Original Article

Evaluation of Effect of Category I Anti-Tuberculosis Therapy on Vitamin D Status of Pulmonary Tuberculosis Patients: A Single Centre, Prospective, Observational Study

K.B. Gupta[1], Abhishek Tandon[1], Veena Ghalaut[2], Vipul Kumar[1] and Yash Alok[3]

Departments of TB and Respiratory Medicine[1] and Biochemistry[2], Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, (Haryana) and Department of Community Medicine[3], All India Institute of Medical Sciences, Bhopal, (Madhya Pradesh), India

Abstract

Objective. Tuberculosis (TB) is a significant cause of mortality and morbidity worldwide. Vitamin D deficiency has been implicated to cause pulmonary TB (PTB). On the other hand, anti-TB drugs, like rifampicin and isoniazid have been hypothesised to cause vitamin D deficiency. The objective of this study was to evaluate and compare serum vitamin D status in patients with PTB before and after anti-tuberculosis therapy (ATT).

Methods. A single centre, prospective, observational, double-blinded study was conducted in 50 patients with PTB on category I ATT. Vitamin D samples were collected at baseline, two months and at the end of six months.

Results. The mean age of the study population was 46.7±21.2 years. The mean serum vitamin D level at baseline was 18.1±6.7 ng/mL (Normal >30ng/mL), 17.9±7.1 ng/mL at two months and, 17.5±7.8 ng/mL at six months. The progressive decline in the mean vitamin D levels was statistically significant (P<0.001). However, it was also observed that in some patients [n=8 (16%)] vitamin D levels increased after ATT; though in majority [n=42 (84%)] vitamin D levels decreased after ATT.

Conclusion. It is postulated that vitamin D supplementation in TB treatment programmes may also have an effect on outcome, thereby the need for further studies to ascertain the exact role, dose and duration of vitamin D augmentation in improving the outcomes. **[Indian J Chest Dis Allied Sci 2021;63:13-16]**

Key words: Vitamin D, Tuberculosis, Smoking

Introduction

Tuberculosis (TB) was reported in one crore cases across the globe of which 16 lakh died from the disease in 2017.[1] Vitamin D deficiency is prevalent among apparently healthy Indians, irrespective of their exposure to sunlight. This has been ascribed to reduced intake of dietary products rich in vitamin D and decreased synthesis of vitamin D as a consequence of darker skin, use of sunscreen and indoor lifestyle.[2]

Vitamin D deficient individuals have a greater susceptibility of developing TB and a worse prognosis on being infected with TB. The likely means through which vitamin D may prevent or limit infection by *Mycobacterium tuberculosis* is through the binding of the bioactive form of vitamin D (1,25-dihydroxycholecalciferol) to the vitamin D receptor (VDR), a polymorphic nuclear receptor that regulates the expression of genes important for immune function and involved in cytokine production. The VDR is present in immune cells, bronchial and pulmonary epithelial cells and is up-regulated following the ligation of specific toll-like receptors during an antimicrobial response. Through this mechanism, vitamin D induces several endogeneous antimicrobial peptides; specifically cathelicidin LL-37 and β defensin and suppresses matrix metalloproteinase enzymes that degrade the pulmonary extracellular matrix.[3]

Vitamin D was used to treat TB during the pre-antibiotic era and even today, trials have continued to assess its role in the treatment and prevention of TB. A systematic review evaluated the results from 21 randomised controlled trials in order to assess the relationship between low vitamin D status and TB, the link between VDR polymorphisms and TB

[Received: August 28, 2019; accepted after revision: September 01, 2020]

Corresponding author: Dr Abhishek Tandon, Department of TB and Respiratory Medicine, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak-124 001 (Haryana), India; E-mail: drabhishektandon07@gmail.com

susceptibility, and the role of vitamin D supplementation in TB treatment and prevention.[4] It concluded that (1) individuals with TB had lower serum vitamin D levels than healthy, age-matched, and sex-matched controls, (2) people with certain VDR polymorphisms (Bacillus stearothermophilus DNA polymerase I(one) [BsmI] and and Flavobacterium okeanokoites DNA polymerase I(one) [FokI]) had increased susceptibility to TB, and (3) TB patients receiving vitamin D supplementation had improved outcomes in a majority of the studies included in the review.[4]

Reports on the interaction between vitamin D and anti-tubercular drugs have shown that rifampicin causes an accelerated loss of vitamin D due to increased clearance, as it acts as an agonist to pregnane X receptor inducing the activity of CYP3A4 and limiting the formation of active one alpha 25(OH)2D3.[5] Isoniazid causes impairment of 25-hydroxylation leading to impaired vitamin D action.[6]

The present study was carried out to evaluate the effects of category I anti-tubercular drugs on the vitamin D profile of patients with active pulmonary tuberculosis (PTB).

Material and Methods

A single centre, prospective, observational, double-blinded study was carried out from August 2017 to March 2018 in the Department of Respiratory Medicine in collaboration with the department of Biochemistry at Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences (PGIMS), Rohtak after obtaining Institutional Ethics Committee approval and written informed consent of all the study participants. Fifty cases of newly diagnosed PTB as defined by Revised National Tuberculosis Control Programme (RNTCP) were included in the study. All subjects above 18 years of age belonging to both genders and residents of Rohtak encompassing different socio-economic backgrounds were included in the study. Multidrug-resistant TB cases, patients with-extra pulmonary TB, co-existing malignancy, diabetes mellitus, chronic kidney disease, human immunodeficiency virus (HIV) infection, patients on vitamin D or mineral supplementation, patients on immunosuppressive drugs, patients who were sputum smear positive at the end of two months and patients with a prior history of anti-tuberculosis therapy (ATT) were excluded from the study. After the start of category I ATT as per directly observed treatment, short-course (DOTS), patients were followed up for the treatment adherence, sputum conversion and completion of treatment as per RNTCP protocol with the help of RNTCP staff.

Estimation of serum vitamin D

Blood sample (5 mL) was collected in a sterile plain vacutainer, under aseptic conditions from all the subjects. Serum was separated, labeled and stored at -20 ^{o}C. Sample collection procedure was repeated after two months (end of initiation phase) and after six months (end of continuation phase). All the samples were stored at -20 ^{o}C and the vitamin D estimation was done after completion of the study to eliminate bias. Vitamin D estimation was done by radio-immunoassay (RIA) using the commercially available kit of Beckman Coulter Inc. (Danaher Corporation company) where at first calibrators, controls and samples were incubated with the incubation buffer, in coated tubes, to release 25OH vitamin D3 and 25OH vitamin D2 from vitamin D binding protein (DBP), then without washing steps, a fixed amount of I^{125}-labeled 25OH vitamin D was added to compete with the 25OH vitamin D3 and 25OH vitamin D2 from samples, controls or calibrators, for a fixed amount of specific monoclonal antibody sites immobilised to the surface of the plastic tubes. After incubation, the content of tubes was aspirated; washed and bound radioactivity was measured. A calibration curve was plotted and the total 25OH vitamin D (D3 and D2) concentrations of the sample were determined by interpolation from the calibration curve.[7]

Statistical Analysis

The data were analysed using the IBM Statistical Package for the Social Sciences (SPSS, version 20). Descriptive statistics were performed initially. Continuous and categorical variables were defined. The Shapiro-Wilk test was carried out for continuous variables for checking normality of distribution. Parametric and non-parametric variables were defined. For association between parametric variables, repeated measures one way analysis of variance (ANOVA) was used. For non-parametric variables, Spearman correlation and Friedman's ANOVA were performed for association and correlation. Post-hoc tests were also performed to find out the exact association between variables. A P value of <0.05 was considered statistically significant.

Results

The mean age of the study population (28 males and 22 females) was 46.7±21.2 years. The mean serum vitamin D values progressively decreased during the course of the treatment (18.1±6.7 ng/mL at the time of starting ATT, 17.9±7.1 ng/mL at the end of 2nd month, 17.5±7.78 ng/mL at the end of 6th month). With a P value of 0.001, the co-relation was statistically significant. On analysing the gender distribution of the mean vitamin D levels on ATT, it was observed that though the levels

progressively decreased for both the groups, the mean vitamin D levels of female patients were much lower than male patients.

To further understand the factors influencing the vitamin D status, the mean vitamin D level at baseline (*i.e.*, at 0 month) was correlated with other study parameters, like number of subjects, gender, degree of anaemia, socio-economic status, severity of disease, alcohol consumption and smoking status.[8] It was found that the study subjects (5 [10%]) with the lowest mean vitamin D levels (*i.e.*, between 0–10 ng/mL) were female patients, non-smokers, did not consume alcohol, had severe disease, severe degree of anaemia, and belonged to the lower socio-economic status; while the study subjects (4 [8%]) with the highest mean vitamin D levels (*i.e.*, between 31–40 ng/mL) were males, smokers, consumed alcohol, had mild disease, were not anaemic, and belonged to the upper middle socio-economic status.

There was a decrease in the mean vitamin D level after treatment of the study subjects with DOTS; but it was observed to have increased in 16% (n=8) of the study subjects as compared to their baseline values. To analyse the variation, the study subjects were divided into two groups. Group A in whom the mean vitamin D increased from their baseline after treatment with DOTS (n=8) and group B in whom the mean vitamin D decreased from their baseline after treatment with DOTS (n=42). The progressive increase in the vitamin D levels in group A and the progressive decrease in the vitamin D levels in group B were statistically significant (P<0.001). The basic characteristics of the two groups were compared and are summarised in the figure.

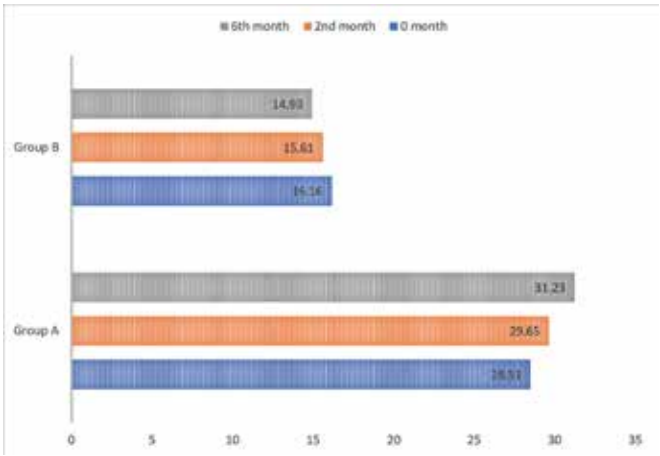

Figure. Comparison of the mean vitamin D levels at 0, 2, 6 months between group A and group B (X axis indicate mean vitamin D values in ng/mL)

Discussion

The mean age of the study population was 46.7±21.2 years; this was in concordance with an earlier study by Bilagi *et al*[9] in whose study of 113 patients of PTB; the mean age was 41.2±16.7 years. Out of the 50 subjects enrolled in this study, there were 28 males and 22 females which is also in concordance accordance with the *WHO Global Tuberculosis Report 2018* stating that most of the TB cases occur in men.[1]

In a study by Afzal *et al*[10], it was found that the risk factors for acquiring PTB were poor nutritional and socio-economic status, houses having inadequate sunlight, bad sanitary conditions, malnutrition, illiteracy and low body mass index. In the present study, 34 out of the 50 (68%) study subjects were anaemic. Similar findings were reported in a study by Hella *et al*[11] who studied 102 TB cases and 98 controls without TB and found that anaemia of chronic disease was more frequent among cases than controls (59.8% *versus* 26.1%). The study subjects were divided into 5 groups based on their socio-economic status according to the modified Kuppuswamy scale, majority of the study participants (68%) were in the upper lower and lower class which is in accordance with a study by Kashyap *et al*[12] which suggests low SES to be an important risk factor for TB infection.

The mean concentration of vitamin D of the study subjects was 18.1±6.7 ng/mL at the time of diagnosis. It was observed that 92% of the study subjects had hypovitaminosis D (less than 30 ng/mL) at baseline. Similar results were also observed by Naik *et al*[13] in 87% of the study subjects. Goswami *et al*[14] reported that vitamin D deficiency increases the probability of progression of TB in subjects infected with TB to active disease; which is also supported the study of Ralph *et al*.[15] The mean vitamin D was lower in female patients (12.9±3.8 ng/mL at 0 month, 12.4±3.6 ng/mL at 2 months and 11.6±3.5 ng/mL at 6 months) when compared to male patients (22.3±5.5 ng/mL at 0 months, 22.1± 6.2 ng/mL at 2 months and 22.2±7.0 ng/mL at 6 months) which is again similar to the results in the study by Talat *et al*.[16]

Mean vitamin D concentration was 17.5±7.8 ng/mL after DOTS which is a significant reduction (P<0.001). Similar trends were also reported in the study by Naik *et al*[13] where the mean vitamin D concentration after DOTS was 17.49 ± 9.7ng/mL (P <0.041). The decrease in vitamin D levels in this study may be attributed to the effects of anti-tubercular drugs; especially rifampicin which causes accelerated loss of vitamin D due to increased clearance as well as isoniazid which causes impairment of 25-hydroxylation leading to impaired vitamin D action.[13]

In the studies reviewed by Ralph *et al*[17], spontaneous improvement in vitamin D during TB treatment was noted in some patients, which was attributed to the elimination of infection. Tostmann *et al*[18] reported

an increase in vitamin D levels after two months of treatment in 81 patients with PTB, which was attributed to improved nutrition and increased sunlight exposure. In our study, 16% of patients showed an increase in vitamin D levels as compared to their baseline values. To analyse the variation, the study subjects were divided into two groups; Group A–patients in whom vitamin D increased after DOTS (n=8) and Group B–patients in whom vitamin D reduced after DOTS (n=42). On analysing the various parameters of the study and comparing it between the two groups, it was hypothesized that younger age, improved socio-economic status and better nutrition was attributed to the statistically significant (P <0.001) improved outcomes in group A as was also reported by Naik *et al*[13] in their study.

Conclusion

It is postulated that vitamin D supplementation in tuberculosis treatment programmes may also have an effect on outcome, thereby, the need for further studies to ascertain the exact role, dose and duration of vitamin D augmentation in tuberculosis patients.

References

1. Global Tuberculosis Report 2018. World Health Organization, Geneva; 2018. *Available at URL:* https://www.who.int/tb/publications/global_report/en/. Accessed on January 20, 2019.
2. Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D, Srinivasarao PV, Sarma KV, *et al*. High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy South Indians. *Am J Clin Nutr* 2007;85:1062–7.
3. Kearns MD, Tangpricha V. The role of vitamin D in tuberculosis. *J Clin Transl Endocrinol* 2014;1:167–9.
4. Sutaria N, Liu CT, Chen TC. Vitamin D status, receptor gene polymorphisms, and supplementation on tuberculosis: a systematic review of case-control studies and randomized controlled trials. *J Clin Transl Endocrinol* 2014;1:151–60.
5. Wang Z, Lin YS, Zheng XE, Senn T, Hashizume T, Scian M, *et al*. An inducible cytochrome P4503A4-dependent vitamin D catabolic pathway. *Mol Pharmacol* 2012;81:498–509.
6. Raviglione MC, O'Brein RJ. Tuberculosis. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, editors *Principles of Internal Medicine by Harrison's;* 18th edition. New York: Mcgraw-Hill; 2011:pp 1340–57.
7. Hollis BW, Kamerud JQ, Kurkowski A, Beaulieu J, Napoli JL. Quantification of circulating 1, 25-dihydroxy vitamin D by radioimmunoassay with I125-labelled tracer. *Clin Chem* 1996;42:586–92.
8. The management of nutrition in major emergencies 2000. World Health Organization, Geneva; 2000. *Available at URL:* http://whqlibdoc.who.int/publications/2000/9241545208.pdf. Accessed on January 28, 2019.
9. Lee SW, Kang YA, Yoon YS, Um SW, Lee SM, Yoo CG, *et al*. The prevalence and evolution of anemia associated with tuberculosis. *J Korean Med Sci* 2006;21:1028–32.
10. Afzal A, Rathore R, Butt NF, Randhawa FA. Efficacy of vitamin D supplementation in achieving an early sputum conversion in smear positive pulmonary tuberculosis. *Pak J Med Sci* 2018;34:849-54.
11. Hella J, Cercamondi CI, Mhimbira F, Sasamalo M, Stoffel N, Zwahlen M, *et al*. Anaemia in tuberculosis cases and household controls from Tanzania: contribution of disease, coinfections, and the role of hepcidin. *PLoS One* 2019;13:e0195985.
12. Rajpal SK, Amit RN, Aliabbas AH, Seema DS, Ashish RS, Ruchika KJ, *et al*. Impact of socioeconomic status and living condition on latent tuberculosis diagnosis among the tribal population of Melghat: a cohort study. *Lung India* 2016;33:372–80.
13. Naik AL, Rajan MG, Manjrekar PA, Shenoy MT, Shreelata S, Srikantiah RM, *et al*. Effect of DOTS treatment on vitamin D levels in pulmonary tuberculosis. *J Clin Diagn Res* 2017;11:BC18–BC22.
14. Goswami R, Mishra SK, Kochupillai N. Prevalence and potential significance of vitamin D deficiency in Asian Indians. *Indian J Med Res* 2008;127:229–38.
15. Ralph AP, Lucas RM, Norval M. Vitamin D and solar ultraviolet radiation in the risk and treatment of tuberculosis. *Lancet Infect Dis* 2013;13:77–88.
16. Talat N, Perry S, Parsonnet J, Dawood G, Hussain R. Vitamin D deficiency and tuberculosis progression. *Emerg Infect Dis* 2010;16:853–5.
17. Ralph AP, Kelly PM, Anstey NM. L-arginine and vitamin D: novel adjunctive immunotherapies in tuberculosis. *Trends Microbiol* 2008;16:336–44.
18. Tostmann A, Wielders JP, Kibiki GS, Verhoef H, Boeree MJ, van der Ven AJ. Serum 25-hydroxy-vitamin D3 concentrations increase during tuberculosis treatment in Tanzania. *Int J Tuberc Lung Dis* 2010;14:1147–52.