Pakistan Journal of Chest Medicine, 2012 July-Sept; 18(3): 34-45

REVIEW ARTICLE

Role of Adjunctive Vitamin D Therapy in Treatment of Tuberculosis

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SUMMARY:

The role of vitamin D in bone homeostasis and calcium metabolism is well documented but some evidences support its immunoregulatory role, particularly in cases of tuberculosis. For many years, vitamin D is being used as an adjunctive therapy in tuberculosis patients though definitive data regarding its importance is lacking. Lack of exposure to sunlight and dietary factors are the most important reasons responsible for vitamin D deficiency. Rifampicin and Isoniazid can also reduce serum vitamin D level. Modern researchers demonstrated probable antimycobacterial action of vitamin D by induction of antimicrobial peptide cathelicidin. But many studies all over the world revealed conflicting results. Mean serum vitamin D level is found to be low in tuberculosis patients in most of the studies but supplementation of vitamin D failed to conclusively prove the necessity of such supplementation. Controversies remained regarding the appropriate dosage of vitamin D and duration of this therapy. With the emergence of drug resistant tuberculosis, addition of vitamin D to antituberculous therapy may be considered to improve outcome as we have limited antituberculous drugs in hand. Further research needed for prevention and management of this vitamin deficiency and to improve outcome of Tuberculosis patients.

Key words:

Vitamin D, tuberculosis, cathelicidin, toll-like receptor

INTRODUCTION:

Historically, vitamin D was used in the treatment of tuberculosis until the advent of modern chemotherapy[1]. Diets rich in egg and milk were recommended prior to introduction of present antituberculous drugs. More than a century ago (1849), the British physician C.J.B. Williams described the use of cod liver oil in the treatment of tuberculosis. He reported that among his tuberculosis patients, 206 out of 234 showed a “marked and unequivocal improvement” after treatment with cod liver oil[2]. Later in the nineteenth century, patients were frequently treated in sanatoriums, which were built in countryside, and were designed to provide sufferers with therapeutic “fresh air” and notably, sunshine[3].

For the intracellular pathogen *Mycobacterium tuberculosis,* a role for vitamin D in the antimicrobial activity of human monocytes and macrophages was first suggested by in vitro experiments in the laboratories of Rook in 1986 [4] and Crowle in 1987 [5]. Vitamin D deficiency is now recognised as widespread[6] and is more common in TB patients than controls[7,8,9]. Patients with tuberculosis have, on average, lower serum concentrations of 25(OH) D than healthy controls[10].

Vitamin D, ‘the sunshine vitamin’, is synthesized in the skin during exposure to ultraviolet light and is also available in the diet, principally from oily fish. Vitamin D is metabolized in the liver to form 25-hydroxyvitamin D [25(OH) D], the major circulating metabolite and measure of vitamin D status[11]. 25[OH] D is then further metabolized by the 1-α-hydroxylase enzyme CYP27B1 to its biologically active metabolite, the steroid hormone 1-α-25 hydroxyvitamin D [12].

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Mechanism of Antimycobacterial Action of Vitamin D:

1-α-25$(OH)_2$ D_3 has no direct antimycobacterial action, but it does induce antituberculous activity in vitro in both monocytes[4] and macrophages[5]. Exogenous 1α, 25$(OH)_2D_3$ induces a superoxide burst[13] and enhances phagolysosome fusion[14] in *Mycobacterium tuberculosis*–infected macrophages; both phenomena are mediated by phosphatidylinositol 3-kinase, suggesting that this response is initiated by ligation of a membrane vitamin D receptor (VDR)[15].

1α-25$(OH)_2D_3$ also modulates immune responses by binding nuclear VDR, where it up-regulates protective innate host responses, including induction of nitric oxide synthase, NOS2A[16]. A recent study found that 1,25$(OH)_2D_3$, in combination with IFN-γ, led to the up-regulation of NOS2 mRNA and NO production by human peripheral blood monocytes/macrophages after stimulation with MTB or PPD, and that this response was attenuated by selective TKR2 antibodies[17].

Recently, 25(OH) D has also been shown to support messenger RNA induction of the antimicrobial peptide cathelicidin LL-37, which possesses antituberculous activity[18]. Cathelicidins are a structurally diverse family of antimicrobial peptide precursors with widespread distribution in mammals, characterized by the presence of a highly conserved cathelin domain of approx 100 residues. Humans express only one, hCAP-18, which is found in alveolar macrophages, lymphocytes, neutrophils and epithelial cells[19]. The promoter of the hCAP-18 gene contains a consensus vitamin D response element, and 1-α-25$(OH)_2D_3$ induces hCAP-18 gene expression in human cell lines[20]. The protein product of hCAP-18 undergoes extracellular cleavage by the neutrophil azurophil granule proteinase 3 to generate a 37-residue peptide LL-37 [21].

LL-37 exerts immunomodulatory activity, being chemo attractant for monocytes, T cells and neutrophils[22] and up regulates IL-8 and MCP-1 in human whole blood[23]. It also possesses broad spectrum bactericidal activity: it kills microbes by disruption of the cell membrane[24]. Therefore, it seemed plausible that 1-α-25$(OH)_2D_3$- induced antimycobacterial activity might be mediated by LL-37.

In vitro studies have shown that vitamin D inhibits the generation of Th1 responses[25] and the production of Interferon-γ by previously activated T cells. It has been suggested that vitamin D may promote the generation of regulatory cells (Treg) [26]. Thus paradoxical effects of vitamin D have been observed in immunity of tuberculosis: vitamin D decreased Th1 mediated immunity, but increased bactericidal activity[27]. Given that the Th1 response is protective, but also causes pathology, vitamin D may provide the ideal response by inducing increased bactericidal activity coupled with a decreased, but present, Th1 response. Vitamin D status may thus have potential as an additional biomarker for protection[28].

In HIV-infected people, vitamin D might also indirectly enhance antimycobacterial immunity by slowing progression of HIV disease[29], although studies investigating the effect of vitamin D metabolites on HIV replication in vitro report conflicting results. But a Californian study found that physiologically relevant concentrations of 1α, 25$(OH)D_3$ induce autophagy in human macrophages which significantly inhibits HIV-1 replication in a dose-dependent manner[30].

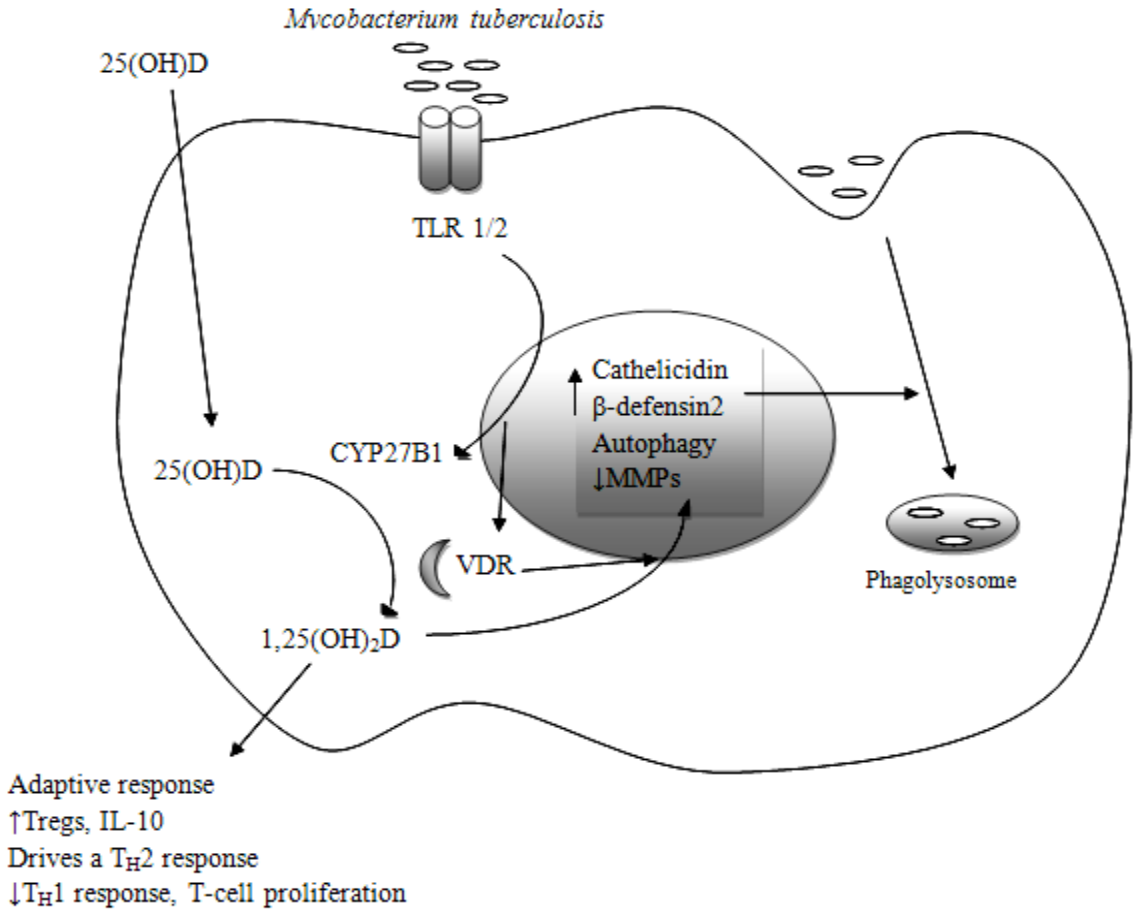

Figure I: ***Mechanism of vitamin-D-induced immunity to Mycobacterium tuberculosis.*** *Stimulation of monocyte Toll-like receptors (TLR1/2) by Mycobacterium tuberculosis (MTB) results in transcriptional induction of the vitamin D receptor (VDR) and 1α-hydroxylase (CYP27B1). Circulating 25-hydroxyvitamin D (25[OH]D) enters the cell and is converted to 1,25-dihydroxyvitamin D (1,25[OH]2D) by the CYP27B1 enzyme. VDR-bound 1,25(OH)2D then induces expression of cathelicidin and β-defensin 2 (DEFB4). In addition 1,25(OH)2D induces autophagy and downregulating metalloproteinases (MMPs), all of which help in the formation of phagolysosomes and the killing of Mtb. 1,25(OH)2D also affects the adaptive immune system and leads to an upregulation of regulatory responses and a skewing towards a Th2 response. IFNγ is thought to induce the expression of the CYP27B1 enzyme suggesting a feedback mechanism between the innate and adaptive response to vitamin D.*

Factors responsible for vitamin D deficiency:

Much remains to be known of the relative contributions of sunlight and diet to body vitamin D levels. A study of indigenous Indonesians suggested that in populations with good year-round sunshine, people could maintain adequate serum levels of vitamin D in spite of poor dietary intake[31]. The prevalence of profound vitamin D deficiency among TB patients in tropical Africa is much lower than in Europe [reported in 0.3–11.2% of patients with TB in tropical Africa[32,33] vs. 64–84% of patients with TB in London[34,35,36]].

Another recently concluded study at Cape Town, South Africa reported a seasonal variation in TB notifications and vitamin D status where a temporal relationship between the summer

peak in vitamin D status and the ensuing autumn dip in TB notifications raised the possibility that seasonal variations in vitamin D status and TB incidence in Cape Town might be causally related[37]. But a study in India however found low vitamin D levels in the study population despite adequate sun exposure, concluding that diet was the most important factor[38]. But the latter study did not take into account the actual time spent outdoors, extent of body exposed to the sun or level of cutaneous pigmentation. On migration away from home, Hindu Asians largely maintain socio-religiously determined adherence to vegetarian diets but exposure to sunshine is reduced. The observation that Asian migrants have lower serum Vitamin D than matched controls in their home countries[39] have led some authors to conclude that the fall in vitamin D levels associated with migration from sunshine-rich to sunshine-poor areas is probably the most important contributory factor as far as migrants are concerned[40]. Most of the south Asian population, including Pakistani immigrants to European countries and south Indians, had <10ng/ml of serum vitamin D level[41] and is consistent with reports from Aga Khan Hospital[42,43]. More recently, case-control studies have demonstrated that a vegetarian diet (low in vitamin D) is an independent risk factor for active TB in South Asians[44] and the patients with TB who are of Gujarati Hindu ethnic origin have significantly higher rates of vitamin D deficiency than ethnically matched tuberculin positive TB contacts[45].

Smoking is a risk factor for tuberculosis disease but, although vitamin D is important for calcium absorption (which is impaired by smoking), there is no evidence to suggest that vitamin D absorption is impaired directly by smoking. However, women who smoke also tend to ingest less vitamin D than non-smoking women[46]. Therefore, if reduced intake of vitamin D caused vitamin D deficiency, smoking would not only be associated with vitamin D deficiency but also be an independent risk factor for tuberculosis, making it an important confounder of the low vitamin D-tuberculosis association.

Although there is good evidence to suggest that a fall in serum vitamin D levels compromises cell mediated immunity and leads to the activation of latent tuberculosis[47], it is also possible that low serum vitamin D levels result from tuberculosis itself. Again, both Rifampicin and Isoniazid have been shown to reduce vitamin D level[48]. In addition, patients with CKD or those who are dialysis-dependent are more likely to have low levels of vitamin D in comparison to those without kidney disease[49]. The incidence of tuberculosis is high in CKD partly as a result of impaired cell-mediated immunity[50], but if low serum vitamin D levels also predispose to tuberculosis, the growing population of people with CKD from underlying causes like DM may need early attention to their body vitamin D levels to mitigate the risk of active tuberculosis[10]. Previously mentioned Cape Town study also revealed an association of vitamin D deficiency with susceptibility to active TB in both the absence and the presence of HIV infection, but the association was stronger in HIV-infected people[37].

Evidences of Association of Vitamin D Deficiency and Tuberculosis:

A study among immigrants from sub-Saharan Africa, attending the infectious disease clinics of a Melbourne tertiary care hospital showed moderate to severe 25(OH)D deficiency (level ≤ 25 nmol/L) in 31 (78%) of 40 patients with TB/past TB, 27(33%) of 81 with LTBI and 2(6%) of 34 with no MTb infection. There was a difference in the geometric mean 25(OH) D levels when patients with LTBI were compared with patients with no MTb infection (37.3 vs. 54.6 nmol/L; p=0.007), patients with TB/past TB were compared with LTBI (16.1 vs. 37.3 nmol/L; p<0.001), and patients with TB/past TB were compared with patients with no MTb infection (16.1 vs. 54.6 nmol/L; p<0.001)[9]. Similar type of conclusion can be drawn from a recent

Spanish study at Castellon which showed mean serum 25(OH) D level in tuberculin sensitivity test (TST) conversion cases among contacts of active TB was lower than controls, 17.5±5.6 ng/ml vs. 25.9±13.7 ng/ml (p=0.041) though size of the study in relation to TST conversion was small[51].

In Vietnam, Ho-Pham and co-authors[52], in a matched case-control study of TB patients and controls, found that low serum 25(OH)D levels (<30 ng/ml) was a risk of tuberculosis in men, but not in women. Again, in Greenland[53], a case-control study of TB patients and controls reported that 25(OH)D levels of <75nmol/L or >140nmol/L were associated with high risk of active TB. Lower level of serum vitamin D was also found among refugee children in Sydney having latent TB and TB infection than in children without TB[54]. An Indonesian clinical trial, that used a considerably higher cumulative dose of 10,000 IU vitamin D daily documented 100% sputum conversion in the vitamin D group against 77% in the placebo group 6 weeks after initiation of antituberculous therapy[55].

Adrian R. Martineau et al showed in their study that Vitamin D supplementation primarily enhances innate responses to mycobacterial infection. A single oral dose of 2.5 mg vit.D corrects profound vit.D deficiency for at least 6 weeks without causing hypercalcemia underlines the potential use of this formulation as a safe, effective & cheap[56].

Philip T. Liu et al demonstrated induction of antimicrobial activity against intracellular M. Tuberculosis H37Ra in THP-1 cells (human acute monocytic leukaemia cell line) by 1,25(OH)D_3. In the above mentioned study, THP-1 cells were infected with the avirulent M.tuberculosis H37Ra at an MOI (multiplicity of infection) of 1 and then treated with 1,25 D3 for 3 days. Subsequently, the intracellular bacteria were harvested and tested for bacterial metabolism according to [$_3$H]uracil uptake or viability according to CFU. The addition of 1,25D3 to the infected THP-1 cells resulted in a 55% decrease in [$_3$H] uracil uptake by the bacterium (p<0.01; Fig.2a) and a 28% decrease in total CFU harvested (p<0.05; Fig.2b)[57,13].

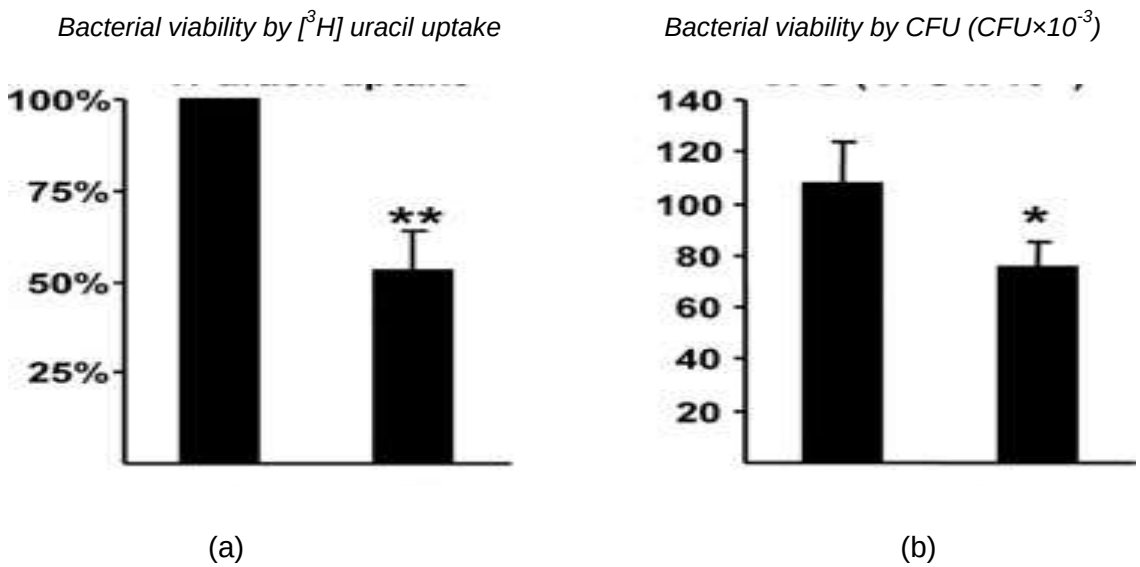

Figure II: *1,25D3 induces antimicrobial activity against intracellular M.tuberculosis H37Ra in THP-1 cells. THP-1 cells were infected with M. tuberculosis H37Ra for 18 hr and treated with 10^{-8} M 1,25D3 for 3 days. Following treatment, the intracellular bacteria were harvested & then assayed for bacterial metabolism according to [^{3}H]uracil uptake (a) and bacterial viability according to CFU (b).Data shown for [^{3}H]uracil uptake represent the average bacterial viability percentage of four individual experiments (n=4±SEM;**,p<0.01). bacterial viability was calculated as the 1,25 D3 value divided by the medium treated value multiplied by 100.CFU experiment is representative of three individual experiments(*,p<0.05). (reproduced with permission)*

A cohort follow-up study from Karachi, Pakistan revealed that low vitamin D levels were associated with progression to active TB disease in healthy household contacts to sputum positive pulmonary TB, though this study had relatively small number of study participants and not taken diet, body mass index, exposure to sunlight into consideration[58].
A randomized two-by-two factorial trial in Mwanza, Tanzania was done to assess effects of multivitamin/mineral (MVM – vitamin A,B,C,D,E and Selenium & Copper) and Zinc(Zn-45mg) supplementation during TB treatment on mortality. Survival status was ascertained at the end of the 8 month TB treatment and supplementation period. The study concluded that supplementation with MVM, including Zn, during treatment of Pulmonary TB may reduce mortality in those co-infected with HIV[59]. Value of nutrition in management of tuberculosis is also evidenced by a wholefood trial comprising a nutritious daily meal in Timor Leste, which was associated with significant improvements in weight gain in the intervention arm in pulmonary TB patients[60].

TABLE I: Systematic review and meta-analysis of some observational studies those examined the association between low serum vitamin D and risk of active tuberculosis can be summarized as follows**:**

Author, Date, Place	**Study population**	**Design**	**Cases**	**controls**	**Parameter measured**	**Results (serum vit. D level)**
Davies PD et al, 1985,UK	UK whites (84%) Indian[a](8%) Others[b](8%)	Prospective study	40 untreated inpatients & outpatients with mainly culture (+) PTB,mean age 43.1 yr	40 controls either members of patients' families or age & sex matched healthy volunteers, mean age 42.2 yr	Serum vit D levels	Median (range) in cases-16.0 nmol/L (2.25-74.25) Controls-27.25 nmol/L(9.0-132.5), P<0.005
Davies et al, 1987, Kenya	Indigenous African population (Kenyans)	Prospective study	15 untreated culture(+)PTB patients presenting to hospital Mean age-33 yr	15 age & sex matched healthy controls selected from patients' families Mean age 35 yr	Serum vit D levels	Median (range) in cases-39.75 nmol/L (16.75-89.25). controls-65.5 nmol/L (26.25-114.75), p<0.05
Davies et al 1988 Thailand	Indigenous Thai population	Prospective study	51 untreated smear(+)PTB patients presenting to a chest clinic. Mean age 30.5 yr	51 age & sex matched healthy controls selected from blood donors attending a hospital blood bank.Mean age 30.4 yr	Serum vit D levels	Mean (SD) in cases-69.5 nmol/L(24.5 nmol/L).controls 95.5 nmol/L (29.25 nmol/L), p<0.001

Chan et al 1994 Hongkong	Indigenous Chinese population	Case-control study	22 untreated hospital in-patients with culture(+)PTB Mean age 56.3 yr	23 in-patients & out-patients receiving care at same time as cases	Serum vit D levels	Mean(SD) in cases-46.5 nmol/L (18.5 nmol/L) controls 52.25 nmol/L(15.75 nmol/L)
Wilkinson et al 2000, UK	Gujrati Hindus resident in London	Case-control study	103 untreated patients with localized[c] & severe[d] TB, recruited from a hospital. Mean age 45.5 yr	42 household multiple contacts of TB patients attending TB contact clinics of same hospital mean age 42.7 yr	Serum vit D deficiency	Odds ratio(CI) for vit D deficiency in cases compared to controls-2.9 (1.3-6.5) p=0.008
Sasidharan et al 2002 India	Indigenous Indian population	Case-control study	35 untreated hospital in-patients with Pulmonary(15) & extra-pulmonary (20)TB. Mean age 37.5 yr	16 healthy age & sex matched controls Mean age 34.1 yr	Serum vit D levels (in fasting blood samples)	Mean(range) in cases-26.75 nmol/L(2.5-75) Controls-48.5 nmol/L(22.5-145) p<0.005

Indian[a] -whose ethnic origin is in the Indian subcontinent (Indian,Pakistani or Bangladeshi)
Others[b] –includes one person each of west Indian,African,Malaysian & Chinese ethnic origin
Localized[c] – disease defined by author as TB confined to one anatomical site
Severe[d] – disease defined by author as Pulmonary or miliary tuberculosis
Source: Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. Int J Epidemiol 37 (reproduced with permission)

Controversies Regarding Vitamin D Supplementation:

But many studies failed to show any promising result between vitamin D supplementation and outcome of tuberculosis patients. Bruce and co-authors indicated[61] that: “the evidence does not support a positive or negative role for vitamin D in host resistance to infection.” A randomized, double blind, placebo-controlled trial by Christian Wejse et al in TB clinics at a demographic surveillance site in Guinea-Bissau including 365 adult TB patients showed reduction in TB score and sputum smear conversion rates did not differ among patients treated with vitamin D (1,00,000 IU of cholecalciferol at inclusion and repeated at 5 and 8 months) or placebo and overall mortality was 15% (54 of 365) at 1 year of follow up and similar in both arms. In this trial, vitamin D supplementation showed no beneficial effect for patients with TB in general, but this may have been due to suboptimal dosage[62]. Again, there were nonsignificant trends toward a beneficial effect in HIV-uninfected patients, with the greatest vitamin D deficiency and a worsening of outcome in HIV-infected patients given vitamin D.

Though reliable and reproducible clinical trial results in both latent and active TB are still lacking, some hypothesise that vitamin D supplementation would only be efficacious as a prophylactic agent for reducing the risk of activation of latent TB (for instance, as life-long therapy in vitamin D-deficient people with latent TB)[63]. Others argue that vitamin D has roles

in immunological pathways other than just latent TB reactivation, thereby warranting trials of vitamin D as an adjunctive agent in active TB[64].

Contrasting with the majority of reports showing that low plasma vitamin D correlates with active TB, Selvaraj et al recently reported that plasma $1,25(OH)_2D_3$ levels were significantly increased among pulmonary TB patients compared to healthy controls. But only $1,25(OH)_2D_3$ was measured in this study, not $25(OH)D_3$. The authors hypothesised that the widely reported finding of low $25(OH)D_3$ in people with active TB may be explained by excessive conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, providing a mechanism for simultaneous findings of low $25(OH)D_3$ and 1α-hydroxylase-driven hypercalcemia which can characterise active TB[65].

Another multicentre randomized control trial of adjunctive vitamin D in adults with sputum smear-positive pulmonary tuberculosis in London, UK by Dr. Adrian R Martineau showed that high dose vitamin D3 (four doses of 2.5 mg at baseline, 2 weeks, 4 weeks and 6 weeks) during intensive phase antimicrobial treatment of Pulmonary tuberculosis did not significantly affect time to sputum culture conversion in the whole study population. The median time to sputum culture conversion was 36 days in the intervention group and 43.5 days in those getting placebo and the result was non-significant at p=0.14. But this study revealed the effect of two polymorphisms of the vitamin D receptor. The t allele of TaqI has been shown in vitro to speed sputum culture conversion, while the f allele of FokI has been shown to delay conversion[66]. Gao and colleagues again reported that among Asians, the FokI ff VDR genotype is associated with increased TB risk, the BsmI bb genotype was protective, and TaqI and ApaI polymorphisms were only marginally significant[67]. A case-control study at Tehran, Iran mentioned combined genotypes AbfT and AabbFfTT were the only statistically significant factors which protected people against pulmonary TB and they could not find any predisposing genotype to TB[68]. But as described by Lewis et al[69], larger studies are required to determine whether VDR polymorphisms play a role in genetic susceptibility to Tuberculosis worldwide.

A famous case-control study(1985) among Indonesian indigenous population by Grange et al failed to show any significant difference of median serum vitamin D level among sputum positive pulmonary Tuberculosis patients and age matched healthy controls. The median serum vitamin D level in that study was found to be 65.75 nmol/L (range 43.75-130.5) in cases compared to 69.5 nmol/L(range 48.5-125) among controls ($p>0.25$)[31].

Vitamin D Supplementation Regimen:

Although the consequences of uncorrected vitamin D deficiency have been well characterized[70], no universally accepted vitamin D repletion regimen has been adopted[70,71]. A recent study suggests that more than 1000 IU of vitamin D daily is necessary to restore vitamin D status[72]. Weekly high dose regimen have been proposed as a safe and effective method to rapidly correct vitamin D deficiency[73,74,75]. In Canada and the USA, new dietary recommendations for vitamin D have recently been published by the Institute of Medicine. The report summarized the evidence about vitamin D and tuberculosis but concluded that : "in the absence of verifiable dose-response data from RCTs [randomized controlled trials], a conclusion about asthma, autoimmune or infectious diseases as indicators for DRI [daily recommended intake] development cannot be reached."[76]

But in Finland, since February 2003, on the basis of recommendations of the Ministry of Social Affairs and Health, vitamin D has been added to commercial milk and dairy products (0.5 µg/100 mL) and to margarine (10 µg/100 g). This fortification is part of a national health

policy aimed at increasing vitamin D intake through diet to 280 IU/d[77]. A double-blind placebo-controlled study among 120 mongol school children reported fewer TST conversions in the vitamin D group who received 800 IU/day vitamin D supplementation for 6 months[78].

Again, according to few researchers, doses of vitamin D administered to patients with active TB may be limited by induction of hypercalcemia[1]. But in one study, Hollis supplemented women during lactation with 4000 IU/day vitamin D_3 without any adverse clinical or laboratory events[79]. In preliminary safety data from another study, 11 TB patients were administered a single dose of 1,00,000 IU ergocalciferol (vitamin D_2, considered less efficacious than D_3), resulting in a significant rise in serum vitamin D_2 and no episode of hypercalcemia at 8 weeks. No rise in serum D_2 occurred in 14 TB patients randomised to placebo[34]. Although the most common time for hypercalcemia occurrence has been reported to be around 2 to 4 weeks[80], these authors only examined hypercalcemia at 8 weeks, thereby potentially underestimating true hypercalcemia occurrence. Therefore, there may be a place for the use of non-calcemic vitamin D analogs as novel adjunctive treatments for active TB.

CONCLUSION:

The recent emergence of extensively drug resistant organisms is likely to have a global impact[81]. Some of these problems could potentially be overcome by adding vitamin D to the treatment of tuberculosis, because a single oral dose increased the killing of mycobacteria in the blood of healthy donors[56], although the currently published studies on the effects of vitamin D supplementation are generally inadequate to evaluate the clinical efficacy of such treatment[1]. In the face of emerging multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of M. tuberculosis for which chemotherapeutic options are restricted, it is necessary to explore immunological approaches to combat tuberculosis[82]. Prospective studies to firmly establish the direction of the relationship between vitamin D and tuberculosis as well as evaluations of vitamin D supplementation in tuberculosis and renal failure patients are needed[10].

As the observational primary studies in this review are unable to conclusively establish the direction of association between low serum vitamin D and tuberculosis, this review highlights the need for larger, well-designed prospective studies clarifying the association.

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