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ASSOCIATION OF VITAMIN D DEFICIENCY AND RISK OF DEVELOPING TUBERCULOSIS MYTH OR REAL!

General Medicine

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ABSTRACT

Introduction: While vitamin D is crucial for calcium homeostasis and bone mineralization, current literature also highlights role of vitamin D deficiency (VDD) as a risk factor for developing tuberculosis (TB).

Aim: To study the association between low serum vitamin D levels and risk of developing active tuberculosis.

Methodology: A sample of forty eight outdoor/ admitted patients with TB (pulmonary or extra-pulmonary) was selected (group I) as per the inclusion and exclusion criteria and was compared with 48 age and gender matched controls (group II). Controls were drawn randomly from patients visiting out-patient department for minor ailments or healthy relatives of the patients. Their routine hematological and biochemical parameters were tested along with vitamin D status, which was assessed by the chemiluminescent method. Serum 25 hydroxyvitamin D3 [25 (OH) D3] levels < 30 ng/ml was considered vitamin D deficiency.

Results: Mean (SD) levels of vitamin D in group 1 was 21.7(±13) whereas mean (SD) levels of group 2 was 40.6(±8.5) ($p = 0.000$). Severe deficiency was seen in 2(4.4%), moderate deficiency was seen in 28(62.2%), mild deficiency was seen in 3(6.7%) and normal levels were seen in 12(26.7%) among the tuberculosis patients. 36 patients in group 1(73.3 %) were deficient in vitamin D as compared to 2 (4.5%) controls (2 out of 48) with $p < 0.001$. Patients with moderate to severe vitamin D deficiency were more likely to be open cases (smear positive) than those with mild deficiency and adequate levels ($P<0.001$).

Conclusion: Patients with TB had a significant deficiency of vitamin D as compared to the controls. Vitamin D deficiency was, thus, associated with higher risk of developing TB. Severity of vitamin D deficiency could be related to the severity of TB.

KEYWORDS

Vitamin D, Tuberculosis, Deficiency, Risk.

Introduction

Tuberculosis (TB) is a global epidemic and a major public health problem in India. More than two billion people across the world (about one-third of the world population) are estimated to be infected with Mycobacterium tuberculosis [1]. In 2016, as per the World Health Organisation (WHO) TB statistics for India, there were an estimated 2.79 million incident cases with a rate of 211/100,000 population [2]. Vitamin D deficiency (VDD) has long been implicated amongst the various risk factors in the causation of TB [3]. Cod liver oil and sun exposure, both sources of vitamin D (vit D), were commonly used in treatment of tuberculosis in the pre-antibiotic era [4]. The active form of vit D-1,25$(OH)_2D_3$ is believed to play an important role in cell mediated immunity through macrophage and monocyte activation and subsequent restriction of the growth of mycobacterium [5,6,7]. Also, it had been shown that, 1,25$(OH)_2D_3$ may act synergistically with pyrazinamide to produce enhanced mycobactericidal effect [8]. Several studies across ethnic backgrounds have shown a positive association between prevalence of TB and VDD. A meta-analysis of seven observational studies noted a reduced risk of active tuberculosis in those with the highest versus the lowest values of vitamin D [9].

Vitamin D deficiency (< 30ng/dL) is specially prevalent in developing countries and its level varies depending on the food fortification policies, demographic features, geographic location and season [7]. India, being a tropical country it was firmly believed that VDD did not exist. Recent literature highlights its high prevalence in the Indian sub-continent (Kanekar et al., 2010; Gulvady and Pingle, 2007) [10,11]. The possible reasons contributing to vitamin D deficiency in a sunlight sufficient country like India are dark skin pigmentation that interferes with ultraviolet ray transmission, insufficient exposure to sun due to social factors or workplace environment and high phytate content in diet that binds with calcium and interferes with its intestinal absorption [11]. Serum vitamin D level is a sensitive measure of vitamin D status of an individual [12].

Despite high burden of pulmonary tuberculosis in our country, only a few studies have been conducted to highlight the issue. The present study was conducted to evaluate the circulating levels of 25-hydroxyvitamin D_3 in newly diagnosed cases of tuberculosis in the indigenous population of Jamshedpur, Jharkhand, a city in eastern part of India.

Aim:

1. To estimate serum vitamin D levels in newly diagnosed cases of tuberculosis (pulmonary and extra-pulmonary).
2. To study the association between low serum vitamin D levels and risk of developing active tuberculosis.

Methods and Materials:

Nature of the study: Prospective, case-controlled study

Duration: January 2016 to June 2017 (18 months)

Sample size: It was calculated using odds ratio of 2, alpha error of 0.05, power of study as 80% and percentage exposed among controls as 70%.

Study population:

The study population constituted patients attending medical and chest outpatient departments or admitted in the medical wards of Tata Main Hospital, Jamshedpur.

Inclusion criteria:

Cases:

1. All adults ≥18 years of age, both sexes
2. Newly diagnosed patients of active tuberculosis, which includes both pulmonary and extra-pulmonary cases

Diagnosis of Tuberculosis was made based on the results of microbiological, radiological, tuberculin test and or histological basis (in case of extra-pulmonary TB) as described below. Examination of ascitic fluid and FNAC (fine needle aspiration cytology) of lymph nodes were done where ever indicated.

Microbiological: At least two baseline sputum samples, including one early morning sample, were obtained from each patient, as per Revised National Tuberculosis Control Programme (RNTCP) guidelines. All samples were examined for the presence of Mycobacterium tuberculosis using Ziehl-Neelsen staining. Bacillary load was graded using World Health Organization (WHO) guidelines.

Tuberculin test: Mantoux test was performed by specifically trained technician using 0.1 mL (10 U) of purified protein derivative (PPD) (CSL); the results were interpreted by them. An induration and erythema of ≥ 10 mm was considered significant.

Histopathological: Diagnosis was established if evidence of caseation necrosis, granulomas with or without M. tuberculosis were found.

Controls:

1. All adults ≥18 years of age, both sexes (age and sex matched)
2. Patients with no history of tuberculosis coming to OPD for other minor ailments.

SSSSSSSSExclusion criteria for cases and controls:

1. Patients with chronic renal failure and chronic liver disease
2. Patients with Diabetes mellitus
3. Co-infection with the human immunodeficiency virus
4. Patients with malabsorption syndromes (chronic pancreatitis and inflammatory bowel diseases, bowel surgery)
5. Patients who are taking drugs which can reduce vitamin D levels or antagonize its actions (eg: phenytoin, diuretics)
6. Patients with overt malnutrition
7. Patients on vitamin D supplementation
8. Pregnant women

Methodology: After taking informed consent of the study participants, all patients were subjected to a detailed medical history which included the demographic details, personal history of and/or contact with TB, alcohol consumption, symptoms suggestive of vitamin D deficiency like muscle pains, bone pains, proximal muscle weakness, questions on type of work (sedentary/outdoor), lifestyle, dietary habits, concomitant illness and medications. A detailed physical assessment and systemic examination were done. All participants also underwent baseline anthropometric measurements of weight and height for calculation of the body mass index (BMI). Cases were subjected to plain chest radiograph to assess radiographic severity of tuberculosis. Prior to initiation of anti-TB treatment, 5ml of venous blood sample was collected in fasting state in plain vials and serum was separated for estimation of serum Vitamin D (25-hydroxyvitamin D) levels by chemiluminescent method using the UniCel DxI Immunoassay Systems. Also other tests like complete blood count, serum albumin and calcium concentrations, blood glucose, renal function tests (serum urea and creatinine) and liver function tests - alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) concentrations, serum proteins, HIV serology and viral markers were done.

Vitamin D status of the study participants was defined as per the Endocrine Society clinical practice guidelines by Hollick et al on evaluation, treatment and prevention of vitamin D deficiency [13].

On the basis of vitamin D levels, patients were divided into the following groups:

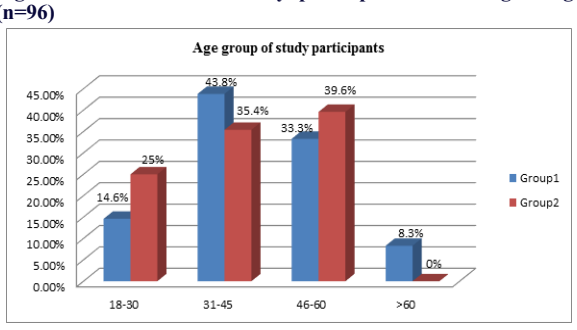
1. Severe deficiency	<10 ng/ml
2. Moderate deficiency	10-19 ng/ml
3. Mild deficiency (insufficiency)	20-29 ng/ml
4. Sufficient	30-39 ng/ml
5. Ideal	40-50 ng/ml

For the purpose of this study, a patient with vitamin D level < 30 ng/ml was considered to be vitamin D deficient. As anti-tuberculosis chemotherapy can lower serum Vitamin D levels, only those patients of tuberculosis were included who were yet to commence treatment.

Statistical analysis: Statistical interpretation of data was performed using Statistical Package for Social Sciences (SPSS IBM) version 21.0. Required univariate and bivariate analysis was done. Results were expressed as mean, standard deviation (±SD) for all continuous variables and frequency and percentage for categorical data. T-test and chi-square test as appropriate to the nature and distribution of the variables were used. A p-value < 0.05 was considered statistically significant.

Results: Amongst the total 96 (100%) study participants - there were two groups with each of 48. Group I included patients with pulmonary or extra-pulmonary tuberculosis while Group II had age and sex matched controls. The mean (±SD) age of cases and controls was 43.7 ± 13.2 years and 40.6 ±13 years respectively. The age distribution of the participants was as shown in the bar graph **(Figure 1)**.

Figure1: Distribution of study participants according to age (n=96)



There were 32 (66.7%) males and 16 (33.3%) females in group I and 30 (62.5%) males and 18 (37.5%) females in group II **(Figure 2)**. 24 males (50%) and 12 females (25%) with TB had VDD, male to female ratio being 2:1.

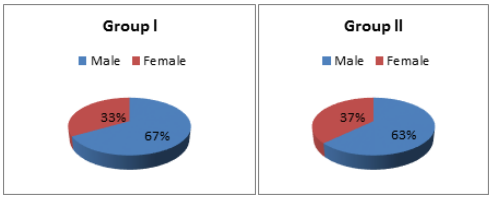


Figure 2: Distribution of study participants according to gender (n=96)

There was no statistically significant difference in age and gender of participants of both groups. Risk factor like overcrowding was seen in 36 (75%) out of the 48 patients with tuberculosis while it was present in 15 (31.3%) patients in group II. Thus, prevalence of overcrowding was significantly higher in patients with tuberculosis than in controls. Prevalence of smoking was also higher in patients with tuberculosis than in the controls [18 (37.5%) vs 10 (20.8%)]. Alcoholism was detected in 13 (27%) and 11 (18.8%) respectively in group I and group II **(Table 1)**. Positive history of contact with a case of tuberculosis was elicited in 7(14.6%) patients in group 1.

Table 1: Demographic profile of study participants (n=96)

Variables	Group I (n=48)	Group II (n=48)	P value
Age (years) mean [SD]	43.7 (±13.2)	40.6 (±13)	0.08
Males n (%)	32 (66.7%)	30 (62.5%)	0.672
Females n (%)	16 (33.3%)	18 (37.5%)	0.667
BMI (kg/m2) mean [SD]	21.5 (±4.7)	24.5 (±3.2)	0.104
Vitamin D_3 (ng/ml) mean [SD]	21.7 (±13.3)	40.6 (±8.5)	0.000
Vitamin D_3 deficiency (n[%])	36 (73.3 %)	2 (4.5%)	0.0001
Smoking n (%)	18 (37.5%)	10 (20.8%)	0.075
Overcrowding n (%)	36 (75%)	15 (31.3%)	0.001
Alcoholism n (%)	13 (27%)	11 (18.8%)	0.637
Socioeconomic class* n (%)	26 (54.2%)	17 (35.5%)	0.38

*Modified BG Prasad scale 2016

The weight (in kg) of the participants ranged from 51.1 to 78.4 kg with a mean (SD) of 63.7(±11.4) in group I and 62.8 to 85 kg with mean (SD) of 72.6(±11.5) in group II while the average BMI (kg/m2) was 21.5(±4.7) and 24.5(±3.2) in group I and group II respectively **(Table 2)**.

Table 2: Anthropometric parameters of study participants (n=96)

S no.	Anthropometric parameter	Group 1 Mean (±SD)	Group ll Mean (±SD)
1.	Weight (kg)	63.7 (±11.4)	72.6 (±11.5)
2.	Height (cm)	159.5 (±9.1)	163.6 (± 9.4)
3.	BMI (kg/m^2)	21.5 (±4.7)	24.5 (±3.2)

Using the criteria of vitamin D deficiency as less than 30 ng/ml, 36 patients of tuberculosis out of 48 (73.3 %) were deficient in vitamin D as compared to 2 (4.5%) controls (2 out of 48) with p < 0.001. Mean vitamin D levels were lower in cases as compared to controls **(Figure 3)**. Mean Vitamin D levels were 21.7 ± 13.3 ng/ml in cases and 40.6 ± 8.5 ng/ml in controls (p < 0.000). Vitamin D deficiency was higher amongst the females (81.3%) when compared to males (71.9%). 23 out of 32 males and 13 out of the 16 females with tuberculosis, were vitamin D deficient.

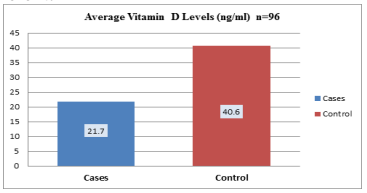


Figure 3: Mean Vitamin D levels in cases and control

Severe deficiency (<10 ng/dl) was found in 2 (4.4%) patients, moderate deficiency in 28 (62.2%) patients and mild deficiency in 6 (12.5%) patients **(Table 3)**. None of the patients except one (2.2%) with severe vitamin D deficiency reported symptoms in form of generalised body aches and pain in the lower back.

Table 3: Distribution of study participants according to vitamin D levels (n=96)

Vitamin D levels values (ng/dl)	Group I - n (%)	Group II – n (%)
Severe deficiency (<10)	2 (4.4)	0
Moderate deficiency (10-19)	28 (62.2)	0
Mild deficiency (20-29)	6 (12.5)	2 (4.5)
Sufficient (30-39)	7 (15.6)	26 (54)
Ideal (40-50)	5 (11.1)	20 (45.5)
Average vitamin D levels	**21.7 (±13)** (ng/dl)	**40.6 (±8.5)**

Table 4: Blood parameters of study participants. (n=96)

S. No.	Blood parameters	Group I Mean (±SD)	Group II Mean (±SD)
1.	Haemoglobin (gm/dl)	10.1 (±1.5)	11.2 (±1.6)
2.	Serum albumin (gm/dl)	3.7 (±0.67)	4.3 (±0.11)
3.	Serum calcium (mg/dl)	8.9 (±0.14)	9.3 (±0.11)
4.	Random blood sugar (gm/dl)	79 (±56)	84 (±14)

The average haemoglobin, serum albumin, calcium, and random blood sugar levels were comparable in both groups as shown in the above **table 4.**

Table 5: Tuberculosis characteristics of study participants (n=48)

S. No.	Tuberculosis- characteristics	Group 1 n (%)
1.	**Tuberculosis - Type**	
	Pulmonary	46 (95.8)
	Extra-pulmonary	2 (4.2)
2.	**Smear status**	
	Smear positive	39 (81.3)
	Smear negative	9 (18.8)
3.	**Chest X ray**	
	Cavitation present	18 (37.5)
	No cavitation	30 (62.5)

In the study group, pulmonary tuberculosis was detected in 46 (95.8%) patients while only 2 (4.2%) patients had extra-pulmonary tuberculosis. Smear positivity was demonstrated in 39 (81.3%) cases of pulmonary tuberculosis while 9 (18.8%) were smear negative. 25 (64.1%) out of 39 cases had vitamin D levels < 20 ng/dl. It was observed that the patients with moderate to severe vitamin D deficiency were more likely to be open cases (smear positive) than those with mild deficiency and adequate levels (P<0.001). The average vitamin levels were 21.02 ng/ml (±11.8) and 23.6 ng/dl (±15.8) respectively in smear positive and smear negative cases. Cavitation was present in 18 (37.5%) out of 48 cases (**Table 5**). The average vitamin D levels were 21.5 ng/ml (±13.1) in patients with cavitation while it was 21.9 ng/ml (±12.1) in those without cavitation.

Discussion:

Following an original observation of VDD in a 14 year old boy with skeletal tuberculosis in 1993, it was hypothesized that it could be a cause rather than the effect contrary to the earlier thinking. Vitamin D plays an important role in host immune defence against Mycobacterium tuberculosis by macrophage activation and restriction of mycobacterial growth. It has been shown by Liu et al that vitamin D supplementation results in increased expression of antimicrobial peptide 'cathelicidin' in the macrophage culture, which could result in killing of the intracellular Mycobacterium tuberculosis [5,6]. Liu (2006) demonstrated that transcriptional regulation of cathelicidin can be mediated by activation of 1,25-dihydroxyvitamin D. Cathelicidins have direct antimicrobial effect like membrane disruption. In fact, according to Liu (2006), serum from donors with insufficient levels of vitamin D supported a lower induction of cathelicidin in monocytes compared to serum of donors with sufficient vitamin D levels [5]. Microbial products stimulate Toll-Like Receptor (TLR2/1) complex on the macrophages and increase expression of vitamin D receptors (VDR), which results in increased conversion from the inactive 25-hydroxyvitamin D_3 to the active 1,25 dihydroxyvitamin D_3[7]. Also, macrophage activation is augmented by tumor necrosis factor–alpha (TNF-α), which acts synergistically vitamin D_3[14]. This is a potential mechanism which could logically explain role of vitamin D in enhancing innate immunity in patients with tuberculosis. However, the in-vivo association between vitamin D status and tuberculosis is still a debatable issue.

Several studies from different parts of our country have shown widespread vitamin D deficiency in Indians of all age groups residing in both rural or urban areas [15-18]. Skin complexion, poor sun-exposure, vegetarian food habits, high phytate content of the diet and lack of vitamin D food fortification programme in the country explain the high prevalence of VDD in India despite its sunny climate [4,19]. The recommended daily dietary intake of vitamin D in adults (19 to 50 years old) varies from 0.5 to 5 mcg (100-200 IU) per day [20].

The possible association between vitamin D and tuberculosis was first reported 20 years ago and has been subsequently shown in various studies. In a study by Sasidharan PK et al (2002), the mean value of 25 hydroxyvitamin D in patients with tuberculosis was 10.7ng/ml in comparison to the apparently healthy control group, which had a mean value of 19.4ng/ml, and the difference was statistically significant [20]. They found low vitamin D levels in the study population despite adequate sun-exposure, concluding that diet was the more important factor. In a meta-analysis done by Nnoaham KE et al, that examined the association between low serum vitamin D and risk of active tuberculosis, found a probability of 70% that a healthy individual would have higher serum vitamin D level than an individual with tuberculosis if both were chosen at random from a population [21]. In a study done by Raheel Iftikhar et al (2017), mean vitamin D levels were 23.23 ± 6.81 ng/ml in cases, and 29.27 ± 8.89 ng/ml in controls ($p< 0.0001$). Vitamin D deficiency was found in 57% of cases and 33% controls ($p< 0.0001$) with an odd's ratio (CI) of 2.67 (1.67–4.25) [7]. Studies in Gujarati Asians living in the United Kingdom found that lower levels of vitamin D were associated with an increased risk of pulmonary tuberculosis [22]. Among African immigrants in Australia, individuals with latent or active tuberculosis were observed to have substantially lower serum Vitamin D levels than those without tuberculosis [23]. Similarly, studies in African residents in London [24] and people of West Africa [25,26] have shown that those tuberculosis had lower levels of vitamin D_3 and higher prevalence of vitamin D deficiency than non-TB individuals. In a study by Zeng J et al, a significantly increased risk of tuberculosis was found with vitamin D level <12.5nmol/L [9]. Another study from India by Rathored et al concluded that vitamin D receptor gene polymorphisms and hypovitaminosis D may predispose to MDR-TB. Lower serum vitamin D may increase the conversion time taken for MDR-TB sputum smear negativity [27].

Iftikar et al (2013) compared vitamin D deficiency in pulmonary versus extra pulmonary TB and it was found that extra-pulmonary TB is associated more frequently with VDD (72%) than Pulmonary TB (52%) [7]. In our study, we had only 2 patients of extra-pulmonary TB, hence, the comparison could not be made.

Overcrowding was observed in 36 (75%) while no overcrowding was seen in 12 (25%) patients of TB. This result was supported by a previous study conducted by Michael Clark et al, [28] which stated that overcrowded housing conditions increases the tuberculosis (TB) transmission from infectious patients to susceptible individuals (like those who are vitamin D deficient). Contact history was present in 7(14.6%) study participants in group I and none in group II participants.

Studies have shown that malnutrition increases the risk of developing TB because of an impaired immune response [29]. TB disease can itself lead to malnourishment because of decreasing appetite and changes in metabolic processes [30]. However, in our study, the mean BMI of the TB cases was 21.5(±4.7), which was not statistically different from that of controls of 24.5(±3.2), thus eliminating malnutrition as a confounding factor. This was similar to the study conducted by Raheel Iftikhar et al [7], where the mean ± SD body mass index of the cases was 20.43 (±2.06) and that of controls was 23.62 (± 2.35). Moreover, the average time to diagnosis of TB in our cases was 15.6 days.

Our study showed male preponderance, which was also seen in a study by Essam Gouda et al [31]. However, female predominance was found in a study by Raheel Iftikhar et al (2013) from Pakistan.

Smoking is considered to be a risk factor for tuberculosis [32]. This association was not to be statistically significant in our study. Vitamin D is important for calcium absorption which is impaired by smoking. However, there is no evidence to suggest that vitamin D absorption is impaired directly by smoking [33].

Considering the role of vitamin D in the immunity against mycobacterium tuberculosis, and its deficiency in the causation of TB, supplementation with vitamin D may have a role in altering the disease course and thus morbidity of this foremost public health problem in developing countries like India. This finding warrants the need for larger prospective studies to determine whether vitamin D supplementation can have a role in the prevention and treatment of tuberculosis. Also in view of this, the potential role of vitamin D supplementation in people with hypovitaminosis D-associated conditions like chronic kidney disease, diabetes mellitus etc should be evaluated [21].

Limitations: 1. We were unable to make a detailed dietary assessment of the intake of vitamin D. 2. Another potential bias in this study was selection bias since it was a hospital-based study that recruited only admitted patients. As the patients in this study were from one center, the results may not be representative of the entire population.

Conclusion: Our study revealed a significantly lower vitamin D level in hospitalised TB patients than the normal subjects and thus, VDD was associated with higher risk of TB. VDD is more likely a risk factor for TB than its consequence, as is evident from the study. Hypovitaminosis D might be related to severity of the tuberculosis and larger studies are needed to determine whether vitamin D supplementation is beneficial to prevent TB.

Conflict of interest: Nil

Source of funding: Nil

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