

A STUDY ON VITAMIN D STATUS IN TUBERCULOSIS

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ABSTRACT

BACKGROUND

India is a country with the highest burden of TB. Vitamin D can lead to activation of immune system and prevent the growth of intracellular Mycobacterium tuberculosis. Good number of studies show that there is deficiency of Vitamin D in tuberculosis patients, though there are limited number of studies to show result on the contrary.

This study was done with an aim to evaluate for any association of vitamin D with tuberculosis.

MATERIALS AND METHODS

The study was undertaken with 52 number of patients newly diagnosed with tuberculosis, diagnosed by clinical criteria and usual laboratory tests. Serum samples were collected and 25-hydroxy vitamin D level was estimated in all of them using chemiluminescent immunoassay technology and compared with that of normal healthy controls.

RESULTS

The study population included 52 patients (34 males and 18 females) diagnosed as TB, and healthy controls. 28 patients had pulmonary and 24 patients had extrapulmonary TB. Our study confirmed a high prevalence of vitamin D deficiency, 69.23% in patients with TB as compared to 34% of the healthy controls. The difference between the means were statistically significant with $t=-2.41731$ and $p=0.008$ ($p<0.05$).

CONCLUSION

Majority of the patients affected by tuberculosis demonstrated hypovitaminosis D in a greater proportion than the healthy controls. Though further large scale studies with patient followup are required to interpret the outcome of supplementation of vitamin D, it may be given as an add-on drug with antitubercular treatment.

KEYWORDS

Tuberculosis, 25-Hydroxyvitamin D, Vitamin D Deficiency.

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BACKGROUND

Tuberculosis (TB) is a global pandemic that mainly affects the low- and middle- income countries. India, followed by Indonesia, China, Nigeria, Pakistan and South Africa account for 60% of the total TB statistics in the world. Despite advances in diagnostic technology and invention of new antituberculous drugs, TB continues to remain a major public health concern. In May 2014, the World Health Assembly at Geneva endorsed "The End TB Strategy" with an aim to end the global TB epidemic by 2035 with targets to reduce TB deaths by 95% and cut down the new cases by 90% and ensure that no family is burdened with catastrophic expenses due to TB. India contributes to one-quarter of the global TB burden. WHO statistics for 2015 gives an estimated incidence and prevalence of 2.2 and 2.5 million respectively for TB in India.⁽¹⁾ TB along with HIV remains as one of the leading causes of morbidity and mortality among general population.

The Revised National Tuberculosis Control Programme (RNTCP) since its inception in the 1990s has made a remarkable progress in reducing the tuberculosis burden in our country through development of comprehensive guidelines for diagnosis and treatment of TB, but still is challenged by various factors such as the emergence of drug-resistant tuberculosis bacilli that hinders the success of RNTCP. Pulmonary TB (PTB) is an infectious disease of the lower respiratory tract caused by an intracellular acid-fast bacillus, Mycobacterium tuberculosis (Mtb). Extrapulmonary TB (EPTB) can virtually affect all organs other than the lungs and has a wide variety of clinical manifestations. Pleural and pericardial serosal membranes, lymph nodes, abdomen, genitourinary tract, joint and bones, meninges and skin are few examples that are involved in EPTB. Only a minority of those infected with the bacilli develop clinical disease which is determined by the host genetic and environmental factors that governs the susceptibility and resistance of the host to M. tuberculosis.

Vitamin D is a fat-soluble vitamin that has functional diversity beyond its role in skeletal system and calcium and phosphorus homeostasis. The biologically active vitamin D (calcitriol) is principally derived from cutaneous 7-dehydrocholesterol on exposure to solar ultraviolet B (UVB) rays. The productive pathway includes double hydroxylation initially to 25-hydroxy vitamin D [25 (OH) D] by 25-hydroxylase enzyme in the liver and later to 1, 25-dihydroxyvitamin D [1, 25(OH)₂D] by 1- α hydroxylase

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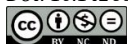
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enzyme in the kidney.⁽²⁻³⁾ 25 (OH)D and 1, 25 (OH)₂D are metabolised to inactive compounds by the enzyme 24-hydroxylase present in the liver. 25-hydroxyvitamin D is the major circulating and storage form of vitamin D with half-life approximately 2-4 weeks. Hence, 25 (OH)D is the most specific and widely used screening test of choice to assess for vitamin D deficiency.⁽⁴⁾

The production of vitamin D from sunlight depends on many factors such as duration and time of exposure, proportion of body surface covered, pigmentation of the skin, atmospheric pollution, latitude and season. The best time of exposure is 10 a.m. to 3 p.m. To produce a similar amount of vitamin D as of the whites, Indians comparatively require three to five times greater exposure to sunlight. Melanin is a pigment present in the skin that competes with UVB and reduces the production of vitamin D; hence explaining the requirement of greater duration of exposure to sunlight by the Indian population. Diet is a minor source available in the form of ergocalciferol from plant sources and cholecalciferol from animal sources although both forms are biologically equivalent. It is also important to ensure normal vitamin D levels in mothers as it is readily transferred from the mother to the foetus transplacentally.

Research in recent years has evidenced association of vitamin D deficiency (VDD) with various diseases such as respiratory diseases including infections, bronchial asthma, chronic obstructive pulmonary disease, diabetes mellitus, hypertension, autoimmune diseases including rheumatoid arthritis, multiple sclerosis, type 1 diabetes mellitus and malignancies including breast, colon and prostate. Certain important causes of vitamin D deficiency include increasing age, reduced sunlight exposure, increased skin pigmentation, low dietary intake, malabsorption, obesity and drugs including antiepileptics, antifungals and antiretroviral drugs. Low calcium and high dietary intake of phytates are one among the causes for increased prevalence of VDD in the Indian subcontinent.

The immunomodulatory roles of vitamin D are very much appreciated in recent years. It plays an important role in innate immunity. Monocyte differentiation into activated macrophages occurs through vitamin D receptors (VDR), which in turn synthesises 1, 25(OH)₂D intracellularly.⁽⁵⁻⁶⁾ VDR expression on monocytes is increased through Toll-like receptors 2/1 (TLR2/1).⁽⁷⁾ The antimicrobial peptide, Cathelicidin is directly up-regulated by 1, 25(OH)₂D⁽⁸⁻⁹⁾ which helps in phagocytosis of Mtb followed by fusion of phagosomes with lysosomes to form autolysosomes and expression of lysosomal enzymes and reactive oxygen species.⁽¹⁰⁾ Vitamin D also enhances the antimicrobial function of macrophages through interferon-gamma.⁽¹¹⁾ Vitamin D inhibits the secretion of matrix metalloproteinases (MMPs) and increases the production of their inhibitors. Vitamin D deficiency favours the extensibility and extrapulmonary dissemination of tubercle bacilli from the pulmonary foci.⁽¹²⁻¹³⁾ The antiviral properties are expressed indirectly through expression of defensins that interferes with membrane fusion of viral particles.⁽¹⁴⁾ The role of 1, 25 (OH)₂D in acquired immunity is evidenced by its direct and indirect regulatory actions on T cells and dendritic cells respectively.⁽¹⁵⁾

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.⁽¹⁶⁾ Marineau et al showed that a single dose of 0.25 mg oral vitamin D significantly enhanced the immunity among Mtb contacts for six weeks.⁽¹⁷⁾ Certain studies demonstrated that vitamin D supplementation in TB patients is associated with faster clinical and radiological improvement and sputum smear conversion.⁽¹⁸⁾ Genetic polymorphisms involving vitamin D receptors may play a role in susceptibility and emergence of drug resistance in TB as evidenced by increased susceptibility among Asians with FokI FF genotype of VDR.⁽¹⁹⁾ However, there are differing opinions and results from studies regarding the correlation of VDD with TB.

MATERIALS AND METHODS

The study is a descriptive study conducted in Department of Pulmonary Medicine and General Medicine in Silchar Medical College and Hospital, Silchar, Assam, India. The sample is selected conveniently from patients attending the out-patient department (OPD) in the time period of six months from February to July 2016. Informed consent was taken from all the study subjects. Newly diagnosed TB cases were included in the study with age and sex matched apparently healthy controls. A New case of TB is defined as a patient who has never had treatment for TB or has taken anti-tuberculosis treatment (ATT) for less than 1 month duration as per RNTCP guidelines. The cases were diagnosed based on clinical features, chest x-ray, sputum smear examination, FNAC, USG, CSF analysis, pleural and ascitic fluid study. Patients with apparently normal nutrition were selected to exclude the effect of malnutrition consequent to TB on vitamin D level.

Children and patients with multidrug-resistant TB were not included in the study. Patients with chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), active diarrhoea, history suggestive of malabsorption, pre-existing liver or renal diseases, human immunodeficiency virus (HIV) infection, diabetes mellitus, osteoporosis, cytotoxic and immunosuppressive drug treatment, malignancy were excluded from the study.

Five millilitres of venous blood samples were collected from each subject and 25-hydroxyvitamin D [25 (OH) D] levels were estimated using chemiluminescent immunoassay technology. Vitamin D status is classified as deficient <20 ng/mL, insufficient 20 to <30 ng/mL, sufficient 30 to 100 ng/mL and intoxication >100 ng/mL.⁽⁴⁾

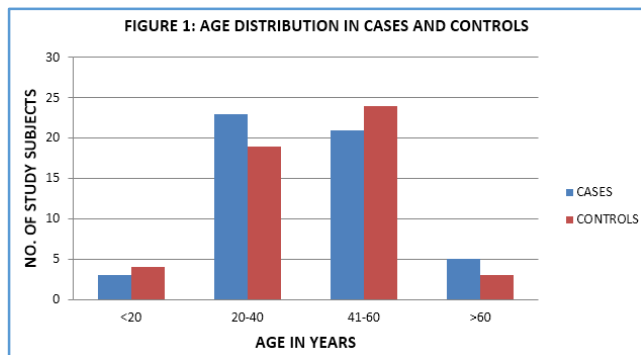
Statistical analysis was done using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean ± standard deviation (SD). The mean 25-hydroxyvitamin D levels were calculated for both cases and controls and also for males and females independently in both cases and controls for comparison. Student t-test was used to compare and find any significant correlation between vitamin D status and Tuberculosis. The p-value of < 0.05 was considered as statistically significant.

RESULTS

The study included 52 newly diagnosed TB cases with 34 males and 18 females and 50 age and sex matched apparently healthy controls. Majority of the patients were in the 20 to 60 years age group (Table 1 & 2).

Age Distribution	< 20 years	20 - 40 years	41 - 60 Years	>60 years
Cases (n=52)	3	23	21	5
Controls (n=50)	4	19	24	3

Table 1. Age Distribution of Subjects in both Cases and Controls



Sex Distribution	Males	Females	Total
Cases	34	18	52
Controls	39	11	50

Table 2. Sex Distribution of Subjects in both Cases and Controls

Among the cases, 28 patients had pulmonary TB and 24 patients had extrapulmonary TB that included 11 cases of pleural effusion, 4 cases of TB lymphadenitis, 5 cases of abdominal Koch's and 4 cases of TB meningitis (Table 3).

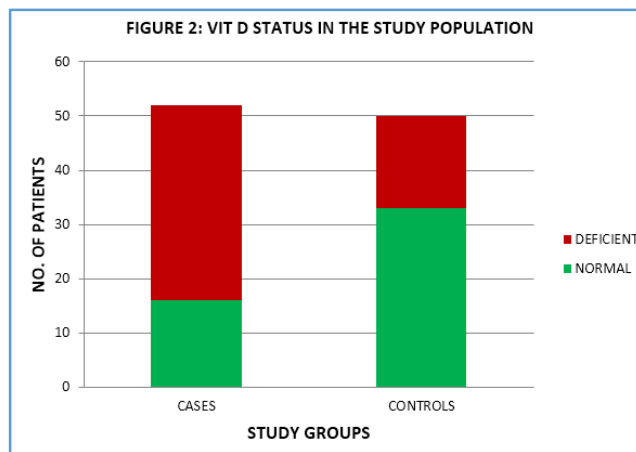
Cases	PTB	Extrapulmonary TB			
		Pleural Effusion	TB lymphadenitis	Abdominal Koch's	TB Meningitis
No. of Patients	28	11	4	5	4

Table 3. Distribution of Number of Patients in Pulmonary and Extrapulmonary TB

25 (OH) D levels were estimated from each subject in both cases and controls group and the mean value was calculated. Among the cases, 36 patients were found to have VDD and 16 patients had normal levels whereas among the controls, 17 subjects had VDD and 33 subjects had normal levels. The sex distribution within the normal and deficient subjects in cases and controls are given in Table 4.

Vitamin D Levels in the Study Population	Normal		Deficient	
	Count (%)	Gender	Count (%)	Gender
Cases (n = 52)	16 (30.77%)	13 males	36 (69.23%)	21 males
		3 females		15 females
Controls (n = 50)	33 (66%)	26 males	17 (34%)	13 males
		7 females		4 Females

Table 4. Distribution of 25(OH) D levels in Cases and Controls



The mean 25(OH) D level of males in cases is 28.44588 ± 17.00878 while in controls it is 34.57308 ± 15.65502. The mean 25(OH) D level of females in cases is 23.09333 ± 13.77367 while in controls it is 32.42455 ± 13.00675. Although the difference in means of males between cases and controls were not statistically significant with t = -1.60226 and p = 0.05677, the difference in mean 25(OH) D levels for females were statistically significant between the cases and controls with t = -1.80679 and p = 0.040976.

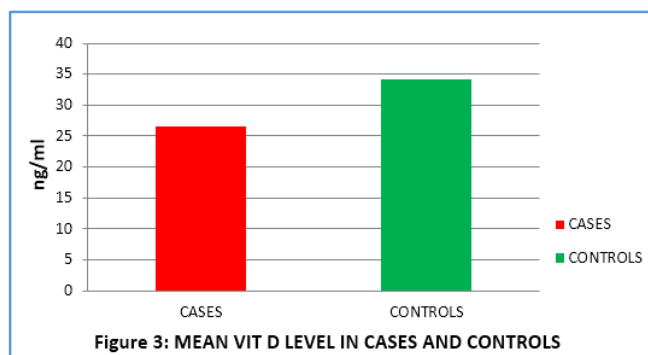
Mean Vit D Levels with Standard Deviation	Cases	Controls
Males	28.44588 ± 17.00878	34.57308 ± 15.65502
Females	23.09333 ± 13.77367	32.42455 ± 13.00675

Table 5. Difference of Mean 25(OH)D Level in Males and Females between Cases and Controls

The mean 25(OH) D level is 26.5925 ± 16.03069 in cases and 34.1004 ± 15.01318 in the controls. The difference between the means of the cases and controls were statistically significant with t = -2.41731 and p = 0.008 (p < 0.05). Our study confirmed a high prevalence of vitamin D deficiency, 69.23% in patients with TB with significant correlation compared to 34% in the healthy controls.

Difference of Mean between Cases and Controls	25 (OH) Vitamin D level	
	Mean	SD
Cases	26.5925	16.03069
Controls	34.1004	15.01318

Table 6. Mean 25(OH) D Level in Cases and Controls



DISCUSSION

Vitamin D has received attention as an important field of research in recent years. Many randomised controlled trials are done and going on to enlighten the association of vitamin D with various diseases with conflicting results. Vitamin D plays an important immunomodulatory role in both innate and adaptive immunity. A meta-analysis of various studies has shown two-fold increased risk of active TB states in individuals with vitamin D deficiency.⁽¹⁶⁾ Sasidharan PK et al from India conducted a study that demonstrated significant vitamin D deficiency in patients with active tuberculosis.⁽²⁰⁾ A study done by B Yuvaraj et al evidenced the association of decreased vitamin D levels with an increase sputum AFB load in patients with tuberculosis.⁽²¹⁾ A study conducted on Indian children with intrathoracic tuberculosis by Khandelwal et al showed that majority of the children demonstrated low serum 25-hydroxyvitamin D levels.⁽²²⁾ 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.⁽²³⁾ 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.⁽²⁴⁾ 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.⁽²⁵⁾ Nursyam et al showed that additional vitamin D therapy with ATT resulted in faster sputum smear conversion and radiological improvement in TB patients⁽²⁶⁾ whereas a study conducted by Marineau et al showed that vitamin D supplementation did not significantly reduce the time to sputum culture conversion.⁽²⁷⁾ A study conducted in India by Peter Daley et al found that adjunctive vitamin D in the treatment of active tuberculosis did not reduce the time to sputum culture conversion.⁽²⁸⁾ In one published case report, simultaneous correction of vitamin D deficiency with ATT in an African-American female who presented with refractory, drug susceptible pulmonary tuberculosis resulted in clinical and microbiological improvement.⁽²⁹⁾ A meta-analysis was done by Lewis et al to assess the association of pulmonary TB with VDR FokI and TaqI polymorphisms.⁽³⁰⁾

Limitations to our study include a small number of patients conducted in only one tertiary health care centre, serum intact parathyroid hormone (iPTH) levels were not estimated, no facility for vitamin D receptor polymorphism testing, no data on amount of sunlight exposure though it is constant and intense in this population, no documentation on the dietary pattern and the body mass index of the study subjects, no followup as most patients were of low socioeconomic status and unable to attend hospitals for regular followup.

CONCLUSION

In our study, majority of the patients affected with tuberculosis demonstrated hypovitaminosis D in a greater proportion than the healthy controls. Literature search shows that vitamin D deficiency is much prevalent in India. Although many studies have evaluated for the association of vitamin D deficiency with tuberculosis, there are no uniform results and hence the correlation remains doubtful. Public health

education should encourage people to spend more time in sunlight and emphasise on adequate dietary intake of vitamin D. It should also give importance to address the aetiologies of vitamin D deficiency and implement effective population based strategies such as vitamin D fortified food products. Further large scale prospective studies with patient followup are required to confirm the association of vitamin D deficiency with tuberculosis, determine the role of vitamin D receptor (VDR) polymorphisms in susceptibility to tuberculosis and to interpret the outcome of vitamin D supplementation along with antitubercular treatment.

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