

## Mini Review

# Vitamin D and Mycobacterial Infections: Basic and Clinical Research

**Miragliotta G and Miragliotta L\***

Department of Interdisciplinary Medicine, University of Bari ALDO MORO, Italy

**\*Corresponding author:** Giuseppe Miragliotta, Department of Interdisciplinary Medicine, University of Bari ALDO MORO, Policlinico, Piazza G Cesare, 70124 Bari, Italy**Received:** August 24, 2015; **Accepted:** October 28, 2015; **Published:** October 30, 2015**Abstract**

Since the early 19<sup>th</sup> century, both environmental (sunlight) and dietary sources (cod liver) of vitamin D have been identified as treatment for tuberculosis. In the last years, it has been recognized that vitamin D modulates a variety of processes and systems concerning host defense, inflammation, immunity. In this context there is a growing evidence that vitamin D boosts antimycobacterial immunity *in vitro*, in particular by stimulating macrophage response to *Mycobacterium tuberculosis*, mainly via the antimicrobial peptide cathelicidin. The epidemiological observations that low vitamin D status is associated with tuberculosis severity or susceptibility have stimulated clinical trials concerning the possibility either to prevent tuberculosis or to improve clinical recovery by supplementation with vitamin D. Although current results seem to suggest a role to vitamin D in prevention or treatment of tuberculosis, more rigorously designed clinical trials are needed for the delineation of necessary changes in clinical practice. Of note, the association between vitamin D and non-tuberculous mycobacterial lung disease (in particular caused by *Mycobacterium avium* complex and *Mycobacterium abscessus*) has been poorly studied. Although a case-control study has ascertained that patients with non-tuberculous mycobacteria infection have a high prevalence of vitamin D deficiency, the causality of the phenomenon needs further studies to be determined.

**Keywords:** Vitamin D; Vitamin D supplementation; Tuberculosis; Non-tuberculous mycobacteria**Abbreviations**

UVB: Short wave Ultraviolet Rays; VDR: Vitamin D Receptor; BMI: Body Mass Index; AMPs: Antimicrobial Peptides; MT: *Mycobacterium Tuberculosis*; NTM: Non-tuberculous Mycobacteria; MAC: Mycobacterium Avium Complex

**Introduction**

Historically, the first observation on the putative role of vitamin D in the host defense to tuberculosis was that by the British physician Williams CBJ, who reported a “marked and unequivocal improvement in patients suffering from tuberculosis after treatment with cod liver oil [1]. On the other hand, until the introduction of tuberculosis chemotherapy, patients with active tuberculosis were treated with either vitamin D enriched food (e.g. fish oil) or by sunlight exposure in sanatoria [2].

In the early 1920s Goldblatt H and Soames KM firstly described the capacity of light rays to either stimulate the growth of rats or increase the degree of calcification of their bones [3], as well as Steenbock H demonstrated the induction of growth promoting and calcifying properties in foods irradiated with ultraviolet light [4]. Nowadays the anecdotal association that patients placed in the sun would see improvement in tuberculosis symptoms can be easily explained if we consider that the exposure to UV sunlight is responsible for the synthesis of 1 $\alpha$ , 25-dihydroxyvitamin D (i.e. sunshine vitamin). A substance necessary for bone formation and later known as vitamin D, was independently identified in cod liver oil by Elmer V. McCollum in 1922 [5].

Vitamin D is available to humans in two different forms: vitamin D<sub>3</sub>, or cholecalciferol, and vitamin D<sub>2</sub>, or ergocalciferol. Cholecalciferol is synthesized in the skin from 7-dehydrocholesterol (7-DHC) under exposure to sunlight (UVB: 290-315 nm) which promotes its non-enzymatic conversion to previtamin D<sub>3</sub>. Vitamin D<sub>3</sub> (cholecalciferol) derives from previtamin D<sub>3</sub> by its conversion by body heat. Excessive sunlight exposure degrades previtamin D<sub>3</sub> and vitamin D<sub>3</sub> to inactive photoproducts in order to avoid excessive production of the vitamin in the skin. Vitamin D<sub>3</sub> is stored in adipose tissue. Ergocalciferol is produced in various plant materials, yeast and fungi when they are exposed to UVB radiation [6]. Humans can obtain both forms from diet by consumption either of animal or plant products. Vitamin D<sub>3</sub> and vitamin D<sub>2</sub> are converted in the liver by the enzyme 25-hydroxylase to 25-hydroxyvitamin D (25[OH] D), also known as calcidiol, the form that circulates in the highest concentration and reflects from both solar and dietary exposure, being therefore a reliable indicator of the vitamin D status [7]. Then it is converted in the kidney to the metabolically active vitamin D hormone, 1 $\alpha$ , 25-dihydroxyvitamin D [1 $\alpha$ , 25(OH) 2D] or calcitriol, by the mitochondrial D-1 $\alpha$ -hydroxylase enzyme (CYP27B1). Although the most 1 $\alpha$ , 25(OH)2D is synthesized in the primary renal tubules of the kidney, extrarenal production of vitamin D may occur in bone, epithelial cells of the skin, lung and colon, parathyroid glands and immune cells, especially activated macrophages [6]. Renal production of 1 $\alpha$ , 25(OH)2D occurs in response to decreased levels of circulating Ca<sup>2+</sup>, which stimulates the production of parathyroid hormone. Parathyroid hormone induces the production of CYP27B1 by primary renal tubules.

## Basic Research

Since the discovery of its role played in rickets prophylaxis in the 1920, vitamin D was for a long period of time only considered in relation to its function in calcium and bone metabolism. The modulation of the immune system was suggested by the presence of VDR in nearly all types of immune cells [8]. Several candidate polymorphisms of VDR and Vitamin D Binding Protein have been studied in their capacity to modulate the development of tuberculosis. In this regard, it is very interesting the hypothesis that VDR polymorphisms may play a role in genetic susceptibility to tuberculosis [9]. A meta-analysis showed a positive association between VDR polymorphism and host susceptibility to tuberculosis [10]. Recently, it has been ascertained that the antimicrobial activity is exerted by vitamin D mainly via its influence on monocytes and macrophages, thus playing a role in the innate immune response to specific infectious agents, including Mt [11]. During bacterial infection, vitamin D has been reported to act in the local tissue response [12], playing an integral role in the production of AMPs [13,14]. In 1986 the 1, 25 (OH) 2D3 treatments of murine and human macrophages was able to potentiate the effects of IFN- $\gamma$  to inhibit Mt in vitro [15,16]. More recently, the critical role exerted by vitamin D in macrophage response to Mt via the AMP cathelicidin has been shown [17,18]. The cathelicidin active fragment LL-37 is produced by phagocytic leukocytes, mucosal epithelial cells, and keratinocytes and is present in both plasma and mucosal secretions [19]. Indeed the intracellular killing of Mt by macrophages, along with the production of nitric oxide [20], is closely related with the cationic antimicrobial peptide human cathelicidin LL-37 [21]. However, the putative synergy of those pathways (nitric oxide and cathelicidin, respectively) needs further exploration [11,22]. The above mentioned studies by Liu [17,18] and other Authors [23,24] have demonstrated the immunomodulatory effects of vitamin D, which promotes LL-37 expression and intracellular killing of Mt. After Mt interaction with Toll-like receptors, the activated macrophages utilize serum circulating 25(OH) D in order to locally produce 1, 25(OH) 2D that, in turn, interacts with VDR. The 1, 25 (OH) 2D-VDR interactions activate the cathelicidin gene, culminating in Mt destruction. These findings are consistent with the notion that low levels of vitamin D are associated with enhanced susceptibility to develop active tuberculosis [17]. Moreover, the capacity of LL-37 to disrupt bacterial membrane integrity, thus inducing autophagy (i.e. intracellular degradation of mycobacteria) has been described [25]. Aside being macrophages the first and primary line of the host cellular defense against the infection, they represent the primary site of bacterial replication. It is noteworthy that vitamin D contributes to the antimicrobial response to Mt by hindering phagosomal progression (i.e. phagosome maturation arrest), through the retention of the host's tryptophan-aspartate-containing coat protein [26,27]. Recently, another role for vitamin D in human tuberculosis has been described. Vitamin D treatment of infected macrophages was indeed demonstrated to be able to suppress the intracellular storage of lipid droplets which are required for the intracellular Mt growth [28].

## Clinical Research on Vitamin D and Mycobacterial Infections

### Mycobacterium tuberculosis infection

Tuberculosis infects approximately a third of the world's

population, causing symptomatic disease in 8.7 million people annually [29]. Furthermore, it is estimated that one third of the world population has latent (asymptomatic) Mt infection, and up to 10% of patients with latent infection will suffer a reactivation of the infectious disease [30]. Therefore, being tuberculosis a major global health problem, the basic science research of vitamin D's effects on mycobacteria has been complemented by clinical studies. Epidemiological data suggested that low vitamin D status is associated with tuberculosis severity or susceptibility [31]. In this regard, defining a level of serum 1 $\alpha$ , 25-hydroxy-vitamin D3 as low or insufficient depends on the level that is defined normal. This interest in vitamin D status was exemplified by the 2011 Institute of Medicine (IOM) report which established a minimum serum 25-hydroxyvitamin D [1  $\alpha$ , 25(OH)D] concentration of 20 ng/mL as the optimal level for skeletal health in the USA [32]. For optimal health benefits, the Endocrine Society recommended a concentration of at least 30 ng/mL [7]. The putative roles played by vitamin D appears to be linked to the VDR polymorphisms [9], although larger studies are required to definitely establish whether the genetic susceptibility to tuberculosis is related to VDR polymorphisms. Over the last years, low vitamin D levels have been demonstrated in association with the increased risk of active tuberculosis [33-36] even if independently of nutritional status [37]. In a Korean population the median 25(OH) D levels in patients with active tuberculosis were significantly lower than those of healthy controls, and the prevalence of vitamin D deficiency was higher in tuberculosis patients than among healthy controls. In both tuberculosis patients and healthy controls low BMI and the seasonality (i.e. spring/winter seasons) represented risk factors for severe vitamin D deficiency [37]. Those findings are in agreement with the reports from Tanzania [38], Uganda [39], and Malawi [36]. In order to explore the association between low serum vitamin D and risk of active tuberculosis in humans, the systematic review and meta-analysis of observational studies between 1980 and July 2006 concerning the association between low serum vitamin D and risk of active tuberculosis concluded that low serum vitamin D levels are associated with higher risk of active tuberculosis [33]. More recently, another study showed that the 1,25(OH) 2D3 concentrations were lower in patients with tuberculosis than in healthy adults, thus providing evidence that lower levels of 1,25(OH)2D3 may predispose to tuberculosis or might represent a risk factor during the development of tuberculosis [40]. To investigate the role played by vitamin D in the pathogenesis of tuberculosis the levels of 25(OH) D were studied in patients who had recovered from Mt disease, in comparison to levels in persons without tuberculosis. Interestingly, individuals who had recovered from tuberculosis had lowered 25(OH)D levels compared to controls without tuberculosis. This finding may reflect pre-morbid levels as well as it might suggest that low 25(OH)D represent a risk factor for tuberculosis rather than a consequence of the disease itself. In addition, low levels of 25(OH) D may be a predisposing factor for developing recurrent tuberculosis [41]. Those findings prompted studies aimed to verify the usefulness of a vitamin D treatment in tuberculosis. In this regard, several randomized, double-blind, placebo-controlled trials are available. In one study, 67 tuberculosis patients received at random vitamin D (0.25 mg/day) or placebo during the 6 initial weeks of treatment. A statistical significant difference in sputum conversion (i.e. the change of detectable to not detectable *Mycobacteria* in the sputum)

was observed in favor of the vitamin D group [42]. In another study, 192 healthy adult tuberculosis contacts were randomized to receive a single oral dose of 2.5 mg vitamin D or placebo and followed-up for 6 months. In comparison with placebo, vitamin D supplementation significantly enhanced the ability of participants' whole blood to restrict BCG-lux luminescence in vitro without affecting antigen-stimulated INTERFERON-lux- $\gamma$ -responses. The results allowed authors to conclude that vitamin D supplementation may primarily enhance innate response (as measured by the ability of whole blood to restrict luminescence) without any polarization of acquired immune response (antigen-stimulated IFN-  $\gamma$ -response) [43]. With the aim to test whether vitamin supplementation to tuberculosis patients might improve clinical outcome and reduce mortality, 365 individuals were studied in a double-blind, placebo-controlled trial. The intervention consisted in 100,000 IU of vitamin D administration or placebo at inclusion and again 5 and 8 months after the start of treatment. Two-hundred-eighty-one patients completed the 12-months follow-up. Vitamin D did not improve clinical outcome among patients and the trial did not show any effect on mortality, although the possibility that the dose used was insufficient should be taken into consideration [44]. In this regard it might be of interest that supplementation with higher doses of vitamin D (600,000 IU) accelerated clinical and radiographic improvement in TB patients as well as increased immune activation in patients with baseline deficient serum vitamin D level (<20 ng/mL) [45]. On the basis of both clinical and ecological-observational studies available to date [46], a therapeutic role for vitamin D treatment of tuberculosis appears of interest. However, the relationship between baseline vitamin D status, dose of vitamin D supplements, and tuberculosis remains to be further investigated. In particular, the complex mechanisms by which vitamin D deficiency or repletion might modify the host response to Mt need further investigation [47].

### Non-tuberculous mycobacteria infection

NTM are a group of more than 100 species of bacteria that are ubiquitous in soil and water. Since NTM require defects in local or systemic host immunity to cause disease, they are opportunists and exhibit varied pathogenicity. The association between vitamin D deficiency and NTM infection has been poorly studied. In this regard, a case-control study evaluating the prevalence and severity of vitamin D deficiency in patients with NTM lung disease has shown that the median 25(OH)D levels for patients with NTM lung disease are significantly lower than those of healthy controls. Moreover, *Mycobacterium avium* complex was found to be independently associated with vitamin D deficiency in patients with NTM lung disease [48]. Bacteria belonging to MAC are frequent cause of disseminated bacterial infection in patients with AIDS and are responsible for high morbidity and mortality. A previous study showed that patients with disseminated MAC infection had severely decreased serum 1,25D levels compared with all other AIDS patients, as well as they exhibited marked activation of the TNF system [49]. The association of activated TNF system and decreased levels of vitamin D might indeed suggest a combination of events leading to strongly impaired defense against intracellular microbes, to enhanced HIV replication, and, finally, to a more rapid progression of immunodeficiency [49].

### Concluding Remarks

Closer attention should be paid to vitamin D status in patients

with mycobacterial diseases. The data available to date on vitamin D from experimental, ecological, case-control, retrospective and observational studies strongly suggest a role of the sunshine vitamin. Even if not yet conclusive, the results of ecological and clinical studies along with the basic research findings strongly support the recommendation to improve the general vitamin D status in children and adults. Maintaining indeed normal serum levels of 25-hydroxyvitamin D may help in the prevention and amelioration of tuberculosis and, more in general, mycobacterial diseases. Meanwhile, we must await the results of further controlled and randomized interventional studies on this very important medical matter.

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