



A Study of Serum Vitamin D Levels in Newly Diagnosed Pulmonary Tuberculosis

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Abstract

Background: Vitamin D was used in tuberculosis management in the pre-antibiotic era. Vitamin D deficiency has been implicated in activation of TB. A meta-analysis published has shown the association of vitamin D deficiency with two-fold higher risk of active TB and certain studies had also demonstrated that vitamin D supplementation in TB patients is associated with faster clinical and radiological improvement and sputum smear conversion.

Aim: To assess the serum vitamin D levels among the patients with pulmonary tuberculosis and its association with the severity of the disease.

Methodology: A cross-sectional study was conducted for a period of one year in Government medical college hospital, Salem. A total of 300 patients with pulmonary tuberculosis confirmed by sputum AFB were included in the study. The vitamin D status of study participants was interpreted based on the serum 25-(OH) Vitamin D concentration following the manufacturer's instructions as deficient (<20 ng/ml), insufficient (20–29 ng/ml), sufficient (30–100 ng/ml) and potential toxicity (>100 ng/ml).

Results: Vitamin D levels were found to be normal in 34% of the subjects and it was insufficient in 11% and found to be deficit in 55% of the study subjects. Multi-logistic regression analysis was applied to identify the factors influencing deficiency of vitamin D among the patients with tuberculosis among males and females. It was proved that age more than 30 years, female gender, sputum results showing 1+ or more, BMI <18.5 and patients with various co-morbidities found to have statistical significant association with reduction in the vitamin D levels among patients with tuberculosis.

Conclusion: Considering the high prevalence of vitamin D deficiency, the present study implicates that further follow-up studies are warranted to determine whether vitamin D supplementation can have a role in the prevention and treatment of tuberculosis.

Keywords: vitamin D, pulmonary tuberculosis, prevalence, multi-logistic regression.

Introduction

Tuberculosis is a major communicable disease and a disease of poor. It's a worldwide health problem.

It is most commonly seen in developing countries due to poverty, malnutrition and economic recession. India accounts for one fourth of the

global TB burden. In 2015, an estimated 28 lakhs cases occurred and 4.8 lakhs people died due to TB. India has highest burden of both TB and MDR TB based on estimates reported in Global TB Report 2016. An estimated 1.3 lakhs incident multi-drug resistant TB patients emerge annually in India which includes 79000 MDR-TB Patients estimates among notified pulmonary cases. India bears second highest number of estimated HIV associated TB in the world. An estimated 1.1 lakh HIV associated TB occurred in 2015 and 37,000 estimated number of patients died among them.¹

The host susceptibility to TB infection depends on a complex interaction between host, bacterial as well as environmental factors, such as poverty, malnutrition, overcrowding, and exposure to other pathogens.^{2,3} In addition to these factors, genetic factors, such as polymorphisms in the interleukin-1 (IL-1) gene cluster and mannose-binding lectin have been shown to influence host susceptibility to TB.⁴ Cell-mediated immunity is important for host protection against mycobacteria infection.⁵

Vitamin D is a fat soluble vitamin. Several studies showed that vitamin D reduces the risk of about 17 types of cancers, auto immune diseases like asthma, multiple sclerosis, rheumatoid arthritis, bacterial infections like dental carries, periodontitis, septicemia, viral infections like Epstein-Barr virus, respiratory tract infections, cardio vascular diseases like coronary heart disease, hypertension, diabetes, hypercholesterolemia, neurological illness like stroke, Alzheimer's dementia, depression, osteoporosis thereby it reduces the overall morbidity, mortality along with longer life expectancy.⁶

Vitamin D plays an important role in activation of 1 α -hydroxylase enzyme to convert 25(OH) D to its active form [1, 25 (OH) 2D] that leads to expression of cathelicidin, a microbicidal peptide for Mycobacterium tuberculosis. Stimulation of Toll-like receptors (TLRs) on monocytes and macrophages by mycobacterium tuberculosis antigens leads to an up-regulation of Vitamin D receptors. Binding of 1, 25(OH) 2D3 activates

vitamin D receptors and induces cathelicidin-mediated killing of Mycobacteria.

Interferon gamma is a pro inflammatory cytokine, which plays a critical role in resistance to mycobacterium tuberculosis infection.⁷ Infection with mycobacterium tuberculosis induces T lymphocytes, natural killer cells and alveolar macrophages to express interferon gamma and induces interferon driven monokines that regulate granuloma formation. IFN-g responses have been shown to be depressed in patients with advanced forms of tuberculosis. Significant improvement in MTBs induced IFN-g responses after 12 weeks of Anti tuberculosis treatment in vitamin D 'Deficient' patients who received 25-hydroxyvitamin.⁸

Vitamin D was used in tuberculosis management in the pre-antibiotic era. Vitamin D deficiency has been implicated in activation of TB. A meta-analysis published has shown the association of vitamin D deficiency with two-fold higher risk of active TB.⁹ Marineau et al showed that a single dose of 0.25 mg oral vitamin D significantly enhanced the immunity among TB contacts for six weeks.¹⁰ Certain studies demonstrated that vitamin D supplementation in TB patients is associated with faster clinical and radiological improvement and sputum smear conversion.¹¹ Lower vitamin D level was reported to be associated with a higher risk of developing active pulmonary tuberculosis.¹² The role of vitamin D in modifying the treatment course of pulmonary tuberculosis is still a matter of debate.^{13,14}

Aim

To assess the serum vitamin D levels among the patients with pulmonary tuberculosis and its association with the severity of the disease.

Methodology

A cross-sectional study was conducted for a period of one year in Government medical college hospital, Salem. The study was started after getting the clearance from the institutional ethical committee and the informed consent was obtained

from all the patients recruited for the study. A total of 300 patients were included in the study. Patients with chronic liver disease, kidney disease, patients on steroid therapy or on epileptic drugs, patients with associated chronic co-morbidity like diabetes, hypertension and patients with HIV infection were excluded from the study. A detailed history related to socio-demographic characteristics, symptoms and personal history were elicited from the patient and a complete general physical examination and systemic examination was conducted and the abnormalities were noted.

Patient's sputum was sent for AFB examination and it was done by ziehl-neelson acid fast stain using standard microscopic examination. Routine blood examinations for monitoring blood sugar, renal function, liver function and ELISA were done to rule out HIV. Serum samples were separated by centrifugation and frozen immediately at -20°C . Serum 25 OH Vitamin D levels were measured using a VIDAS 25 OH Vitamin D Total testing kits (Biomerieux, Marcy l'Etoile, France) on mini VIDAS automated immunoassay platform. VIDAS 25 OH Vitamin D Total is a quantitative test using Enzyme Linked Fluorescent Assay (ELFA) technology. The vitamin D status of study participants was interpreted based on the serum 25-(OH) Vitamin D concentration following the manufacturer's instructions as deficient (<20 ng/ml), insufficient (20–29 ng/ml), sufficient (30–100 ng/ml) and potential toxicity (>100 ng/ml). The VIDAS 25-OH Vitamin D Total assay showed excellent performance with correlation of $r = 0.93$ compared with the reference standard liquid chromatography/mass spectrometry methods (LC-MS/MS).¹⁵

Data were entered and analysed using SPSS version 21. Chi-square test was used to assess the association between two categorical variables. Multiple logistic regression analysis was used to assess the factors influencing vitamin D deficiency among the tuberculosis patients considering p value $<.05$ to be statistically significant.

Results

The age wise distribution of the study subjects shows that majority of the subjects both in males and females were in the age group between 40 and 60 years and the mean age was 42.6 years and the male: female ratio was 1.27: 1 (table 1). Vitamin D levels was found to be normal in 34% of the subjects and it was insufficient (20-29 ng/ml) in 11% and found to be deficit (<20 ng/ml) in 55% of the study subjects (table 2). Grading of the sputum AFB shows that only 9% of the subjects had scanty sputum and majority of the subjects (43%) had sputum 3+ results and the remaining had sputum results as 1+ or 2+ (table 3). The BMI status of the study subjects shows that majority (69%) of them had BMI <18.5 and only 3% of the subjects were obese or overweight with a BMI of more than 30 (table 4). The prevalence of co-morbidity among the patients with tuberculosis was 30.3% with majority of them were having either diabetes or hypertension and the other less common co-morbid conditions were coronary artery disease, cerebrovascular disease and obesity (table 5). Multi-logistic regression analysis was applied to identify the factors influencing deficiency of vitamin D among the patients with tuberculosis among males and females. It was proved that age more than 30 years, female gender, sputum results showing 1+ or more, BMI <18.5 and patients with various co-morbidities found to have statistical significant association with reduction in the vitamin D levels among patients with tuberculosis (table 6).

Table 1: Age and sex wise distribution of the study subjects

Age group	Male	Female	Total	P value
<20	9 (5.3%)	2 (1.5%)	11 (3.6%)	0.418
20 – 30	18 (10.7%)	16 (12.1%)	34 (11.3%)	
31 – 40	30 (17.8%)	19 (14.3%)	49 (16.3%)	
41 – 50	42 (25%)	41 (31%)	83 (27.6%)	
51 – 60	41 (24.4%)	38 (28.7%)	79 (26.3%)	
>60	28 (16.6%)	16 (12.1%)	44 (14.6%)	
Total	168 (100%)	132 (100%)	300 (100%)	
Mean ± SD	41.6 ± 7.8	43.8 ± 8.2	42.6 ± 6.8	

P value derived by applying chi-square test

Table 2: Vitamin D levels among the study subjects

Vitamin D levels	Frequency	Percentage
Normal (30 -100ng/ml)	102	34%
Insufficient (20 – 29ng/ml)	33	11%
Deficit (<20ng/ml)	165	55%
Total	300	100%

Table 3: Distribution of the study subjects based on their sputum AFB results

Sputum AFB results	Frequency	Percentage
Scanty	24	9%
1+	75	25%
2+	72	24%
3+	129	43%
Total	300	100%

Table 4: BMI status of the study subjects

BMI	Frequency	Percentage
<18.5	207	69%
18.5 – 24.9	57	19%
25 – 29.9	27	9%
>30	9	3%
Total	300	100%

Table 5: Distribution of the study subjects based on the various associated co-morbidities

Co-morbidities	Frequency	Percentage
Diabetes	29	9.6%
Hypertension	41	13.6%
CAD	7	2.3%
CVA	5	1.6%
Obesity	9	3%
Total	91	30.3%

Table 6: Multi-logistic regression analysis for factors influencing vitamin D deficiency among TB patients

Factor	Vitamin D levels		OR	95% CI	P value
	Deficient (<20ng/ml) (n=165)	Not deficient (>20 ng/ml) (n=135)			
Age	<30 (n=45)	12 (7.2%)	3.45	3.1 – 4.02	<.001
	>30 (n=255)	153 (92.7%)			
Gender	Male (n=168)	74 (44.8%)	2.81	2.2 – 3.5	<.001
	Female (n=132)	91 (55.2%)			
Sputum	<=1+ (n=99)	21 (12.7%)	4.91	4.5 – 5.46	<.001
	>1+ (n=201)	144 (87.3%)			
BMI	<18.5 (n=207)	142 (86%)	3.57	2.8 – 4.32	<.001
	>18.5 (n=93)	23 (14%)			
Comorbidities	Present (n=91)	74 (44.8%)	2.79	2.5 – 3.36	<.001
	Absent (n=209)	91 (55.2%)			

Discussion

In recent years Vitamin D has received attention as an important field of research. Many randomised controlled trials which were conducted earlier had shown both types of results few studies had shown association between TB and vitamin D levels and few of them with conflicting results. Vitamin D plays an important immune-modulatory role in both innate and adaptive immunity. A meta-analysis study had shown a two-fold increased risk of active TB in individuals with vitamin D deficiency¹⁶ and another study by Sasidharan PK et al from India demonstrated significant vitamin D deficiency in patients with active tuberculosis.¹⁷

Further substantiating our results a study done by B Yuvaraj et al¹⁸ on adult population and a study done by Khandelwal et al¹⁹ among children had proved a strong association between decreased vitamin D levels with an increase sputum AFB load in patients with tuberculosis. A retrospective study conducted on Indian children with intra-thoracic tuberculosis had demonstrated low serum 25-hydroxyvitamin D levels.²⁰ Studies conducted outside India had also proven association between vitamin D deficiency and tuberculosis. A cohort study conducted in Pakistan found that vitamin D deficiency is associated with progression of latent TB to active disease in healthy household contacts.²¹ A study done in China by Wei-Wei Gao et al found that patients with pulmonary tuberculosis had lower 1, 25-dihydroxyvitamin D concentrations than the healthy controls.²² A study done on adult TB patients in Vietnam by Ho-Pham et al showed the prevalence of vitamin D deficiency in 35.4 and 45.3 percent of males and females respectively.²³

In our study after controlling for potential confounders using multivariable logistic regression model, low BMI level and being positive for TB were significant predictors of severe vitamin D deficiency. Similar to the current study, reports from Tanzania,²⁴ Uganda²⁵ and Malawi²⁶ show that a low BMI, high sputum positivity and associated co-morbidities were

associated with Vitamin D deficiency in TB patients. This may be explained as patients with low BMI and poor immune response usually have a little adipose tissue so they are unable to store vitamin D, and they have no reserves when there is poor dietary intake of foods that are rich with vitamin D. More TB patients were unemployed, which indicated a potential lower socioeconomic status of TB patients. It has been suggested that vitamin D deficiency in TB patients might lead to impaired immune control of mycobacteria.^{27,28} In line with this, we found that TB was independently associated with severe vitamin D deficiency. Our results are consistent with studies indicating vitamin D deficiency as a risk factor for developing tuberculosis.²⁹

Sunlight being an important source of vitamin D, and sun exposure in India is usually higher when compared to other western countries and so the serum vitamin D level of our study subjects was found to be much lower when compared to subjects in Greenland³⁰ and West London³¹ which might be due to differences in skin pigmentation, as melanin efficiently absorbs UVB radiation and dark skin persons require 3 to 4 times longer sun exposure. Many studies have reported that the prevalence of vitamin D deficiency in tuberculosis patients varies depending on the season³² although our study was carried out during the sunny months; the vitamin D deficiency was still common in TB patients in the current study. One of the major limitations of our study which is similar to few other studies is that a direct causal relationship between low vitamin D levels and the risk of developing TB needs further investigation with higher number of study subjects.

Conclusion

High prevalence of vitamin D deficiency was found among newly diagnosed TB patients. Factors such as low body mass index, high sputum positivity, associated co-morbidities and female gender had shown a strong association for vitamin D deficiency among TB patients. Considering the high prevalence of vitamin D deficiency, the

present study implicates that further follow-up studies are warranted to determine whether vitamin D supplementation can have a role in the prevention and treatment of tuberculosis.

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