times more likely to have an exacerbation of asthma that those children with a stable or increased 25(OH)D level.

In contrast to these favourable findings, Hyppönen and colleagues have reported that regular vitamin D supplementation (≥2000 IU/day) of Finnish infants increases the risk of developing atopy, allergic rhinitis, and asthma at the age of 31.[32] In a separate study in the UK, they have also linked deficient (<25 nmol/L) and excessively high (>135 nmol/L) serum 25(OH)D_3 levels with elevated serum IgE levels.[9] The relationships of vitamin D with asthma and allergy appear complex. **Vitamin D might influence asthma at least at two levels: 1) predisposition towards / protection against asthma, and 2) prevention of infection-related asthma exacerbations.**

Practical advice for clinicians

Because of the risks of skin damage and skin cancer, many people and health professionals prefer **oral vitamin D supplementation** when treating vitamin D deficient patients. A loading dose is often useful, as plateau levels are not reached for 2-5 months with regular oral supplementation. Humans can obtain vitamin D orally in two forms - vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Most experts consider ergocalciferol less potent than cholecalciferol for raising 25(OH)D levels. As a general rule, vitamin D levels increase by approximately

1.75 nmol/L for each 100 IU/day (0.70 nmol/L for each microgram /day) of vitamin D_3 consumed by adults.[33] In many vitamin D deficient people, current vitamin D supplementation recommendations, even to reach levels of only 50 nmol/L or higher, may be inadequate. Vitamin D requirements in various disease states are not known. A standard oral vitamin D supplementation recommendation in the presence of ongoing infection or absorption problems might not suit all people. The influence of active infection on vitamin D requirements is unknown. The recent work by Martineau and colleagues indicates that vitamin D genetic polymorphisms would also appear to influence the efficacy of vitamin D treatment.[27]

Many laboratories use 150 nmol/L or 200 nmol/L as the safe upper limit for 25(OH)D. However a safe 25(OH)D level may not necessarily be an optimal level. Excessively high (>135 nmol/L) serum levels of 25(OH)D are associated with elevated serum IgE levels. Further research is necessary to determine optimal vitamin D levels. Currently the optimal 25(OH)D range would appear between 100-125 nmol/L. Higher levels may not be beneficial in a number of disease states.

Conclusions

Vitamin D has important links to both innate and acquired immune systems. Many clinical studies, from around the world, suggest that **Vitamin D may have an important role in the prevention and treatment of a number of infectious diseases**.[1] Appropriate double-blind RCT data have just started to be published (**Table 1**). For most situations, the optimal vitamin D treatment regimes, as well as appropriate circulating 25(OH)D levels remain to be determined. Studies suggest, however, that many **people may benefit from vitamin D supplementation to improve their vitamin D status**.

References

Because of the extensive literature concerning vitamin D and infectious disease, the references have been limited to those that relate to recent papers and reviews. If there are researchers whose work is indirectly referred to but not listed below, please accept the authors' apologies.

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