Adjunctive vitamin D for treatment of active tuberculosis in India: a randomised, double-blind, placebo-controlled trial

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Summary

Background **Vitamin D has immunomodulatory effects that might aid clearance of mycobacterial infection. We aimed to assess whether vitamin D supplementation would reduce time to sputum culture conversion in patients with active tuberculosis.**

Methods **We did this randomised, double-blind, placebo-controlled, superiority trial at 13 sites in India. Treatment-naive patients who were sputum-smear positive, HIV negative, and had pulmonary tuberculosis were randomly assigned (1:1), with centrally labelled, serially numbered bottles, to receive standard active tuberculosis treatment with either supplemental high-dose oral vitamin D_3 (four doses of 2·5 mg at weeks 0, 2, 4, and 6) or placebo. Neither the patients nor the clinical and laboratory investigators and personnel were aware of treatment assignment. The primary efficacy outcome was time to sputum culture conversion. Analysis was by modified intention to treat. This trial is registered with ClinicalTrials.gov, number NCT00366470.**

Findings **Between Jan 20, 2010, and Aug 23, 2011, we randomly assigned 247 participants to the vitamin D group (n=121) or the placebo group (n=126), of whom 211 participants (n=101 and n=110, respectively) were included in the primary efficacy analysis. Median time to culture conversion in the vitamin D group was 43·0 days (95% CI 33·3–52·8) versus 42·0 days (33·9–50·1) in the placebo group (log-rank p=0·95). Three (2%) patients died in the vitamin D group and one (1%) patient died in the placebo group; no death was considered attributable to the study intervention. No patients had hypercalcaemia.**

Interpretation **Our findings show that vitamin D supplementation did not reduce time to sputum culture conversion. Further studies should investigate the role of vitamin D in prevention or reactivation of tuberculosis infection.**

Funding **Dalhousie University and Infectious Diseases Training and Research Centre.**

Introduction

Chemotherapy for active *Mycobacterium tuberculosis* infection includes a prolonged course of combination antibiotic drugs.[1] Use of vitamin D for tuberculosis treatment began in 1849, with the observation that oil from fish liver improved appetite and strength.[2] Vitamin D has immunoregulatory functions that might have a role in the treatment of many diseases.[3–5] 1,25-dihydroxyvitamin D (1,25-$[OH]_2$D) enhances killing of mycobacteria by macrophages and accelerates resolution of inflammatory responses during tuberculosis treatment.[6]

Eight randomised trials reporting the effectiveness or safety of the addition of vitamin D supplementation to standard tuberculosis treatment have been published; however, methods, dosage, and outcomes have varied substantially.[7–13] Only one of the trials used a recognised surrogate outcome marker—time to culture conversion.[11] In this trial, 146 patients with pulmonary tuberculosis in the UK were allocated to receive a placebo-matched dose of 2·5 mg of oral vitamin D once every 2 weeks, given four times during the first 8 weeks of treatments. 62 patients assigned to vitamin D and 64 patients assigned to placebo were included in the primary efficacy analysis. Although median time to sputum culture conversion did not differ significantly between the groups (36·0 days in the vitamin D group and 43·5 days in the placebo group [adjusted hazard ratio 1·39, 95% CI 0·90–2·16; p=0·14]), vitamin D significantly accelerated culture conversion in a patient subgroup defined by vitamin D receptor polymorphism genotype.[11]

A high dose of vitamin D could be associated with harm from hypercalcaemia. Three randomised trials[8,9,14] assessed calcium concentrations during treatment with vitamin D, and one reported a significant increase. A 2·5 mg dose of vitamin D_3 (100 000 IU) induced a mean increase of 109·5 nmol/L in concentrations of 25-hydroxyvitamin D at 1 week in 11 vitamin D deficient patients with tuberculosis, without hypercalcaemia.[14]

In view of the in-vitro evidence of benefit, with little evidence of toxic effects, and low cost, a clinical trial investigating use of adjunctive vitamin D seemed justified. Because vitamin D receptor genotypes are ethnically divergent, benefit could vary between different geographical locations. Furthermore, whether patients with tuberculosis would benefit most from a restoration of normal vitamin D concentrations during deficiency, or whether supraphysiological concentrations would be more beneficial, is unclear. We postulated that addition of vitamin D to standard tuberculosis treatment would

reduce time to sputum culture conversion in an Indian population.

Methods

Study design and patients

We did this randomised, double-blind, placebo-controlled superiority trial at 13 sites in the Vellore (tuberculosis case notification rate of 136 cases per 100 000 population per year) and Krishnagiri (79 cases per 100 000 population per year) districts of Tamil Nadu, India.[15] The local climate is sunny throughout the year, with rainy seasons in July, August, and October. Recruitment sites were local Revised National Tuberculosis Control Programme clinics, which were visited by study personnel. Participants (aged 18–75 years) who were HIV negative and had pulmonary tuberculosis, with at least one documented positive sputum smear, were eligible for inclusion if they had taken one dose of tuberculosis treatment or fewer. We excluded paritipants with pre-existing liver or kidney disease, concurrent steroid or cytotoxic drug treatment, metastatic malignant disease, pregnancy or lactation, active diarrhoea, hypercalcaemia (corrected serum calcium >2·62 mmol/L), or those who were not expected to survive for 180 days. Patients determined to have multidrug-resistant tuberculosis were removed from the trial and given second-line treatment. Patients were followed up in the tuberculosis clinic or visited in their homes after recruitment at the clinic.

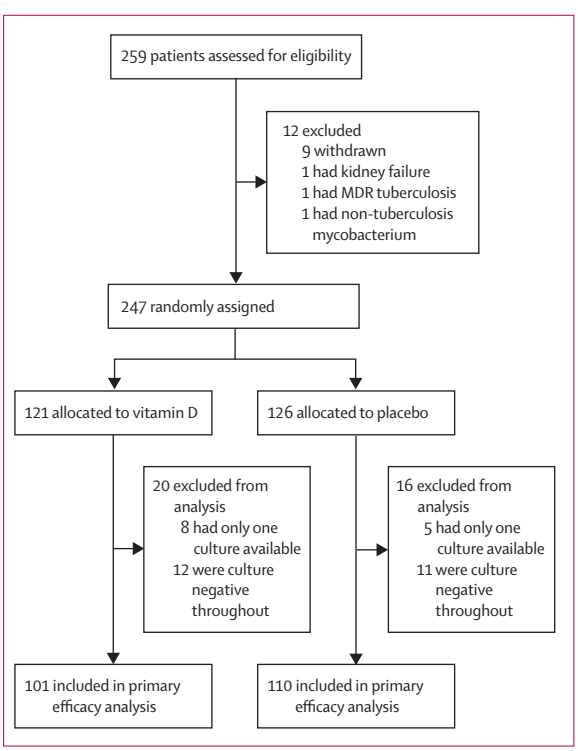

***Figure 1*: Trial profile**
MDR=multidrug-resistant.

The protocol followed Good Clinical Practice standards and national ethical guidelines, with no deviation from protocol except for expansion from four recruitment sites to 13 sites to increase recruitment. The protocol was approved by the Drugs Controller

	Vitamin D group (n=121)	Placebo group (n=126)
Sex		
Male	88 (73%)	101 (80%)
Female	33 (27%)	25 (20%)
Age (years)	41·6 (15·1)	43·7 (14·3)
Body-mass index	18·0 (2·9)	17·8 (3·0)
Occupation		
Professional	5 (4%)	3 (2%)
Skilled worker	23 (19%)	30 (24%)
Clerical, shop owner, or farm owner	6 (5%)	6 (5%)
Unskilled worker or manual labourer	41 (34%)	43 (34%)
Retired or pensioner	2 (2%)	2 (2%)
Unemployed	42 (35%)	39 (31%)
Other	2 (2%)	3 (2%)
Monthly income (rupees [US$])		
<5000 (<$95)	110 (91%)	119 (94%)
5000–10 000 ($95–185)	7 (6%)	5 (4%)
1000–20 000 ($185–370)	4 (3%)	2 (2%)
Education completed		
None	24 (20%)	32 (25%)
Primary school	39 (32%)	37 (29%)
Class VI-IX	25 (21%)	25 (20%)
Class X	20 (16%)	19 (15%)
Diploma or bachelor's degree	12 (10%)	8 (6%)
Master's degree	1 (1%)	3 (2%)
Professional or doctoral degree	..	2 (2%)
Karnofsky score		
50	4 (3%)	1 (1%)
60	20 (16%)	16 (13%)
70	61 (50%)	65 (52%)
80	33 (27%)	40 (32%)
90	3 (2%)	4 (3%)
Presently smokes cigarettes		
Yes	27 (22%)	39 (31%)
Presently chews betel nuts		
Yes	2 (2%)	4 (3%)
Presently consumes alcohol		
Yes	25 (21%)	30 (24%)
Monoresistant to isoniazid	14 (12%)	8 (6%)
Monoresistant to rifampicin	1 (1%)	1 (1%)
No data for rifampicin susceptibility	5 (4%)	20 (16%)
Corrected calcium (mmol/L)	2·27 (0·15)	2·28 (0·17)
25-hydroxyvitamin-D concentration (nmol/L)	63·1 (46·6)	62·2 (51·0)

Data are n (%) or mean (SD).

***Table 1*: Baseline and demographic characteristics**

General of India, the Health Ministry Screening Committee of the Government of India, and the ethics committees of Christian Medical College Vellore, India, and Dalhousie University, Canada. All patients provided written informed consent.

Randomisation and masking

Patients were randomly assigned (1:1), with centrally labelled, serially numbered bottles, to receive standard active tuberculosis treatment with either supplemental high-dose vitamin D_3 or placebo. 200 bottles containing vitamin D and 200 bottles containing placebo were randomised by computer into permuted blocks of four without stratification, then labelled with serial study numbers in their randomised order. As each patient was recruited, the next serially numbered bottle was assigned by the study personnel.

Neither the patient nor the clinical and laboratory investigators and personnel in India were aware of treatment assignment. The randomisation code was maintained in Canada by one investigator (RV). After the database was locked, the code was broken and analysis was done with knowledge of assignment.

Procedures

Data were obtained by two study personnel who were trained in study protocol and Good Clinical Practice standards. Study personnel worked directly with government medical officers, who were responsible for providing diagnosis and treatment, but who did not participate in study data collection or intervention.

Both groups received standard category one tuberculosis treatment according to Indian national guidelines.[15] Patients assigned to the vitamin D group received four doses of tasteless, odourless 2·5 mg vitamin D_3 oil (100 000 IU per dose) orally, once every 2 weeks for 8 weeks, given by direct observation by study personnel, starting with the first dose of tuberculosis treatment. This dose was chosen on the basis of previous evidence of change in vitamin D concentration from insufficient to sufficient in patients with tuberculosis,[14] and an enhancement in anti-tuberculosis immunity in patients with this disease.[16] The daily dose was regarded as less than the amount of vitamin D created in healthy skin after 30 min of sun exposure.[17] The intervention was the same as that done by Martineau and colleagues,[11] but ethnic origins and levels of sun exposure differed between the populations. Patients assigned to the placebo group received identical Miglyol oil. Both treatments were stored in identical dark glass dropper bottles at room temperature and protected from light. At the end of the trial, RV tested one vial for vitamin D content and recorded it to contain more than 95% of the original concentration of active ingredient (Vieth R, unpublished). No advice was given to patients to change their usual diet or sun exposure.

Sputa were collected by spontaneous expectoration, according to instructions from study personnel, at treatment days 0, 14, 28, 42, 56, and 180, with additional sputum collected at day 90 if the day 56 sputum was culture positive.

At the screening visit, we collected information about patient demographics and Karnofsky performance index status.[18] Sputum acid-fast smear and liquid mycobacterial culture with susceptibility to isoniazid and rifampicin, HIV serology (including pre-test and post-test counselling), serum 25-hydroxyvitamin D_3, calcium, albumin, creatinine, alanine aminotransferase, and, when indicated, urine pregnancy test, were undertaken. Serum calcium tests were repeated at days 4 and 28, and 25-hydroxyvitamin D_3 tests at day 180. Sputum smears were done with fluorescent microscopy. Sputum was pretreated with 4% N-acetyl-L-cysteine with sodium hydroxide for 15 min, and culture was done with BACTEC 9000 MB (Becton-Dickenson, Sparks, MD, USA), with 42 days incubation. Contaminated cultures were re-treated and reinoculated. For susceptibility testing we used the 1% proportion method. Resistant isolates only were confirmed with the Gene Xpert system (Cepheid, Sunnyvale, CA, USA). One investigator (SS, a trained technologist) did all testing at a single laboratory. The laboratory that undertook all mycobacterial testing is certified by the Revised National Tuberculosis Control Programme as an intermediate reference laboratory. We tested total concentrations of 25-hydroxyvitamin D_3 with the Cobas e analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Outcomes

Our primary efficacy outcome was time to sputum culture conversion (time to first negative smear). Secondary efficacy outcomes were time to culture conversion, time to smear conversion, proportion of patients who had positive cultures at 56 days, Karnofsky

	N	Events (%)	Patients censored (%)	Median time to culture conversion in days (95% CI)	Log-rank p value*
Primary outcome					
Time to culture conversion (first negative culture)					
Vitamin D	101	85 (84%)	16 (16%)	43·0 (33·3–52·8)	0·952
Placebo	110	87 (79%)	23 (21%)	42·0 (33·9–50·1)	..
Secondary outcomes					
Time to culture conversion (first of two consecutive negative cultures)					
Vitamin D	81	69 (85%)	12 (15%)	33·0 (21·5–44·5)	0·331
Placebo	82	66 (80%)	16 (19%)	40·0 (29·5–50·5)	..
Time to smear conversion (first negative smear)					
Vitamin D	101	85 (84%)	16 (16%)	43·0 (33·3–52·7)	0·949
Placebo	110	88 (80%)	22 (20%)	43·0 (36·1–49·9)	..
Time to smear conversion (first of two consecutive negative smears)					
Vitamin D	85	69 (81%)	16 (19%)	41·0 (21·5–44·5)	0·445
Placebo	87	66 (76%)	21 (24%)	45·0 (29·4–52·6)	..

*For difference between vitamin D and placebo groups.

Table 2: **Primary and secondary outcomes**

performance status and body-mass index (BMI) at 56 days, rate of rise in time to detection in culture,[19] and 25-hydroxyvitamin-D concentration at day 180.

The primary safety outcome was incidence of hypercalcaemia (corrected serum calcium >2·62 mmol/L). Secondary safety outcomes were rate of serious adverse events (death, admission to hospital, life-threatening illness, persistent disability, congenital anomaly, or predefined disease-related complications of tuberculosis infection), and adverse events (any untoward medical occurrence after study drug). Serious adverse events were reported within 24 h to the ethics committees of the Christian Medical College (Vellore, India) and Dalhousie University (NS, Canada). Deaths were investigated by interview of family members. Because positive cultures (or smears) were noted after negative tests, we additionally considered an alternative survival analysis as a secondary outcome: time to first of two consecutive negative cultures (or smears).

Stastical analysis

The effect-size estimate was a reduction in median time to culture conversion from 42 days to 28 days.[11] With a power of 0·80, a two-sided analysis, a significance of 0·05, accrual time of 365 days, follow-up time of 180 days, and an equal assignment into two groups, 96 patients in each group were required. Loss to follow-up and contaminated cultures were estimated to cause a loss of 30% of results; therefore, we recruited 250 patients.

We used a log-rank test (SPSS version 19.0.0) to compare time to culture conversion for primary and secondary outcomes. No interim analysis was done. We analysed efficacy in the modified intention-to-treat population (all patients who received one dose of intervention and had at least two sputum culture results available, including those who were culture negative at the first visit but culture positive at the second visit). We analysed safety in all patients who received at least one dose of intervention.

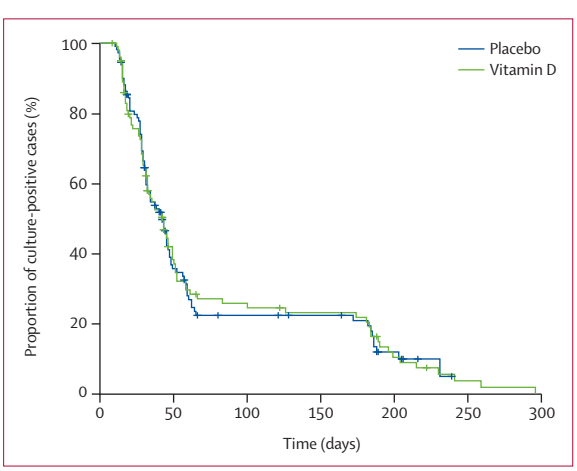

Figure 2: **Time to culture conversion (first negative culture)**
Crosses indicate censored patients.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility to submit for publication.

Results

Figure 1 shows the trial profile. Between Jan 20, 2010, and Aug 23, 2011, we randomly assigned 247 participants

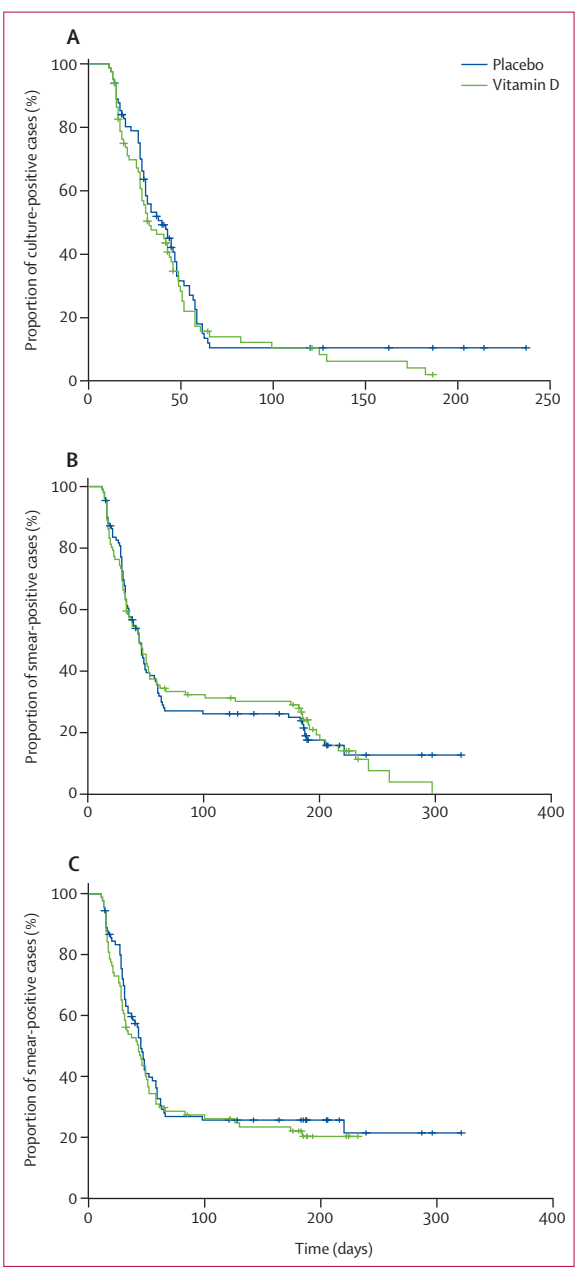

Figure 3: **Time to first of two consecutive negative cultures (A), time to first negative smear (B), and time to first of two consecutive negative smears (C)**
Crosses indicate censored patients.

	Nature of event	Date reported to ethics committee*	Intervention	Event considered intervention related?	Description
March 28, 2010	Hospital admission because of seizure	April 3, 2010	Placebo	No	Patient had first dose of placebo on day 1 and the second dose on day 20. Visited at home on day 33 and was drowsy. Admitted to hospital from day 34 to day 39. All metabolic measures and CT scan of head were normal. Corrected serum calcium was 2·05 mmol/L
May 15, 2010	Death (same patient as above)	July 7, 2010	Placebo	No	Final study dose given on day 42. Patient was in a good medical condition at a follow-up visit on day 50. Investigators were informed that patient died during his sleep on day 114
Sept 23, 2010	Death	Dec 23, 2010	Vitamin D	No	Patient had first dose of study drug on day 1. Withdrew consent and did not receive further study drug. Followed up as per protocol for 2 months. Investigators were informed on day 161 that he had died on day 72. Massive alcohol consumption just before death
March 3, 2011	Death	June 1, 2011	Vitamin D	No	Investigators were informed on day 227 that the patient died on day 138
March 10, 2011	Hospital admission because of hydropneumothorax	March 14, 2011	Placebo	No	Patient had first dose of study drug on day 1. On the same day he complained of acute breathlessness. Admitted with massive right-sided hydropneumothorax, which was drained with an intercostal tube, and discharged home on day 12
May 21, 2011	Death	May 27, 2011	Vitamin D	No	Patient was well at day 58, but died at day 60

*Ethics committee of the Christian Medical College, Vellore, India.

***Table 3*: Serious adverse events in chronological order**

to the vitamin D group (n=121) or the placebo group (n=126), of whom 211 participants (n=101 and n=110, respectively) were included in the primary efficacy analysis (figure 1). Patient follow-up was completed on Feb 20, 2012. All patients were Tamil or Telugu in ethnic origin. Baseline and demographic characteristics were mostly similar between groups, but the placebo group had slightly more men than the vitamin D group, and there were slightly more patients with isoniazid monoresistance in the vitamin D group (table 1). Mean baseline vitamin D concentration was less than the threshold of sufficiency (table 1), considered to be 75 nmol/L by most sources.[20] The appendix provides data for attrition.

See Online for appendix

Median time to culture conversion did not differ significantly between patients in the vitamin D group (43·0 days [95% CI 33·2–52·8]) and those in the placebo group (42·0 days [33·9–50·1]; table 2, figure 2). Likewise, we noted no significant difference between groups in any of the secondary outcomes of median time to first of two consecutive negative cultures, median time to first negative smear, or median time to first of two consecutive negative smears (table 2, figure 3).

The proportion of sputum culture negativity at day 56 did not differ significantly and was similar between groups, at 80·8% in the vitamin D group and 82·9% in the placebo group (appendix). The rate of rise in time to detection of growth was 0·17 in the vitamin D group and 0·11 in the placebo group (difference 0·066, p=0·59; appendix). Vitamin D concentration increased by 6·66 nmol/L (p=0·15) in the placebo group, for which vitamin D concentrations were available for 124 (98%) of 126 patients at baseline and for 79 (63%) patients at day 180, providing comparison data for 77 patients (appendix). By contrast, the concentration in the vitamin D group increased significantly by 14·2 nmol/L (p=0·001) from baseline, when data were available for 119 (98%) of 121 patients, to day 180, when data were available for 67 (55%) patients (comparison data for 65 patients; appendix).

Baseline BMI was available for all patients, with a mean of 17·9 kg/m² (SD 2·9; table 1). BMI was available at day 58 for 213 (86%) of 247 patients, with a mean of 18·7 kg/m² (SD 3·1; p<0·0001 by *t* test), showing a significant weight gain over the course of the study. The increase in BMI was 0·087 kg/m² greater in the placebo group than in the vitamin D group (p=0·597). Baseline Karnofsky performance index score was available for all patients, with a mean of 71·7 (SD 7·8; table 1). Scores were available at day 58 for 212 (86%) of 249 patients, with a mean of 83·4 (SD 8·1; p<0·0001 by *t* test), showing a significant performance improvement. The improvement in score was 1·90 greater in the vitamin D group than in the placebo group (p=0·115).

No hypercalcaemia was detected by calcium monitoring or symptoms (appendix). Table 3 summarises serious adverse events. Four (2%) patients died during the study: three in the vitamin D group and one in the placebo group; no death was considered directly attributable to study intervention (table 3). We recorded four adverse events in the vitamin D group and three adverse events in the placebo group, none of which required a change in medical therapy (appendix).

Discussion

Vitamin D supplementation did not reduce time to sputum culture conversion, nor did it reduce time to detection in culture, in line with findings from one previous randomised trial using similar methods.[11] We did not have the capacity to do vitamin D receptor polymorphism testing, although on the basis of the common ethnic origin of our patients, we might not have detected much variety.

Patients with active tuberculosis in south India have mild vitamin D deficiency (mean 62·6 nmol/L [SD 48·8], sufficiency concentration 75 nmol/L). This deficiency might be explained by the vegetarian diet, which does not provide adequate vitamin D intake, because sun exposure in this population is intense and constant. Increases from baseline in concentrations of vitamin D in patients with available comparison data in our study show that tuberculosis treatment improved vitamin D deficiency. We noted a significant increase in vitamin D concentrations in patients in the vitamin D group, but not in those in the placebo group; however, patients who received vitamin D did not achieve sufficiency. Sampling at day 0 and day 180 alone might have failed to identify a significant mid-study increase in vitamin D concentrations, which were reduced towards normal by day 180.

Change in time to culture conversion is an outcome that shows success of early sterilisation, which might contribute to prevention of tuberculosis relapse after treatment. Improved sterilisation might enable a reduction in treatment duration, without loss of efficacy,[21] but exactly how much reduction in time to conversion would justify a reduction in treatment duration is unknown. The sample size in our study was based on the estimated effect size reported by a previous trial[11] (reduction from 42 days to 28 days), and was considered to represent a clinically significant improvement.

The main limitation of our study was missing data (roughly a quarter of cultures), which could have affected the outcome. Missing data was within the 30% expected loss accounted for in the sample-size calculation. Patients with active tuberculosis are among groups with low socioeconomic status and are often migratory or have additional social or mental health problems, making trial compliance challenging. There were an unexpected number of patients who were smear positive and culture negative at baseline, which might show inadequate sputum collection, a single culture inoculation instead of duplicate inoculations, or unreported previous treatment. Sputum transportation was within 1 day of collection, so loss of viability during transportation was not likely. Additionally, persistence of culture positivity up to day 180, despite exclusion of multidrug-resistant isolates and pretreated patients at baseline, was concerning. Such persistence could suggest that resistance might have emerged during the trial, although repeat susceptibility testing was not done. Treatment was provided by direct observation. A limitation of our study design was the absence of sputum collection between 28 days and 42 days. Culture conversion might have happened earlier if data points were available at more timepoints. Our survival curves show a flat section between day 56 and day 180, when culture data were not available. Future studies should consider the affect of frequency of sampling on time to conversion.

The survival analysis technique can compensate for censored data and variable dates of collection, but the conversion from culture positive to negative to positive, or from negative to positive to negative, is problematic for analysis. To correct this weakness we did two survival analyses, based on two interpretations of the endpoint, and the conclusion remained the same with both methods. Previous studies have defined the outcome as time to all cultures negative,[22] or the midpoint between the last positive and the first negative culture.[11] The definition of this endpoint determines the comparability of effect sizes between studies, so future studies should concur.

Addition of high-dose vitamin D to standard tuberculosis treatment does not seem toxic. No patients had hypercalcaemia, and none of the serious adverse events were related to the intervention. Adverse events in our study were greatly under-reported compared with in trials in North America, which might indicate cultural differences in reporting, or missing data. The success of recruitment in our trial was high, showing a culture in which patients generally accept the suggestions of their health-care provider. Substantial effort was taken to ensure adequate informed consent.

In view of the absence of benefit shown in our trial and the trial done by Marineau and colleagues[11]—two randomised trials with a similar study design—future studies would have to address specific weaknesses in

Panel: Research in context

Systematic review

We searched PubMed with the search terms "tuberculosis", "vitamin D" and limited by "randomized controlled trial", with no language or date limits. Eight randomised trials[7–14,23] have been published about the role of adjunctive vitamin D for treatment of active tuberculosis. Dosages and outcomes were not similar between the trials, so we did not do a meta-analysis. Only one trial, by Martineau and colleagues,[11] used the widely accepted outcome of time to culture conversion. The findings showed that vitamin D did not significantly affect time to sputum culture conversion in the whole study population.[11]

Interpretation

Our trial was designed using the same intervention and outcome as Martineau and colleagues' 2011 trial,[11] but was applied in a tuberculosis endemic country, where vitamin D concentrations might be different on the basis of sun exposure and diet. Although our trial was larger than Martineau and colleagues', the findings were likewise negative, showing that vitamin D supplementation did not reduce time to sputum culture conversion or time to detection in culture. On the basis of these findings, vitamin D supplementation does seem to be safe, but does not seem to provide benefit to patients with active tuberculosis. These two trials could be combined in a meta-analysis. Future studies could consider the role of vitamin D in patients with latent tuberculosis or for prevention of acquisition of tuberculosis.

published designs to be justified (panel). Our observations regarding clinical trial design (definition of time of endpoint and analysis of time to first negative culture and to first of two consecutive negative cultures) could be applied to other tuberculosis treatments to standardise reporting.

Contributors

PD wrote the protocol; acquired ethics permissions and funding; did the literature search; wrote the manuscript; and supervised data collection, data analysis, and data interpretation. VJ and AL collected data. KRJ supervised data collection and was acted as a government interface. JS supervised laboratory testing. RV provided the intervention, did randomisation, designed the study, and interpreted data. SS did laboratory testing. LJ analysed data. DJC supervised data collection and provided permission to recruit from the directly observed treatment strategy clinic of the Christian Medical Centre (Vellore, India). MS designed the study and interpreted data. DM supervised the study, acted as a government interface, and acquired funding.

Declaration of interests

RV co-owns Ddrops, a company that promotes and sells vitamin D. All other authors declare no competing interests.

Acknowledgments

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