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Immunomodulation by vitamin D: implications for TB

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Abstract

TB remains a major cause of mortality throughout the world. Low vitamin D status has been linked to increased risk of TB and other immune disorders. These observations suggest a role for vitamin D as a modulator of normal human immune function. This article will detail the cellular and molecular mechanisms by which vitamin D regulates the immune system and how vitamin D insufficiency may lead to immune dysregulation. The importance of vitamin D bioavailability as a mechanism for defining the immunomodulatory actions of vitamin D and its impact on TB will also be discussed. The overall aim will be to provide a fresh perspective on the potential benefits of vitamin D supplementation in the prevention and treatment of TB.

Keywords

cathelicidin; CYP24A1; CYP27B1; defensins; monocyte; neutrophil; TB; Toll-like receptor; vitamin D; vitamin D receptor

TB is caused by the infectious pathogen *Mycobacterium tuberculosis* (Mtb) and is one of the major causes of death throughout the world. Antibiotics can be used to treat TB but the cost of these drugs and the rise of antibiotic resistance have prompted renewed efforts to identify additional tools to combat the worldwide health challenges of this disease. Prominent among these is vitamin D and this article will discuss the various findings supporting a beneficial role for vitamin D in TB. In particular, the article will focus on the two concepts that have underpinned the link between vitamin D and the immune system, and thus the potential impact on TB outcome: the mechanisms for innate and adaptive immune responses to vitamin D and new perspectives on what constitutes vitamin D sufficiency, notably the bioavailability of vitamin D to target cells in the immune system. These two concepts are schematically represented in **FIGURE 1**.

Mechanisms for vitamin D metabolism & function

The machinery involved in vitamin D metabolism and action has been summarized in detail previously [1–4]. In humans, circulating levels of vitamin D are derived primarily from the action of sunlight on the skin following photolytic conversion of 7-dehydrocholesterol to

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vitamin D (cholecalciferol), although some vitamin D can be obtained via dietary supplementation. To achieve physiological activity, vitamin D is first hydroxylated by the enzyme vitamin D-25-hydroxylase to yield 25-hydroxyvitamin D (25D). This is the major serum form of vitamin D, which circulates bound primarily to vitamin D-binding protein (DBP) with a small amount of 25D being bound to albumin. The latter is more abundant than DBP but has much lower affinity for vitamin D metabolites [5,6]. DBP and its 25D ligand are rescued from urinary excretion by a mechanism involving receptor-mediated uptake of DBP-25D by the protein megalin in the proximal tubules of the kidney [7]. In most other tissues, it appears that 25D enters target cells by diffusion as a free molecule unbound to DBP [8–10] and the potential importance of this as a mechanism for immune responses to vitamin D is discussed later in the article.

In classical vitamin D endocrinology, 25D reclaimed by renal proximal tubule cells is converted into active 1,25-dihydroxyvitamin D (1,25D) catalyzed by the enzyme 25-hydroxyvitamin D-1 α -hydroxylase (CYP27B1). The sensitivity of this reaction is illustrated by the fact that proximal tubular cells also express the vitamin D catabolic enzyme vitamin D-24-hydroxylase (CYP24A1), which converts either 25D or 1,25D to less active 24-hydroxylated metabolites. After release into the circulatory system 1,25D acts on peripheral tissues by binding to nuclear vitamin D receptors (VDR) in target cells. This process involves heterodimerization with the retinoid X receptor, and interaction with other coactivators and corepressors [11,12]. The resulting receptor complex can regulate transcription by binding to vitamin D-responsive elements (VDRE) in the promoter regions of target genes. Thus, 1,25D synthesized by the kidney acts in an endocrine fashion. However, expression of CYP27B1 has been reported for many other nonrenal tissues [13–16]. It is therefore possible that locally synthesized 1,25D can act in a paracrine fashion on neighboring cells, or in an intracrine fashion on the same cell, depending on the localized expression of VDR. Numerous genes in various cell types are regulated by 1,25D, including targets associated with established actions on calcium homeostasis and bone metabolism. However, recent attention has focused on the ability of vitamin D to regulate nonclassical responses, notably its effects on innate and adaptive immunity (reviewed in [17–19]), and these will be discussed in the following sections.

Vitamin D & antimicrobial innate immunity

A role for vitamin D in the management of TB was suggested by the work of Niels Ryberg Finsen who received the Nobel Prize for Medicine in 1903 for showing that he could cure *Lupus vulgaris* (the epidermal form of TB) with light from an electric arc lamp. Historically, the other evidence for the benefit of vitamin D stemmed from the usage of cod liver oil as a TB treatment [20]. However, the current interest in the health benefits of vitamin D for TB stems from more recent work describing the cellular and molecular machinery that underpins the actions of vitamin D on Mtb. In 1986, Rook *et al.* provided the first evidence of a role for vitamin D in the immunological control of Mtb by showing reduced proliferation of Mtb in macrophages treated with 1,25D [21]. This effect was enhanced by concurrent addition of the cytokine, IFN- γ , a known stimulator of macrophage CYP27B1 [13,22]. Despite these observations, it was another 20 years before significant new studies on the antibacterial actions of vitamin D were published. In 2006, Liu and colleagues showed that Mtb-sensing by the Toll-like receptor 2/1 (TLR2/1) complex increases expression of VDR and CYP27B1 in monocytes [23]. The resulting intracrine synthesis of 1,25D promoted VDR-mediated trans-activation of the antimicrobial peptide, cathelicidin (LL37), and killing of Mtb in monocytes provided that sufficient 25D was available for CYP27B1 [23]. Recently, the potential power of this kind of intracrine activation by CYP27B1 in innate immune response to infection was confirmed *in vivo* when cells isolated from bacterially infected bovine mammary tissue showed a substantial increase in CYP27B1

when compared with cells isolated from mock infection [24]. Transcriptional regulation of LL37 by 1,25D had been described previously [25,26], but the major advance provided by Liu and colleagues was in demonstrating that similar responses could be mediated via intracrine activation of 25D. Serum from vitamin D-insufficient donors with low levels of 25D supported lower induction of monocyte LL37 after TLR2/1 challenge when compared with serum from vitamin D-sufficient donors [23]. Similar results have also been reported using serum from vitamin D-insufficient patients before and after supplementation with vitamin D [27].

Antimicrobial responses to vitamin D are not restricted to LL37. Consensus VDRE have been detected in the gene promoters of both LL37 [28] and γ -defensin 2 (DEFB4) [25]. Although the latter does not appear to be directly regulated by 1,25D in the same manner as LL37 [23], subsequent studies have shown induction of DEFB4 expression in the presence of 1,25D and IL-1 γ [25,29]. This costimulatory mechanism involves activation of NF- κ B responsive motifs adjacent to the VDRE of the *DEFB4* promoter [29]. NF κ B-VDRE induction of *DEFB4* has also been described following activation of the intracellular pathogen recognition receptor NOD2 by its ligand muramyl dipeptide [30]. The importance of this mechanism is underlined by the fact that Muramyl dipeptide is a cell wall product of both Gram-positive and Gram-negative bacteria, and NOD2 is potently induced by 1,25D in a wide variety of cell types [30]. It is therefore possible that immune response to bacterial infection is mediated by vitamin D through a variety of molecular mechanisms and target genes.

The antibacterial effects of vitamin D are not restricted to induction of antibacterial proteins. Recent studies have demonstrated a role for vitamin D in promoting the environment associated with antibacterial activity. Autophagy is the cellular process of degrading cytosolic components including decaying organelles and nonfunctional proteins, and has also been linked to immune processes [31–33] and TB disease [34,35]. It is therefore interesting to note studies showing that 1,25D promotes autophagy in monocytes, with inhibitors of autophagosome formation suppressing antibacterial activity [36,37]. Other innate antibacterial mechanisms that may be targeted by vitamin D include the generation of reactive oxygen species (ROS), and monocytes treated with combined Mtb and 1,25D showed increased ROS generation [38,39]. Another ROS, nitric oxide (NO) is known to play a key role in the killing of bacteria in mice [40,41], and a similar mechanism may also be present in humans [42]. In one study, vitamin D status was linked to NO-mediated killing of mycobacteria. Specifically, vitamin D-deficient mice fared less well than their vitamin D replete littermates following infection with *Mycobacterium bovis* [43]. By contrast, mice lacking the enzyme that synthesizes NO showed a poor response to infection regardless of vitamin D status [43]. The evidence for a similar vitamin D-mediated NO mechanism in humans is less clear, although NO production stimulated by 1,25D has been shown to be sufficient to suppress Mtb proliferation in cultured monocytes [44].

Vitamin D, adaptive immunity & TB

Although the innate immune system is important for bacterial killing by monocytes and related cell types [45] such as neutrophils [46], the ability of a host to contain and eradicate pathogens such as Mtb also requires the adaptive or acquired immune system. Interaction between vitamin D and the adaptive immune system works on two levels: first, factors produced by the adaptive immune system may influence innate immune responses to vitamin D and second, modulation of the adaptive immune system by vitamin D.

The T lymphocyte (T-cell) cytokine, IFN- γ , has been recognized for many years as a potent inducer of monocyte CYP27B1 activity [13,22]. However, more recent studies have shown

that IFN- γ also enhances intracrine antibacterial responses to 25D by monocytes [47]. Similar responses have also been demonstrated with another cytokine, IL-15 [48], indicating that the antibacterial activity of vitamin D is not exclusively determined by pathogen-TLR interaction but may also involve a milieu of cytokines associated with adaptive immunity. The effects of IFN- γ on promoting intracrine responses to 25D have been contrasted with suppressive responses to another cytokine, IL-4, which impaired vitamin D-mediated induction of LL37 by promoting the catabolic vitamin D enzyme CYP24A1 [47]. Other examples of adaptive immune system influence on innate immune responses to vitamin D include the costimulatory effects of IL-1 β on *DEFB4* expression outlined above [29]. Although these recent data support a role for cytokines from the adaptive immune system as modulators of vitamin D responses within the innate immune system, it is important to recognize that many T-cell cytokines are themselves able to exert powerful antibacterial effects. For example, IL-1 β promotes phagosome maturation [49–52] and autophagy [34,53,54], while IL-4 has been shown to inhibit starvation and IFN- γ -induced autophagy [55].

Distinct from its ability to utilize T-cell cytokines to promote antibacterial responses, vitamin D has also been shown to modulate the adaptive immune system itself. Initial studies suggested that the principal action of 1,25D in this regard is to inhibit the proliferation of activated T cells that express the VDR [56–58]. However, more recent studies have shown that vitamin D is also a potent modulator of the T-cell phenotype. Experiments *in vitro* indicate that 1,25D inhibits the T-helper (Th) 1 T cells associated with cellular immune responses (reviewed in [59,60]), whilst conversely enhancing humoral Th2 cell responses [61,62]. Some investigators have suggested that Th1 immunity has a greater role in controlling TB infection relative to Th2 immunity (reviewed in [63,64]), while others have reassessed this perspective (reviewed in [65,66]), suggesting that a balance between pro- (Th1) and anti- (Th2) inflammatory responses is optimal for control of TB [66]. Another class of T cells known to be regulated by vitamin D are regulatory T cells (Tregs) that act to suppress the activity of other T cells (reviewed in [67]). Initial evidence for the induction of Treg by 1,25D arose from studies of naïve T cells co-incubated with 1,25D and immunosuppressive drugs [68,69]. However, subsequent reports have shown that 1,25D alone can induce Tregs [70], and it appears that preferential differentiation of Tregs is a pivotal mechanism linking vitamin D and adaptive immunity. This immunosuppressive mechanism may occur via effects on antigen presentation [68,71,72] but direct effects on T cells have also been described [73]. Although the induction of Tregs by vitamin D has important implications for autoimmune disease and host-graft rejection [74–76], it is also relevant to diseases such as TB. Analysis of T cells from TB patients showed that increased levels of Tregs were inversely related to production of IFN- γ , a marker of anti-TB immune activity [77].

Another subpopulation of inflammatory T-cells distinct from Th1 cells are Th17 cells [78]. Vitamin D appears to reduce Th17 responses in murine models of inflammatory bowel disease [79] and multiple sclerosis [80], as well as in patients with rheumatoid arthritis [81]. However, in TB, transfer of Th17 cells to mice lacking T or B cells decreased levels of Mtb [82]. In a study comparing tuberculin skin test (TST)-positive versus TST-negative patients, TST-positive patients exhibited higher Treg and lower Th17 activities as assayed by cytokine levels [83]. The precise role of vitamin D in mediating adaptive immune responses to TB remains to be determined. However, the ability of 1,25D to promote antibacterial activity while simultaneously modulating T-cell function underlines the potential importance of vitamin D in maintaining a balanced immune response to infection, while minimizing tissue damage within the host.

Vitamin D & TB in clinical studies

Vitamin D status & TB

The observations linking vitamin D and innate immune response to infection suggest a possible link between vitamin D status and susceptibility to TB. Epidemiology has shown that serum levels of 25D of less than 75 nM are associated with higher incidence of TB [84–88]. Although in some studies, this level of serum 25D has been proposed as a target for optimal vitamin D status, this may vary according to health outcome. The recent Institute of Medicine report indicates that a lower level of serum 25D (50 nM) is optimal but emphasized that this was based solely on parameters of bone health [89,90]. As yet, it is unclear which concentration of 25D will reflect vitamin D sufficiency/insufficiency with regard to diseases such as TB.

Meta-analyses and reviews of prior studies support the benefits of vitamin D as preventative against TB [91,92]. The possible use of vitamin D as therapy for TB is less clear. Vitamin D-rich cod liver oil was originally used as therapy for TB prior to the discovery of antibiotics but it is difficult to interpret the impact of such strategies as there are no accompanying data for vitamin D status. Indeed, the therapeutic effects of vitamin D on TB have only been properly studied in the last few years. A double-blind randomized trial of patients supplemented with vitamin D (single dose of 2.5 mg vitamin D) yielded immune cells that showed enhanced control of bacillus Calmette–Guérin levels *in vitro* when compared with cells from patients receiving a placebo [93]. In another study, AIDS-negative TB patients were treated with vitamin D (0.25 mg/day) as an adjunct to standard TB therapy. This resulted in greater sputum conversion from acid fast bacteria positive status to acid fast bacteria negative status and improvement as evaluated by radiology when compared with placebo-treated patients [94]. Two recent double-blind randomized control studies have assessed vitamin D administration and TB. One study from the UK using four doses of 2.5 mg vitamin D showed no overall difference in sputum conversion time between treatment and placebo groups [95] and another in TB clinics in Guinea-Bissau using three doses of 100,000 International Units of vitamin D did not improve clinical outcome [96]. The latter study is difficult to interpret because the supplementation group did not show higher levels of serum vitamin D compared with the placebo group [96]. It is also possible that inherited factors may influence responses to vitamin D supplementation. In the UK study, a significant improvement in sputum conversion was observed in patients with polymorphic variants associated with the *VDR* gene [95]. The potential impact of these genetic variations on vitamin D-mediated immune function is detailed in the following sections.

Impact of genetic variations on TB responses to vitamin D

The vitamin D system is characterized by important genetic variations associated with both the vitamin D metabolic and signaling systems. A large number of single nucleotide polymorphisms have been identified for the *VDR* gene (reviewed in [97]), which may cause alterations in the molecular biology of VDR. For example, the Fok I polymorphism ‘F’ yields a VDR that has three fewer amino acids than the ‘f’ form but nevertheless appears to be more active [98]. Studies of various populations have shown that the ‘ff’ genotype is more commonly observed in TB patients [87,99,100] but other studies showed no association with *VDR* genotypes [101,102]. However, a recent double-blind randomized controlled trial with high-dose vitamin D showed no difference in sputum conversion time when assessed relative to the FokI genotype [95]. The 3’ UTR region of the *VDR* gene has Apa I, Bsm I and Taq I polymorphisms that are believed to affect mRNA stability, thus yielding variation in VDR activity similar to that observed in the human glucocorticoid receptor [103]. A recent study from Turkey reported that the ‘B’ Bsm I allele was over-represented in TB patients compared with healthy controls [104], whilst other studies have

described prevalence of the 'BB' genotype in TB [100]. Improved sputum conversion rates in TB patients treated with vitamin D were observed in patients with the 'tt' TaqI genotype [95]. Taken together, these studies suggest that TB susceptibility and the therapeutic impact of vitamin D on TB may depend on both patient genetics and vitamin D status.

Historically, the most well-recognized inherited variations within the vitamin D system are provided by the gene for *DBP*. Variations in the *DBP* gene, originally referred to as group-specific component (Gc) 1F, 1S and 2, produce proteins with different affinities for vitamin D metabolites [105]. These affinity differences could impact bioavailability of 25D and thus immune regulation (FIGURE 1; arrow linking the two domains). Studies by our group have highlighted a role for DBP in modulating the bioavailability of 25D to target cells such as monocytes [9]. In this instance, antibacterial responses to 25D were more pronounced in the presence of low affinity forms of DBP, suggesting that the antibacterial actions of vitamin D are due to 25D that is not bound to DBP [9] or in other words 'free' 25D. Likewise, a higher affinity form of DBP such as GC1F/1F may lead to decreased free 25D. This may be a factor for the ineffectiveness of vitamin D in the Guinea-Bissau clinical trial where GC1F/1F is most likely the predominant *DBP* allelic combination [106]. Gene variants of *DBP* may also act as an inherited determinant of serum vitamin D status by influencing the serum concentrations of DBP, which are known to be linked to serum levels of 25D [107,108]. Finally, the DBP protein is known to be glycosylated at several sites, and this change in the DBP protein is known to be associated with the ability of DBP to act as a macrophage-activation factor (MAF) [109,110]. One recent study has shown that DBP-MAF activity in lung disease could be beneficial in protecting against chronic obstructive pulmonary disease [111]. To date, direct assessment of *DBP* genotype and TB has only been described in one report, which showed an association between the GC2 allele and active TB [112]. However, this was only observed for a cohort of patients with extremely low vitamin D status and so the contribution of *DBP* genotype to disease risk is difficult to interpret in this setting.

Five-year view

The last 5 years have witnessed remarkable advances in our understanding of the link between vitamin D and the immune system. This has been driven in part by a change in our perspective on what constitutes vitamin D sufficiency and insufficiency. However, recent statements from the Institute of Medicine (IOM) suggest that over the next few years there may be further modifications to our concept of optimal vitamin D. The IOM report on vitamin D released in late 2010 [90,201] reiterated the importance of adequate serum levels of vitamin D in maintaining good bone health and stated that serum levels of 50 nM 25D were probably optimal for adequate skeletal function. This is much lower than the consensus level for optimal vitamin D status previously reported for vitamin D, which was 75–80 nM serum 25D [2,113,114]. The IOM indicated that they could make no recommendation for the levels of vitamin D associated with nonclassical actions of vitamin D such as its immunomodulatory properties. This statement was based primarily on the absence of appropriately controlled trials for vitamin D supplementation in a nonskeletal setting. The IOM emphasized that more targeted research is required to further clarify the role of vitamin D in non-classical responses. Thus, a major priority for vitamin D research over the next 5 years will be to initiate and/or complete clinical trials that will address the beneficial effects of vitamin D supplementation on human health problems such as TB and other immune disorders.

With specific reference to the prevention and treatment of TB, it is clear that several other facets of vitamin D research will also be prevalent over the next 5 years. For example, current association studies and clinical trials have been based on the relationship between vitamin D status (serum 25D) and disease parameters. However, based on the data outlined

earlier, it seems likely that future studies will also need to consider the impact of inherited factors, notably *DBP* gene variants, on peripheral cell responses to 25D. In particular, there is current discussion on the possible use of 'free' 25D (that which is not bound to DBP) as a marker of vitamin D bioavailability. The amount of free 25D can be calculated [115] based on the total serum level of 25D, the serum concentration of DBP and the affinity of DBP, all of which can vary with genotypes [105,116]. In addition to defining the bioavailability of 25D, the role of DBP as a MAF requires clarification. The effect of 25D on DBP-MAF function, as well as the influence of DBP concentration and genotype on MAF function is as yet unclear.

The last 5 years have been characterized by a remarkable increase in our understanding of how the vitamin D-metabolizing enzymes, CYP27B1 and CYP24A1, interact to control intracrine responses to vitamin D in the immune system. What is less clear is whether these responses require systemic production of precursor 25D or whether parental vitamin D itself can be metabolized by cells from the immune system. The specific enzyme that synthesizes 25D from vitamin D still needs to be determined but putative candidates such as CYP2R1 are known to be expressed by monocytes and may provide an additional pathway by which vitamin D can regulate the immune system. Finally, although TLR-mediated induction of CYP27B1 and VDR is central to our current understanding of vitamin D and bacterial killing, the precise molecular mechanisms by which this occurs are unclear and will need to be fully defined.

The current link between vitamin D and TB centers primarily on the induction of bacterial killing through combined innate and adaptive immune responses. However, there are other facets of TB where the effects of vitamin D have been less well studied. This includes the possible effects of serum 25D on the progression of latent TB to active disease [117]. Likewise, it is possible that some beneficial effects of vitamin D with respect to TB will stem from the suppression of localized inflammatory tissue damage via immunosuppressive Tregs. Although no current data have been reported for this aspect of vitamin D-mediated immunity in the setting of TB disease, this is likely to be a key feature of future studies. Finally, it has been recognized for many years that granulomatous diseases such as TB and sarcoidosis can themselves promote dysregulation of the vitamin D system. Although overproduction of active 1,25D in granulomatous diseases was one of the initial observations linking vitamin D and the immune system, the mechanisms for this disease complication are unclear. Future studies will be needed to address this problem with particular emphasis on a possible role for vitamin D in the pathophysiology of granuloma formation.

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Key issues

- Although the bone health benefits of vitamin D are well established, research in the last 5 years has highlighted new links between vitamin D and infectious and autoimmune diseases.
- The immunological benefits of vitamin D stem from studies demonstrating its role as a modulator of innate and adaptive immune activities.
- Vitamin D appears to play a major role in the innate immune system by stimulating the expression of antimicrobial proteins and by enhancing the formation of autophagosomes.
- Vitamin D appears to play a major role in the adaptive immune system by stimulating the development of suppressive regulatory T cells while suppressing the development of inflammatory Th17 cells.
- Response to pathogens such as *Mycobacterium tuberculosis* require both innate and adaptive immunity and vitamin D may be an excellent candidate for coordinating these two arms of the immune system, particularly in diseases such as TB.
- Immune responses to vitamin D are driven primarily by intracrine metabolism of 25-hydroxyvitamin D (25D) – the main marker of vitamin D status. As serum 25D levels vary considerably across the globe, it is likely that immune responses will also vary. Vitamin D insufficiency has been linked to TB.
- Bioavailability of vitamin D may not be fully reflected by total serum 25D levels alone. Serum levels and genotype of the vitamin D carrier protein, vitamin D-binding protein, may also need to be taken into consideration, together with other genetic variants in the vitamin D system.
- Current clinical trials for the use of supplementary vitamin D as treatment for TB have been inconclusive but the latest studies have emphasized the need for analysis of both serum 25D levels and genetic variants of vitamin D receptor.
- Once established, diseases such as TB can lead to dysregulation of the vitamin D system, notably overexpression of 25-hydroxyvitamin D-1 α -hydroxylase in disease-affected tissues.

Figure 1. Vitamin D, immune function and TB

Vitamin D is generated in the skin by UVB exposure through photoconversion of 7-DHC, produced from cholesterol by the action of DHCR7. Vitamin D can also be obtained by dietary supplementation. Vitamin D is converted into 25D by CYP2R1 in the liver. Total 25D levels (vitamin D status) in the blood is primarily determined by the 25D bound to DBP, although some 25D is bound to other serum proteins, such as albumin, or present as 'free' 25D. Glycosylation of DBP generates a MAF (DBP-MAF). Free 25D can enter into immune cells by simple diffusion and can then be activated via the enzyme CYP27B1. 1,25D generated in this way can then act in an intracrine fashion within the same cell if the VDR is expressed. Alternatively, 1,25D produced by immune cells may act in a paracrine fashion on neighboring cells expressing VDR. The enzyme CYP24A1 attenuates both intracrine and paracrine responses to vitamin D by catabolizing both 25D and 1,25D. Interaction between 1,25D and VDR acts to promote the transcriptional regulation of vitamin D target genes. These include genes associated with innate and adaptive immunity. Thus, sufficient vitamin D (bioavailability) may be a pivotal factor in appropriate and adequate immune responses to pathogens such as *Mycobacterium tuberculosis*, enabling protection against or by providing treatment for TB.

7-DHC: 7-dehydrocholesterol; 25D: 25-hydroxyvitamin D;

1,25D: 1,25-dihydroxyvitamin D; CYP2R1: Vitamin D-25-hydroxylase;

CYP27B1: 25-hydroxyvitamin D-1 α -hydroxylase; CYP24A1: Vitamin D-24-hydroxylase

DHCR7: 7-dehydrocholesterol reductase; DBP: Vitamin D-binding protein;

MAF: Macrophage-activating factor; NO: Nitric oxide; ROS: Reactive oxygen species;

Treg: Regulatory T cell; UVB: Ultraviolet B; VDR: Vitamin D receptor.