doi.org/10.36721/PJPS.2023.36.6.SP.1901-1908.1

The role of vitamin D supplementation in augmenting IFN-γ production in response to mycobacterium tuberculosis Infection: A randomized controlled trial

Muhammad Imran Hussain, Ramisha Ibtisam, Tayyaba Fatima, Huba Khalid, Ayesha Aziz, Khansa*, Adan Sitara, Anam Shahzad and Aymen Jabeen
Department of Allied Health Science, Nutrition and Dietetics, Riphah Unternational University, Lahore, Pakistan

ABSTRACT: Vitamin D supports the immune system fight TB by inhibiting Interferon-gamma (IFN-γ) and lowering host inflammation. The purpose of the research was to see if giving the vitamin D supplements to TB patients affected their prognosis. A randomized placebo control study of 200 TB patients was performed among which 106 received 400,000 IU of injectable vitamin D3 and 94 received placebo for 2 doses. Assessment was carried out at the end of every month for 3 months. IFN-γ responses to whole blood stimulation generated by the Mycobacterium tuberculosis sonicate (MTBs) antigen and early secreted and T cell activated 6 kDa (ESAT6) were assessed at 0 and 12 weeks. The statistical analysis used descriptive statistics (mean and standard deviation), Friedman's test and Fisher's test. The vitamin D group gained significantly more weight (+3.90 pounds) and had less persistent lung disease on imaging (1.33 zones vs. 1.84 zones). They also had a 50% decrease in cavity size. Additionally, patients with low baseline serum concentrations of 25-(OH)D had a significant increase in MTB-induced IFN-γ production after taking vitamin D supplements. Vitamin D administration in large amounts can hasten the recovery of TB patients. The findings point is a therapeutically useful activity of Vitamin D's in the management for tuberculosis.

Keywords: Tuberculosis, vitamin D, interferon gamma, protein, infection.

INTRODUCTION

Tuberculosis (TB) is a contagious bacterial infection caused by Mycobacterium tuberculosis (MTB) that has been present for approximately 3000-5000 years BC. According to the World Health Organization (WHO), tuberculosis (TB) is a worldwide health crisis and a major contributor to premature deaths among young adults. Approximately one-third of the global population is susceptible to tuberculosis, according to estimates. Previously, tuberculosis (TB) was believed to mostly affect third world countries. However, during the past decade, the incidence of TB has also risen in industrialized countries. The prevalence of tuberculosis (TB) in the population of South East Asia is as high as 44%. Pakistan is ranked fifth out of 22 highly ranked countries (Hussain *et al.,* 2019). Tuberculosis patients experience a persistent decrease in levels of both macro- and micro-nutrients, which significantly impairs their muscle strength and immune system function. Patients in hospitals require urgent food support along with the required treatment. The lack of certain micronutrients is leading to secondary immunodeficiency, which is the main factor contributing to increased susceptibility to infectious diseases and associated morbidity in persons. Individuals have a higher susceptibility to TB (Hussain *et al*., 2019). Two epidemiological studies have shown a considerable correlation between seasonal fluctuations in serum vitamin D levels and the occurrence of tuberculosis (TB) (WHO 2013, Martineau *et al.,* 2011). A meta-analysis revealed a significant correlation between low levels of vitamin D in the bloodstream and an elevated susceptibility to tuberculosis (Maclachlan *et al.,* 2012). These findings indicate that vitamin D supplementation is likely to have a primary preventive effect on the likelihood of developing tuberculosis and also has a positive impact on the effectiveness of tuberculosis therapy.

The utilization of vitamin D in the treatment of tuberculosis commenced in 1849, following the finding that the consumption of fish liver oil resulted in enhanced appetite and physical vigor (Nnoaham *et al.,* 2008).

Vitamin D significantly contributes to the innate immune response against Mycobacterium tuberculosis (MTB) infection, as well as its activation and progression. Moreover, vitamin D insufficiency is a recognized predisposing factor for the acquisition of both MTB infection and active tuberculosis (TB). Vitamin D activates VDR (25-hydroxyvitamin D receptors) in the body's immune cells. These proteins are essential for killing mycobacterium tuberculosis (MTB) (Ganmaa *et al*., 2020). MTB antigens activate VDR on macrophages and monocytes, which leads to the production of cathelicidin and 1,25 dihydroxy vitamin D (Liu *et al*., 2007, Liu *et al*., 2006). These molecules help to destroy MTB. People with low vitamin D levels are more likely to develop tuberculosis (Gibney *et al*., 2008, Nnoaham *et*

**Corresponding author:* e-mail: khansamubeen123@gmail.com

al., 2008). This is because vitamin D helps in boosting immunity and infection protection. In the past, sunlight and cod liver oil were used to treat tuberculosis because they are good sources of vitamin D. In recent years, there have been a number of studies that have investigated the role of vitamin D in the treatment of tuberculosis (Ayelign *et al*., 2020). A recent cohort study has failed to prove that vitamin D supplementation can improve clinical and microbial responses to traditional antibiotic treatment for tuberculosis perhaps due to low dose (Xiong *et al*., 2020). However, other studies have found benefit. The authors of another study investigated whether vitamin D supplementation can improve the symptoms and immunological reactivity of people with active tuberculosis (Ayelign *et al*., 2020). They believe that vitamin D may be beneficial in these patients, even though previous studies have not found a clear benefit.

MATERIALS AND METHODS

Research design

The clinical investigation was randomized, placebo-controlled study. The Riphah International University Lahore Research Council supported the trial and it was authorized by Riphah International University Lahore institutional review boards. The study was conducted for one year from April 2022 to April 2023. Before taking part, all patients gave their signed, informed permission. Patients registered at outpatient TB clinics with positive QuantiFERON-tuberculosis (TB) Gold Plus (QFT-Plus), smear-positive and Invasive pulmonary TB identified within a week were included. A previous record of prior latent TB infection, or any other severe disease instead of an (with a diagnosis of HIV, whether recognized or as assumed) active pulmonary TB were considered exclusion criteria of the research.

Primary and *secondary outcome variables*

The primary results were variations in gaining weight, skin fold as well as the elimination in chest scan anomalies. The QuantiFERON-TB Gold Plus (QFT-Plus) test, variations in sputum converting, along with variations of Interferon-gamma (IFN-γ) responses were secondary goals. Improvements in the TB score, Urine and serum level of Ca^{+}, albumin protein and creatinine are the primary findings. An ad hoc research assessed variations within an inflammatory reaction or clinical improvement related to initial vitamin D level.

Clinical evaluation

Baseline data, lung imaging, sputum specimens, serum sample and cytokine analyses were collected. The pharmacy department at the University of Veterinary & Animal Sciences Lahore was in charge of providing the study drug/placebo in order to produce a randomization sequence. The research coordinator and specialist were on-site to recruit eligible participants. Patients continued to receive shorter treatment regimens (STR) which included 2 months of 4 antituberculosis drugs (Ethambutol, Isoniazid, Rifampicin and Pyrazinamide), followed by 6 months of Ethambutol and Isoniazid. To screen, enroll and randomly select eligible patients a computer-generated list of classified, random assignments was used. The two trial arms received either an equal amount of color-matched normal saline or 400,000 International Units of injectable cholecalciferol delivered twice a month for one month.

Patients with tuberculosis were evaluated clinically and by sputum, chest X-ray and blood tests at 0, 2, 3 and 4 months of treatment. Tuberculosis grading was used to assess the severity of the disease (Wejse *et al*., 2008). 25-(OH) D levels were measured to assess vitamin D status. Tuberculosis grading has become a recognized evaluation measure designed to properly monitor changes in the diagnostic state of an individual's tuberculosis. The features include Self-reported complaints (cough, difficulty in breathing, sleep hyperhidrosis, etc.) and clinical indicators (tachycardia, paleness, a high temperature, listening abnormalities, chest tightness, hemoptysis), height and weight measurement and MUAC (mid-upper arm circumference). MUAC was measured with a stiff measuring tool on the bicep muscles for the lower arm at the intersection of the olecranon and acromion processes. Height was measured in meters and weight was assessed in kg at each visit. The seriousness of tuberculosis was isolated with the tuberculosis grading. Patients were segregate into three severity catalogues based on their tuberculosis grade: Category 1 (grade 0-5), Category 2 (grade 6-7) and Category 3 (grade 8 or higher).

The severity of tuberculosis was assessed by two consultant pulmonary physicians using three methods: radiography, cavity size and zone involvement (table 1). Patients were also asked if they had experienced any symptoms of hyperkalemia and those with a BMI under 18 had their calcium and albumin levels tested.

QuantiFERON-TB Gold Plus (QFT-Plus) analysis and *IFN-Y measurements*

The University of Veterinary & Animal Sciences Lahore created recombinant antigen [Early released and activate targeting-6 kDa and Mycobacterium TB virulent strain H37Rv (virulent)] entire sonicate *Mycobacterium tuberculosis* for the National Institutes of Health (NIH) TB vaccine testing and laboratory material collaboration. M. Antigen-induced reactions in research participants were assessed before starting any anti-tuberculosis medication or supplementation and again after placebo therapy (n = 90) and vitamin D treatment groups (n = 106). IFN-γ was found in the supernatants of cell cultures collected six days following stimulation with 6 mcg/ml ESAT6 and 12 mcg/ml MTB in diluting QuantiFERON-

tuberculosis (TB) Gold Plus (QFT-Plus). Each sample had a duplicate made. Samples are centrifuged to eliminate any cellular trash.

Biochemical test

A radio immunoassay (RIA) was used to evaluate 25-hydroxy vitamin D, Ca^{+} and protein in the blood. Evaluations in the clinic and microscopic testing of sputum were done during the first, second and third months of treatment. Chest X-rays and specimens of blood were taken in the first and third months for cytokine testing. Patients were asked about any hyperkalemia-related symptoms. Serum 25-(OH) D, Ca^{+} and protein levels in the individuals who have a Body Mass Index less than 18 were measured 3 months after enrollment. To determine the required sample size, an increase in pounds was expected (with the vitamin D treatment) that was at least 8% greater than the mean increase in pounds in the placebo group as well as a 12% variation in radiograph performance among both groups. With a 5% level of statistical significance, the research projected 106 participants in both groups who rejected the null hypothesis. With a 14 percent incidence of elevated calcium levels in 25-(OH) D group, 40 participants were required. Weight increase, pulmonary radiography defects, the cytokines actions, sputum rates of conversion and Tuberculosis grade improvements were the primary objectives.

Ethical approval

Ethical approval was obtained from Research & Ethics Committee of Riphah International University, Lahore with reference number of REC/RCR & AHS/22/34.

STATISTICAL ANALYSIS

Intention-to-treat Statistical analyses were performed to compare two groups at 0- and 4-months using Fisher's exact tests for categorical variables and for continuous data descriptive stats mean and the standard deviation are used. Result variations were described with means and medians, ranges, or standard deviations. A Friedman non-parametric experiment was employed for the comparison of Interferon-gamma actions before and after therapy among 25-(OH) vitamin D and Tuberculosis in serious groups. Each-sided p-value <0.05 was determined to be significant. Version 27.0 in SPSS was employed to analyze the data. The institutional research department conducted an interim safety study for 3 months to look for changes in mortality. There were no variations in death rates among both study groups.

RESULTS

Explanation of the research

As seen in fig. 1, 300 patients were evaluated for inclusion; 200 were admitted into the research and randomly assigned to one of two treatments, with 106 receiving 25-hydroxyvitamin D supplementation and 94 receiving a placebo. The probe began in April 2022, with the first person registering in and the last person joining in December 2022. During the course of the study, two patients within the 25-(OH) D treatment group and two within the placebo group died. A solitary death occurred in the vitamin D group two weeks after that it was administered due to a rapidly worsening respiratory disease of unidentified cause and one died in an automobile accident. Malaria and Dengue claimed the life of single participant each in the control class.

Study findings

Table 1 shows the initial parameters of the two research populations. With the anomaly of a higher number of individuals with high temperature within the placebo group 38 vs 59, the two arms did not vary substantially. The average age of the entire population was 27 years and 67 individuals had + 3 AFB on sputum microbial burden. Mean 25-(OH) vitamin D levels for the all population were in the 'meager range; 32.57 ng/mL, $\pm$ 7.52. Initial 25(OH)D levels did not correlate with sputum AFB load, or Tuberculosis B severity grade. Hemoptysis and low vitamin D levels appeared to be more closely related than previously thought.

Table 2 shows Statistical Comparisons of Baseline Characteristics between the Cholecalciferol and Placebo Groups. Only Unstimulated interferon-gamma levels show significant results.

Table 3 displays the changes in medical variables that were assessed after four months of anti-TB treatment. The 25-(OH) vitamin D intake group showed a mean increase in pounds of + 3.90 (95% CI 3.06–3.24) after the end of 4^{th} month of anti-tuberculous therapy v/s + 2.20 (95% CI 1.27–3.08) in the placebo group (p-0.006) and a mean Body mass index of + 0.72 (95% CI 0.69 - 1.19) in the placebo arm (p 0.009). According to the analysis of the chest radiographs at end of 4^{th} month, the mean no, of zones implicated in the vitamin D group was 1.33 vs. 1.84 zones in the placebo group (p-0.003) (SD= 1.09, 1.28). Patients in the vitamin D group demonstrated a 49% improvement.

By week 4 of treatment, 128 (65%) of the trial participants' sputum samples were found to be negative. In general, no appreciable sputum changes, the rate of converting smear were discovered. The difference between the two research arms' Tuberculosis grade at weeks 4 (p -0.20), 8 (p-0.97), or 12 (p-0.18) was statistically significant. Participants in the vitamin D arm who were recruited exhibited substantially bigger changes in Tuberculosis severity scores when contrasted with those with normal baseline 25(OH)D (p-0.015). Furthermore, the supplementary treatment category with

Table 1: Parameters at the beginning of the cholecalciferol and placebo research groups (N = 200)

Initial parameters	Randomized	
	Drug treatment (n= 106)	Placebo treatment (n = 94)
Age (in years), Mean ± SD	27.7 ± 13.3	28.3 ± 14.3
Sex, Male	69 (42.6)	69 (44.3)
Weight (kg), Mean ± SD	45.3 ± 7.5	45.6 ± 9.0
Body Mass Index, Mean (range)	17.4 (10–24)	17.3 (10–26)
MUAC (cm), (range)	21.4 (13–32)	21.3 (14–33)
Existing illness Signs		
Cough	130 (88.3)	140 (89.3)
High Temperature	38 (27.2)	59 (49.4)
Coughing up blood	20 (32.7)	23 (20.7)
Tachycardia	86 (56.7)	85 (76.6)
Dyspnea	74 (53.7)	79 (55.7)
Sleep hyperhidrosis	59 (43.2)	54 (39.2)
Chest ache	74 (53.7)	78 (59.2)
Conjunctival pallor	72 (54)	68 (49.7)
Tuberculosis grade, Mean ± SD, 95 CI	6.67 ± 2.03, 6.3-7.02	6.84 ± 2.50, 6.4-7.28
Distribution by Severity of Tuberculosis		
Category I/0 – 5	22 (20.3)	39 (29.4)
Category 2/6 – 7	49 (28.5)	39 (28.2)
Category 3/≥ 8	39 (28.2)	38 (29.3)
Serum-25-(OH) D_3 levels; Mean, (SD)	32.57 ± 7.52	33.88 ± 10.34
25(OH)D_3< 20 ng/ml (inadequate)	70 (49)	48 (39)
25(OH)D_3 20-30 ng/ml (Insufficient)	46 (34.8)	54 (42.5)
25(OH)D_3>30 ng/ml (Optimal)	17 (13.5)	24 (19.8)
Chest imaging Classification		
minimal Advanced disease	10 (7.6)	13 (10.2)
Moderately Advanced disease	77 (58.3)	62 (48.8)
Far Advanced disease	45 (34.1)	52 (40.9)
No Cavity	9 (4.2)	7 (2.3)
Cavity size less than 3cm	59 (39.8)	49 (39.4)
Cavity size greater than and equal to 3 cm	58 (38.9)	59 (48.8)
No. of zones involved, Mean ± SD	3.61 ± 1.42	3.64 ± 1.47
Sputum microbial burden (microscopy)		
Sufficient, 2–9 AFB/100 fields	3 (2.0)	2 (1.6)
+1, 10–99 AFB/100 fields,	30 (27.3)	24 (23.8)
+2, 1–10 AFB/50 fields,	20 (18.9)	23 (18.1)
+3, >10 AFB/20 fields,	67 (50.8)	59 (49.9)
Interferon-gamma levels pg/ ml mean ± SD		
Unstimulated-levels	0.4 ± 3.8	0 ± 0
ESAT6-stimulated	412 ± 978	302 ± 805
MTBs-stimulated	2825 ± 1392	2857 ± 1338
MUAC: Mid Upper Arm Circumference; BMI: Body Mass Index; and s-25-(OH) D3 stands for serum 25-hydroxy vitamin D3.		

initial "Insufficient" values had a tendency towards fewer chest radiographic zones involved (p-0.045) and greater sputum smear clearing (p-0.05).

Mycobacterial-antigen stimulated IFN-Y responses in placebo* and *treatment groups

The assessed baseline M. antigen-stimulated IFN-Гretaliation in the placebo group to measure potential effects on cytokine secretion profiles resulting from patient-specific changes in disease severity. It was found that patients with Class I, II, or III disease did not have different ESAT6-induced interferon-gamma responses (IFN-γ) (p-0.989, Friedman test). (p - 0.248, Friedman's test) Interferon-gamma (IFN-γ) induced by (MTBs) in patients with Category 1, 2 and 3 TB was similar.

Because vitamin D reduces Interferon- production in the host, the study participants' blood levels of 25-

Table 2: Differences in clinical Parameters from start to end of research

Disease parameter(s)	Randomized	*p*-value
TB Severity	Drug treatment (n = 106) Placebo treatment (n = 94)	95% CI
Average change in TB score ± SD, (95% CI)	- 3.07 ± 2.19, (−3.43, -2.32) - 2.08 ± 2.39, (−3.18, -2.29)	0.209
Average change in weight (kg), (95% CI)	+ 3.90, (3.06 – 3.24) + 2.20, (1.27 – 3.08)	0.006
Average change in BMI, (95% CI)	+ 1.39, (1.05 – 1.39) + 0.72, (0.69 – 1.19)	0.009
Average change in MUAC (cm), (95% CI)	+ 1.29, (0.68 – 1.70) + 0.89, (0.71 – 1.19)	0.068
Chest X-ray Involvement Average no. of zones[1] involved ± SD	1.33 ± 1.09 1.84 ± 1.28	0.003
Sputum Smear Smear Conversion, no. (%) [2]	110 (80.9) 101 (82.0)	0.41
IFN-Y levels (pg/ml) mean ± SD		
Unstimulated levels	4.1 ± 29.2 3.9 ± 44.9	0.198
ESAT6-stimulated*	376 ± 917 203 ± 672	0.081
MTBs-stimulated*	3071 ± 1357 2789 ± 1498	0.503

95% CI=95% Confidence Interval, SD=Standard Deviation, [1] Bilateral lung fields are divided into six zones in total, three for each lung. "Zone involvement" refers to active cavity and parenchymal illness, excluding older fibrotic scarring, [2] Includes all patients who reported at treatment's end, 12 weeks later, that they were "unable to expectorate" despite having been able to do so at baseline, ESAT6=early secreted and T cell activated antigen-6 kDa, MTB=Mycobacterium tuberculosis whole sonicate antigen, * shows the number of cytokines after background from unstimulated cells has been subtracted.

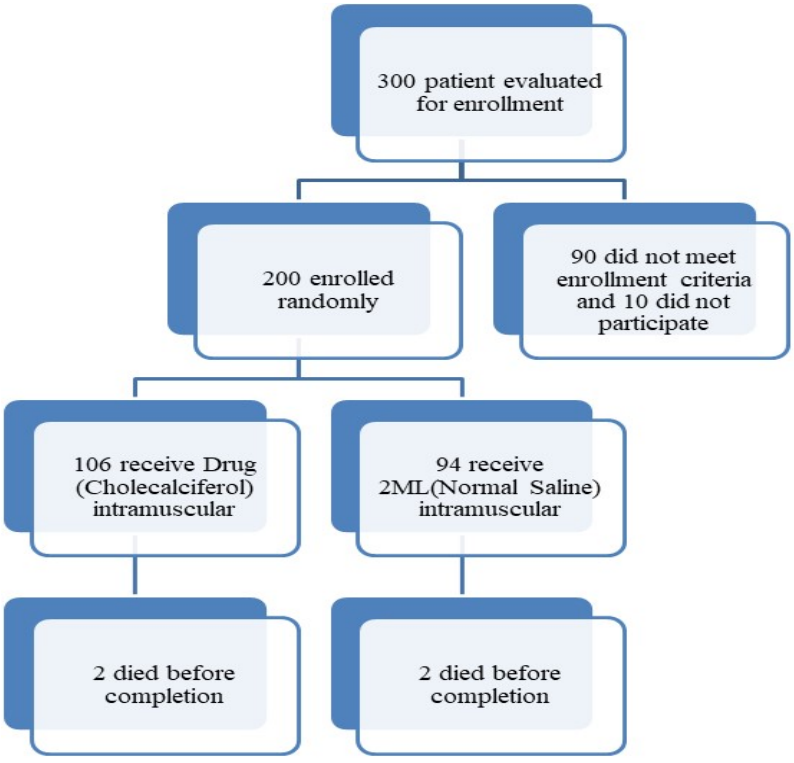

Fig. 1: Research Flowchart. The experimental drug (cholecalciferol) and the placebo (normal saline) were equal in color and volume.

hydroxyvitamin D varied. The participants were splitted to see if there was a correlation among plasma 25 hydroxyvitamin D amounts with the cytokines reactions in the placebo group. The participants were divided into those with optimum, inadequate, or insufficient 25(OH)D levels.

Supplementation increased in *Mycobacterium tuberculosis* produced interferon-gamma (IFN-Γ) responses in patients with low 25-hydroxy vitamin D levels. Following that, the mycobacterial antigen-restorative IFN-γ retaliation were measured before and after 4 month of medication in each the placebo and

treatment group of the research. MTBs-induced Interferon-gamma (IFN-Γ) levels in the 25-(OH) vitamin D treatment group were higher after treatment p=0.33. Although, there was no disctinction in Mycobacterium tuberculosis-producing Interferon-gamma (IFN-γ) levels among 0 and 4 months in the placebo group. When ESAT6-stimulated Interferon-gamma (IFN-Γ) reactions were evaluated, there was no change among zero and end of 4th month results in the placebo or treatment groups.

Furthermore, the elements that might be influencing the rise in *Mycobacterium tuberculosis* induce Interferon-gamma (IFN-Γ) reactions were checked in the treatment class after therapy and were subsequently examined through the data in sub-classifying individuals based on illness seriousness and initial serum 25-(OH) D levels.

In individuals with more severe TB, treatment boosts the IFN-Γresponses generated by *Mycobacterium tuberculosis* sonicate antigen. After 4-month of anti-tuberculous therapy, *Mycobacterium tuberculosis* induced Interferon-responses were considerably higher among the group of III cases illness in each the placebo p -0.002 and 25-(OH) vitamin D therapy groups. No similar post-therapy improve was observed in study participants' ESAT6m produce Interferon reactions. Supplementing patients with *M. tuberculosis* sonicate increases Interferon-gamma (IFN-Γ) production reactions in people who have a low 25 hydroxyvitamin D value examined participants in both research arms who were divided into those with optimal, insufficient and inadequate 25-(OH) D value to examine the impact of 25-(OH) vitamin D supplements in convalescent with various plasma 25-(OH) vitamin D value.

DISCUSSION

It was discovered that the therapeutic amounts of vitamin D supplements given to those with active tuberculosis twice a month in the form of vitamin D3, led to proportionately higher pounds and quicker radiological recovery than placebo.

Recent theories inform that vitamin D expedites healing of host inflammation, which explained why vitamin D levels have increased. Vitamin D supplementation increases the production of the immune response mediated by cells against M. tuberculosis elicited by mycobacterial antigens in people with low 25-(OH) vitamin D value.

It is well understood that vitamin D (25-hydroxyvitamin D) plays an important immune-modulating role in tuberculosis. While a lack of 25-OH vitamin D raises sensitivity to tuberculosis (Gibney *et al*., 2008, Panda *et al* 2019). Binding of 1, 25 hydroxy vitamin D3 and its receptors induces cathelicidin-mediated mycobacterial killing (Liu *et al*., 2007, Liu *et al.,* 2006).

Interferon gamma (IFN-Γ) is a cytokine that promotes inflammation that is required for MTB resistance (Sudfeld *et al*., 2020). The T lymphocytes, killer cells from the body, macrophages in the airway and MTB infection all produce IFN-γ production and the generation of IFN--driven monokines which govern granuloma formation (Saunders *et al*., 2007). It has been found that those suffering from advanced TB have decreased IFN-γ responses. On twelve weeks of ATT for patients found to be categorized as vitamin D "deficient," it was detected that a substantial rise in Mycobacterium tuberculosis-produce interferon gamma (IFN-Γ) in responses for the individuals that took 25-hydroxy vitamin D.

This is the first vitamin D supplementation research which separated participants into groups based on their sickness severity as well as their baseline serum the vitamin D-25 value. Previous studies have shown that patients with tuberculosis have higher levels of interferon-gamma (IFN-γ) producing cells against ESAT6 and that these levels increase after treatment (Ulrichs *et al*., 2000). However, these studies did not distinguish between pre-treatment and post-treatment IFN-γ responses. A study by Coussens (Coussens *et al*., 2012) found that patient levels of IFN-γ producing cells against ESAT6 remained stable after anti-tubercular treatment (ATT). This suggests that IFN-γ responses may not be a good indicator of treatment response. Mycobacterium tuberculosis and virulent *M. bovis* have ESAT6 and CFP10 encoded by RD1, whereas *M. bovis* BCG and the environment. Mycobacteria do not (Gurjav *et al*., 2019). Due to exposure to ambient mycobacterial and Mycobacterium tuberculosis, IGRAs have varied sensitivity, which leads to T cell IFN-γ responses (Mustafa *et al*., 2021). M. tuberculosis whole sonicate (MTBs) can stimulate T cells, macrophages and other cells and has been used to determine the severity of tuberculosis. Patients with more severe disease have lower IFN-γ responses (Hasan *et al*., 2012). The study found that patients in the group with the most severe disease (group 3) had significantly higher IFN-γ responses to MTBs after 4 months of ATT. This suggests that IFN-γ responses may be a better indicator of host defense in patients with severe tuberculosis. The study found that vitamin D supplements may help boost the host defense system in patients with low 25-(OH) (20ng/mL) at the start of treatment. This was evident by the increased production of interferon-gamma (IFN-γ) following 12 weeks of anti-tuberculosis treatment. However, no improvement was seen in patients with insufficient vitamin D (25-hydroxyvitamin D) levels at the start of treatment.

The study also found that vitamin D supplements may help improve clinical condition, as evidenced by weight

gain. Weight gain is commonly studied in outpatients as an indicator of clinical progress in tuberculosis (Ahmad *et al*., 2021). The study's findings are consistent with previous reports of the positive impact of vitamin D for TB patients. In 1848, it was reported that cod liver oil helped TB patients recover from their illness, gain weight and reduce their risk of death. Martineau found that a oral unit dose of 2.5 milligrams (100,000 IU) of the supplement ergocalciferol significantly delayed mycobacterial productivity (Martineau *et al*., 2007).

In a 6-week study of 67 Indonesian patients, 420,000 IU of vitamin D dramatically reduced pulmonary tuberculosis individuals' sputum conversion rates (Nursyam *et al*., 2006). Furthermore, supplementing with 100,000 IUs of 25-hydroxyvitamin D3 enhanced sputum conversion rates in individuals with Taq1 25-hydroxyvitamin D receptor polymorphism, probably due to its broader effects on muscle, vascular and homeostasis (Martineau *et al*., 2011, Moreira *et al*., 2009, Eftekhari *et al*., 2011).

After 12 weeks of treatment, the vitamin D treatment group had less ailment than the placebo group, which is consistent with a small Egyptian trial that found higher clinical and radiographic recovery after 1000 international units of vitamin D supplementation orally (Cervantes *et al*., 2019), as well as a reported case of an African-American patient with resistant, drug-sensitive TB who ameliorate after receiving 1,200,000 International Units of ergoclaciferol (Yamshchikov *et al*., 2009). Martineau and Wejse discovered no change in clinical outcomes or death in 146 pulmonic tuberculosis patients in London and Guinea-Bissau with 10 mg of 25-hydroxyvitamin D3 and 300,000 international units of cholecalciferol or placebo. Changes might be caused by VDR polymorphisms, doses, or initial blood levels (Martineau *et al*., 2011, Wejse *et al*., 2009).

The study also found that vitamin D supplementation was relatively safe, even in patients who were not deficient. Only 2 participants in the group receiving vitamin D died in the study and one of these deaths happened during the first two weeks with anti-tuberculous treatments due to respiratory failure.

The study's limitations include the inability to monitor all patients at the completion of their medical care (6 months) and the lack of data on food intake. However, the study groups were well-matched at enrollment and included people from a hetrogeneity of socio-economic and cultural backgrounds, which might have reduced the likelihood that one group has better nutritional intake than the other.

CONCLUSION

In conclusion, this study demonstrates that persons that are 25-hydroxyvitamin D deprived had a better clinical reaction to high dosage vitamin D therapy and radiographic recovery, as well as improved host immunological responses.

REFERENCES

Ahmad S, Arora S, Khan S, Mohsin M, Mohan A, Manda K and Syed MA (2021). Vitamin D and its therapeutic relevance in pulmonary diseases. *J. Nutr. Biochem.,* **90**: 108571.

Ayelign B, Workneh M, Molla MD and Dessie G (2020). Role of Vitamin-D supplementation in TB/HIV co-infected patients. *Infect. Drug Resist.,* **13**: 111-118.

Cervantes JL, Oak E, Garcia J, Liu H, Lorenzini PA, Batra D, Chhabra A, Salazar JC and Roca X (2019). Vitamin D modulates human macrophage response to Mycobacterium tuberculosis DNA. *Tuberculosis*, **116**: S131-S137.

Coussens AK, Wilkinson RJ, Hanifa Y, Nikolayevskyy V, Elkington PT, Islam K, Timms PM, Venton TR, Bothamley GH, Packe GE and Darmalingam M (2012). Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. *Proc. Natl. Acad. Sci.,* **109**(38): 15449-15454.

Eftekhari MH, Akbarzadeh M, Dabbaghmanesh MH and Hasanzadeh J (2011). Impact of treatment with oral calcitriol on glucose indices in type 2 diabetes mellitus patients. *Asia Pac. J. Clin.,* **20**(4): 521-526.

Ganmaa D, Uyanga B, Zhou X, Gantsetseg G, Delgerekh B, Enkhmaa D, Khulan D, Ariunzaya S, Sumiya E, Bolortuya B and Yanjmaa J (2020). Vitamin D supplements for prevention of tuberculosis infection and disease. *N. Engl. J. Med.,* **383**(4): 359-368.

Gibney KB, MacGregor L, Leder K, Torresi J, Marshall C, Ebeling PR and Biggs BA (2008). Vitamin D deficiency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. *Clin. Infect. Dis,* **46**(3): 443-446.

Gurjav U, Ankhbat M, Ganbaatar G, Batjarga K, Ochirbat B, Baigal D, Jargalsaikhan B, Munkhjargal O, Bolormaa S, Yansanjav N and Luvsanyandan N (2019). Vitamin D deficiency is associated with tuberculosis infection among household contacts in Ulaanbaatar, Mongolia. *Int. J. Tuberc. Lung Dis.,* **23**(8): 919-923.

Hasan Z, Rao N, Salahuddin N, Islam M, Ashraf M, Rottenberg ME and Hussain R (2012). Mycobacterium tuberculosis Sonicate–Induced IFNγ, CXCL10 and IL10 can Differentiate Severity in Tuberculosis. *Scand. J. Immunol.,* **75**(2): 220-226.

Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C and Kamen DL (2006). Toll-like receptor triggering of a vitamin

D-mediated human antimicrobial response. *Science*, **311**(5768): 1770-1773.

Liu PT, Stenger S, Tang DH and Modlin RL (2007). Cutting edge: Vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. *J. Immunol.,* **179**(4): 2060-2063.

Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, Packe GE, Moore-Gillon JC, Darmalingam M, Davidson RN and Milburn HJ (2011). High-dose vitamin D3 during intensive-phase antimicrobial treatment of pulmonary tuberculosis: A double-blind randomised controlled trial. *The Lancet*, **377**(9761): 242-250.

Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, Packe GE, Davidson RN, Eldridge SM, Maunsell ZJ and Rainbow SJ (2007). A single dose of vitamin D enhances immunity to mycobacteria. *Am. J. Respir. Crit. Care Med.,* **176**(2): 208-213.

Moreira-Pfrimer LD, Pedrosa MA, Teixeira L and Lazaretti-Castro M (2009). Treatment of vitamin D deficiency increases lower limb muscle strength in institutionalized older people independently of regular physical activity: A randomized double-blind controlled trial. *Ann. Nutr. Metab.,* **54**(4): 291-300.

Mustafa AS (2021). Immunological characterization of proteins expressed by genes located in *Mycobacterium tuberculosis*-specific genomic regions encoding the ESAT6-like proteins. *Vaccines*, **9**(1): 27.

Nnoaham KE and Clarke A (2008). Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. *Int. J. Epidemiol.,* **37**(1): 113-119.

Nursyam EW, Amin Z and Rumende CM (2006). The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. *Acta Med. Indones*., **38**(1): 3-5.

Panda S, Tiwari A, Luthra K, Sharma SK and Singh A (2019). Status of vitamin D and the associated host factors in pulmonary tuberculosis patients and their household contacts: A cross sectional study. *J. Steroid Biochem. Mol. Biol.,* **193**: 105419.

Saunders BM and Britton WJ (2007). Life and death in the granuloma: Immunopathology of tuberculosis. *Immunol. Cell Biol.,* **85**(2): 103-111.

Sudfeld CR, Mugusi F, Muhihi A, Aboud S, Nagu TJ, Ulenga N, Hong B, Wang M and Fawzi WW (2020). Efficacy of vitamin D3 supplementation for the prevention of pulmonary tuberculosis and mortality in HIV: A randomised, double-blind, placebo-controlled trial. *The Lancet HIV*, **7**(7): e463-e471.

Ulrichs T, Anding P, Kaufmann SHE and Munk ME (2000). Numbers of IFN-γ-producing cells against ESAT-6 increase in tuberculosis patients during chemotherapy. *Int. J. Tuberc. Lung Dis.,* **4**(12): 1181-1183.

Wejse C, Gomes VF, Rabna P, Gustafson P, Aaby P, Lisse IM, Andersen PL, Glerup H and Sodemann M (2009). Vitamin D as supplementary treatment for tuberculosis: A double-blind, randomized, placebo-controlled trial. *Am. J. Respir. Crit. Care Med,* **179**(9): 843-850.

Wejse C, Gustafson P, Nielsen J, Gomes VF, Aaby P, Andersen PL and Sodemann M (2008). TBscore: Signs and symptoms from tuberculosis patients in a low-resource setting have predictive value and may be used to assess clinical course. *Scand. J. Infect. Dis.,* **40**(2): 111-120.

Xiong K, Wang J, Zhang J, Hao H, Wang Q, Cai J and Ma A (2020). Association of dietary micronutrient intake with pulmonary tuberculosis treatment failure rate: ACohort study. *Nutrients*, **12**(9): 2491.

Yamshchikov AV, Oladele A, Leonard Jr MK, Blumberg HM, Ziegler TR and Tangpricha V (2009). Vitamin D as adjunctive therapy in refractory pulmonary tuberculosis: A case report. *South. Med. J*., **102**(6): 649.