Impact of Vitamin D in the Treatment of Tuberculosis

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Abstract: Tuberculosis (TB) is a major global health problem and often coincides with nutritional deficiency. In fact, vitamin D deficiency has been reported among TB patients, and vitamin D receptor polymorphisms are associated with susceptibility to Mycobacterium tuberculosis. High doses of vitamin D were widely used to treat TB patients in the preantibiotic era. This approach was successful: vitamin D can suppress intracellular growth of M tuberculosis in vitro. Vitamin D also induced the expression of cathelicidin, which is involved in the first line of defense in TB patients. Thus, vitamin D may have a role in TB treatment, and further investigation is needed.


Vitamin D is essential for both normal bone structure and serum calcium maintenance through the regulation of calcium absorption in the gut and reabsorption in the kidney, mediated by the vitamin D receptor (VDR). Many reports have described other nontraditional roles of vitamin D, including roles in experimental autoimmune encephalomyelitis, 1 multiple sclerosis, 2 collagen-induced arthritis, 3 diabetes mellitus, 4 cardiovascular disease 5 and cancer. 6 Vitamin D metabolites are important immunomodulatory hormones that activate monocytes and suppress lymphocyte proliferation, immunoglobulin production and cytokine synthesis. The presence of VDR in peripheral blood monocytes and activated T cells 7,8 suggests a relationship between vitamin D and the immune system. Studies have shown defective macrophage functions; such as impaired chemotaxis, phagocytosis and increased production of proinflammatory cytokines with vitamin D deficiency. 9 An association between low serum 25-hydroxyvitamin D 3 (25OHD 3 ) levels and acute respiratory infections has also been shown in young army recruits. 10 Cell-mediated immunity is crucial to the host response to infection with Mycobacterium tuberculosis. 11,12 Malnutrition is known to suppress immunity, 13 and a possible link between vitamin D deficiency and impaired host defense against M tuberculosis has been suggested. 14 In 2007, a meta-analysis of randomized controlled trials suggested that vitamin D supplements were associated with a decreased risk of mortality due to any cause. 15 In this article, we will review the role of vitamin D in tuberculosis (TB).

VITAMIN D POLYMORPHISM AND TB

Genetic studies provide excellent opportunities to link molecular variations with epidemiological data. DNA sequence variations, as polymorphisms, have modest and subtle but true biological effects. Receptors play a crucial role in the regulation of cellular function, and small changes in their structure might influence various intracellular signal-transduction pathways. 1,25-Dihydroxyvitamin D 3 (1,25OHD 3 ) binds to a nuclear receptor, VDR, which is associated with specific recognition sequences called vitamin D-responsive elements.

The commonly occurring linked single nucleotide genetic markers (polymorphisms) at the 3’ end of VDRs are restriction fragment length polymorphism of BsmI, ApaI and TaqI and the exon 2 splice site FokI polymorphism.

Polymorphisms in the VDR gene have been reported to be associated with susceptibility to M tuberculosis. In fact, a TaqI polymorphism in VDR gene has been shown to be associated with resistance to TB and might provide protection against TB in West Africa and native Paraguayans. 16,17 However, no evidence for this association exists in the Tibetan population. 18 An increased probability of culture conversion during TB treatment has been independently associated with the TaqI Tt genotype. 19 M tuberculosis-infected monocytes secrete matrix metalloproteinase (MMP)-9, 20 which is correlated with the severity of TB. 21 Interestingly, the TaqI T allele has been associated with decreased production of an antiproteinase (tissue inhibitor of metalloproteinase) 1 that is a natural inhibitor of MMP-9. 22 Calcitriol also modulates tissue MMP expression under experimental conditions. 23 Selvaraj et al 24,25 found a higher frequency of the tt genotype in female TB patients, whereas the Bb and FF genotypes were more frequent in male TB patients. A FokI polymorphism in VDR has been linked to an increased risk of clinical TB in Tibetan patients and has been associated with low serum vitamin D levels among Indian immigrants in England; however, this polymorphism is protected against active TB diseases in native Paraguayans. 17,26 Babb et al 26 reported that smoking status and VDR genotype contribute independently to smear conversion time and that the ApaI AA genotype and TaqI T-containing genotypes predictive of a faster response to TB chemotherapy.

However, that case–control study did not identify an association between FDR genotype and TB. Polymorphisms in the VDR gene and NRAMP1 gene (natural resistance-associated macrophage protein 1, now renamed SLC11a1—solute carrier family 11a member 1) have been statistically associated with TB susceptibility in Han Chinese and Iranian populations. 27,28 The NRAMP1 gene has been shown to regulate the concentration of divalent cations in the phagosomes of macrophage. 29 Furthermore, an association between TB resistance and NRAMP1 gene variants has been shown in a Cambodian population. 30 However, no association has been found between NRAMP1 gene polymorphisms and TB in various other racial groups (Denmark, Taiwan and Morocco). 31–33 A polymorphism in Toll-like receptor (TLR) 2 can result in predisposition to the development of TB in humans. 34 Genetic variants in interleukin (IL)-1β, VDR FokI and TLR2 have also been found to be associated with an increased risk of extra pulmonary TB. 35 Lewis et al 36 conducted a meta-analysis of VDR polymorphisms and TB risk using studies of different ethnic populations published before August 2004. They found that the results of these studies were inconclusive and that the studies themselves were underpowered. VDR genotype might affect the severity of TB, but no association was found between TB and VDR polymorphisms in a South African population. 37
In a family-based study, the transmission-disequilibrium test analysis showed a significant global association of the single-nucleotide polymorphism combinations FokI-BsmI-TaqI and FokI-ApaI with TB greater than that of individual single-nucleotide polymorphism.37 Recently, Gao et al38 reviewed 23 studies with regard to the relationship between VDR polymorphisms and TB. They noted that the FokI ff genotype showed a pronounced positive association with TB. On the other hand, a significant inverse correlation with TB was identified for the BsmI bb genotype, and marginally significant associations with TB were also found for the TaqI and ApaI polymorphisms. VDR gene variants may regulate cytotoxic T-cell responses via 1,25(OH)2D3-mediated suppression of granzyme A expression in TB.39 A Gc gene variant of the vitamin D binding protein has been shown to be related to decreased circulating levels of 25(OH)D3, 1,25(OH)2D3 and vitamin D binding protein40,41 and was found to be strongly associated with susceptibility to active TB in Tibetans.40 The relationship between allelic variations in VDR and TB has been summarized in Table 1.

### ROLE OF VITAMIN D IN TB

High doses of vitamin D were widely used to treat active TB in the preantibiotic era. In 1847, Charpy and Dowling42 discussed the role of vitamin D in cutaneous TB. Vitamin D was used to treat TB of the bone and offered some improvement.43 Williams44 then reported the beneficial effects of fish liver oil in TB patients. In addition, the importance of sunlight has been observed throughout the history of TB treatment. In 1854, Hermann Brehmer, a Silesian botany student who was suffering from TB, traveled to the Himalayan mountains to pursue his botanical studies and cured his TB.45 Ultraviolet (UV) light B exposure is sufficient to double the circulating 25(OH)D levels, but no significant change has been observed in antinociceptor immunity.46 Association between vitamin D deficiency and TB has been reported in a number of studies. A vegetarian diet, which is associated with a low plasma vitamin D level, is an independent risk factor for active TB among Asian immigrants living in south London.47 TB patients also have significantly lower mean concentrations of serum 25(OH)D3 compared with healthy subjects.48,49 A correlation between serum levels of vitamin D and risk for latent TB infection has been noted among African immigrants living in Australia.50 Furthermore, low vitamin D levels have been associated with a 5-fold increased risk for progression to TB.51 Patients with chronic renal failure are also at an increased risk for developing TB,52 because certain uremic toxins can suppress 1,25(OH)2D3 synthesis and its biological activity.53 To study the association between low vitamin D serum levels and the risk of active TB in humans, Nnoaham and Clarke54 conducted a systematic review and meta-analysis of observational studies published between 1980 and July 2006. They found that low vitamin D serum levels were associated with higher risk of active TB.

Vitamin D is known to suppress the intracellular growth of "M. tuberculosis" in vitro.55 Crowle et al56 demonstrated that a concentration of 4 μg/mL of 1,25(OH)2D3 in vivio could inhibit the multiplication of virulent tubercle bacilli in cultured human macrophages. In 1969, Brincourt57 reported that vitamin D supplements were able to dissolve cavities in TB patients. Furthermore, in a random trial, multivitamin supplements (including vitamin D) reduced mortality by 50% among human immunodeficiency virus-infected TB patients.58 Morcos et al59 treated 24 cases of TB in children and noted clinical and radiological improvement after vitamin D treatment. In another study, vitamin D supplements resulted in more rapid sputum clearance of acid-fast bacilli and radiological improvement among Indonesian TB patients.60 Anti-TB therapy and correction of a vitamin D deficiency have also resulted in clinical and microbiologic improvements in a refractory drug-susceptible TB patient.61 On the other hand, Wejse et al62 reported that vitamin D did not improve the clinical outcomes of TB patients as their trial demonstrated no overall effect on mortality in TB patients. However, this finding may have been due to a suboptimal dosage.

### MECHANISM OF VITAMIN D IN TB

The gene encoding for the tryptophan-aspartate-containing coat proteins has been recognized to play a crucial role in the "M. tuberculosis" survival within human macrophages.63 The combination of vitamin D and retinoic acid has been reported to down-regulate tryptophan-aspartate-containing coat proteins

### Table 1. Relationship between allelic variations in vitamin D receptor (VDR) gene and tuberculosis (TB)

<table>
<thead>
<tr>
<th>Polymorphism in the VDR gene</th>
<th>Linked to</th>
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<tbody>
<tr>
<td>TaqI</td>
<td>Resistance to TB in West Africa and native Paraguayans</td>
</tr>
<tr>
<td>Tt allele</td>
<td>Increased culture conversion during TB treatment</td>
</tr>
<tr>
<td>T allele</td>
<td>Decreased tissue inhibitor of metalloproteinase (TIMP)-1</td>
</tr>
<tr>
<td>tt genotype</td>
<td>Higher frequency in female TB patients</td>
</tr>
<tr>
<td>Bb and FF genotype</td>
<td>Higher frequency in male TB patients</td>
</tr>
<tr>
<td>FokI</td>
<td>Increased risk TB in Tibetan patients</td>
</tr>
<tr>
<td>ff genotype</td>
<td>Decreased vitamin D levels in Indian immigrants in England</td>
</tr>
<tr>
<td>Apal AA genotype</td>
<td>Against active TB in native Paraguayans</td>
</tr>
<tr>
<td>BsmI bb genotype</td>
<td>Increased risk of extra pulmonary TB</td>
</tr>
<tr>
<td>VDR gene variants</td>
<td>Predictive of a faster response to TB chemotherapy</td>
</tr>
<tr>
<td>Vitamin D binding protein</td>
<td>Significant associations with TB</td>
</tr>
<tr>
<td>Gc gene variant</td>
<td>May regulate cytotoxic T-cell response by suppression of granzyme A in TB</td>
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25(OH)D3, vitamin D binding protein (DBP), against active TB in native Paraguayans, increased risk of extra pulmonary TB, increased culture conversion during TB treatment, decreased tissue inhibitor of metalloproteinase (TIMP)-1, higher frequency in female TB patients, higher frequency in male TB patients, increased risk TB in Tibetan patients, decreased vitamin D levels in Indian immigrants in England, initial risk of TB to West Africa and native Paraguayans, resistance to active TB in native Paraguayans, increased risk of extra pulmonary TB, significant associations with TB, may regulate cytotoxic T-cell response by suppression of granzyme A in TB, no TB association in a South African population, vitamin D binding protein, against active TB among Gujarati Asians in London.
transcription in a dose-dependent manner\(^6\) and inhibits \textit{M tuberculosis} entry into and survival within macrophages.\(^6\)

1,25OHD\(_3\) reduces the viability of \textit{M tuberculosis} by enhancing the fusion of phagosomes and lysosomes in the infected macrophages.\(^6\)

The role of 1,25OHD\(_3\) is important in normal innate immune responses. TLRs are a part of the innate immune system and detect foreign invaders. TLR2 activation leads to direct antimicrobial activity against intracellular \textit{M tuberculosis} infection in human and murine macrophages.\(^6\)

TLR activation results in the expression of VDR and 1α-vitamin D hydroxylase in human monocytes.\(^6\)

1,25OHD\(_3\) can cause vitamin D-induced expression of cathelicidin in bronchial epithelial cells\(^8\) and may also enhance the production of leucine-enein-37, an antimicrobial peptide of the cathelicidin, which acts as a first line of defense in TB prevention.\(^7\)

Liu et al\(^8\) demonstrated that poor vitamin intake may increase susceptibility to \textit{M tuberculosis} infection by inefficiently supporting the induction of cathelicidin mRNA expression in monocytes. The addition of a VDR antagonist inhibited this induction of cathelicidin mRNA by more than 80%; consequently, the antimicrobial activity was reduced by approximately 70%.\(^6\)

1,25OHD\(_3\) can directly induce antimicrobial gene expression and activity through vitamin D-responsive elements located in the promoters of cathelicidin and defensin.\(^7,2\)

Furthermore, knockdown of either defensin or cathelicidin in primary monocytes results in a loss of TLR-mediated antimicrobial activity against intracellular mycobacteria.\(^7,2\)

1,25OHD\(_3\) also induces autophagy in human monocytes via cathelicidin and leads to the localization of mycobacterial phagosomes with autophagosomes in human macrophages in a cathelicidin-dependent manner.\(^7\)

African Americans seem to be more susceptible to \textit{M tuberculosis} infection in rural communities of southeastern America.\(^7\)

Crowle and Elkins\(^7\) reported that the tubercle bacilli grew significantly faster in infected macrophages from African Americans and 1,25OHD\(_3\) gave less protection against bacilli-infected macrophages from African Americans than from white donors. Furthermore, vitamin D insufficiency is more prevalent among African Americans than other Americans in North America.\(^7\)

African Americans may have an intestinal resistance to the actions of 1,25OHD\(_3\).\(^7,7\)

MMP enzymes can degrade all components of the pulmonary extracellular matrix. \textit{M tuberculosis} induces MMP expression in infected human macrophages,\(^8,10\) and these enzymes have been implicated in the pulmonary cavitation observed in TB patients. In terms of treatment, 1,25OHD\(_3\) has been reported to inhibit MMP secretion by human monocytes\(^5,8,8\) and enhances the level of its inhibitors (tissue inhibitor of metalloproteinase-1) in TB.\(^8\)

Vitamin D-induced monocyte resistance to \textit{M tuberculosis} is regulated by PI 3-K (class I phosphatidylinositol 3-kinase) and is mediated by NADPH-dependent phagocyte oxidase.\(^5\)

PI 3-K is a multifunctional signaling molecule that has been implicated in a wide range of cellular processes.\(^8\)

In vitamin D-deficient mice, macrophages function abnormally, but these defects can be corrected by 1,25OHD\(_3\) restoration in vitro and in vivo.\(^8\) In vitro, 1,25OHD\(_3\) stimulates macrophage differentiation and hydrogen peroxide (H\(_2\)O\(_2\)) production\(^8\) and inhibits bacterial growth in macrophages infected with \textit{M tuberculosis}.\(^9,1,1,2\)

1,25OHD\(_3\) enhances mycobacterial killing by increasing nitric oxide production, a potent antimicrobial mechanism in activated macrophages. Furthermore, 1,25OHD\(_3\) may limit host damage by decreasing \textit{M bovis}-induced gamma interferon (IFN-γ) production.\(^9\)

TB-infected mice deficient in nitric oxide synthetase 2 develop severe necrotizing pyogranulomatous inflammation of the lungs with heavy TB bacilli colonization and systematic dissemination.\(^9,4\) 1,25OHD\(_3\) reduces the growth of \textit{M tuberculosis} in cultured human peripheral blood mononuclear cells by 75%.\(^4\)

Furthermore, lymphocyte apoptosis is diminished by the addition of 1,25OHD\(_3\), to cultured TB-infected mononuclear cells.\(^9\)

\textit{In vitro} and \textit{in vivo}, IFN-γ is a potent enhancer of mononuclear phagocyte H\(_2\)O\(_2\) secretion in murine cells; however, no additive or synergistic effects of 1,25OHD\(_3\) and IFN-γ have been shown with regard to H\(_2\)O\(_2\) production.\(^9\)

The presence of the VDR in peripheral blood monocytes and activated T cells\(^7–\text{97}\) provides evidence for relationship between vitamin D and the immune system. Vitamin D has a significant role in TB, along with its associated immune-regulatory activities. 1,25OHD\(_3\) up-regulates innate immunity via phagocytosis by monocyte/macrophage populations and down-regulates acquired immunity by inhibiting major histocompatibility complex class II antigen expression by antigen-presenting cells.\(^9\)

In mice, TB-infected lungs have been shown to produce high levels of tumor necrosis factor α, IFN-γ and IL-6 mRNA.\(^9\)

1,25OHD\(_3\) differentially modulates the production of cytokines in response to \textit{M tuberculosis} antigens predominantly by suppressing the production of IL-12 and IFN-γ.\(^9\)

IL-12 is a key cytokine required for T\(_h\)1 development,\(^10\) a prominent feature in TB lesions.\(^10\)

Pyrazinamide (PZA) is an important TB drug and plays a key role in shortening TB therapy from 9 to 12 months to 6 months.\(^10\)

Wade and Yang reported that UV light enhanced the activity of PZA against \textit{M tuberculosis} \textit{in vitro}\(^10,10\) and suggested that UV light can generate free radicals that cause damage to macromolecules—such as DNA—and affect membrane integrity. 1,25OHD\(_3\) has been reported to have a synergistic effect with PZA to kill tubercle bacilli in cultured human macrophages.\(^10,1\)

A modest but significant abnormality in the regulation of circulating 1,25OHD\(_3\) has been observed in normocalcemic TB patients.\(^10,1\)

Increased 1,25OHD\(_3\) levels have been reported in TB patients, and, consequently, hypercalcemia has also been observed in TB patients, which could lead to down-regulation of VDR expression.\(^10,1,10\)

This 1,25OHD\(_3\) level is produced by bronchoalveolar macrophages and T lymphocytes in the blood in TB patients.\(^10,1,10\)

Selvaraj et al\(^10,10\) suggested that high plasma 1,25OHD\(_3\) levels in TB patients might be because of up-regulated expression of \textit{CYP27b1}, which is involved in the conversion of 25OHD\(_3\) to 1,25OHD\(_3\). Previous studies have reported that \textit{M tuberculosis} lipoproteins stimulate the up-regulation of \textit{CYP27b1} through dendritic cells and monocytes.\(^7\)

Adams et al\(^10,10\) suggested that diminished availability of the 25OHD\(_3\) substrate, available for \textit{CYP27b1} compromises host’s responses to \textit{M tuberculosis}. The addition of 1,25OHD\(_3\) can lead to increased serum 25OHD\(_3\) levels, normal serum calcium levels\(^10,10\) and increased expression of cathelicidin, which may enhance immunity against TB.\(^10,10\)

On the other hand, 1,25OHD\(_3\) does not result in a concomitant increase in 24-hydroxylation enzyme activity.\(^10,10\)

Chloroquine has been used to control hypercalcemia in granulomatous patients,\(^11\) and chloroquine therapy has been associated with a significant reduction in the serum levels of 1,25OHD\(_3\) and urinary calcium, perhaps via inhibition of the conversion of 25OHD\(_3\) to 1,25OHD\(_3\).\(^1\)

However, a combination of three drugs (chloroquine, 1,25OHD\(_3\) and PZA) kills TB faster than the combination of 1,25OHD\(_3\) and PZA in cultured human macrophages.\(^11\)

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Ketoconazole has been reported to decrease the serum levels of ionized calcium and 1,25(OH)2D in TB-associated hypercalceemia113 and has also been shown to act against M tuberculosis in vitro and in a mouse model.114

CONCLUSION

The relationship between vitamin D and TB has been discussed previously, and vitamin D may have a role in TB treatment. Vitamin D supplementation may be beneficial to individuals with insufficient vitamin D levels. Additional studies into the role of vitamin D in TB treatment are warranted.

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