ORIGINAL ARTICLE

Comparative Effect of ATT Alone and in Combination with Vitamin D on Physiological and Laboratory Parameters in Pulmonary TB

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ABSTRACT

Objectives: To evaluate the role of adjuvant Vitamin D therapy on pulmonary tuberculosis with respect to disease severity. Objectives of this study were to assess and compare the levels of Vitamin D, C -reactive protein and ESR within and between both groups before and after therapy and time taken for sputum microscopy conversion. We also compared the effect of therapy on the severity of the disease within and between both groups after therapy.

Study Design & Methods: 86 tuberculosis patients were enrolled after obtaining a written informed consent. They were divided into two groups; namely Group D (Patients with ATT and adjuvant Vitamin D) and Group P (Placebo group receiving normal saline injection). Two doses of Vitamin D I/M (0.6 million units) at baseline and at week 6 of the study along with standard ATT were given to the patients in D group. Normal saline injection along with standard therapy was given to the patients in Group P. Laboratory investigations were conducted at baseline and at Day 75 of the therapy and sputum microscopy examination fortnightly till sputum conversion.

Results: In the D group, at Day 75 of therapy, adjuvant Vitamin D treatment significantly increased serum Vitamin D, serum calcium, Hemoglobin, BMI and significantly decreased ESR, CRP and total white blood cell count. In the Placebo group, anti tuberculous therapy significantly increased serum Calcium, BMI and significantly reduced ESR, CRP and total white blood cell count at Day 75 of therapy. The mean number of days for sputum conversion in Group D and P was 49 days and 61 days respectively.

Conclusion: Adjuvant Vitamin D therapy plays a prominent role in the treatment of pulmonary tuberculosis as in treatment group it profoundly produced early sputum conversion by 12 days in comparison to Placebo group which received ATT alone.

How to cite this article: Azam F, Shaheen A, Zuberi FF. Comparative effect of ATT alone and in combination with Vitamin D on physiological and laboratory parameters in pulmonary TB. J Dow Uni Health Sci 2015; 9(3): 92-98.

INTRODUCTION

Mycobacterium tuberculosis is the usual cause of tuberculosis which primarily affects respiratory system. Other less often causes include M. africanum and M. bovis which can affect other organs of the body[1]. There are many strategies that can cure prevent this disease but still it is one of the most significant health concerns of the world. Presently the number of people infected with this disease exceeds more than 30 percent of the world's population. The prevalence of this disease is remarkably high in densely populated regions such as South East Asia and South Asia and especially higher in countries with high population such as China, India, Bangladesh and Pakistan where more than 50 percent of the new tuberculosis cases are diagnosed[2].

Major categories of risk factors for this disease are natural, life style related and medical factors. One of the important medical factors which increase susceptibility of contracting tuberculosis is weakened immune system which is mostly caused by diabetes, malignancies, hemodialysis, immune suppressant treatment and HIV / AIDS. High rates of death associated with tuberculosis makes quick medical intervention very significant. The first line of therapy for treating tuberculsios includes rifampin, ethambutol, isoniazid, pyrazinamide and streptomycin. Drugs used as second line of treatment include p-aminosalicylic acid, cycloserine and fluroquinolones[3,4].

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Unfortunately the emergence of resistance and the low compliance of tuberculosis patients has resulted because of its very long duration of treatment[5,6]. Despite the continuous struggle of international health organizations to limit the spread of this disease, the number of newly diagnosed patients with tuberculosis continues to increase as the resistance against anti tuberculosis drugs such as rifampin and isoniazid is increasing[7,8].

Resistance against anti tuberculosis drugs is being reported in Pakistan, and, presently it is included in one of the countries with a very high burden of multi drug resistant (MDR) tuberculosis. In this scenario one of the strategies to prevent the poor compliance and therefore growing resistance against anti tuberculosis drugs could be to shorten the duration of treatment of active disease. Research on innovative agents and therapies is required keeping in mind the aspect of immunological correlation with etiology and pathogenesis of this disease[9]. One more important factor which would be necessary for any new agents to decrease the duration of treatment would be that it accelerates the sterilizing action which is the rate at which mycobacterium bacilli are killed in the lesions.

With this background, adjuvant Vitamin D therapy appears to be one of the most useful options for this purpose as both immune system and most tissues affected by this disease have receptors for this vitamin and respond to active form of vitamin D that is 1,25 dihydroxyvitamin D. Presence of prior evidence that vitamin D deficiency is induced by drugs used in the treatment of tuberculosis such as isoniazid and rifampin suggests that along with standard treatment for tuberculosis, this vitamin could be a very good option as adjunct therapy.

Data suggests that there exist mechanisms through which Vitamin D can suppress the activity of mycobacteria to initiate macrophage-mediated antimicrobial processes through acquired immunity[10].

Recent studies have also shown that vitamin D deficiency and tuberculosis are strongly associated with each other[11,12]. Some studies have also given evidence that administering varying doses of this vitamin can result in early sputum microscopic conversion in patients with tuberculosis[13,14].

The purpose of this study was to evaluate the role of Vitamin D as adjuvant therapy in the treatment of pulmonary tuberculosis along with standard anti tuberculous therapy and to assess and compare the time taken for sputum microscopic conversion within and between treatment and placebo groups after therapy.

MATERIALS & METHODS

Study Design, Setting and Ethical Approval: It was an observational clinical study. After obtaining a written and informed consent, eighty six newly diagnosed tuberculosis patients were recruited in the study.

This research project was performed at Dow University of Health Sciences, Karachi (Ojha Institute of Chest Diseases) after obtaining approval from funding committee, Institutional Review Board (IRB) and Board of Advanced Studies and Research (BASR) of Dow University of Health Sciences.

The time period of the study was almost eight months and it was conducted between October 2010 and July 2011. For each patient, the individual study period was approximately seventy days. The study patients were informed of the nature, importance, potential adverse effects and outcomes of the study.

Inclusion and Exclusion Criteria: To diagnose sputum smear positive tuberculosis patients, the WHO criteria was used which defined sputum smear positive tuberculosis as testing at least two initial sputum smear examinations positive for acid fast bacilli (AFB +); or one sputum examination positive for AFB+ and radiographic abnormalities consistent with active pulmonary tuberculosis; or one sputum specimen positive for AFB+ and culture positive for AFB+. The patients enrolled had previously not taken anti tuberculosis treatment or Vitamin D supplementation. Patients of both genders aged between eighteen to fifty years were recruited in the study if they gave written informed consent to participate.

Patients who were excluded from our study included patients with negative AFB sputum smear, extra pulmonary tuberculosis and pregnant and lactating females. Those patients who were found to have known intolerance to anti tuberculosis drugs or Vitamin D were also excluded. Presence of other concomitant diseases such as known kidney disease and infectious diseases was also an exclusion criteria. Patients with history of using immunosuppressants, cytotoxic drugs, corticosteroids or benzothiadiazine derivatives in the year preceding the recruitment in the study were also excluded from the study. Furthermore, patients presenting with increased levels of serum calcium and serum Vitamin D levels or patients who presented with near normal, normal or upper normal range of Vitamin D or calcium or as per the criteria of Dow Diagnostic and Research Laboratory (DDRL) were also excluded from the study.

In all, eighty six patients were enrolled in the study and were divided into two groups namely group D (patients receiving both ATT and adjuvant vitamin D therapy) and group P (patients receiving normal saline as placebo treatment) by pair wise randomization according to disease severity. During the study, detailed family and past medical and surgical history along with information regarding demographic details were collected from both treatment and placebo groups.

The independent variables of the study comprised of gender,age, weight, heigh, BMI, ethnicity, formal education received and socio economic status whereas the dependent variables of the study were days required for sputum conversion, ESR, Hs-CRP, serum vitamin D levels and serum calcium levels.

At the beginning of the study all the laboratory investigations were conducted and vital signs were recorded. For laboratory investigations, blood sample of 10 ml for each subject was obtained at the Dow Diagnostic and Research Laboratory under sterile conditions after a 12-hr overnight fast. Blood samples for high sensitivity C reactive protein, ESR, Vitamin D serum levels and calcium serum levels estimation were collected in four separate gel tubes for each patient.

During the study period, standard anti tuberculosis DOT therapy was given to both additionally vitamin D treatment and placebo groups and patients in the Vitamin D group (D) were also administered two Vitamin D doses of 0.6 million IU through I/M route at day 7 and at week six of the study. Patients in Placebo group (P) were only administered placebo normal saline at seventh day and end of week 6 along with regular DOT therapy. At day 70, laboratory investigations were repeated whereas sputum microscopy was performed after every two weeks till sputum conversion. All medications and laboratory investigations were provided to the patients free of cost.

Sampling Technique (Pairwise Randomization): The sample size was calculated to be 70 by using statistical software SPSS with 95% confidence interval and 80% power of the test. Using pairwise randomization, the total number of enrolled patients was divided into two equal groups. The purpose of pairwise randomization was to make sure that both groups were equal in composition regarding the number of patients of sputum smear severity (grade +1, +2 and or +3). Anticipating that some patients might be lost to follow up or might not be able to complete the study due to any reason, eighty six patients were enrolled in the study.

Statistical Analysis: All the data were recorded on and analyzed by SPSS version 17. Numerical variables have been expressed as mean and standard deviation whereas categorical variables have been expressed using frequency and percentage. Paired and unpaired Student's T tests were used for analysis of variation in different variables between and among the different groups.

RESULTS

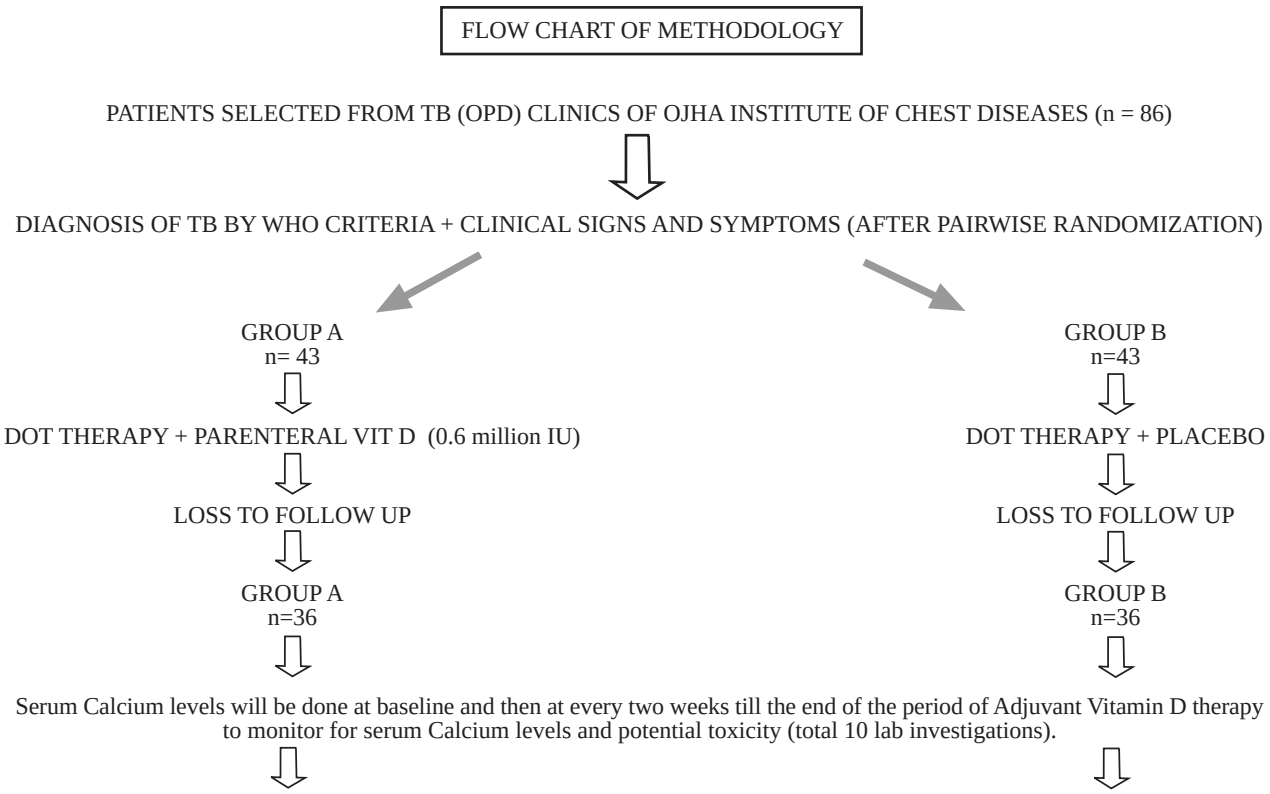
Eighty six newly diagnosed tuberculosis patients were recruited for this study out of which 72 patients completed the study and remaining fourteen patients could not complete the study because of various reasons. (six patients were lost to follow up as they had come from flood affected areas and went back to their villages during the study period while one patient died in a road traffic accident during the study period; subsequent to loss to follow up of these patients, data of their pair wise randomized controls was also not analyzed). These patients were placed in two groups namely Group-P (Control group which was given standard anti tuberculosis treatment) and Group D (Treatment group which was given Vitamin D along with standard anti tuberculosis treatment). At baseline, the two groups of patients had similar composition in terms of age, gender, ethnicity, literacy, socio economic status and BMI.

According to disease severity as per sputum microscopy, the two groups of patients were both further subdivided into three groups which were P1 with AFB sputum 1+ in placebo group, P2 with AFB sputum 2+ in placebo group , P3 with AFB sputum 3+ in placebo group, D1 with AFB sputum 1+ in Vitamin D treatment group, D2 with AFB sputum 2+ in Vitamin D treatment group and D3 with AFB sputum 3+ in Vitamin D treatment group. There were 12 patients in each subgroup (Table 1).

After statistically analyzing the variations in the mean values of the laboratory investigations and other variables in the two groups at baseline and at day 70, following results were seen. Statistically significant results were obtained with changes in serum Vitamin D, ESR and BMI with p values of 0.000, 0.038 and 0.000 respectively. However, adjunct Vitamin D therapy administration did not produce significant changes in Hemoglobin, Calcium and Hs-CRP.

Statistically significant changes were seen in the mean number of days required for AFB sputum microscopic conversion in the Vitamin D treatment and placebo groups which were 49 days and 61 days respectively. ($p = 0.032$). (Table 2)

For sputum conversion, significantly different number of days was required by patients in the Vitamin D treatment group (D) and placebo group (P) which were 49.21 and 61.33 days respectively. Similarly for sputum conversion of patients in group D1, P1, D2, P2, D3 and P3 significantly different mean number of days were required which were 52.02, 60.32, 49.12, 61.48, 45.98 and 61.86 days respectively. (Table 3)



DISCUSSION

This study was carried out to evaluate role of Vitamin D in patients with pulmonary tuberculosis in our local setting. The predisposing factors for acquiring tuberculosis (TB) are poor socioeconomic and nutritional status, poor sanitary conditions, houses with inadequate sunlight, low BMI, malnutrition and lack of education[15-17].

All these factors contribute to increased likelihood for tuberculosis by reduced hemoglobin levels in addition to developing other diseases[13,18]. Mean body mass index (BMI) and hemoglobin level of enrolled TB patients in our study was less than the normal reference range supporting poor nutritional status of the patients. Our study found no association of gender with TB which are in close collaboration with other studies[19,20]. However, 53 out of 72 patients were not educated and the rest of the patients had education level upto matric and intermediate which clearly shows that poor socio-economic status and illiteracy are strong indicators of the disease. Furthermore, 72.22% had very poor belongings with monthly income less than or equal to Rs. 6000. We found that Pushto speaking community represented significant number of patients in our study and this finding has already been reported in a study conducted in Karachi reporting a similar ethnic proportion[17].

Our research study found higher mean ESR and CRP levels at baseline in both control and treatment groups confirming the importance of these markers as an evidence of inflammation. (Table 1B) A number of studies have reported significant drop in ESR and CRP, being important markers of disease activity, after anti tuberculous treatment[21-23]. The findings of our research showed a significant decline in ESR and CRP from 74.94 ± 32.01 mm/Hr to 28.75 ±21.12mm/Hr (p <0.001) and from 56.03 ±40.52 mg/L to 23.24 ±31.97 mg/L (p <0.001), respectively, in tuberculosis patients after commencement of treatment. The results of our study imply that ESR is a more specific indicator of severity of tuberculosis as compared to different other markers in our local setup.

A number of studies have revealed association between lower than normal serum vitamin D and pulmonary TB[11,12,24-26]. Our study supported this evidence as the mean value of serum calcium level (<8.6 mg/dl) and Vitamin D (< 10.0 ng/ml) at baseline was well below the reference range, indicating vitamin D deficiency leading to hypocalcemia.

Table 1: Disease Severity and Demographics (N=72)

Variables	Group D Vitamin D along with standard ATT	Group P Standard ATT	p-value
Age, years (mean ±SD)	25.67±6.33	23.559.53	0.54
Body mass index, kg/m^2 (mean ±SD)	17.38(±2.7)	16.86(±2.13)	0.19
Gender			0.24
Male	21 (58.3%)	18 (50.0%)	
Female	15 (41.6%)	18 (50.0%)	
Ethnic group			0.17
Pushto	11(30.5%)	12(33.3%)	
Punjabi	10(27.7%)	4(11.1%)	
Siraiki/Kashmiri/ Hazara	6(16.6%)	7(19.4%)	
Sindhi	5(13.8%)	7(19.4%)	
Urdu Speaking	3(8.33%)	6(16.6%)	
Socioeconomic status			0.53
Income less than 2 US dollars per day or Rs 6000 / month	24(66.6%)	27(75.0%)	
Income > Rs. 6000–12,000 / month	12(33.3%)	9(25.0%)	
Education			0.48
Illiterate	25(69.4%)	26(72.2%)	
Under grade 10 (matriculate)	7(19.4%)	6(16.6%)	
Grade 10 (Matriculate)	2(5.5%%)	2(5.5%)	
Grade 12 (Intermediate)	2(5.5%)	2(5.5%)	
Disease Severity			
1^+	12(33.33%)	12(33.33%)	
2^+	12(33.33%)	12(33.33%)	
3^+	12(33.33%)	12(33.33%)	

Group D = Treatment Group (ATT + Adjunct Vitamin D)
Group P = Control Group [ATT + placebo (normal saline)]

Table 3: Days Required for Conversion of Sputum

Group name (number of patients)	Days required for conversion of sputum	p value
Group D (36)	49.21(2.31)	0.032*
Group P (36)	61.33(2.68)	
Group D1 (12)	52.02 (2.44)	0.034*
Group P1 (12)	60.32(2.56)	
Group D2 (12)	49.12 (2.29)	0.028*
Group P2 (12)	61.48(2.70)	
Group D3 (12)	45.98	0.019*
Group P3 (12)	61.86	

* = Statistically significant

Table 2: Adjuvant Vitmain D Group vs Standard ATT (Placebo) Group BMI and Laboratory Investigations at Baseline and Day 70 (N=72)

	Group D ATT with adjuvant Vitamin D (36) Mean ± SD		Group P ATT with placebo (36) Mean ± SD		p-value
	Day 0	Day 70	Day 0	Day 70	
Vitamin D ng/ml	8.19(4.56)	76.90(23.68)	7.92(4.64)	6.39(3.81)	<0.001 *
Calcium mg/dl	8.69(0.41)	9.23(0.54)	8.49(0.58)	8.91(0.54)	0.92
Hemoglobin g/dl	11.53(1.73)	12.52(2.12)	11.38(2.05)	11.82(1.94)	0.134
ESR mm/hr	75.12(32.03)	29.35(21.23)	82.45(32.31)	48.64(33.98)	0.038 *
Hs-CRP mg/L	55.98(39.44)	24.11(30.88)	67.41(37.31)	22.11(31.44)	0.28
BMI kg/m^2	17.61(2.70)	19.89(2.51)	16.76(2.34)	18.12(2.30)	<0.001 *
Days for conversion of sputum	49.21(2.31)		61.33(2.68)		0.032 *

Group D = Treatment Group (ATT + Adjuvant Vitamin D therapy)
Group P = Placebo Group (control) [ATT + placebo (normal saline)]
* = Statistically significant

This has also been detected in different studies that first line anti tuberculous drugs especially isoniazid and rifampicin influence Vitamin D metabolism causing low levels of vitamin D during course of the treatment[27,28]. These low levels of Vitamin D induced by anti tuberculosis therapy might even slightly increase the serum calcium levels during the treatment regardless of the regulation of calcium levels by Vitamin D[29,30].

We found in our study that among 36 patients, mean level of Vitamin D in the Placebo group reduced from 7.90 ±4.58 ng/ml at baseline to 6.45 ±3.77 ng/ml at day 70 whereas the mean level of serum calcium had a non significant increase corroborating the earlier evidence from different studies. In terms of days required for microscopic sputum conversion, in our study P1 among three subgroups required the minimum mean number of days i.e., 60 days substantiating the direct association of disease severity with delayed sputum conversion[14].

Scarce data is available regarding the comparison of results with baseline parameters after adjuvant vitamin D treatment along with standard anti tuberculous treatment. In our research study, 600,000 IU[31] of adjuvant Vitamin D (IM) was administered at baseline (week 1) and at Day 40 (week 6) of the anti tuberculous treatment. The underlying principle for preferring the IM route was that Vitamin D capsules of only 400 IU and 800 IU available in our local setting were very costly and available only at few places. Furthermore, a large number of tablets (25 to 50 tablets per day) were required to meet the daily requirement, making it unfeasible and difficult for patients. It was also

possible to directly monitor the administration of vitamin D by the principle investigator by selecting the IM route.

Vitamin D mean values at baseline and at day 70 showed a significant increase from 8.20 ± 4.45 ng/ml to 77.20 ± 23.71 ng/ml ($p < 0.001$) whereas the mean calcium levels from 8.75 ± 0.45 ng/ml to 9.12 ±0.52 ng/ml ($p < 0.001$). The mean hemoglobin level was also observed to increase significantly from 11.62 ±1.70 ng/ml at baseline to 12.61 ± 2.10 ng/ml at day 70 ($p < 0.001$) signifying improvement of nutrition as a marker for disease control. A significant increase in BMI was observed on the other hand associating the importance of improved nutritional status and possibly immune system with disease resolution in treatment group. An inverse relationship was observed between the severity of the disease and the time period for AFB sputum conversion with administration of adjuvant vitamin D in adjuvant vitamin D groups i.e., 52 days (D1), 49 days(D2) and 46 (D3).

Administration of adjuvant Vitamin D therapy along with standard treatment helped in regulating the serum Vitamin D levels within normal range in group D whereas this deficiency was further aggravated in group P receiving placebo treatment with anti tuberculous treatment. Likewise the variation in the mean ESR values at day 0 and day 70 in group D group was statistically significant as compared to group P. On the contrary, the difference in the mean values of calcium, Hs-CRP and hemoglobin at day 0 and at day 70 in the vitamin D and placebo groups was not statistically significant suggesting the role of higher dose to produce some better outcome. A multi centered study conducted in Pakistan during 2009-2010 evaluating the role of adjuvant Vitamin D therapy also reported overall accelerated clinical and radiographic improvement in pulmonary tuberculosis patients[32].

Limitations of the study were that more specific bio markers of tuberculosis such as cathelicidin and interferons could not be used due to financial constraints. Oral route for administering high dose Vitamin D could not be used as Vitamin D capsules in our local setting were only available in strengths of 400 IU and 800 IU at few selected places.

CONCLUSION

In conclusion, an increase in severity of disease resulted in earlier sputum conversion in vitamin D treatment subgroups D1, D2, and D3 whereas in placebo subgroups P1, P2 and P3, the association of severity of disease was associated with delayed sputum conversion. Furthermore an inverse effect was observed on sputum conversion time that is higher the disease severity earlier the sputum conversion indicating more potent disease severity control. Adjuvant Vitamin D treatment could have short term effects by shortening the treatment duration and improving the patient adherence to treatment, and long term effects by decreasing the emergence of drug resistance due to better compliance.

Acknowledgements: Dr. Fahad Azam designed, conducted research, wrote paper. Dr. Nasim Karim designed research. Dr. Abida Shaheen analyzed data, wrote paper, had primary responsibility for final content. Dr. Faisal Faiyaz Zuberi conducted research. Dr. Rabia Arshad conducted research. Dr. Syed Hafiz Imran-ul-Haque conducted research.

Conflict of interest: The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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