

## Molecular Interplay between Vitamin D and Immunity can Aid Antitubercular Treatment: Vitamin D in Immunomodulation of TB

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### ABSTRACT

Tuberculosis (TB) causes maximum mortality and morbidity worldwide. 25 per cent of the global population harbour *Mycobacterium tuberculosis* (Mtb) and therefore are at risk of developing active disease. Of late, the disseminated diseases of TB are on the increase. Nearly one-third of all TB infections can be classified as extrapulmonary-TB (EPTB). TB can spread to the bone, brain, intestine, peritoneum, genitourinary system, and female genital sites leading to problems of conception. Therefore undoubtedly, TB has turned out to be a tremendous public health problem globally. The emergence of drug-resistant bacteria calls for new anti-tuberculous drugs to enhance response to antimicrobial therapy for active TB. However, discoveries of very effective anti-TB new medicines have not materialised yet. Thus, nutritional anti-TB intervention is highly important. In the pre-antibiotic era, Vitamin D was used for the treatment of TB. Its active component 1,25-dihydroxy-vitamin D<sub>3</sub> was shown to display anti-TB activity *in vitro*. Vitamin D deficient humans display greater susceptibility to TB. Vitamin D deficiency induces worse disease progression in TB cases as observed in many clinical trials. The efficacy of the addition of vitamin D supplements in TB treatment has also been estimated. Thus, by now, the role of vitamin D in TB prevention and treatment is well established. Knowledge of the molecular mechanism of vitamin D is crucially vital for new anti-TB drug design. This review article discusses the recent advancement regarding the molecular mechanism of vitamin D-related anti-TB action. Further elucidation of this area may help novel anti-TB drug development.

**Keywords:** Tuberculosis; *Mycobacterium tuberculosis*; Drug-resistant organisms; Vitamin D; Anti-TB chemotherapeutics

### 1. INTRODUCTION

A leading cause of infectious deaths worldwide is Tuberculosis (TB). The increasing rate of emergence of drug-resistant TB poses a grave challenge to the global public health system.<sup>1</sup> Primarily, TB is a respiratory illness that usually affects the pulmonary niches. But it can also affect the brain, spine, kidney, and genitourinary system. TB can either be dormant or active. Dormant TB is when an individual becomes infected with the Mtb bacteria but usually does not display symptoms and is not contagious. Generally, dormant TB becomes activated in individuals having weak immune systems.

Vitamin D<sub>3</sub> (i.e., cholecalciferol) is a steroidal hormone that is obtained from diet. However, it can primarily be synthesised in the skin from 7-dehydroxycholesterol in presence of UV light. Cholecalciferol attaches vitamin D-binding protein (DBP) in order to be carried to the liver. In the liver, cholecalciferol is hydroxylated to produce 25-hydroxycholecalciferol by the enzyme 25-hydroxylase. Subsequently, DBP transports 25-hydroxycholecalciferol to

the kidneys, where the Cyp27B1 and the help of the molecule TLR convert 25-hydroxycholecalciferol into 1,25-dihydroxycholecalciferol, [1,25(OH)<sub>2</sub>D<sub>3</sub>] active form of vitamin D. Vitamin D plays an important role in augmenting immunity are shown in Fig.1.

Recent studies carried out on different populations have suggested that deficiency of vitamin D is related to an increased risk of TB<sup>2</sup>. It has been shown that immune cells produce the hormonally active vitamin D, [1,25(OH)<sub>2</sub>D<sub>3</sub>]. Macrophages and other immune cells can express 1- $\alpha$ -hydroxylase (CYP27B1), which converts circulating 25-hydroxyvitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] (calcifediol or calcidiol) into 1,25(OH)<sub>2</sub>D<sub>3</sub>. Furthermore, Mtb infection leads to activation of Toll-like receptors (TLR1/2), which stimulates different cells of the innate immune system and their cytokines expression patterns as well as antimicrobial peptides.<sup>3</sup>

Mtb is an obligate aerobic bacterium requiring oxygen in its mechanism. It is an intracellular parasite that is transmitted by inhalation of just 1–5 bacilli. Following inhalation, the first immune response to Mtb infection starts with the innate immune system, involving the epithelial

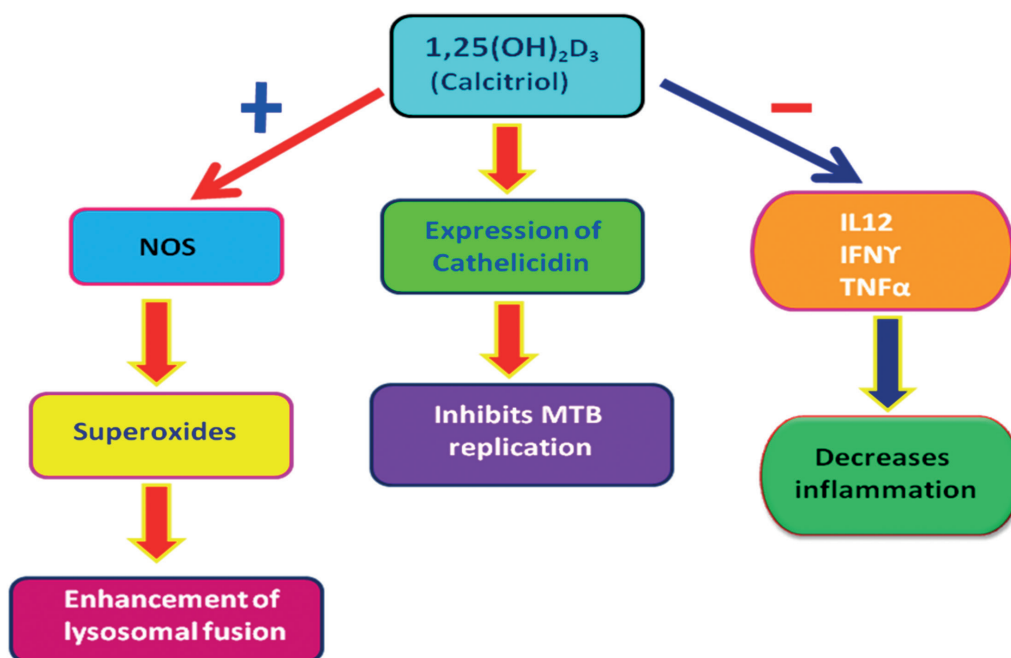


Figure 1. Vitamin D is an immunoregulatory molecule. (+ sign) indicate p-regulation (- sign) indicates down-regulation; NOS: Nitric Oxide Synthase.

cells of the airways and alveolar macrophages. This early response is facilitated by the recruitment of neutrophils, which are one of the first cells to arrive at the site of action<sup>4</sup>. Now, micronutrients are considered a potential adjunctive immunotherapeutic agent. This discipline is a growing field of keen medical attention because recent scientific evidence established the antimicrobial action of vitamin D<sub>3</sub> inside macrophages. The emergence of TB and HIV co-epidemics presents a crucial setback to TB control programs worldwide. Vitamin D's low intake in a population has been identified as a major risk factor for tuberculous development. Due to this reason, supplementation of vitamin D in the diet can be a novel strategy for TB prevention which could substantially shorten the course of TB treatment, particularly in case of increasing drug resistance.

Tribal regions are abundantly located throughout India, and there was no research study about the molecular mechanisms of vitamin D on immunity of the tribal populations of India. The dose of Vitamin D for different populations depends upon their genetic make. Since we are located in tribal-dominated geography, our research group focuses on tribal people's food habits and deficiency of vitamin D in their diet, which happens mainly due to their dietary restrictions. We observed that there is a strong correlation between vitamin D deficient dietary intake and the occurrence of TB among tribal populations.

To understand the details of the molecular mechanism of vitamin D as an anti-TB agent, further research is necessary. It would help in the designing of novel antitubercular drugs. Therefore, we want to understand the molecular mechanisms via which vitamin D plays a

role in inhibiting the Mtb infection. [1,25(OH)<sub>2</sub>D<sub>3</sub>] gets accumulated in the nuclei of the target organs via a receptor-based mechanism. It initiates the transcription of DNA, which encodes phosphorous and calcium transporter proteins, whose nature remains undetermined yet.

## 2. APPROACH OF THIS STUDY

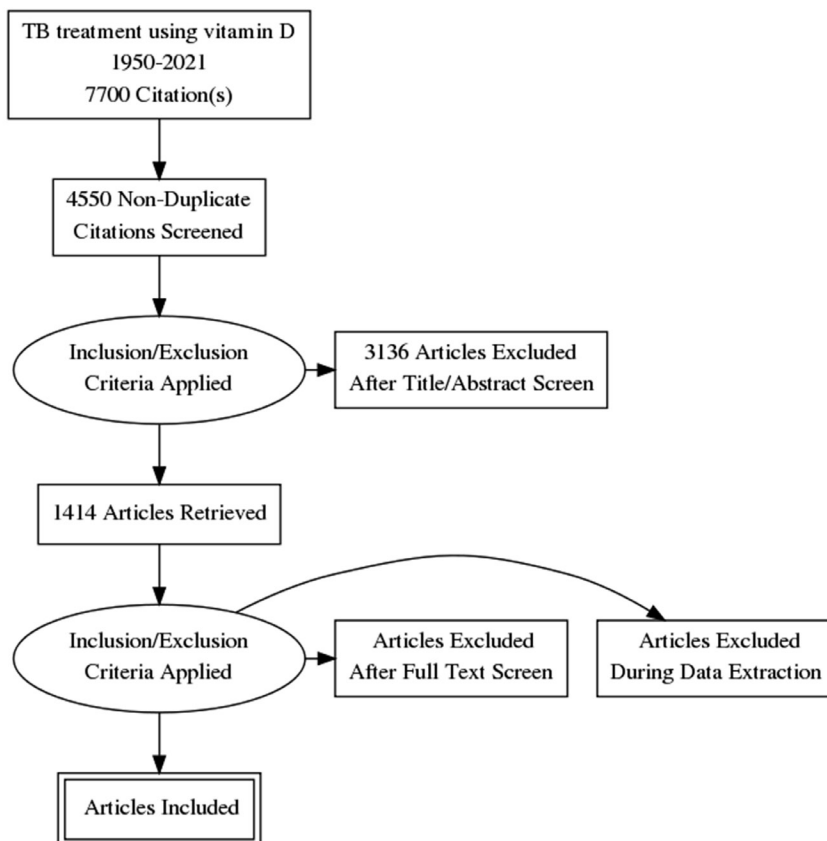
This systematic review was written according to the meta-analyses guidelines, systematic reviews, and checklists throughout the design and execution which is shown in Fig. 2.

### 2.1. Search Strategy and Criteria for Data Selection

Different search engines like Pub-Med, MEDLINE are used. Search sites are for collection of data for this review, and references from relevant articles using the search terms "vitamin D," "tuberculosis," "molecular mechanisms of vitamin D," "antibacterial action," and "structure and mode of action of VDR," "antitubercular drugs" and "management of TB." Articles between 1981–2021 were included in this study.

### 2.2. Molecular Mechanism of Vitamin D's Role in Immunity

In the year 1969, the nuclear vitamin D receptor (VDR) for [1,25(OH)<sub>2</sub>D<sub>3</sub>] was identified. This prompted a two-decade-long proliferation of publications describing the broad area of influence of vitamin D on the endocrine system. This system is marked by the presence of VDR in more than 30 tissue or organs present in the human body. Now, the genomic frontiers of the cellular presence of VDR encompass B and T cells of the immune system, hair follicle, bone marrow, muscles, adipose tissue, and



**Figure 2.** Flow diagram that represents the selection of data.

cancer cells. In the middle of the 1980s, the whole new area of  $[1,25(\text{OH})_2\text{D}_3]$  triggered rapid responses (RRs) were discovered. RRs were responses that occurred too rapidly (within minutes to an hour). These RRs can be interpreted based on the result of the nuclear VDR regulating gene transcription.

The VDR is a steroid receptor that is related to the Pregnane X Receptor (NR1I2, PXR) and constitutive androstane receptor (CARs, NR1I3 and NR1I4); these receptors were the first described as orphan receptors. VDR binds with  $[1,25(\text{OH})_2\text{D}_3]$ , the hormone involved in calcium homeostasis. This VDR-cholecalciferol interaction subsequently leads to cathelicidin production. Thereafter, cathelicidins action lead to phagolysosomal killing of MTB (Figs 3 and 4).

Cytochrome p450 27B1 (CYP27B1 or simply  $1\alpha$ -hydroxylase) is a cytochrome  $\text{P}_{450}$  enzyme in humans; CYP27B1 gene encodes this enzyme. These genes encode the enzyme  $1\alpha$ -hydroxylase. This enzyme helps to generate vitamin D's active form, namely  $[1,25(\text{OH})_2\text{D}_3]$ . It can also directly synthesise in the body with the help of sunlight or can be obtained from foods in the diet.

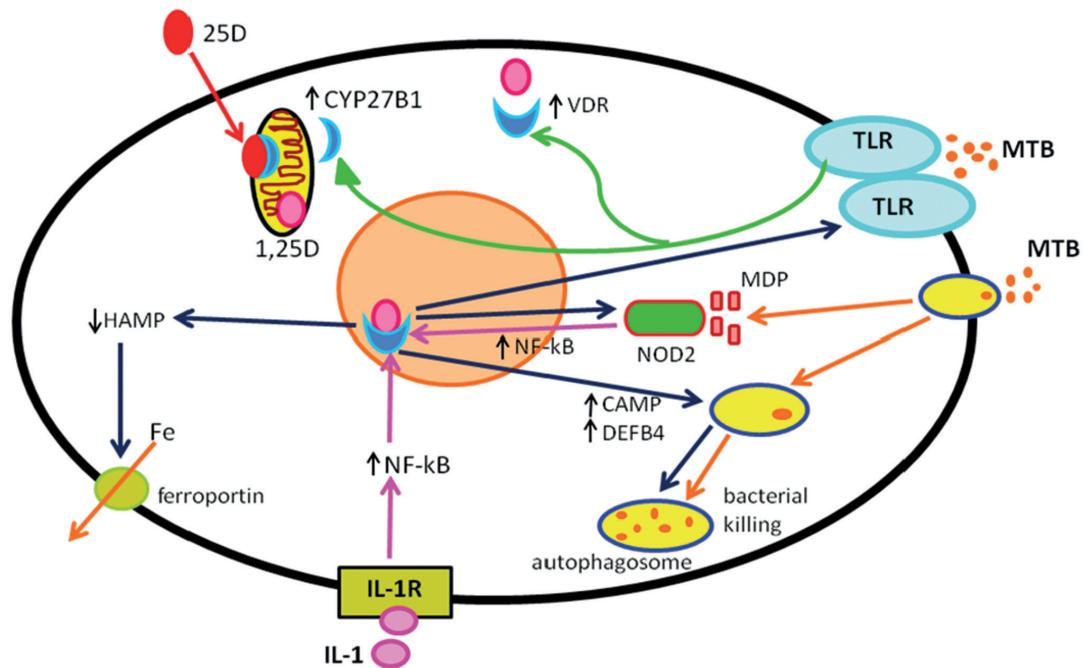
The mutations in CYP27B1 gene cause the deformation of teeth and bones by reducing or eliminating the action of  $1\alpha$ -hydroxylase. All these physiological problems happen because vitamin D cannot be converted to its active form and thus cannot help in the absorption of minerals. More importantly, elimination of  $1\alpha$ -hydroxylase

prevents formation of VDR-cholecalciferol complex. Hence, cathelicidin mediated phagolysosomal killing of Mtb gets down-regulated.

TLRs display crucial roles in the action of the innate immune system. TLRs are non-catalytic receptors, which span single membranes. These receptors are expressed on macrophages, dendritic cells, and other sentinel cells. The aforementioned cells recognize structurally conserved pathogen-derived molecules.

Cathelicidins are synthesised as a pre-propeptide and has three parts: an N-terminal signal peptide, a cathelin domain (the conserved pro-sequence), and a C-terminal cationic antimicrobial peptide (AMP)<sup>5</sup> which has a highly variable sequence. In neutrophils<sup>6</sup>, proteolytic cleavage helps to generate the mature AMP. A single cathelicidin gene called *CAMP* (*hCAP18/LL-37/FALL39*) is expressed in humans, whereas some mammals, such as mice, possess numerous cathelicidins (*CRAMP/ CNLP/ MCLP*).<sup>5</sup> The human gene and the mRNA are both denoted as *CAMP*. On the other hand, the pre-protein is called cathelicidin or CRAMP (mouse) or hCAP18 (human); the processed peptides is indicated as LL-37 (in humans). The mature AMP containing cationic surface interacts with negatively charged bacterial cell envelope to damage and puncture Mtb cells.

Lysosomes present in macrophages and polymorphonuclear leukocytes (PMNs) mainly store a polypeptide family; cathelicidin-related antimicrobial peptides<sup>7</sup>. Cathelicidins



**Figure 3. Overview model of vitamin D's action on *M. tuberculosis*.**  
(Adapted from Chun RF, Liu PT, Modlin RL, Adams JS, Hewison M. Impact of vitamin D on immune function: Lessons learned from genome-wide analysis. *Front Physiol*, 2014, 5,151.)

**MDP: Muramyl dipeptide; NF-κB: nuclear factor-κB; HAMP: iron-regulatory hepcidin; IL-1R: interleukin- responsiveness; NOD2: Nucleotide-binding oligomerization domain protein 2. Blue lines: induction of CAMP and DEF4; reduced expression of HAMP; autophagy augmentation; NOD2 expression induction; TLR gene regulation by feedback mechanism; the enhanced killing of bacteria. Green lines: generation of 1,25(OH)<sub>2</sub>D from 25D; 1,25(OH)<sub>2</sub>D then binds to VDR and this leads to transcriptional regulation. Pink lines: accessory immune signals (NOD2 binding by MDP and IL-1 responsiveness) for some responses (e.g., induction of DEF4) co-operate with intracrine vitamin D through NF- κB.**

perform a crucial role in the innate immune defense of mammals against invading bacterial infections.<sup>8</sup> The cathelicidin family of peptides falls in the class named AMPs. Defensins are members of this class. Cathelicidins were first discovered in neutrophils. Later, many other cells like epithelial cells and macrophages have also been found to express cathelicidin after activation by viruses, bacteria, fungi, or 1,25(OH)<sub>2</sub>D. In humans, cathelicidin gene-encoded polypeptide is cleaved to generate the mature LL-37<sup>10</sup> peptide, which has many immunological functions.

Cathelicidin's genetic expression is regulated upwards by the help of vitamin D. Cathelicidin is found to exhibit microbicidal activity over a broad spectrum of viruses, fungi, and bacteria.<sup>9</sup> Following fusion with lysosomes, cathelicidin promptly perforates lipoprotein membranes of engulfed microbes. The mechanism of action of cathelicidin involves damaging and puncturing the microbial cell envelope.<sup>10</sup>

Innate immunity against *Mtb* and other intracellular pathogens involves the action of Cathelicidin (LL-37). After infection of *Mtb*, induction, and production of LL-37 have been observed in human cells. Alveolar macrophages (AM) and Monocyte-derived macrophages (MDM), which are parts of innate immunity, are the most significant participants in anti-*Mtb* response<sup>11</sup>. LL-37 production in MDMs and AMs has been observed

during *Mtb* infection. LL-37 is primarily produced during acute *Mtb* infection and probably also at the time of the early infection period. However, it has no participation or very limited role in the bacterial activity of chronic granulomatous inflammation.

The innate immune system elicits a host response of rapid nature against the pathogenic microbes. TLRs mediate the innate immunity of drosophila. The expression of AMPs is induced by TLR.<sup>12</sup> Mammalian TLR homologs such as TLR2 and TLR1 heterodimer<sup>13</sup> recognize a variety of ligands derived from microbes. TLRs' activation brings about an antimicrobial response of direct nature in macrophages and monocytes *in vitro*. This murine activity is mainly mediated via nitric oxide<sup>14</sup> generation. But, in human macrophages, TLR2/1-induced antimicrobial activity remains unaffected in presence of inhibitors of reactive oxygen intermediates or nitric oxide.<sup>15</sup> However, the mechanism for microbicidal activity of humans is yet unknown.

Investigations of resistance to *Mtb* show that the activation of TLR2/1 decreased the sustainability of intracellular bacteria in human macrophages and monocytes; however, the same is not true in monocyte-derived dendritic cells (DCs).<sup>15</sup> DNA microarrays were used to study gene expression profiles of DCs and monocytes which were stimulated with 19-kD *Mtb*-derived lipopeptide (TLR2/1L); whereas a negative control was treated with

medium only. Microarray data were statistically analysed to score for differentially-expressed genes in the two types of cells after TLR2/1L treatment.

Genes that are not up-regulated in DCs, but are overexpressed in monocytes were matched against a list of genes associated with recognised antimicrobial properties. This identified VDR and S100A12 (calcium-binding pro-inflammatory molecule) as candidates.<sup>16</sup> Philip T. Liu *et al.* showed that Cyp27B1 was up-regulated 12 and 24 hours after TLR2/1 stimulation.<sup>10</sup> But, other VDR downstream target genes like cathelicidin antimicrobial peptide (CAMP),  $\beta$ -defensin 4 (DEFB4), [1,25(OH)<sub>2</sub>D<sub>3</sub>]-regulated VDR-specific Cyp24 hydroxylase gene. But, no mRNA up-regulation was observed for other VDR downstream target genes like CAMP, DEFB4, [1,25(OH)<sub>2</sub>D<sub>3</sub>] regulated VDR-specific Cyp24 hydroxylase gene.<sup>17</sup> The data obtained from microarray experiments were verified by quantitative PCR (qPCR). These aforementioned data indicate that TLR brings on overexpression of VDR and CYP27B1 genes in macrophages and monocytes are shown in Fig.3.

Production of [1,25(OH)<sub>2</sub>D<sub>3</sub>] in kidneys takes place in response to decreased levels of Ca<sup>2+</sup> in blood. Low Ca<sup>2+</sup> concentration in the blood stimulates parathyroid hormone (PTH) production. In primary renal tubules, PTH induces CYP27B1 expression. With rising levels [1,25(OH)<sub>2</sub>D<sub>3</sub>] its production gets suppressed through negative feedback. In this feedback loop, the VDR attaches to the promoter of CYP27B1 to down-regulate the expression of this gene. [1,25(OH)<sub>2</sub>D<sub>3</sub>] enhances uptake of intestinal Ca<sup>2+</sup>. It results in decreased PTH levels. Additionally, in osteocytes, [1,25(OH)<sub>2</sub>D<sub>3</sub>] induces the production of FGF-23; this down regulates PTH production. Moreover, CYP24 production is induced by vitamin D. This active form of vitamin D<sub>3</sub> is a cytochrome P450 enzyme of mitochondria. Both [25(OH)D<sub>3</sub>] and [1,25(OH)<sub>2</sub>D<sub>3</sub>] are catabolised by it; thereby limiting CYP24's expression.<sup>18</sup>

Vitamin D production outside the kidney occurs in many tissues such as the lung, epithelial cells of the skin, parathyroid glands, colon, bone, and immune cells, as well as in activated macrophages. Control of Vitamin D production in macrophages is quite different from that of renal tissues. Macrophage activation by IFN- $\gamma$  or TLR ligands results in the induction of CYP27B1 and production of [1,25(OH)<sub>2</sub>D<sub>3</sub>]. This process is reliant on the availability of [25(OH)D<sub>3</sub>]. FGF-23 or PTH does not regulate CYP27B1 activity in macrophages. Macrophages, unlike renal tubules, do not limit the production of [1,25(OH)<sub>2</sub>D<sub>3</sub>]. Continuous activation of macrophages may result in accumulation of [1,25(OH)<sub>2</sub>D<sub>3</sub>] and can cause diseases in humans as shown in Fig.4.<sup>19</sup>

### 2.3 Antibacterial Activity of Vitamin D

Early studies put attention on the [1,25(OH)<sub>2</sub>D<sub>3</sub>] binding capacity in the lymphocytes of adaptive immune systems like T and B cells.<sup>20</sup> Subsequent research described intracellular attachment of [1,25(OH)<sub>2</sub>D<sub>3</sub>] in innate immune cells such as macrophages, monocytes, neutrophils, and dendritic cells.<sup>21</sup> Increased levels of [1,25(OH)<sub>2</sub>D<sub>3</sub>] in serum were found in some patients of sarcoidosis, which is a granulomatous disease; these elevated serum levels were attributed to the generation of the active form [1,25(OH)<sub>2</sub>D<sub>3</sub>] from its pro-hormone [25(OH)D<sub>3</sub>]. It happens in the tissue macrophages as well as systemic macrophages of the patients mentioned above.<sup>22</sup> Similar observations were reported from other inflammatory and granulomatous diseases<sup>23</sup>. These findings indicated the immune activity of the CYP27B1 enzyme (1 $\alpha$ -hydroxylase) that catalyzes the conversion of [25(OH)D<sub>3</sub>] to [1,25(OH)<sub>2</sub>D<sub>3</sub>]; such were disease-related occurrences.

Genome-search of DNA sequences that can bind liganded VDR indicated the presence of consensus Vitamin D-Responsive Elements (VDREs) located inside the promoters of the gene encoding two antibacterial

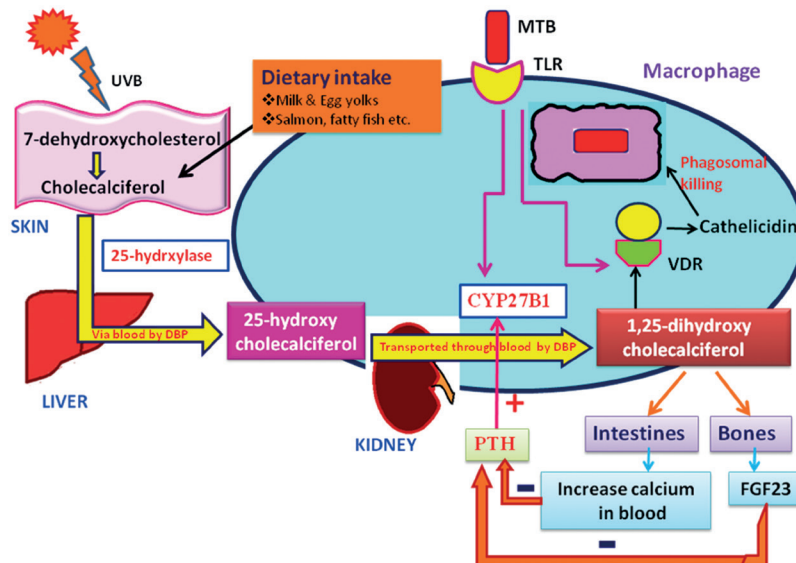


Figure 4. Overview of vitamin D's role in the killing of MTB.

proteins, *DEFB4* and *CAMP*<sup>44</sup>. Intriguingly, although both *CAMP* and *DEFB4* genes display proximal promoter direct-repeat 3 (DR3), consensus VDREs and *CAMP* can only be induced transcriptionally via  $[1,25(\text{OH})_2\text{D}_3]$  in monocytes<sup>24</sup>. In subsequent studies, the mechanism for the differential regulation of *DEFB4* and *CAMP* by  $[1,25(\text{OH})_2\text{D}_3]$  in monocytes was explained; the first study demonstrated enhanced expression of *DEFB4* in monocyte after co-treatment using interleukin-1 (IL-1) and  $[1,25(\text{OH})_2\text{D}_3]$ .<sup>25</sup> The significant role of VDR and NF- $\kappa$ B in co-induction of *DEFB4* transcription has been highlighted by investigations of Nucleotide-binding oligomerisation domain-containing protein 2 (NOD2), which can sense intracellular pathogen. Interestingly,  $[1,25(\text{OH})_2\text{D}_3]$  transcriptionally up-regulates NOD2.<sup>26</sup>

To explain the role of TLR2/1 in the mediation of innate immune response to *Mtb*, Liu and his colleagues performed DNA microarray gene expression pattern analysis using DCs and macrophages; this was done after treatment with one of the pattern recognition receptors (PRRs) for *Mtb*.<sup>10</sup>

Notably, the TLR stimulus influences the expression of *VDR* and *CYP27B1*. It suggests that macrophage responses to *Mtb* implicate an intracrine, endogenous, vitamin D system. Functional read-outs obtained from TLR2/1 *Mtb* studies indicated expression of mRNAs expressed from *CYP24A1* and *CAMP*.<sup>10</sup> *Mtb* can subvert phagosome-to-phagolysosome transition to evade the antibacterial process and maintain intracellular viability.<sup>27</sup> Promotion of autophagy via the ability of  $[1,25(\text{OH})_2\text{D}_3]$  and in synthetic analog is well established.<sup>28</sup> However, induction of autophagy can be significant for vitamin D-induced antibacterial *Mtb* response; this was suggested by recent data.<sup>29</sup> Monocyte autophagy resulting from TLR2/1 activation involves enhancing the expression of *CYP27B1* and *VDR*.<sup>29</sup> It is a common effect with the expression of antibacterial proteins. It further highlights the vital role of intracrine  $[25(\text{OH})\text{D}_3]$  metabolism and its action in normal human innate immunity.

For epithelial keratinocytes, a similar TLR response has been described. Here the basal expression of *CYP27B1*

is not sufficient for the facilitation of serum 25D-mediated intracrine antibacterial proteins secretions. Yet, after wounding of skin, proximally generated transforming growth factor  $\beta$  (TGF $\beta$ ) up-regulates *CYP27B1* expression.<sup>30</sup> Consequently, *CYP27B1* expression driven by TGF $\beta$  can then lead to the generation of intracrine antibacterial proteins like *CAMP*, which fight pathogens infecting epidermal injury.<sup>52</sup> Recent studies employing *CYP27B1* knock-out mice have proposed that CD8<sup>+</sup> T-cells are the main repositories of extrarenal 1,25D within the murine immune system.<sup>31</sup>

#### 2.4 Structure and Mode of Action of VDR

VDR is a member of the nuclear receptor superfamily.  $[1,25(\text{OH})_2\text{D}_3]$  hormone-related biological responses are mediated by VDR; which was initially identified as a chromatin-associated protein<sup>32</sup> are shown in Fig.5. VDR binds  $[1,25(\text{OH})_2\text{D}_3]$  with specificity and high affinity. A tight aggregation of VDR and RXR (which is a retinoid X receptor isoform and is a heterodimeric partner of the former) is mediated by VDRE (.6). This ligand-bound VDR-RXR heterodimer gets conformationally reorganised to recognize the VDREs, which interact with the DNA sequences of genes regulated with vitamin D.<sup>33</sup> The zinc fingers of the VDR-RXR (Fig.6) combine with the DNA binding domain (DBD). The C-terminal extensions (CTEs) of the zinc fingers recognize and bind the target genes.

Generally, VDREs are either a spacer of six nucleotides (ER6) with an inverted repetition of two half-elements or a direct repetition of two half-elements made of hexanucleotide having a spacer of DR3s. In the case of positive DR3 VDREs, RXR binds to the 50 half-sites on the right side, whereas VDR occupies the 30 half-elements on the left are shown in Fig.6.<sup>34</sup>

VDREs are the various gene regions with key vitamin responsive elements. The expression of these key elements that are modulated directly by  $[1,25(\text{OH})_2\text{D}_3]$ . VDREs are present in many genes *rBGP*, *hCYP24A1*, *hCAMP*, *cPTH*, *rPTHrP*, etc.; those genes are either positively or negatively regulated by the help of  $[1,25(\text{OH})_2\text{D}_3]$ .

Several VDREs require all VDR-RXR binding sites

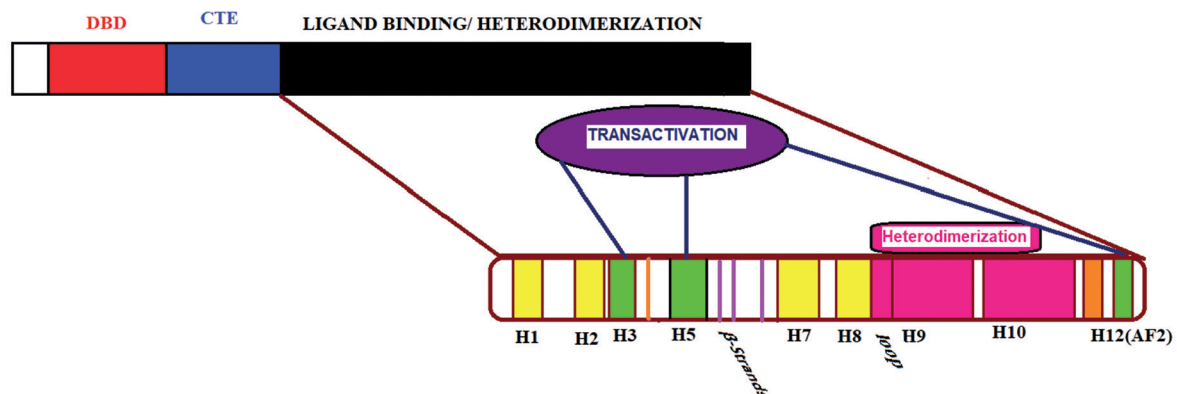
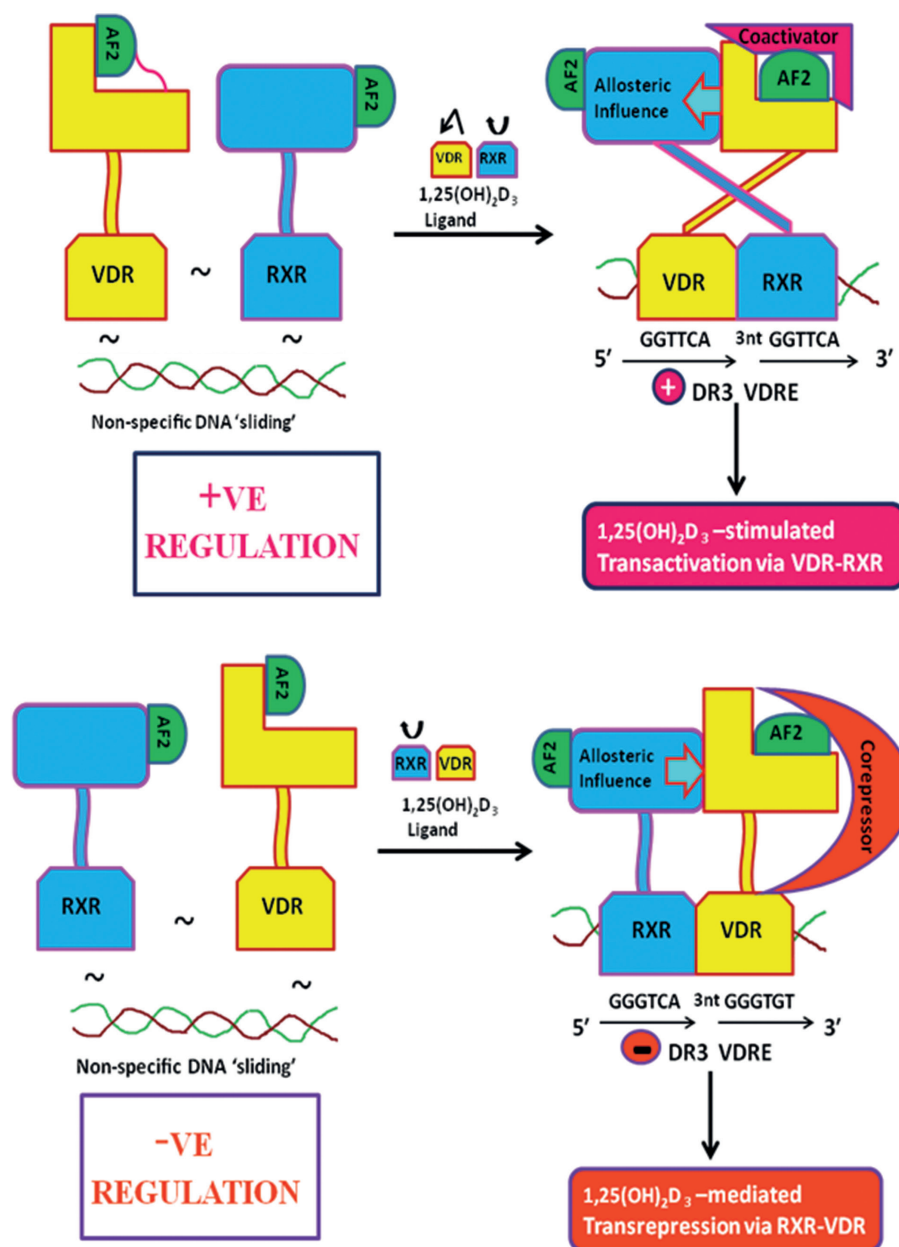


Figure 5. VDR structure-function relationship and gene organization. (Adapted from Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh JC, Jurutka P.W. Molecular mechanisms of vitamin D action. *Calcif Tissue Int.*, 2013, 92(2), 77-98.)



**Figure 6. Ligand dependent activation and repression.**

(Adapted from Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh JC, Jurutka P.W. Molecular mechanisms of vitamin D action. *Calcif Tissue Int.*, 2013, 92(2), 77-98.)

for maximum induction via [1,25(OH)<sub>2</sub>D<sub>3</sub>]. Individual VDRE-containing genes seem to function in a synergistic manner to attract basal factors and co-activators which lead to transactivation. A VDR ligand-binding domain (LBD) for heterodimerisation is a sandwich-like structure that consists of at least 12  $\alpha$ -helices.<sup>35</sup> LBD presents VDR surfaces (predominantly the loop between H8 and H9 and helices H9 and H10) for heterodimerisation with RXR as well as interaction with co-activators. Co-activator interfaces of VDR, as shown in Fig.5, are made of regions of H3, H5, and H12 (the last domain constitutes the activation function-2 (AF-2 domain).

During the innate immune response, activation of TLR sets off direct antimicrobial activity.<sup>36</sup> Therefore, after

stimulation, the production of LL-37 was examined. TLR ligands, including TLR-9, were used for the stimulation of MDMs. Mtb DNA is a ligand for TLR-9. Hence, when cells were stimulated with purified Mtb DNA, the production of high levels of LL-37 was observed. TLR-9 ligands present in lung Epithelial Cells (ECs) induced AMPs like human beta-defensin-2 (HBD-2).<sup>37</sup> Conversely, TLR-9 was suggested by several publications to be an important receptor for innate immune responses against Mtb.<sup>38</sup> The expression of VDR and CYP27B1 genes are regulated upwards by the means of TLR activation in human macrophages. This causes the killing of intracellular Mtb due to the induction of cathelicidin activity.

## 2.5 Role of vitamin D in Preventing TB

[1,25(OH)<sub>2</sub>D<sub>3</sub>] the activated form of vitamin D, has multifaceted actions on the immune system. In 2006, Liu *et al.* demonstrated Mtb's interaction with the help of the TLR2/1 complex boosts the VDR and CYP27B1 expression in monocytes. [1,25(OH)<sub>2</sub>D<sub>3</sub>]’s synthesis leads to VDR-mediated trans-activation of cathelicidin and killing of intracellular Mtb. Cathelicidins have a direct antibacterial role through membrane disruption. Cathelicidins have a direct bactericidal action via membrane disruption. Additionally, cathelicidins display antiviral activity, and it inhibits retrovirus, adenovirus, and herpes simplex viruses. It has been shown that macrophages are the most proficient producers of LL-37 peptide following Mtb infection. This suggests that, during early infection, LL-37 from macrophages plays a vital role in the innate immune response. Liu and colleagues (2006) showed that transcriptional regulation of cathelicidin is mediated by [1,25(OH)<sub>2</sub>D<sub>3</sub>] activation. Microbial products mediated stimulation of TLRs inside of macrophages. This leads to enhanced conversion of inactive [25(OH)D<sub>3</sub>] into active [1,25(OH)<sub>2</sub>D<sub>3</sub>].<sup>39</sup> Adams, *et al.*, demonstrated that the production of cathelicidin and defensin-2 is a consequence of TLR activation. [1,25(OH)<sub>2</sub>D<sub>3</sub>] up-regulates these two antimicrobial peptides production by a large degree.

Liu *et al.* 2009 showed that sera from individuals with low levels of [1,25(OH)<sub>2</sub>D<sub>3</sub>] produced a low level of cathelicidin in monocytes as compared to sera of donors with a high level of vitamin D. Adams *et al.* reach the same conclusion using sera from individuals with insufficient vitamin D levels before and after supplementation of vitamin D. Further experiment showed that 4 µg/ml of vitamin D was able to protect infected human macrophages reproducibly; *in vitro*, this restricts mycobacterial growth. The crucial role of Vitamin D in the immune response against Mtb requires LL-37 production; these promote phagolysosome formation.<sup>40</sup> Additionally, [1,25(OH)<sub>2</sub>D<sub>3</sub>] has also been demonstrated to promote autophagy in monocytes.

The balance towards Cyp27B1 is tilted by PTH which leads to the activation of vitamin D signaling. FGF23 causes the inactivation of vitamin D signaling and can be induced by high concentrations of calcitriol and low serum phosphate. Calcitriol goes and binds to VDR eliciting the expression of cathelicidin, an antimicrobial peptide. Cathelicidin goes to phagosome producing killing of phagosomal located tuberculous bacilli.

## 2.6 Management of Drug-Resistant Tb

Over the past few decades, antitubercular drug resistance is an emerging global public health problem. Mtb isolates at least resistant to both isoniazid and rifampin (INH-R+RIF-R) is called multidrug-resistant TB (MDR-TB). More recently, Mtb isolates with greater resistance to anti-TB drugs have been generated. Extensively drug-resistant TB (XDR-TB) is a type of MDR-TB that displays resistance to rifampin and isoniazid as well as to one of three injectable second-line drugs (*e.g.*, amikacin,

capreomycin, or kanamycin) and any fluoroquinolone, *i.e.*, [INH-R + RIF-R + any FQ-R+AMI-R/KAN-R/CAP-R].

MDR-TB is tough to treat. XDR-TB is complicated to treat. That is why MDR-TB and XDR-TB display high mortality rates as the treatment options available to them are minimal; their prognosis is also poor. MDR-TB can be cured by prolonged treatments using a combination of first-line drugs [such as ethambutol (EMB), pyrazinamide (PZA)<sup>41</sup>, streptomycin (SM), and second-line drugs [any FQ-R+AMI-R/KAN-R/CAP-R+clofazamine+prothionamid e+ethionamide+etc.]. However, these alternative regimens are more expensive than the common combination of first-line drugs and are used against PAN-sensitive TB (INZ+RIF+EMB+PZA+SM). Anti-MDR-TB regimens are administrative for a much longer period (~18 months) and have more adverse effects.<sup>42,43</sup>

To overcome this, the directly observed therapy (DOT) was initiated as an ambulatory treatment under the supervision of WHO in the 1960s. Directly observed therapy short-course (DOTS), was introduced by WHO. This is the short course (6-9 months) chemotherapy regimen that uses a combination of the first-line anti-TB drugs for emphasizing the importance of direct observation in TB chemotherapy; thus, DOTS was introduced in 1993. DOTS indicates that the patient infected with TB swallows a short regimen of anti-TB drugs under direct supervision and observation of healthcare workers or related professionals. The entire course is divided into two phases - the intensive phase followed by a continuation phase. A methodology for the management of MDR-TB outbreaks is illustrated in Fig. 7.

## 3. DISCUSSION

Vitamin D plays a vital role in immunological effects<sup>44</sup> as well as prevention and cure of Mtb. At present, TB has become a severe problem especially due to a large increase in MDR-TB and XDR-TB cases. Therefore, new antitubercular drugs are badly needed. By studying the antitubercular mechanism of vitamin D, some new chemotherapeutic agents against TB can be developed.<sup>45</sup> Some of the natural compounds and intermediates generated by this vitamin D-mediated process have to be characterised thoroughly. Such characterisation may lead to discoveries of analogs and modifiers that can be used as new anti-TB drugs. Such drug-development strategies have been attained by various pharmaceutical companies and research units. From this approach, we can expect the development of new types of drugs against TB in upcoming generations. It also has to be noted that not all TB drugs (new generation and old) are optimal from the point of view of potency, side effects, and cost. Therefore, the additional discovery of new antitubercular drugs is much needed even today. That is why a thorough understanding of this vitamin D-related pathway is very much important for a complete cure of TB.

In this content of TB controls strategy, two facts that are generated by a large body of vitamin D-related research are of particular importance. Firstly, vitamin



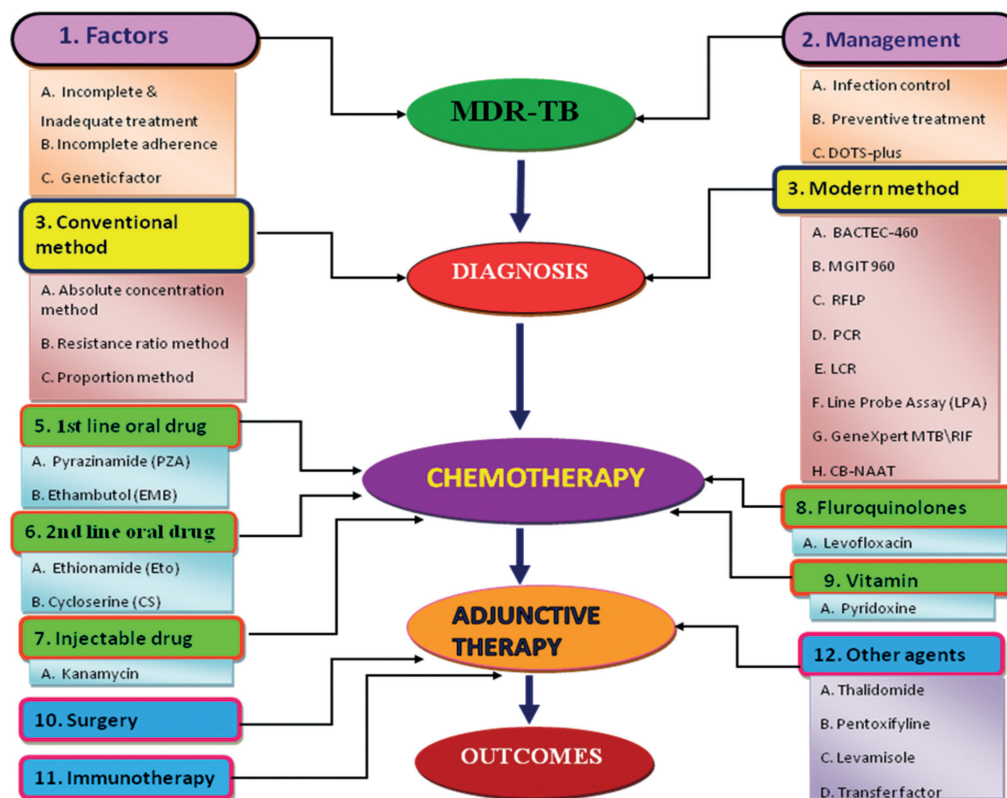


Figure 7. Overview of diagnostic and therapeutic strategies against MDR-TB.

(Adapted from author's work; Bhunia SK, Sarkar M, Banerjee A, Giri B. *Asian Pacific Journal of Tropical Disease*, 2015, 5(9), 673-686.)

MGIT, Mycobacteria growth indicator tube; RFLP, Restriction fragment length polymorphism; LCR, Ligase chain reaction; LPA, Line probe assay; CB-NAAT: Cartridge-based nucleic acid amplification test.

D's active form boosts the capability of macrophages to inhibit the intracellular growth of *Mtb*. Secondly, on activation of TLRs by *Mtb* surface molecules, the cathelicidin production gets impaired, when adequate vitamin D is absent in serum.<sup>46</sup>

The risk of tuberculosis can heavily be enhanced by smoking. Although vitamin D is needed for the absorption of calcium, no evidence suggests that the absorption of vitamin D is directly impaired due to smoking<sup>47</sup>. Although in India, in spite of adequate sun exposure, it was observed that levels of vitamin D were low in the population. This observation helps to conclude that diet was the more important factor in determining the body's vitamin D levels.

The association between the chances of tuberculosis and the deficiency of vitamin D can be explained in two ways. Firstly, low levels of vitamin D in TB patients may decrease further at the beginning of the disease. Further drops in vitamin D levels can predispose the patient to another vitamin D deficiency-related condition. Secondly, people with diabetes mellitus are 4 to 5 times more probable (than people without diabetes mellitus) to suffer from chronic kidney diseases because [1,25(OH)D] - which is an active form of vitamin D- is generated in the kidney. In cases of renal failure, the supplementation of vitamin D helps in the normalisation of bone metabolism. This

is done by correcting the elevated levels of parathyroid hormone. Some research groups have shown that the supplementation of vitamin D remarkably improves symptoms of pulmonary TB in the course of the first month of treatment.<sup>48</sup>

Bedquiline (BDQ) used against tuberculosis is an inhibitor of the enzyme adenosine triphosphate synthase of *MTB*. It is a bactericidal and long-acting drug. BDQ and other newly discovered medications like Delamanid are used especially to treat active drug-resistant organisms like MDR-TB and XDR-TB. During its developmental stage and early use, BDQ was hailed as a "magic bullet" against TB and not much resistance was expected to arise against this new drug. However, soon thereafter, BDQ resistant-TB was reported as the "Dark Clouds on the Horizon" in the treatment of MDR-TB and XDR-TB.

Due to the advent of drug-resistant TB it is very important to find new drugs. This cholecalciferol-VDR mediated pathway is the de-novo mechanism, which inhibits TB naturally (Fig 3 and 4). Therefore, reaction intermediates and active components isolated from different points of this pathway may function as new drugs against TB. This is a good pathway for improving the probability of drug discovery against TB. It was known long since that vitamin D suppressed TB. Now, the anti-TB role of vitamin D can be confirmed from

its molecular mechanism. Cholecalciferol-VDR complex induces cathelicidin expression. Cathelicidin enters the lysosome and generates LL37 active form which punctures TB to stop bacterial colonisation. Therefore, molecular players of the vitamin D-mediated pathway and their homologs/analogues/mimics have a good chance to kill TB; these are viable candidates for development of novel anti-TB drugs.

#### 4. CONCLUSION

In this manuscript, we have emphasised the latest developments regarding vitamin D's working mechanism and its biological consequences for both cure and prevention of TB. A new understanding of the molecular mechanism of vitamin D-mediated bioactivation has been unraveled. Induction of this pathway by the two molecules, cathelicidin, and CYP27B1, is of particular importance. The latest mechanistic studies of all these intermediate molecular actions confirm the highly potent antitubercular activity of vitamin D and indicate the key molecular players of this pathway.

Thus, analogs and structurally related small compounds (like these isosteres and bioisosteres molecules) of these molecular entities of the vitamin D and their subparts can be used as potent novel antitubercular drugs which are in great demand.

Vitamin D, a known immunomodulator possessing anti-inflammatory and dual antimicrobial properties, can be used in improving immunity, and thereby, it can help TB patients withstand the prolonged treatment schedule of TB physiologically more confidently. Thus, it can reduce TB morbidity throughout the world. Vitamin D is widely available and relatively cheap. It has extensive benefits for human health. The only hindrance to our capacity to translate these advantages to the clinic is due to inadequate evidence from a well-controlled clinical trial. Although no current research study has addressed this aspect of immunity mediated by vitamin D in the context of TB disease, which is expected to be a key feature for our studies in the future. This approach should generate new anti-TB drugs and other interventions against TB as well as other mycobacterial diseases.

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