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CAN VITAMIN D SUPPLEMENTATION HASTEN RECOVERY OF TUBERCULOSIS

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ABSTRACT

Background: Vitamin D has been shown to be involved in the host immune response toward Mycobacterium tuberculosis. **Aim:** To test whether vitamin D supplementation in treatment of patients with tuberculosis improved clinical outcome and could influence recovery. **Methods:** The randomized, double-blind, placebo controlled trial was conducted in TB clinics at a demographic surveillance site in Burdwan under control of Burdwan medical College. Six hundred and twenty two patients with pulmonary TB were randomized to receive either 600,000 IU of Intramuscular vitamin D3 or placebo for 2 doses. Assessments were performed in each month upto 6 months. **Results:** Reduction in TB score and sputum smear conversion rates were significantly differ among patients treated with vitamin D or placebo. **Conclusions:** Supplementation with high doses of vitamin D accelerated clinical, sputum conversion rate in all TB patients and increased host immune activation in patients. These results suggest a therapeutic role for vitamin D in the treatment of TB.

KEYWORDS: Vitamin D supplementaton, Tuberculosis, Antitubercular treatment.

1. INTRODUCTION

Despite the widespread availability of antimicrobials, bacterial respiratory infections remain a major global cause of death. [1] Mortality is associated with infection with antibiotic-resistant organisms[2, 3] and with failure to resolve immunopathological inflammatory responses.[4–6] Immunomodulatory agents that augment antimicrobial immune responses and accelerate resolution of pulmonary inflammation could be used as adjuncts to antimicrobial therapy to improve treatment outcomes.[7]

Calcitriol, the active metabolite of vitamin D, induces innate antimicrobial responses and suppresses pro-inflammatory cytokine responses in vitro.[8] Calcitriol's antimicrobial activity is mediated via induction of reactive nitrogen intermediates, reactive oxygen intermediates, antimicrobial peptides, and autophagy.[9] Calcitriol is synthesized by the vitamin D 1-α hydroxylase enzyme, the expression of which is up-regulated in leukocytes and pulmonary epithelium following ligation of Toll-like receptors by pathogen-associated ligands.[10, 11] Extrarenal generation of calcitriol is dependent on the availability of its precursor calcidiol, the major circulating vitamin D metabolite that supports induction of antimicrobial responses in vitro[10, 12] and the concentrations of which are often low in patients with pulmonary infection.[13–15]

Vitamin D was used to treat tuberculosis in the preantibiotic era,[16] and vitamin D supplementation has been shown to enhance healthy tuberculosis contacts' immunity to mycobacteria.[17] These observations prompted us to conduct a randomized controlled trial evaluating the influence of adjunctive high-dose vitamin D on time to bacterial clearance in patients receiving antimicrobial therapy for smear-positive pulmonary tuberculosis.

2. MATERIAL AND METHODS

2.1 Study area

This randomized, double-blind placebo-controlled clinical trial was conducted in the TB clinics at a demographic surveillance site in Burdwan under control of Burdwan medical College, Burdwan, West Bengal, India.

2.2 Selection of subjects

A total of 622 from 1846 patients aged 18-60 years who had been newly diagnosed with pulmonary tuberculosis who attended Burdwan Medical College of Burdwan district were selected as study population by simple random sampling after informed consent had been received between February 2011 and October 2013. Pulmonary TB was diagnosed if at least one of the following criteria were met: (1) Isolation of Mycobacterium tuberculosis from sputum or other clinical specimens. (2) A positive polymerase chain reaction test for TB in sputum or other clinical specimens. (3) Presence of caseation granulomas in tissue. (4) Lymphocyte predominant exudative effusion with an adenosine deaminase level >40 IU/L. Based on clinical history taking and clinical records, patients with extrapulmonary TB, human immunodeficiency virus (HIV) infection, hepatic disease, renal failure, malignancy, diabetes mellitus, pregnancy, sarcoidosis, hyperparathyroidism or those taking any

corticosteroids, immunosuppressive agents, thiazide diuretics or drugs known to interfere with vitamin D levels (phenytoin, phenobarbital, carbamazepine, theophylline) were excluded from the study. All the tubercular patients continued to receive Directly Observed Therapy (DOTS) with 2 months of 4 antitubercular drugs (Isoniazid, Rifampicin, Ethambutol and Pyrazinamide) followed by 6 months of Isoniazid and Ethambutol.

2.3 Study design

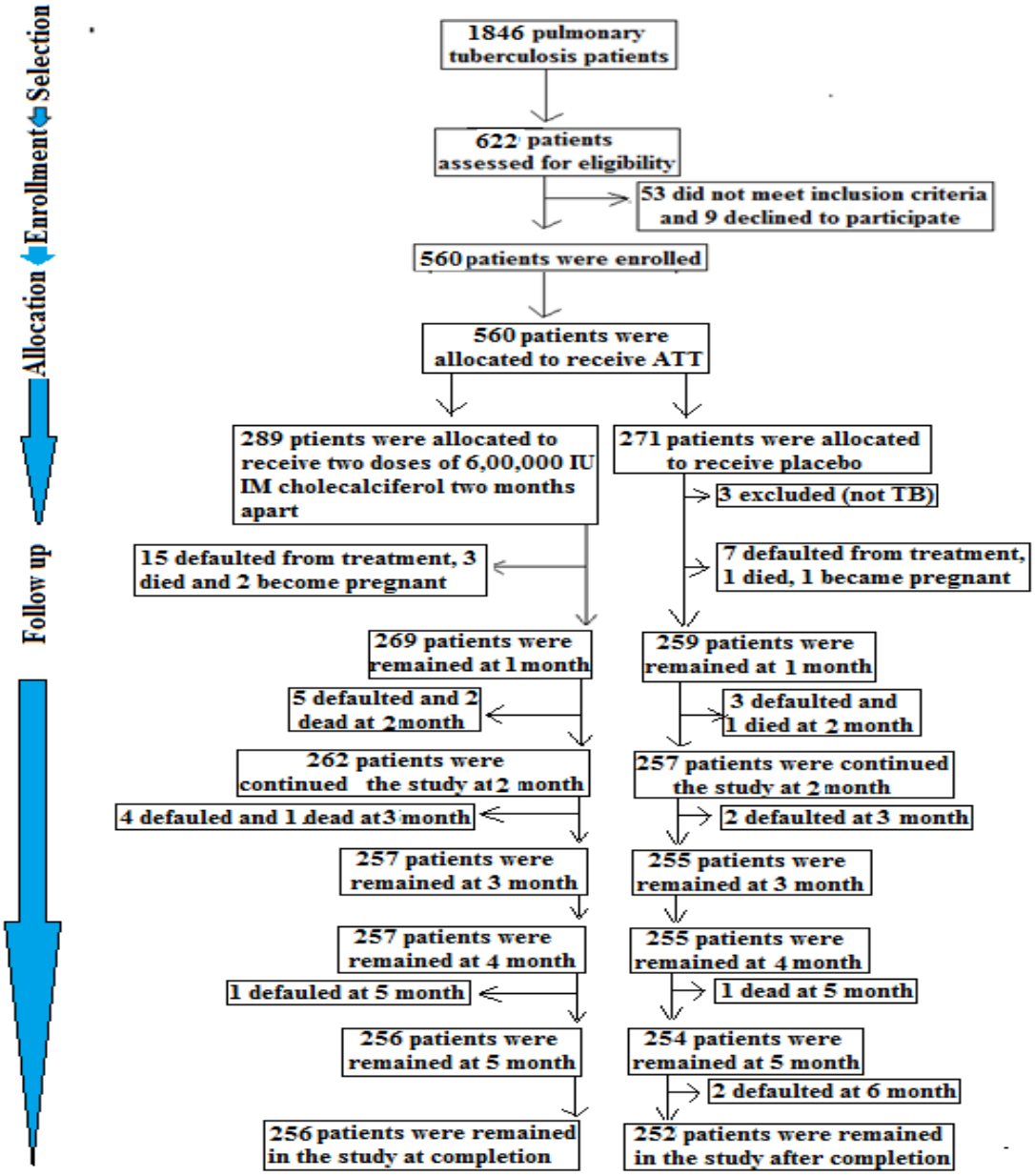

Figure 1: Study design of the present study

2.3 Enrolment Procedures

Six hundred and forty six twenty two patients were screened for eligibility. Five hundred and sixty patients were enrolled and randomized to the two treatment arms. Two hundred and

eighty nine in the cholecalciferol supplementation arm and 271 in the placebo arm, as shown in Figure 1.

The one study arms were either administered 600,000 IU of intramuscular vitamin D3 (cholecalciferol) for 2 doses two months apart within three months study period or an equivalent volume of placebo (normal saline) matched for colour. The rationale behind the choice of dosage and time points was a concern that vitamin D might induce hypercalcemia, and therefore no intervention was given when patients were seen at 2 months. Instead, samples were collected for calcium measurement and hypercalcemia was assessed at the first interim analysis. We chose administration of a few large doses so as not to further increase the substantial pill burden on the patients and because it was a simple intervention applicable to low-resource settings. A single large dose has been shown as effective as daily administration, and the half-life is 2 months.[18] The dosage was known to be efficient for treatment of vitamin D deficiency and also safe to give with no deficiency present and even in pregnancy.[19]

2.4 Randomization Procedures

The random allocation sequence was computer generated a list of continuous study numbers was generated with a random allocation to treatment 1 or 2. Study numbers were consecutive and given to patients by the field assistant at inclusion, and patients were recorded in a book with prewritten study numbers and allocation sequence numbers 1 or 2. Study medicine was provided in identical containers labelled lot 311 (allocation sequence number 1) or lot 312 (allocation sequence number 2). A physician gave the trial information, obtained patient's consent, and conducted the clinical examination; a trial nurse administered study medicine according to sequence number.

2.5 Blinding

The patient, primary physicians, investigator physicians, study coordinator, site assistants were blinded to the treatment allocation. Trial medicine was available in two lots and for logistical reasons it was not concealed whether a patient was on lot 1 or lot 2 during the trial. Blinding was not assessed among patients, but we assessed blinding among 10 field assistants and investigators through tasting both lots. Six voted in favour of lot 312 being vitamin D, seven voted in favour of lot 311. The randomization code was broken by the primary investigator in November 2013 on completion of data analysis. The randomization sequence

was generated by the Department of Pharmacology, Burdwan Medical College, WBHUS University, who were responsible for dispensing the study drug.

2.6 Follow up

The outcome of study groups was assessed by clinical assessments, differences in sputum conversion rates, using differences in anthropometric parameters and resolution of chest radiograph abnormalities. The outcome variables were measured at 0, 1,2,3,4,5 and 6 months of treatment followed by a household visit at least one visit in a month after initiation of treatment followed by a household visit 16 months. The patients were asked for the following adverse effects related to hypercalcemia such as nausea, vomiting, excessive thirst, anorexia, symptoms of kidney stones, and confusion. To show the safety of vitamin D supplementation in patients with TB, we investigated whether vitamin D increased serum calcium concentrations significantly. A significant change in serum calcium was defined as albumin-corrected serum calcium increasing to a concentration greater than 2.75 mmol/L in a patient with no hypercalcemia before vitamin D supplementation. Calcium concentrations were measured in patients seen at 2 months, and completing 6 months of treatment. After the first interim analysis of calcium concentrations at 2 months, it was proceeded to give vitamin D at 2 months.

2.7 Interim Analysis

A data and safety monitoring board was established to monitor the study. Interim safety analyses were done at 2 and 16 months for calcium concentrations and mortality, and were blinded to treatment group. No clinical outcomes were analyzed, only safety; the predefined stopping rule was a difference in mortality between the two treatment arms at the 5% significance level.

2.8 Clinical assessment

Clinical examination was used to calculate a TB score for estimation of TB severity [20] for all visits. The TB score is a validated assessment tool developed to objectively measure change in the clinical status of TB patients. Its components include self-reported symptoms (cough, shortness of breath, night sweats, chest pain, haemoptysis), clinical signs (tachycardia, pallor, fever, auscultatory findings). The TB score achieved can range from 0–13. TB scores were further divided into 3 severity classes; Severity Class I (TB score 0 to 5), Class II (TB score 6 – 7) and Class III (TB score ≥ 8).

2.9Anthropometric measurements

BMI: Weight and height measurements were obtained, using standardized technique.[21] BMI was calculated as the weight in kilograms divided by the square of height in meters. Body mass index (BMI) was calculated as the weight (kg) divided by the square of height (m^2).

Mid Upper Arm Circumference (MUAC): It was recorded at the mid-point of the acromion and the olecranon process over the biceps muscle of the non-dominant arm, using a non-stretchable measuring tape (TALC, Guilford, UK) to the nearest 0.2 cm.[22]

2.10 Laboratory investigation

Peripheral venous blood was drawn at 0 and 12 weeks of study and allowed to coagulate at room temperature for 30–45 min, followed by centrifugation at 2500Xg for 15 min. All serum samples were stored at -70^{0}C and kept under these conditions until chemical analysis was performed. Serum 25(OH)D was estimated by enhanced chemiluminescent assay (ECI) using instrument VITROS *eci* (Johnson &Johnson) & dedicated reagent.[23] The inter- and intra-assay coefficients of variation were 6.4 and 5.7%. Vitamin D estimation should be done in each monthly visit. Serum calcium and albumin were measured by absorbance (COBAS Integra; Roche Diagnostics, Mannheim, Germany). Corrected total serum calcium for individual variations in albumin was measured by the following equation: adjusted serum calcium (mmol/L) = serum calcium total (mmol/L) X 0.00086 X (650 - serum albumin [mmol/L]).

2.11 Statistical analysis

It was expressed the variables by their means or medians and standard deviations or range. The Pearson chi-square (x^2) was used to assess statistical differences in proportions between groups ($P < 0.05$). The Student's t test was to assess differences in means between two groups when a normal distribution was present and the Wilcoxon rank-sum test when nonparametric analysis was needed. The data for biochemical analysis was subjected to standard statistical analysis using the Statistical Package for Social Science (SPSS) 11.5 software for windows.

3. RESULT

3.1 The characteristics of the study population

Baseline characteristics of the two study populations are shown in Table 1. The two arms did not statistically differ significantly.

Table 1: Baseline personal profile and clinical details of two study groups.

Demographic profiles	Randomization		p value
	Drug intervention group (n = 289)	Placebo group (n = 271)	
Age (Years)	35.71 ± 11.84	34.67 ± 9.58	0.182
Sex Male Female	 150 (51.9) 139 (48.1)	 141 (52) 130 (48)	0.381
BMI (Kg/m^2)	18.29 ± 5.14	17.98 ± 4.92	0.279
MUAC (cm.)	20.7 ± 5.36	21.11 ± 4.87	0.109
TB score	7.54 ± 1.98	7.34 ± 1.81	0.224
Sputum smear positive	246 (85)	236 (87)	0.145
Albumin-corrected serum calcium (mmol/L)	2.01 ± 0.29	2.02 ± 0.25	0.098
Serum 25(OH)D concentration in ng/ml	30.02 ± 6.91	29.56 ± 5.12	0.195

Data are expressed as numbers (group percentages in parentheses) for categorical variables and mean values ± SD for continuous variables; $p < 0.05$ consider statistically significant.

3.2 Changes in measured clinical variables before and at various time points of the antitubercular therapy

In the Figure 1, it was observed that the group with high dose vitamin D supplementation had faster recovery from pulmonary TB as reflected by TB score than placebo arm.

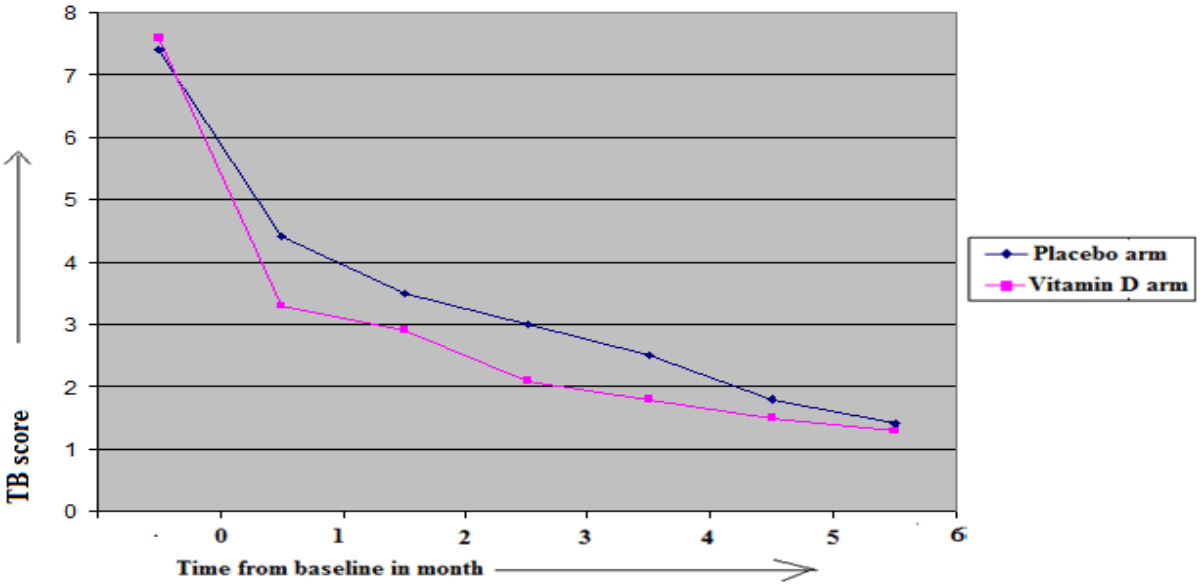

Figure 2: TB score at various time points for the two treatment arms.

Evaluation of sputum microscopy data for the study subjects showed that high dose vitamin D supplementation had faster sputum conversion rate than placebo arm as shown in the Figure 2.

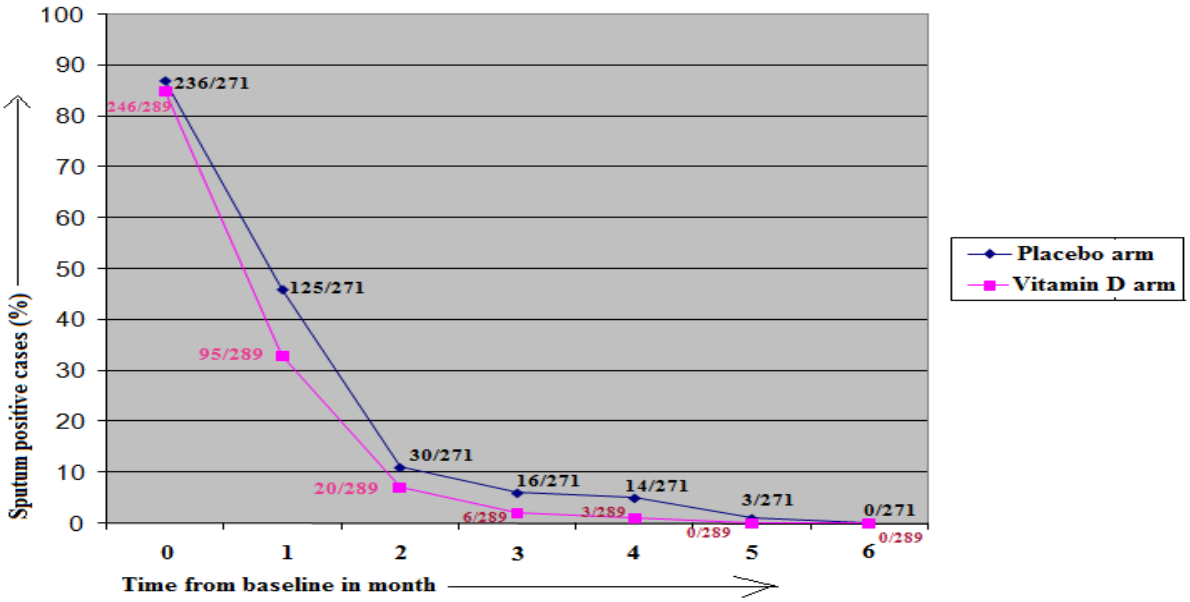

Figure 3: Proportion of patients with positive acid-fast bacteria sputum examination at various time points for the two treatment arms.

3.3 Serum 25(OH)D status in different time points of the study among two groups

When serum 25(OH)D was measured in both groups of the present study it was found that vitamin D concentration was significantly decreased gradually as time proceed in group receiving only ATT but group receiving vitamin D supplementation along with ATT concentration of vitamin D did not differ significantly with time span of ATT.

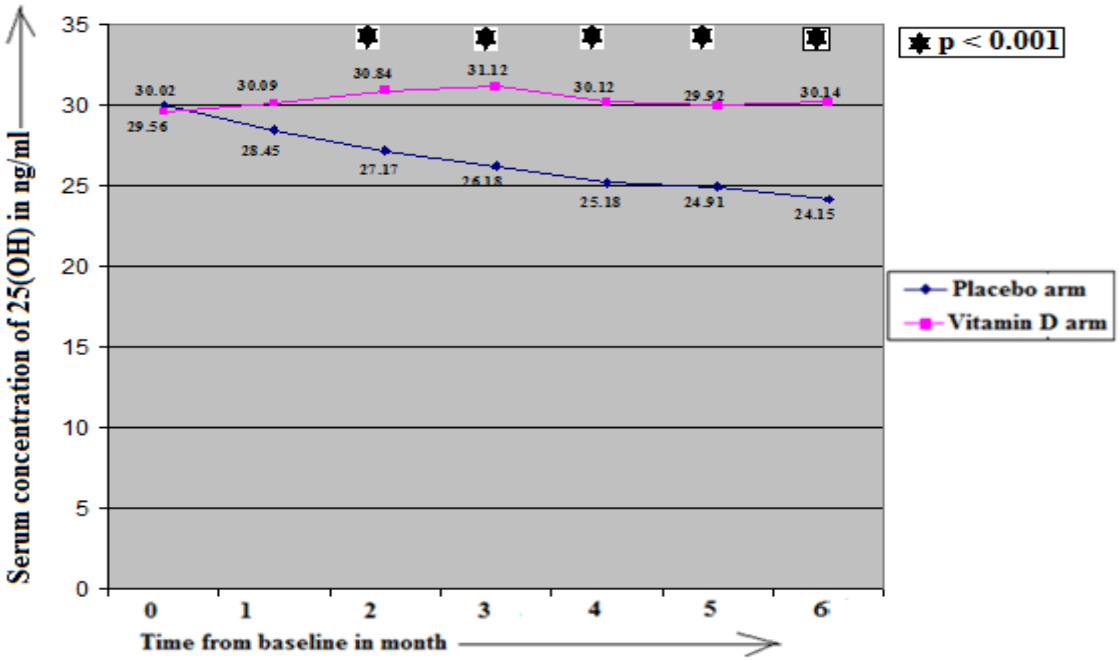

Figure 4: Mean vitamin D serum levels before and during study of two study arms.

In vitamin D arm, the mean 25-OHD levels on follow up were not significantly different from those of the baseline value as Figure 4(a). In placebo arm, although the mean 25-OHD levels on first month follow up (28.45ng/ml) were not significantly different from those of the baseline data, average 25-OHD levels further decreased with increasing months of follow up. Indeed, from second month of follow up onwards, 25-OHD levels differed significantly from those of baseline value (27.17, 26.17, 25.18, 24.91 and 24.15 ng/ml for 2,3,4,5 and 6 month, respectively; $p<0.001$; figure 4b). Mean 25-OHD levels were almost 20% lower in 6th month follow up compared with baseline value.

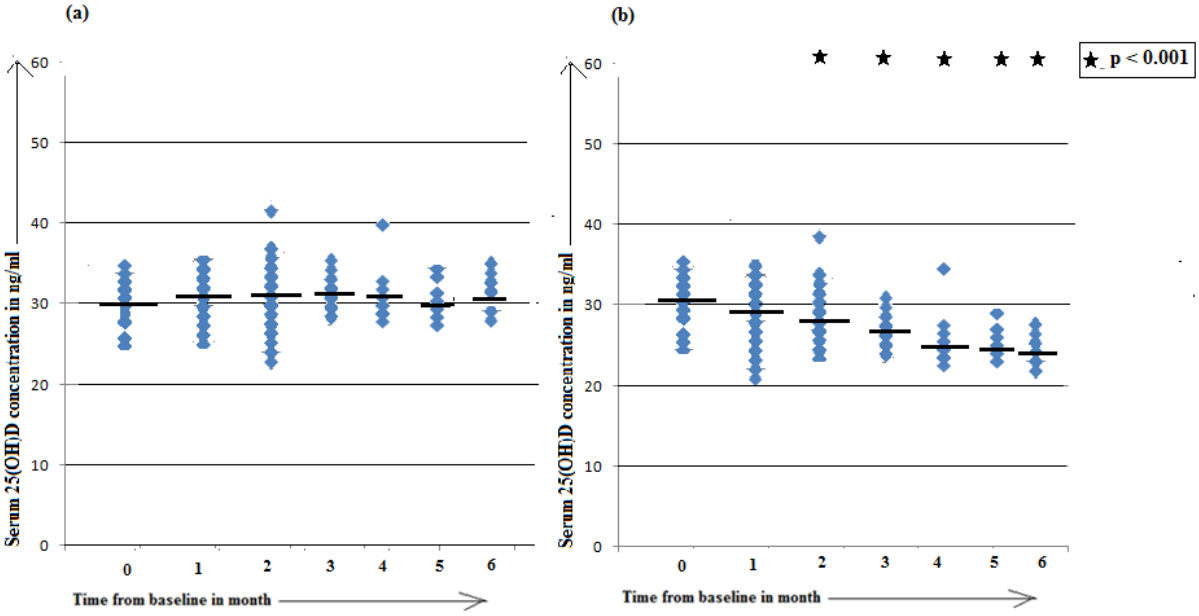

Figure 5: (a) Plot showing 25-OHD levels according to the various time points in vitamin D arm. (b) Plot showing 25-OHD levels according to the various time points in placebo arm. An analysis of variance (ANOVA) univariate test followed by a Tukeye Kramer posthoc test for testing multiple groups was used to calculate significances. Asterisks indicate p<0.0001.

4. DISCUSSION

Vitamin D was used for treatment of tuberculosis (TB) in the preantibiotic era[24–26] and before then cod liver oil, rich in vitamin D, was used as well as sun exposure.[27] Then it was proved that Vitamin D has been attributed an important role in host immune defence against Mycobacterium tuberculosis.[28,29] So, the present study was conducted to evaluate the influence of adjunctive high-dose vitamin D on time to bacterial clearance in patients receiving antimicrobial therapy for smear-positive pulmonary tuberculosis. It was shown that high doses of Vitamin D given to patients with active pulmonary TB can lead to

proportionately greater improvement of TB score and more rapid sputum clearing for mycobacterium as compared to placebo as some recent studies.[30-32] A possible mechanism of vitamin D–mediated effect on TB treatment efficacy is that 1,25-dihydroxyvitamin D (calcitriol) has been shown to induce antimycobacterial activity in macrophages in vitro, to upregulate protective innate host responses, and to trigger antimicrobial peptides such as cathelicidin.[28] No data are available on dose dependent effects of vitamin D on mycobacterial activity, but it has been speculated that pharmacologic doses of vitamin D may elevate serum 25-hydroxyvitamin D [25(OH)D] concentrations to levels saturating the ability of vitamin D–binding protein to bind vitamin D metabolites, leading to an increase in biological availability of 1,25-dihydroxyvitamin D in infected tissues.[33] However, Wejse et al. found no effect of supplementation on disease outcome.[34] The study addressed the use of 100,000 IU of vitamin D given as an adjunctive treatment at the time of commencement of anti-TB therapy, and again at 5 and 8 months after starting treatment. The authors note that the dose may have been insufficient, and the response to vitamin D dependent on the immune status of the individual patient.

Another interesting finding our study was that though the baseline serum vitamin D value was not significantly differ among the two groups but after 6 month complete course of ATT in pulmonary tuberculosis vitamin D concentration was significantly low in TB receiving only ATT than patient taking ATT along with perenteral vitamin D. Some previous reports have also shown a definite decline in vitamin D levels after prolonged TB treatment[13-15,35] which is in agreement with the present findings. The decline in vitamin D levels with treatment may be explained by enhanced vitamin D metabolism due to the influence of two of the standard first-line anti-tuberculosis drugs, isoniazid and rifampicin on cytochrome P450 activity.[36,37] Isoniazid reduces 25[OH]D and 1,25[OH]2D concentrations by the inhibition of 25-hydroxylase, as has been shown in vitro studies, animal studies and human volunteers.[36,38,39] Rifampicin is a strong inducer of CYP3A4. Induction of these enzymes increases the enzymatic conversion of 25[OH]D to the inactive metabolite 24,25[OH]2D and results in decreased 25[OH]D and 1,25[OH]2D concentrations, as shown in studies in human volunteers.[40,41] Combined use of Isoniazid (INH) and rifampicin reduces 25[OH] D and 1,25[OH]2D concentrations in both human volunteers and TB patients. That may be one of important reason of delayed clinical and sputum conversion. Vitamin D supplementation elevates circulating calcitriol concentrations, and may therefore enhance response to antimicrobial therapy for respiratory infections.

5. CONCLUSION

This study proposes that high dose vitamin D supplementation can lead to a more marked clinical recovery as well as sputum clearance in all patients with pulmonary TB.

REFERENCES

1. WHO Global Burden of Disease: 2004 Update (WHO, Geneva), 2008.
2. Tleyjeh IM, Tlaygeh HM, Hejal R, Montori VM, Baddour LM. The impact of penicillin resistance on short-term mortality in hospitalized adults with pneumococcal pneumonia: A systematic review and meta-analysis. Clin Infect Dis, 2006; 42: 788–797.
3. Gandhi NR et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet, 2006; 368: 1575–1580.
4. Barnes PF et al. Predictors of short-term prognosis in patients with pulmonary tuberculosis. J Infect Dis, 1988; 158: 366–371.
5. Kellum JA et al.; GenIMS Investigators Understanding the inflammatory cytokine response in pneumonia and sepsis: Results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med, 2007; 167: 1655–1663.
6. Yende S, et al. GenIMS Investigators. Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis. Am J Respir Crit Care Med, 2008; 177: 1242–1247.
7. Siempos II, Vardakas KZ, Kopterides P, Falagas ME. Adjunctive therapies for community-acquired pneumonia: a systematic review. J Antimicrob Chemother, 2008; 62: 661–668.
8. Martineau AR, et al. IFN-γ- and TNF-independent vitamin D-inducible human suppression of mycobacteria: The role of cathelicidin LL-37. J Immunol, 2007; 178: 7190–7198.
9. Hewison M. Antibacterial effects of vitamin D. Nat Rev Endocrinol, 2011; 7: 337–345.
10. Liu PT et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science, 2006; 311: 1770–1773.
11. Hansdottir S et al. Respiratory epithelial cells convert inactive vitamin D to its active form: Potential effects on host defense. J Immunol, 2008; 181: 7090–7099.
12. Fabri M et al. Vitamin D is required for IFN-γ-mediated antimicrobial activity of human macrophages. Sci Transl Med, 2011; 3(104): 104ra102.

13. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. Eur J Clin Nutr, 2004; 58: 563–567.
14. Martineau AR, et al. Association between Gc genotype and susceptibility to TB is dependent on vitamin D status. Eur Respir J, 2010; 35: 1106–1112.
15. Martineau AR, et al. Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. Proc Natl Acad Sci USA, 2011; 108: 19013–19017.
16. Martineau AR, Honecker FU, Wilkinson RJ, Griffiths CJ. Vitamin D in the treatment of pulmonary tuberculosis. J Steroid Biochem Mol Biol, 2007; 103: 793–798.
17. Martineau AR, et al. A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med, 2007; 176: 208–213.
18. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr, 1999; 69: 842–856.
19. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ, 2003; 326: 469.
20. Wejse C, Gustafson P, Nielsen J, Gomes VF, Aaby P, Andersen PL, Sodemann M: TBscore: Signs and symptoms from tuberculosis patients in a low-resource setting have predictive value and may be used to assess clinical course. Scand J Infect Dis, 2008; 40(2): 111–120.
21. Deepa M, Pradeepa R, Rema M, Mohan A, Deepa R, Shanthi Rani S, Mohan V. The Chennai Urban Rural Epidemiology Study (CURES): Study design and Methodolgy (Urban component) CURES -1 J. Assoc. Physicians India, 2003; 51: 863-870.
22. Powell-Tuck J, Hennessy EM. A comparison of mid upper arm circumference, body mass index and weight loss as indices of undernutrition in acutely hospitalized patients. Clin Nutr, 2003; 22: 307–312.
23. Wagner D, Hanwell HEC, Vieth R. An evaluation of automated methods for measurement of serum 25-hydroxyvitamin D. Clinical Biochemistry, 2009; 42: 1549–1556.
24. Dowling GB, Prosser Thomas EW. Treatment of lupus vulgaris with calciferol. Lancet, 1946; 1: 919–922.
25. Ellman P, Anderson K. Calciferol in tuberculosis peritonitis with disseminated tuberculosis. BMJ, 1948; 1: 394.

26. Brincourt J. Liquefying effect on suppurations of an oral dose of calciferol. Presse Med, 1969; 77: 467–470.
27. Bennett JH. The pathology and treatment of pulmonary tuberculosis, 1st ed. Edinburgh: Sutherland and Knox, 1853; 138.
28. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, et al. Toll-like receptor triggering of a vitamin D–mediated human antimicrobial response. Science, 2006; 311: 1770–1773.
29. Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, Packe GE, Davidson RN, Eldridge SM, Maunsell ZJ, et al. A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med, 2007; 176: 208–213.
30. Salahuddin N, Ali F, Hasan Z, Rao N, Aqeel M, Mahmood F. Vitamin D accelerates clinical recovery from tuberculosis: results of the SUCCINCT Study [Supplementary Cholecalciferol in recovery from tuberculosis]. A randomized, placebo-controlled, clinical trial of vitamin D supplementation in patients with pulmonary tuberculosis BMC Infectious Diseases, 2013; 13:22.
31. Nursyam EW, Amin Z, Rumende CM. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. Acta Med Indones, 2006; 38: 3–5.
32. Morcos MM, Gabr AA, Samuel S, Kamel M, el Baz M, el Beshry M, Michail RR. Vitamin D administration to tuberculous children and its value. Boll Chim Farm, 1998; 137: 157–164.
33. Martineau AR, Honecker FU, Wilkinson RJ, Griffiths CJ. Vitamin D in the treatment of pulmonary tuberculosis. J Steroid Biochem Mol Biol, 2007; 103: 793–798.
34. Wejse C, Gomes VF, Rabna P, Gustafson P, Aaby P, Lisse IM, Andersen PL, Glerup H, Sodemann M. Vitamin D as Supplementary Treatment for Tuberculosis: A Double-blind, Randomized, Placebo-controlled Trial Am J Respir Crit Care Med, 2009; 179: 843–850.
35. Hughes B. Serum 25-hydroxyvitamin D and function outcomes in the elderly. Am. J. Clin. Nutr, 2008; 88: 537S–40S.
36. Desta SZ, Flockhart DA. Inhibition of cytochromeP450 (CYP450) isoforms by isoniazid: potent inhibition ofCYP2C19 and CYP3A. Antimicrob. Agents Chemother, 2001; 45: 382–92.
37. Martineau AR, Honecker FU, Wilkinson RJ, Griffiths CJ. Vitamin D in the treatment of pulmonary tuberculosis. J Steroid BiochemMol Biol, 2007; 103: 793–798.

38. Bengoa JM, Bolt MJ, Rosenberg IH. Hepatic vitamin D 25- hydroxylase inhibition by cimetidine and isoniazid. J Lab Clin Med, 1984; 104: 546–552.
39. Gupta RP, He YA, Patrick KS, Halpert JR, Bell NH. CYP3A4 is a vitamin D-24- and 25-hydroxylase: analysis of structure function by site-directed mutagenesis. J Clin Endocrinol Metab, 2005; 90: 1210–1219.
40. Goodwin B, Hodgson E, Liddle C. The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module. MolPharmacol, 1999; 56: 1329–1339.
41. Brodie MJ, Boobis AR, Dollery CT, *et al.* Rifampicin and vitamin D metabolism. ClinPharmacol The, 1980; 27: 810–814.