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Original Research Article

A study of vitamin D supplementation with directly observed treatment short course for Pulmonary Tuberculosis

Gurpreet Kaur Randhawa*, Suneet Jindal, Jaswant Rai, Nirmal Chand Kajal

Department of Pharmacology, Government Medical College, Amritsar, Punjab 143001, India

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***Correspondence to:**
Dr. Gurpreet Kaur Randhawa,
Email: kullar.g@gmail.com

ABSTRACT

Background: Tuberculosis remains one of major global health problems due to growing resistance in TB bacilli against anti-tubercular treatment (ATT). Vitamin D_3 has been reported to have immunostimulatory effect. Aim was to study effect of Vitamin D_3 on efficacy and safety of ATT / Directly Observed Treatment, short course regimen.

Methods: Prospective, randomized and interventional study of 90 days was carried out in 60 newly diagnosed sputum positive pulmonary tuberculosis patients on DOTS strategy. Study was conducted in Government Medical College, Amritsar, Punjab, India. 30 patients each were randomly divided into two groups, A and B, with group A - vitamin D_3 and DOTS regimen and Group B - DOTS alone. Patients were evaluated on day 0, 30, 60 and 90 by TB score, sputum microscopy, laboratory investigations, and adverse drug events. At the end of 90 days, results were tabulated and data analyzed statistically applying relevant tests.

Results: Statistically non-significant improvement in symptoms, sputum conversion and decrease in mean TB scores was seen in Group A vs B at 90 days. Significant increase in mean Vitamin D levels was seen in Group A at end of study. Insignificant difference in safety profile was observed in group A which showed additional adverse events suspected to be due to Vitamin D. Equivocal hepatoprotective effect of Vitamin D was observed.

Conclusions: Vitamin D as adjuvant to ATT does not confer additional benefit to newly diagnosed pulmonary tuberculosis patients. Large multi-centric trials are required to find any benefit of Vitamin D supplementation with ATT.

Keywords: Anti-tubercular treatment, Adverse drug event, Sputum conversion, TB score

INTRODUCTION

Pulmonary tuberculosis is a highly communicable infectious, chronic granulomatous disease of lungs caused by Mycobacterium tuberculosis bacilli (Mtb). Pulmonary Tuberculosis is associated with high morbidity and mortality. Globally, there were an estimated 10.4 million new TB cases with 1.4 million TB deaths in 2015.[1] In 2015, there were an estimated 480 000 new cases of multidrug-resistant TB (MDR-TB) and an additional 100 000 people with rifampicin-resistant TB (RR-TB) who were also newly eligible for MDR-TB treatment.[1] In India, an incidence of 2.2 million cases, and prevalence of 2.5mn of TB were reported in 2014. Pulmonary bacteriologically confirmed new TB cases notified in 2014 were 754,268 in India[2], and 23,864 in Punjab.[3] Approximately 1.44 million patients are registered for treatment with Revised National Tuberculosis Control Programme (RNTCP) of India in 2014.[4]

World Health Organization (WHO) recognizes India as one of the 22 high-burden countries (HBC's) that account for about 80% of the world's TB cases.[5] India alone accounts for the 26% of TB cases globally, and ranks 17th among 22 HBC's in terms of TB incidence rate. It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB.[6]

India had been implementing a scientifically evidence-based TB control programme since 1962.[7] The Revised

National Tuberculosis Control Programme (RNTCP) (1993) is based on the globally recommended Directly Observed Treatment, Short-course (DOTS) strategy for TB control promoted by WHO.[7] Newly diagnosed sputum smear-positive patients are categorized in Category I. These patients have a high bacillary load and therefore have a higher risk of having drug resistant mutants in their bacillary population. They are given four drugs in the initial intensive phase. The treatment consists of a two-month intensive phase of four drugs- Rifampicin (R), Isoniazid (H), Ethambutol (E) and Pyrazinamide (Z) –followed by a four-month continuation phase of RH. In India, treatment success for new smear-positive cases was 88% with cohort size of 642,000 cases studied in 2011.[8]

In India, 91.2% subjects more than 50 years of age had vitamin D_3 deficiency.[9] A series of studies from different parts of India have pointed towards widespread Vitamin D_3 deficiency in Asian Indians of all age groups residing in rural or urban areas.[10] For 25-hydroxyvitamin D_3 levels, cut-off of ≤50 nmol/l (≤20 ng/ml) is defined as deficient, 50-75 nmol/l (20-30 ng/ml) as insufficient and ≥75 nmol/l (≥30 ng/ml) as optimal level.[11] Vitamin D deficiency has been correlated with an increased incidence of severity of TB and Multi-drug resistant TB (MDR-TB). There is also 5-fold increased incidence of development of TB in vitamin D_3 deficient contacts of active TB patients with each relative 1-log decrement in 25-hydroxyvitamin D_3 levels.[12] Vitamin D_3 has been found to have an immunomodulatory role and enhances autophagosome formation, helping host cells in killing Mycobacteria tuberculosis bacteria.[13]

The evidence for role of Vitamin D_3 on the course of tubercular disease is inconclusive and different studies show both favorable and unfavorable responses. In SUCCINT (Supplementary Cholecalciferol in recovery from Tuberculosis) study, a randomized, double-blind, placebo controlled trial, 259 patients with pulmonary TB were randomized to receive either 600,000 IU of intramuscular vitamin D_3 or placebo for 2 doses one month apart. After 12 weeks, the vitamin D_3 supplemented arm demonstrated significantly greater mean weight gain and lesser residual disease by chest radiograph, greater reduction in cavity size.[14] Supplementation with high doses of vitamin D_3 accelerated clinical and radiographic improvement in all TB patients.

No data is available on dose-dependent effects of vitamin D_3 on mycobacterial activity.[15] The effect of vitamin D_3 supplementation on early sputum conversion to reduce the spread of TB needs to be evaluated. Scarce and incomplete data is available for adverse events during vitamin D3 supplementation with DOTS strategy.

Present study aims to evaluate the role of adjunctive vitamin D_3 for an early sputum conversion with an improvement in clinical and laboratory parameters, and its effect on tolerability of DOTS regimen. It also correlates the effect of vitamin D_3 supplementation on parameters as Erythrocyte Sedimentation Rate (ESR), Complete Blood Count (CBC).

METHODS

A prospective, randomized, interventional study of 90 days duration was conducted in the Department of Pharmacology in collaboration with Department of Tuberculosis and Respiratory Diseases, Government Medical College, Amritsar. The study enrolled 60 newly diagnosed sputum positive pulmonary tuberculosis Category I patients of either sex of ≥16 years of age, attending the out-patient department. Patients were divided randomly in two groups A and B, comprising of 30 patients each (Figure 1).

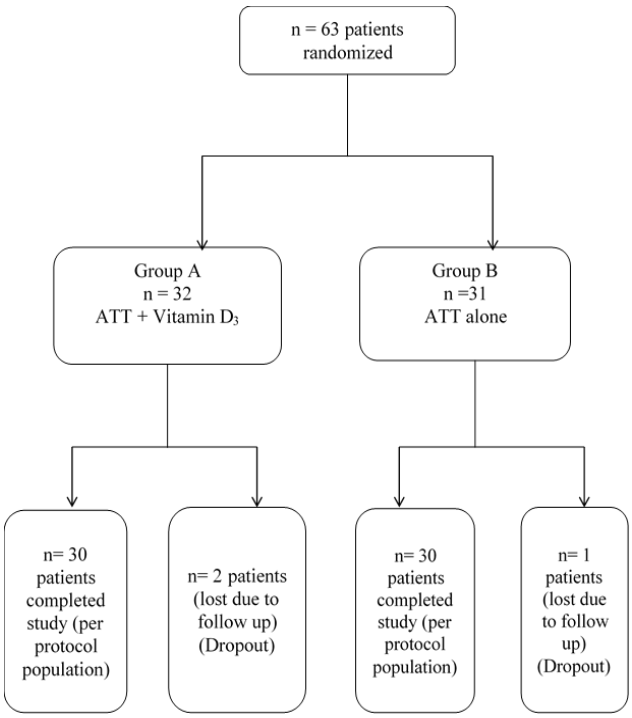

Figure 1: Patient recruitment and plan of study.

Group A

In this group, the patients received standard Anti-tubercular therapy.

(Directly Observed Therapy, Short-course regimen DOTS) in combination with Vitamin D_3 600,000 IU intramuscularly on Day 0, 30 and 60.

Group B

In this group, the patients received standard DOTS regime alone.

The approval of thesis and ethics committee was taken before start of the study. The patients were recruited in

study after written informed consent. Any adverse effect with drug treatment was managed accordingly.

Based upon clinical history, the patients with the following conditions were excluded from study. Extra-pulmonary TB, Human Immunodeficiency Virus (HIV) infection, Pregnant and lactating women, Hepatic and renal diseases, Cardiac disorders, Concomitant Diabetes Mellitus, Overweight, Gout, consumption of vitamin D within last two months, Patients on drugs interacting with metabolism of Vitamin D_3, immuosuppressives, Recent trauma or surgery, Hyperthyroidism, Malignancy, Sarcoidosis, and in case of requirement of modification of DOTS therapy.

Clinical assessment /examination

Demographic profile

This includes basic patient parameters like name, age, sex, marital status, educational qualification, occupation, total income per month, socioeconomic status, religion, rural or urban, family type, family size.

Routine investigations

Will be carried out on Day 0, 30, 60 and 90.

- Body Temperature Chart (In Fahrenheit)
- Body Weight (in Kg)
- Mid Upper arm circumference (MUAC) in millimeters (mm)
- Hemoglobin concentration, white blood cell count (WBC/TLC), WBC differential count (DLC) including neutrophil, lymphocyte
- Erythrocyte Sedimentation Rate (ESR)
- Serum Glutamic Oxaloacetate Transaminase/ Aspartate aminotransferase (SGOT/AST) and Serum Glutamate Pyruvate Transaminase/ Alanine Transaminase (SGPT/ALT) levels
- Serum Calcium (Ca+2), serum Phosphate (PO4-3) levels and Alkaline Phosphatase Enzyme (ALP)
- Serum Uric acid estimation

These investigations (e-i) were conducted in the Department of Clinical Biochemistry of Government Medical College, Amritsar.

Study parameters includes:

- TB Score - 0, 30, 60 and 90 days.16
- Sputum microscopy using Ziehl- Neelsen staining (ZN staining) - 0, 30, 60 and 90 days
- Vitamin D estimation using Sandwich- ELISA technique - 0 and 90days
- Adverse Drug reactions monitoring - 0, 30, 60 and 90 days.

Based on various signs and symptoms parameters, an objective index called TB Score was used, which took into consideration the pre-defined parameters like Cough, Hemoptysis, Dsypnoea, Chest pain, Night sweating, Fever, Lung Auscultatory findings, Body Mass Index and MUAC giving each a score of 1 if they are present. A total score was derived using this scheme out of 13. Patients were grouped into 3 classes based on clinical severity. This also predicted the morbidity. These classes are: Class I (0-5), Class II (6-7) and Class III (≥8).[16]

All the included patients completed the study and were statistically analyzed. The observations were tabulated in the form of mean ± standard deviation (SD) and analyzed using 't' test; paired `t`-test for intra-group comparison and unpaired `t` test for inter-group comparison and level of significance was determined as its 'p' value with $p>0.05$ as insignificant and $p<0.05$ as significant. Sputum conversion was analyzed using Fisher's exact test (Chi square test) using 2x2 contingency table, and the same levels of significance as mentioned above will be considered significant / non-significant.

RESULTS

Table 1: Baseline parameters of study groups.

Characteristics*	Group A	Group B
Demographic profile		
Number of patients	30	30
Age (yrs.)	33.9 (±11.42)	31.53 (±13.16)
Sex (Male: Female)	19 Males: 11 Females	18 Males: 12 Females
Body weight (Kg)	50.7 (±10.87)	47.93 (±11.15)
MUAC (in mm)	231.66 (±37.23)	229.33 (±37.95)
BMI (kg/m^2)	19.42 (±4.08)	18.57 (±3.21)
Body Temperature (^{o}F)	98.96 (±0.6)	99.06 (±0.65)
Blood and other general parameters		
Hemoglobin (g/dl)	10.39 (±2.08)	10.24 (±1.95)
Neutrophils %	73.06 (±9.77)	61.46 (±12.07)
Lymphocytes %	1.73 (±1.99)	4.4 (±4.21)
ESR (mm after 1st hour)	76.4 (±32.55)	80.83 (±25.45)
Toxicity parameters		
SGOT (IU/L)	33.47 (±12.01)	32.6 (±11.08)
SGPT (IU/L)	40.56 (±11.63)	40.95 (±10.47)
ALP (IU/L)	87.73 (±21.49)	85.06 (±22.19)
Serum uric acid (mg/dl)	4.24 (±0.54)	3.87 (± 0.65)
Vitamin d_3 related		
Serum Calcium (mg %)	9.15 (±0.52)	8.8 (±0.4)
Serum phosphate (mg%)	3.09 (±0.69)	2.78 (±0.69)
Average sputum positivity	2.26 (±0.9)	2.26 (±0.86)
Serum Vitamin D3 estimation (nmol/L)	29.93 (±10.35)	26.52 (±13.88)

* Study parameters of both the groups at 0 week (Mean ± SD)

The baseline characteristics in group A and B were comparable at the beginning of study. The mean age was found to be comparable, which was 33.9±11.42 years in group A and 31.53±13.16 years in group B. The gender ratio at baseline was also comparable i.e. 63.3% Males: 36.67% Females and 60% Males: 40% Females in group A and group B respectively. All other demographic parameters like body weight, MUAC, BMI and urban/rural distribution were also comparable (Table 1).

In Group A and Group B, there was statistically significant increase (p-value <0.05) in mean difference in body mass index at 30, 60 and 90 days from the baseline within the groups (Table 2). No difference was found in 0 to 90 days among Group A and Group B (p-value = 0.31).

Table 2: Mean change in body mass index at 0, 30, 60 and 90 days.

Body Mass Index (kg/m^2)				
	Group A		Group B	
	Mean	SD	Mean	SD
Day 0	19.42	4.08	18.57	3.21
Day 30	19.80	3.99	18.82	3.20
Day 60	20.01	3.92	19.08	3.21
Day 90	20.29	3.92	19.34	3.22
	p-value			
	Group A		Group B	
0-30 days	<0.05*		<0.05*	
0-60 days	<0.05*		<0.05*	
0-90 days	<0.05*		<0.05*	

* p value <0.05 is significant

Statistically significant improvement (p-value <0.05) in mean TB score was found at 30, 60 and 90 days when compared with baseline values in both Group A and Group B, but no statistically significant mean difference was observed between Group A and Group B at 0 and 90 days (p-value = 0.92) (Table 3).

Table 3: Mean change in TB score at 0, 30, 60 and 90 days.

TB Score				
	Group A		Group B	
	Mean	SD	Mean	SD
Day 0	6.06	1.87	5.56	2.40
Day 30	4.00	1.66	3.66	2.38
Day 60	2.23	1.56	2.13	1.75
Day 90	0.93	1.14	0.96	1.42
	p-value			
	Group A		Group B	
0-30 days	<0.05*		<0.05*	
0-60 days	<0.05*		<0.05*	
0-90 days	<0.05*		<0.05*	

* p value <0.05 is significant

At the beginning of the study, all the patients in both the groups were sputum positive i.e. 100% positive. At 30 days, 17% patients in Group A became sputum negative, and 7% in Group B became negative. Similarly, 93% and 73% patients became negative at 60 days in Group A and Group B respectively. At 90 days, all the patients attained sputum negativity in both the groups (Table 4, Figure 2). There was no significance observed in the sputum conversion at 30 days (p-value 0.4238) and 60 days (p-value 0.0797), as observed using Fisher's exact test.

Table 4: Sputum negativity at 0, 30, 60 and 90 days.

Attainment of Sputum Negativity (in %)				
	Group A		Group B	
	Negative	%	Negative	%
Day 30	5	17%	2	7%
Day 60	28	93%	22	73%
Day 90	30	100%	30	100%
	p-value (using Fisher's exact test) Group A vs Group B			
Day 30	0.4238			
Day 60	0.0797			

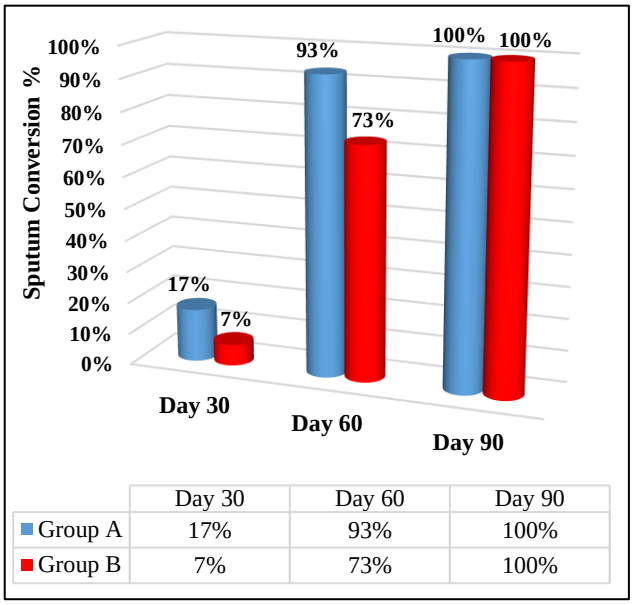

Figure 2: Comparison of sputum conversion at 30, 60 and 90 days.

In Group A and Group B, there was statistically significant (p-value <0.05) decrease in ESR at 30, 60 and 90 days from the baseline within the groups. No difference was found in 0 to 90 days among Group A and Group B (p-value = 0.632) (Table 5).

In Group A only, there was statistically significant (p-value <0.05) decrease in neutrophil fraction at 30, 60 and 90 days from the baseline within the group. No difference was found in 0 to 90 days between Group A and Group B (p-value = 0.182) (Table 6).

Table 5: Mean change in ESR at 0, 30, 60 and 90 days.

ESR (mm after first hour)				
	Group A		Group B	
	Mean	SD	Mean	SD
Day 0	76.4	32.55	80.83	25.45
Day 30	47.03	22.06	50.60	18.96
Day 60	29.8	12.58	33.43	12.60
Day 90	20.4	7.22	21.33	7.80
	p-value			
	Group A		Group B	
0-30 days	<0.05*		<0.05*	
0-60 days	<0.05*		<0.05*	
0-90 days	<0.05*		<0.05*	

* p value <0.05 is significant

Table 6: Mean change in neutrophil % at 0, 30, 60 and 90 days.

Neutrophils %				
	Group A		Group B	
	Mean	SD	Mean	SD
Day 0	73.06	9.77	61.46	12.07
Day 30	69.03	5.93	61.63	7.88
Day 60	66	5.69	60.33	6.48
Day 90	64.03	5.1	62.23	5.23
	p-value			
	Group A		Group B	
0-30 days	<0.05*		0.9120	
0-60 days	<0.05*		0.5680	
0-90 days	<0.05*		0.7410	

* p value <0.05 is significant

In Group A only, there was statistically significant (p-value <0.05) increase in lymphocyte fraction at 30, 60 and 90 days from the baseline within the group. No difference was found in 0 to 90 days between Group A and Group B (p-value=0.189) (Table 7).

Table 7: Mean change in lymphocyte % at 0, 30, 60 and 90 days.

Lymphocytes %				
	Group A		Group B	
	Mean	SD	Mean	SD
Day 0	24.26	7.49	32.93	10.79
Day 30	29.06	5.16	34.26	7.43
Day 60	32	4.33	37.00	6.91
Day 90	34.6	4.44	36.26	5.23
	p-value			
	Group A		Group B	
0-30 days	<0.05*		0.4000	
0-60 days	<0.05*		<0.05*	
0-90 days	<0.05*		0.1140	

* p value < 0.05 is significant

There was statistically significant increase in Vitamin D levels in Group A only (p-value <0.05) on 90 days. At 90 days, there was significant difference in Vitamin D levels between Group A and Group B (p-value <0.05) (Table 8).

Table 8: Mean change in vitamin D at 0, 30, 60 and 90 days.

Vitamin D levels (nmol/L)				
	Group A		Group B	
	Mean	SD	Mean	SD
Day 0	29.93833	10.36	26.52373	13.89
Day 90	49.76901	8.448995	28.08	12.71
	p-value			
	Group A		Group B	
0-90 days	<0.05*		0.1290	

* p value <0.05 is significant

In Group A only, there was statistically significant increase in serum calcium levels at 30, 60 and 90 days from the baseline within the group. Statistically significant increase in serum calcium levels was observed between Group A and Group B at 90 days (Table 9).

Table 9: Mean change in serum calcium at 0, 30, 60 and 90 days.

Serum calcium (mg %)				
	Group A		Group B	
	Mean	SD	Mean	SD
Day 0	9.15	0.52	8.8	0.40
Day 30	9.39	0.46	8.93	0.42
Day 60	9.67	0.67	11.82	14.77
Day 90	9.93	0.66	9.27	0.53
	p-value			
	Group A		Group B	
0-30 days	<0.05*		0.1042	
0-60 days	<0.05*		0.2710	
0-90 days	<0.05*		0.2449	

* p value <0.05 is significant

A total of 147 adverse events were reported in Group A in the total 90 days study duration, which were suspected with Vitamin D supplementation. Additionally, 159 events were reported in the same group, which were suspected with ATT. Similarly, 171 adverse events suspected with ATT were reported in Group B. Hence, there is no sufficient evidence to prove the safety of Vitamin D; and reduction of adverse events with supplementation of Vitamin D along with ATT (Table 10).

No statistically significant mean difference of various toxicity biomarkers like SGOT, SGPT, ALP and uric acid were found between Group A and Group B at the end of the study. Hence, there was no hepato-protective role of Vitamin D (Table 11).

Table 10: Total adverse events reported in group A and group B in total 90 days.

Suspected ADRs with Vitamin D (600,000 IU IM once monthly for 3 months) therapy		
Adverse Drug Reactions	Group A	
Nausea	59	
Vomiting	35	
Excessive Thirst	5	
Anorexia	38	
Symptoms related to kidney stones	3	
Confusion	6	
Other Neurological deficits	1	
Total events	147	
Suspected ADRs with Anti Tubercular Therapy (ATT)		
Adverse Drug Reactions	Group A	Group B
Rash	9	12
Fever	29	32
Paresthesia/Numbness/Altered Sensorium	20	20
Abdominal pain/distension	39	32
Body aches/Joint pains	28	40
Discoloration of body fluids	29	27
Decreased visual acuity/Blurring of vision	0	0
Pruritis	5	8
Total events	159	171

Table 11: Mean change in hepatic toxicity biomarkers % at 0 and 90 days.

Parameter	Group A mean	Group B mean	Mean difference	p-value
SGOT (IU/L)	40.18 (±11.54)	40.56 (±9.84)	-0.38	0.89
SGPT (IU/L)	47.4 (±11.31)	51.26 (±12.31)	-3.86	0.21
ALP (IU/L)	98.16 (±13.38)	102.81 (±14.71)	-4.64	0.205
Uric Acid (mg/dl)	4.55 (±0.57)	4.68 (±0.66)	-0.13	0.406

DISCUSSION

The general baseline and demographic characteristics were similar in both groups A and B (Table 1). In the present study, a statistically significant increase in BMI was observed at 30, 60 and 90 days respectively from baseline in both groups (p-value <0.05 Table 2). But there was no statistically significant mean difference (0.94 Kg/m^2) over 90 days in BMI among Group A and B (p-value 0.31 Table 2).

The above findings are similar to a double-blind, randomized placebo-controlled study conducted by Daley et al in which 211 South Indian patients were studied. An increase of 0·087 kg/m^2 in BMI was observed in the placebo group which was more than in the vitamin D group (p-value 0·597).[16]

In the present study, statistically significant improvement in mean TB score was found at 30, 60 and 90 days when compared with baseline values in Group A and B (p-value <0.05 Table 3), but no statistically significant mean difference was observed between Group A and B at 0 and 90 days (p-value 0.92). Thus, clinical improvement can be due to ATT with no additional advantage of vitamin D.

These observations are similar to those found by Wejse et al where there was no change in TB score and time to clinical improvement (progression to low-severity class) in 365 patients randomized to vitamin D supplemented and placebo groups.[15]

A similar clinical outcome score was designed in a randomized, double-blind, placebo-controlled study conducted by Ralph et al (2013) where change in body weight, $\%FEV_1$ change, cough, sputum, hemoptysis was included. This study also did not observe any significant change in the above mentioned clinical score with vitamin D in PTB patients.[18]

The similar non-significant findings were also reported by Salahuddin et al inTB score, with Vitamin D supplemented and placebo groups at 4 weeks (p-value 0.16), 8 weeks (p-value 0.89) and 12 weeks (p-value 0.16).[14]

Farazi et al found an association between TB severity score with lower levels of serum calcidiol (p-value = 0.043) in Vitamin D (single dose of 450,000 IU) supplemented and placebo groups over 3 months.[19]

Fisher's exact test [Chi-square (χ^2)] analysis didn't demonstrate any significant difference in TB conversion between Group A and Group B at both day 30 (p-value 0.4238) and day 60 (p-value 0.0797). Thus, there is no sufficient evidence to say that vitamin D hastens the sputum conversion in smear positive PTB patients. Sputum conversion is the most important parameter to be considered to stop the disease spread in the community.

These observations are in concordance with Daley et al who noted no significant difference between intervention and placebo groups in median time to first negative smear [43.0 (95% CI 33.3 - 52.7) vs 43.0 (95% CI 36.1 - 49.9) in Group A and B respectively, with log-rank [p-value 0.949], or median time to first of two consecutive negative smears.[16]

Another double-blind, randomized, placebo-controlled trial was conducted by Martineau et al where 126 participants were included for the primary efficacy analysis. In this study, median time to sputum culture conversion was 36.0 days in the intervention arm and 43.5 days in the placebo arm (adjusted HR 1.39; 95% CI

0.90-2.16, p-value 0.14).[18] The similar findings were observed by Wejse et al where sputum smear conversion rates did not differ among patients treated with vitamin D or placebo.[20]

Another double-blind, randomized placebo-controlled study conducted by Ralph et al also observed that time to sputum microscopy clearance did not differ among study arms.[18] According to study conducted by Salahuddin et al, no significant differences were observed in sputum smear conversion rates (81.8% vs 81.1%; p-value 0.39).[14]

On the contrary, another randomized, placebo controlled study on 67 Indonesian patients, by Nursyam et al which reported that pulmonary TB patients when given 420,000 IU of vitamin D for 6 weeks had significantly higher sputum conversion rates as compared to placebo (p-value 0.002).[21] Another randomized placebo-controlled study conducted by Coussens et al in which 95 patients were randomized to vitamin D supplementation and placebo groups, observed that median time to sputum smear conversion in the intervention arm was significantly shorter than that in the control arm (23 days vs. 36 days; hazard ratio 1.69, 95% CI 1.02-2.79, p-value 0.04).[22]

Similarly, Hassanein et al found a significant decrease in sputum conversion time in group I (cases - received vitamin D (200,000 IU) intramuscular injection once besides antituberculous drugs) compared to group II (control - only antituberculous drugs) (p <0.001). Sputum of most patients (56.7%) in group I were converted at the 3rd week of initiation of anti-TB drugs while in group II, sputum of most patients (46.7%) were converted at the 8th week after the start of anti-TB therapy.[23]

These contrasting findings in various studies regarding sputum conversion time could be due differences in vitamin D3 dosage, achieved vitamin D3 levels and applied statistical methods.

In Group A and B, there was statistically significant decrease in ESR at 30, 60 and 90 days from baseline within the groups (Table 5). No statically significant difference was observed at 0 and 90 days between Group A and B (p-value <0.05).

These findings are in concordance with a study conducted by Coussens et al which showed decrease in both vitamin D and placebo groups of ESR with p-value <0.0001.[22]

In the present study, we observed a decrease in neutrophil count fraction and an increase in lymphocytes count fraction from baseline at 30, 60 and 90 days, only in Group A (p-value <0.05 Table 6 and 7). These findings are also in concordance with a study conducted by Coussens et al.[22]

There was statistically significant increase in Vitamin D levels in Group A over 90 days duration 29.94 nmol/l (±10.36 nmol/l)] vs 49.77 nmol/l (±8.45 nmol/l) at 0 and 90 days respectively with p-value <0.05 (Table 8). At 90 days, there was significant difference in Vitamin D levels between Group A and B [49.77 nmol/l (±8.44 nmol/l) vs 28.08 nmol/l (±12.71 nmol/l) with p-value <0.05] (Table 8). Correspondingly, Serum calcium levels were significantly better in Group A only over 90 days (Table 9). Patients with active pulmonary TB responded well to vitamin D supplementation regardless of the vitamin D state in serum before initiation of vitamin D therapy. Hypercalcemia did not occur at (600,000 IU IM once monthly for three months) dose used in this study and the patients still remained deficient (≤50 nmol/l) in vitamin D3 levels even after treatment.

The above observations are similar with a study conducted by Daley et al where 100,000 IU of Vitamin D_3 (2·5 mg dose of vitamin D_3 orally) was given to test group. The concentration in the vitamin D group increased significantly by 14.2 nmol/l (p-value 0.001) from baseline vs 6·66 nmol/l in the placebo group (p-value 0·15), though, even the patients who received vitamin D were unable to achieve sufficiency.[17] Another double-blind randomized study conducted by Martineau et al where same dose was used as by Daley et al, but was given at 0, 2, 4 and 6 weeks along with ATT in test group vs placebo in control group; reported mean serum Vitamin D at 8 weeks to be 101.4 nmol/l vs. 22.8 nmol/l in intervention vs. placebo arms respectively (95% CI for difference 68.6-88.2 nmol/l, p-value <0.001).[20]

On assessment of adverse effect profile, the patients of both the groups under study tolerated the drugs well and in none of the patients the adverse effects were serious enough to discontinue the drug therapy (Table 10).

In this present study, we measured adverse effects as adverse events i.e. the total number of times any adverse events reported by all the patients in total 90 days of study, based on pre-defined questionnaire, and also other events not mentioned in the questionnaire were observed.

In group A, adverse events reported were 147 suspected due to Vitamin D; and 159 suspected of ATT, a net of 306 adverse events. In group B, total adverse events suspected of ATT were reported to be 171. Majority of the adverse events suspected of Vitamin D in Group A were nausea (59 events), vomiting (35 events) and anorexia (38 events). Discontinuation of therapy was not warranted in any of the cases and no death occurred during the study period in the patients. ADRs related to Vitamin D supplementation along with ATT has not been clearly mentioned in the medical literature. The present study was an endeavor to study toxicity patterns of both groups A and B in detail.

These adverse events could also be suspected due to ATT. In both groups, adverse events suspected of ATT mainly includes fever, paresthesia/numbness, abdominal pain/distension, body aches/joint pains and discoloration of body fluids.

No significant (p-value >0.05) change was seen in investigations carried out to monitor vitamin D + ATT toxicity vs ATT alone toxicity i.e. SGOT, SGPT, ALP and uric acid levels in both the groups (Table 11). Equivocal result was observed.

CONCLUSION

Vitamin D as an adjuvant to ATT in DOTS regimen has not shown any additional advantages over ATT alone in improving the symptomatology over a period of 90 days and early sputum conversion. There was also no effect at improving the safety of ATT by addition of Vitamin D. In fact, there were additional adverse events suspected due to vitamin D in addition to those due to ATT. Safety profile of both the groups is similar, no additional advantage of vitamin D supplementation was found. Equivocal hepato-protective role of Vitamin D was observed. Vitamin D is known to have immunomodulatory action but it failed to provide any additional advantage over ATT alone in the present study.

The present study recommends that Vitamin D (600,000 IU units IM on 0, 30 and 60 days) as an adjuvant to ATT in DOTS regimen is not beneficial in newly diagnosed pulmonary tuberculosis patients in Punjab, India. Genetic polymorphisms of genes like Taq1 Tt, tt, TT genotype and others like toll-like receptor, vitamin D binding protein which are responsible for vitamin D immunomodulation might also be related to therapeutic response to vitamin D supplementation in TB, need to be studied in Punjab.[24] Vitamin D plays an important role in activation of 1 α-hydroxylase to convert 25(OH) D to its active form [1, 25 (OH) 2D] that leads to expression of cathelicidin, a microbicidal peptide for *M. tuberculosis.* Serum levels >30 ng/mL (sufficient levels) provide an adequate substrate for this enzyme.[25] Serum levels <20 ng/mL (deficient levels) may therefore impair the macrophage-initiated innate immune response to *M. tuberculosis* and offer a possible explanation for geographic and ethnic variations in susceptibility to TB.[26] Large multi-centric trials of longer duration are required to find out benefits of Vitamin D supplementation with ATT, if any.

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