


# Effect of DOTs Treatment on Vitamin D level in Pulmonary Tuberculosis Patients

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**Abstract**
**Background:** Pulmonary Tuberculosis (PTB) is a chronic infectious disease that caused by *Mycobacterium tuberculosis* which primarily affect lungs but can also involve other organs. PTB spreads through air with symptoms like chronic cough, chest pain, fever, weight loss, and night sweats and TB remains a major cause of death, especially in developing countries. Vitamin D plays a vital role in immunity, and its deficiency lead to increased susceptibility to PTB and drugs like Anti-TB drugs like rifampicin can worsen the Vitamin D deficiency by altering its metabolism. Despite abundant sunlight, India faces widespread Vitamin D deficiency (70–100%) due to poor diet, low sun exposure, and lifestyle factors.
**Aim:** To assess and compare circulatory Vit-D status of newly diagnosed PTB patients before and after DOTs.
**Method:** This study was conducted on 75 newly diagnosed sputum smear-positive PTB patients aged 18–60 years from the Department of Medicine, Dr. KKBM Subharti Hospital Government Dehradun. All biochemical investigations were carried out in the Clinical Biochemistry Laboratory at Dr. KKBM Subharti Hospital, Jhajra Campus, Dehradun. The study period was from September to December 2023 and followed a non-randomized trial design. Both male and female patients were included.
**Results:** The study found that after 3 months of DOTs therapy, Vitamin D levels significantly decreased (P value=0.001) while urea and creatinine levels increased, suggesting that anti-TB drugs, particularly rifampicin, may impair Vitamin D metabolism and kidney function.
**Conclusion:** Present study shows that Vitamin D deficiency is highly prevalent in PTB patients and is linked to increased disease severity. DOTs therapy may further lower Vitamin D levels, highlighting the need for regular monitoring. Vitamin D supplementation should be considered to improve treatment outcomes, and future studies are needed to determine its optimal dose and duration in TB management.

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**Keywords:** Pulmonary Tuberculosis, Vitamin D, Urea, Creatnine, DOTs

## Introduction

Pulmonary Tuberculosis is a chronic airborne infectious disease caused by *Mycobacterium tuberculosis*. Although the lungs are the primary site of infection, TB can also affect other organs such as brain, kidneys, bones, intestines, and spine except nail and hair and also vary depending on the affected organ, but in pulmonary TB, common signs include chronic cough, chest pain, haemoptysis, fatigue, fever, weight loss, and night sweats [1].

In developing countries, TB accounts for nearly 7% of all deaths and remains the leading cause of mortality from a single infectious agent among adults [2]. The World Health Organization (WHO) was the first to declare TB a global health emergency. In 2007, WHO reported an 9.27 million new cases, 13.7 million prevalent cases, and 1.77 million deaths, with Asia and Africa contributing to about 86% of the total global burden [3].

The global male-to-female ratio of TB incidence is approximately 0.67:0.47 [4]. The disease is closely linked with poverty, as overcrowded living conditions, inadequate ventilation, poor hygiene, and low educational status increase the risk of transmission [5] and also Malnutrition further weakens immune defences, while poor living conditions promote the spread of infection [6].

The drug resistance in PTB is critical for controlling the disease. The emergence of MDR-TB and extensively XDR-TB strains poses a major global health threat [7]. According to WHO, about half a million MDR-TB cases occur each year worldwide. In Bangladesh, MDR-TB prevalence is around 3.5% in new cases and 20% in previously treated patients, with a mortality rate of 50–60% [8].

Factors that are contributing to the development MDR-TB include irregular treatment, poor adherence to therapy, unsupervised medication intake, substandard drug quality, interrupted drug supply, and weak national control programs [9]. Regular MDR surveillance and implementation of the DOTs-Plus program are essential for effective disease management. Strong collaboration between government, private, and non-governmental sectors is necessary for successful TB control. Diagnosing the paediatric TB remains challenging for clinician as children are often unable to produce sputum; hence, diagnosis depends on clinical evaluation, scoring systems, and advanced techniques such as culture and nucleic acid amplification tests [10].

The deficiency of Vit-D recognized as a potential risk factor for TB susceptibility and progression [11]. Vit-D (calciferol), a fat-soluble vitamin, plays a key role in calcium balance and immune regulation. It metabolized in liver to 25(OH)D and further converted in kidneys to its active form which is $1,25(OH)_2D$. This active form promotes the synthesis of cathelicidin, an antimicrobial peptide that restricts *M. tuberculosis* growth inside macrophages. Deficiency of Vit-D is therefore linked with increased vulnerability to PTB [12].

The DOTs remain the basic treatment for TB. However, anti-TB drugs such as rifampicin activate the pregnane X receptor, which induces CYP3A4 enzyme activity which enhance Vit-D catabolism and reducing its active form [13]. whereas, hepatotoxic effects of drugs like isoniazid, pyrazinamide, and rifampicin further impair Vit-D metabolism and calcium balance. Additional factors such as malnutrition, alcohol consumption, HIV infection, and liver dysfunction contribute to Vit-D depletion in PTB patients [14].

Vit-D deficiency is a widespread health problem, affecting nearly one billion individuals worldwide, with serum 25(OH)D levels below 20 ng/mL considered deficient. Despite its sunny climate, India reports a high prevalence (70–100%) of Vit-D deficiency. This widespread deficiency is attributed to inadequate dietary intake, vegetarian diets, limited sun exposure, air pollution, and indoor lifestyles. In rural populations, protein malnutrition and lack of animal-derived foods are major contributors, while in urban settings, limited sunlight exposure and sedentary habits worsen the issue. Therefore, improving dietary practices and adequate Vit-D supplementation are vital for TB prevention and overall health enhancement [15].

## Materials and Methods

Present study consists of patients with PTB from the Department of Medicine at Dr. KKBM Subharti Hospital, this study was conducted from July 2023 to June 2024. All biochemical investigations were performed in the clinical biochemistry laboratory at Dr. KKBM Subharti Hospital, Jhajra Campus Dehradun.

## Objectives of the Study

1. To measure the levels of Vit-D in pulmonary tuberculosis patients before and after 3 months of DOTs treatment
2. To assess the correlation between Vit-D levels and the effectiveness of DOTs therapy in these patients.

## Inclusion Criteria

We recruited male and female patients aged from 18-60 with newly diagnosed sputum smear-positive pulmonary Tuberculosis were included in the study group.

## Exclusion Criteria

Cases of multidrug resistance, extrapulmonary Pulmonary Tuberculosis, malignant tumours, diabetes mellitus, renal failure, patients with HIV infection, and patients who are not giving their consent will be not included in this study.

## Sample Collection

Since the purpose of present study was to know the effect of DOTs on Vit-D Deficiency status in a PTB patient. Serum samples were collected between 7 and 9 AM after at least 12 h night fasting. In each patient, at least two Vit-D serum samples were determined and this included a sample before the onset of anti TB treatment and another one taken 3 months after treatment onset if they respond to treatment.

## Statistical Analysis

Data were analyzed by using appropriate statistical methods by computing percentage, mean, standard deviation, p test and Spearman rank correlation analysis to assess the correlation between Vit-D levels and the effectiveness of DOTs therapy. Wherever necessary, the results were depicted in the form of graphical and tabular format. All the statistical tests will be performed using SPSS software version 20.0.

## Result

To evaluate the effect of standard DOTs therapy on Vit-D status in PTB patients, baseline and post-treatment biochemical profiles were analyzed in 75 newly diagnosed cases. The findings demonstrate significant post-treatment shifts across serum Vit-D, liver, and renal parameters.

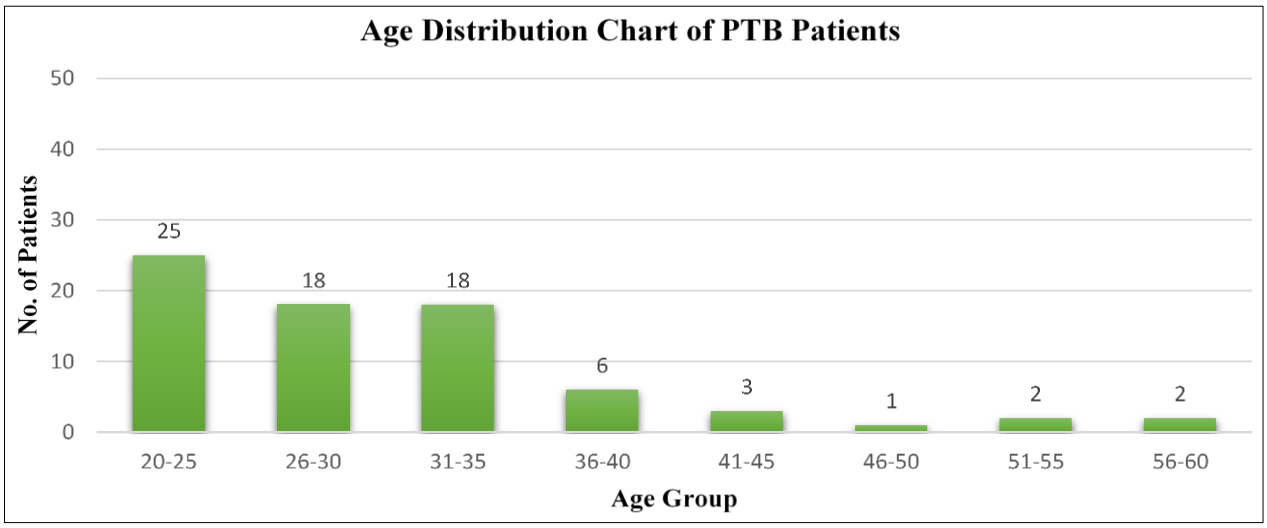



**Fig 1:** Age Distribution Chart of Pulmonary Tuberculosis Patient

Age distribution of the study participants is presented in Figure: 1. The majority of PTB patients belonged to the 20–25 years age group (25 patients, 32.5%), followed by the 26–30 years and 31–35 years age groups, each comprising 18 patients (23.4%). Six patients (7.8%) were in the 36–40 years age group, while 3 patients (3.9%) belonged to the 41–45 years age group. Only 1 patient (1.3%) was in the 46–50 years age group. The 51–55 years and 56–60 years age groups each included 2 patients (2.6%). These findings indicate that PTB was most prevalent among young adults, particularly those aged 20–35 years.

**Table 1:** Biochemical parameters of pulmonary tuberculosis patients before and after DOTs therapy.

| Parameter | Before DOTs (Mean ± SD) | After DOTs (Mean ± SD) | Mean Difference | p value |
|---|---|---|---|---|
| Vit-D | 22.04 ± 3.34 | 14.06 ± 2.88 | 7.98 | <0.001 |
| ALP | 132.71 ± 11.22 | 126.98 ± 11.27 | 5.73 | <0.001 |
| AST | 45.37 ± 5.07 | 40.56 ± 4.28 | 4.81 | <0.001 |
| ALT | 50.24 ± 5.69 | 48.14 ± 5.58 | 2.1 | <0.001 |
| Total Protein | 6.98 ± 0.69 | 6.51 ± 0.64 | 0.47 | <0.001 |
| Urea | 36.61 ± 4.28 | 41.94 ± 3.69 | -5.33 | <0.001 |
| Creatinine | 1.14 ± 0.17 | 1.40 ± 0.14 | -0.26 | <0.001 |

Table 1 shows biochemical parameters of PTB patients before and after DOTs therapy. A significant reduction was observed in the mean serum Vit-D level, which decreased from 22.04 ± 3.34 ng/mL before treatment to 14.06 ± 2.88 ng/mL after DOTs therapy (mean difference = 7.98 ng/mL, $p < 0.001$). Similarly, significant decreases were noted in serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total protein levels following treatment (all $p < 0.001$). In contrast, mean serum urea and creatinine levels increased after DOTs therapy, with urea rising from 36.61 ± 4.28 mg/dL to 41.94 ± 3.69 mg/dL and creatinine increasing from 1.14 ± 0.17 mg/dL to 1.40 ± 0.14 mg/dL. These changes were also statistically significant ($p < 0.001$).

In Table2, Gender-wise comparison of serum Vit-D levels showed that the mean Vit-D concentration decreased following DOTs therapy in both male and female PTB patients. In males (n = 45), the mean Vit-D level declined from 16.92 ng/mL before treatment to 11.68 ng/mL after DOTs therapy. In females (n = 32), the corresponding values decreased from 16.29 ng/mL to 10.28 ng/mL. Overall, female patients had slightly lower mean Vit-D levels than male patients at both time points.

**Table 2:** Gender-Wise Comparison of Serum Vitamin D Levels

| Row Labels | Vit D before DOTs (mean) | Vit D after DOTs (mean) |
|---|---|---|
| Female (n=32) | 16.29 | 10.28 |
| Male (n=45) | 16.92 | 11.68 |

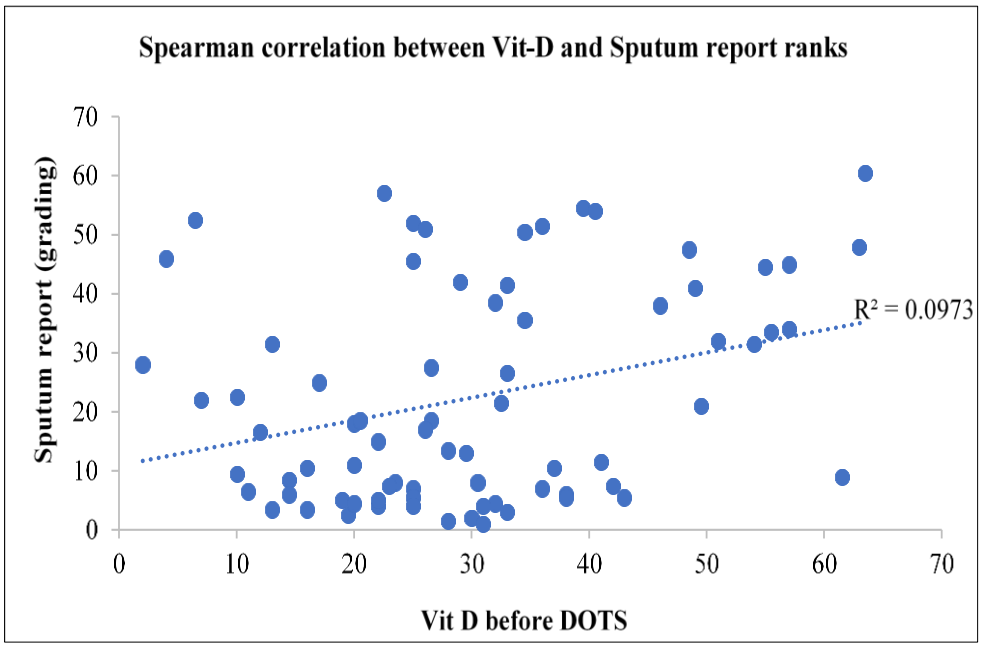



**Fig 2:** Spearman rank correlation scatter plot showing the relationship between baseline Vitamin D levels (ranks) and Sputum report grading (ranks) before DOTs therapy initiation (R2=0.0973).

To assess the correlation between Vit-D levels and the effectiveness of DOTs therapy in these patients, the relationship between Vit-D levels and Sputum report grading prior to treatment, a Spearman rank correlation analysis was performed. This yielded a coefficient determination of R2=0.0973, corresponding to a Spearman correlation coefficient of rs≈0.312. This indicates moderate positive monotonic relationship between Vit-D levels before DOTs treatment and Sputum report grades. Although higher level of Vit-D ranks showed align with higher Sputum report ranks, the low R2 value demonstrates substantial dispersion among individual patient data points, suggesting that baseline Vit-D alone does not strongly predict sputum grading status.(Figure:2)

**Discussion**

The present study conducted on newly diagnosed PTB patients before and after taking DOTs therapy, showed that the prevalence of Vit-D Deficiency was significantly higher in patients who had undergone three months of DOTs treatment.

The Mean of Vit-D level before DOTs is 22.04 and the Mean of Vit-D after DOTs is 14.06 which is statistically significant, Vit-D plays an important role in macrophage activation and restriction of mycobacterial growth. Several biological studies to detect effects of Vit-D on immune system of the body show that Vit-D has a definitive role in suppression of proliferation of *Mycobacterium* TB and generalized inflammatory response produced secondary to it. [69,70] Similarly, on triggering of toll-like receptors by molecules of the tubercle bacillus, the production of microbe-killing cathelicidin is impaired in the absence of adequate serum Vit-D [16]

It was observed that 87% of the study subjects had hypovitaminosis D (less than 30 ng/ml) at baseline, whereas Friis H *et al*., with a study group of 655 patients diagnosed with PTB reported hypovitaminosis D in 41.2% patients [17]. This study confirms previous studies that, patients presenting with active TB have significantly lower mean concentrations of serum 25- hydroxycholecalciferol [73]. Goswami R *et al*., reviewed and reported that Vit -D deficiency increases the susceptibility of individuals infected with TB, turning it into an infective disease [18], which is supported by Ralph AP *et al*., (19). Vit-D Deficiency has been implicated as a risk factor for the development of TB, though a recent supplementation trial showed no clinical impact [20].

According to this study the level of urea and creatinine raised after DOTs therapy, The Mean of urea before DOTs is 36.61 and the Mean of Urea after DOTs is 41.94. Urea and Creatinine levels rose after DOTs from their baseline values in both the groups which may be caused by the toxic effect of rifampicin on the kidneys, reported by some studies.

The Study by Chandra *et al*. which evaluated high-dose adjunctive Vit-D3 (100,000 IU × 4 doses) alongside standard ATT. Epidemiologically, present study demonstrated a heavy concentration of active PTB in young adults, with 32.5% of cases in the 20–25 age group and 55.9% falling between 20 and 35 years. Present study has similar finding, like Chandra *et al*. which suggest that reduction in CRP & ALP indicates that inflammation throughout the body is decreasing. As the MTB bacteria are eliminated by treatment, the granulomatous inflammation caused by tuberculosis subsides, and the liver's acute-phase inflammatory response also returns toward normal. Therefore, the falling CRP and ALP levels are evidence of a good response to anti-tubercular therapy. [21]

In Present study Spearman rank correlation revealed a moderate positive monotonic association between Vit-D ranks and before DOTs treatment, sputum report grades (RS 0.312, R2 = 0.0973). While the study by Chandra *et al*., reveled the low $R^2$ value (0.10) indicates that serum Vit-D is a moderate independent predictor of initial sputum bacillary load. However, Vit-D supplementation may still be beneficial

because it strengthens the immune system by stimulating cathelicidin production, which can enhance the clearance of *Mycobacterium tuberculosis* and improve treatment outcomes. Because baseline Vit-D alone does not strongly predict disease severity, future prospective studies should evaluate whether Vit-D supplementation during treatment can improve outcomes. Vit-D enhances the immune response by increasing the production of cathelicidin, an antimicrobial peptide that helps immune cells kill *Mycobacterium tuberculosis*. This may promote more effective bacterial clearance and improve recovery. [22]

The present study findings align closely with the observational trial by Esma Seda et.al, which investigated the relationship between serum Vit-D levels, initial disease severity, and clinical progression during ATT., similarly reported a high prevalence of baseline hypovitaminosis D among active PTB patients, observing an inverse relationship between serum 25(OH)D levels and clinical/radiological severity at presentation. Furthermore, Karaca documented a progressive decline or failure of endogenously low Vit D levels to normalize during standard ATT without exogenous supplementation, mirroring our observed drop post-DOTs. Both studies collectively demonstrate that low Vit-D status acts as an underlying immunological vulnerability factor which associated with active disease, standard rifampicin-containing DOTs regimens further deplete host Vit D reserves due to enzymatic induction (CYP24A1). Which means that giving vit-D supplements along with standard anti-tuberculosis treatment may help prevent vit-D deficiency after treatment and support the immune system in fighting and clearing *Mycobacterium tuberculosis*. Vit-D can enhance immune functions, including the production of antimicrobial peptides such as cathelicidin, which may aid bacterial clearance. [22] and the study by Hassen *et al*., suggested malnutrition as a risk factor for anti TB drugs induced hepatotoxicity, which leads to depletion of glutathione stores, rendering patients more vulnerable to oxidative injuries, and lowers the pace at which the liver metabolizes drugs. This could be a reason for impaired 25 hydroxylation of Vit-D in the liver and thereby decrease in mean Vit D after DOTs [23]

## Conclusion

In present study Vit-D Deficiency is highly prevalent among the patients with Pulmonary Tuberculosis compared to healthy individuals. Hypovitaminosis Vit-D is associated with more severe clinical symptoms in patients with Pulmonary Tuberculosis. The reduced levels of Vit-D in circulation can be linked to increase risk of Pulmonary Tuberculosis. As a result, Vit-D level in Tb patients necessary to control, as Vit-D Deficiency may develop without symptoms and intensify once TB (DOTs Treatment) starts. Also, there is some limitation, the study is Conducted at a single hospital in Dehradun, which may restrict generalizability to broader & demographically, diverse populations with sample size (n = 75) may limit the statistical power for detailed sub-group analyses (e.g., sex or age interactions) and also duration of 4-month study period prevent long term follow up to observe after treatment Vit-D recovery and also Unmeasured Confounders like Seasonal variations, daily sunlight exposure, dietary intake, and baseline BMI were not strictly controlled. However, there is view great need for comprehensive studies in TB patients.

The findings of this study highlight the importance of monitoring vit-D levels during DOTs therapy in PTB patients, emphasizing the need for timely nutritional assessment and appropriate supplementation to support better treatment outcomes.

## Abbreviations

$1,25(OH)_2D$ = 1,25-dihydroxyvitamin D, 25(OH)D =25-hydroxyvitamin, XDR-TB =extensively drug-resistant, MDR-TB = multidrug-resistant, CYP3A4= cytochrome P450 3A4

## Declarations

Ethics approval and consent to participate: The Institutional ethical committee approved the research. (GBCM/IEC/2023/06-12) Conflict of interest: There is no conflicts of interest

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