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Hypovitaminosis D and Pulmonary Tuberculosis: A Study in a Tertiary Care Institute of Mewat

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Original Research Article

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Abstract: Tuberculosis (TB) remains a major challenge to global public health. Vitamin D is an immunoregulatory hormone. Epidemiological evidence also suggests a link between vitamin D deficiency, serum calcium, serum phosphate and TB but the relation is not clear. Our study was aimed to assess the levels of serum Vitamin D, serum calcium and phosphorus in pulmonary tuberculosis (PTB) patients of Mewat. Blood samples were collected from 100 patients with newly diagnosed PTB (PTB-0) and PTB cases taking treatment or have completed treatment (PTB-Rx). Age and sex matched controls with same ethnic background, dietary habits and sun exposure were taken. Serum was analysed for vitamin D, calcium and phosphorus. Serum vitamin D was significantly decreased in both groups of cases (PTB-0=13.9±5.8 ng/ml)(PTB-Rx=13.6±5.1ng/ml) as compared to controls (29.5± 6.5 ng/ml). 86% of cases had vitamin D value <20ng/ml. Calcium was comparable in all the groups. Serum phosphorus was significantly increased in newly diagnosed PTB cases (5.3±1.2 mg/dl) but was within normophosphatemic limits as compared to controls (4.1±0.4 mg/dl) and was comparable with that of PTB cases on treatment or completed treatment (5.2±1.7 mg/dl). Hypovitaminosis D was significantly associated with TB infection in our study. Since it has a role in Cell Mediated Immunity it could be one of the causes for the infection. Deficiency was noted in 86% of the cases, the importance of improving the nutrition and if required even supplementing vitamin D by food fortification.

Keywords: Vitamin D, tuberculosis, Mewat, normocalcemia, hyperphosphatemia

INTRODUCTION

Tuberculosis (TB) remains a major challenge to global public health[1]. Tuberculosis is the second most common cause of death from infectious disease (after those due to HIV/AIDS). One third of the world's population is thought to have been infected with mycobacterium tuberculosis (MTB), with new infections occurring in about 1% of the population each year.[2] Approximately 3 million fatalities reported in 2014 occur yearly and approximately five deaths happen in each minute[3]. Growing frequency of TB is an alarming situation for the community wellbeing of underdeveloped and industrial nations. 75% of tuberculosis cases in developing countries are in the economically productive age group (15-50 years)[2]. Researches stated that globally about 1/3 of the inhabitants are contaminated and three million individuals pass away in each year due to TB. Approximately 342 losses occur in a single hour because of this infected disease[4]. Pulmonary tuberculosis (PTB) is spreading worldwide.

The largest incidence, with an estimated 2.0 million new cases in India.[2] In addition to HIV infection, other factors that contribute to susceptibility and progression of TB are still controversial[1]. It has been suggested that susceptibility to TB is associated with immune response of host that could be influenced by environmental and genetic factors or by gene-environment interactions[2].

Vitamin D, an immunomodulatory effector, has been proven to play an essential role in inducing antimycobacterial activity by inhibiting the growth of MTB and up-regulating protective innate immunity[6,7]. Vitamin D has been shown to modulate monocyte-macrophage activity by binding to Vitamin D receptors, which both act as antigen presenting cells causing destruction of MTB and are responsible for

intracellular replication of MTB[8]. Previous clinical studies have indicated that "Vitamin D deficiency" is associated with an increased risk of tuberculosis; however, the criteria for "Vitamin D deficiency" differed among these studies. Some of these studies used a concentration of vitamin D < 50 nmol/L as a cutoff value for Vitamin D deficiency [9-11] while some other studies used a concentration below 25 nmol/L [12, 13]. Some studies also used other criteria, including 30 nmol/L and 62.5 nmol/L in some studies. Meanwhile, although a meta-analysis in 2008[14] found that low serum Vitamin D levels are associated with a higher risk of active tuberculosis for the exact range of serum Vitamin D for low serum Vitamin D was not defined. Explicit "dangerous" serum Vitamin D should be more practical in clinical and referable for future studies.

Hypercalcemia is acknowledged in granulomatous disease like TB but varied calcium abnormalities have been reported in studies related to tuberculosis, with some studies reporting hypercalcemia and a few others reporting hypocalcemia as a major biochemical finding. Some workers have detected hypercalcemia in 25% or more of their study population[15,16] with PTB, with symptoms of hypercalcaemia present in only 5–12 % of these patients. Hypercalcaemia in PTB has also been reported by Meuthen *et al.* [17] and Chan *et al.* [18]. have reported that albumin corrected calcium was found significantly higher in PTB patients despite a lower calcium intake but comparatively low percentage of hypercalcaemia was found in another related study. Contrary to all the above reports, pulmonary TB has been found to be associated with hypocalcaemia in some studies[19-22].

Similarly, alterations in serum phosphorus levels have been variedly reported in TB. Wells *et al*.[23] referred to a few early reports of phosphorus retention and reduced excretion and also to a few reports of increased urinary levels of lipid-bound phosphorus in patients with tuberculosis. Sweany *et al.*[24] found that phosphorus levels varied considerably from patient to patient and they suggested that some of the phosphorus might be associated with lipoproteins liberated by the destruction of cell membranes. Sharma[25] observed elevated phosphorus levels in Indian patients who were hypercalcaemic but not in those who were normocalcaemic. Studies have shown that there was a significant change in total serum calcium and inorganic phosphate levels of subjects studied especially hypocalcaemia and hypophosphatemia which is common in PTB[26].

Thus, we performed this study to define the precise range of serum Vitamin D, levels of serum calcium and phosphorus that contributes to TB infection in the PTB (IPD of SHKM GMC, Mewat) of Mewat district of Haryana which falls under subtropical semiarid zone where the incidence of tuberculosis is very high.

MATERIAL AND METHODS

100 cases of recently diagnosed pulmonary (PTB-0) and PTB on DOTS treatment or who have finished the course (PTB-Rx) were taken and divided into two groups. The diagnosis of TB was based on clinical, radiological, sputum Acid Fast Bacilli (AFB) smear positivity and gene expert analysis. DOTS (Direct observed treatment, short-course) therapy comprises of combination of drugs – Rifampicin, Isoniazid, Pyrazinamide and Ethambutol. The regimen was administered as per the RNTCP (DOTS) guidelines[27]. Comparison was done with 100 healthy controls that were age, sex matched with similar ethnic and social backgrounds and with comparable pattern of diet intake and sun exposure i.e. generally either a concerned family member or friend was taken. The clinical characteristics of cases like skin colour, exposure to sunlight, eating habits were noted. Skin colour of patients was defined as ''dark'', ie, black, ''mid'', ie, brown and ''light'', ie, white. Ultraviolet-B (UV-B) rays from the sun trigger the synthesis of vitamin D3 in the skin. In order to measure the exposure to sunlight a questionnaire was developed based on previous studies that have determined the variables affecting cutaneous vitamin D production from sun exposure (ie, season, time and hours of day, parts of body covered and sunscreens). Sunscreens with a sun protection factor of 8 or more will block UV-B rays[28]. Adequacy of sunlight exposure was assessed based on whether or not the subjects spent at least ten hours a week outdoors in daylight. Dietary pattern was also noted. Their demographic data are shown in table 1.

Venous blood samples were obtained from the patients and healthy controls. Biochemical characteristics including serum vitamin D levels, serum calcium and phosphorous levels were estimated by ELISA technique, arsenazo III[27] and phosphomolybdate[27] method respectively and were compared.

Cases excluded were infants under 2 years, women over 55 years, significant smokers (10/day), the clinically obese, those who were otherwise clinically prone for vitamin D deficiency like malabsorption, liver or renal disorders and those on drugs which can reduce Vitamin D levels or antagonize its actions[29]. Those who were predisposed to develop tuberculosis due to other obvious causes also were excluded (HIV infection, Diabetes, on immunosuppressive treatment, severe Protein Energy malnutrition and patients with concurrent disease, eg, carcinoma of the prostate, any

disseminated carcinoma, uncorrected thyroid disease or renal disease. These factors can themselves either depress or elevate serum levels of vitamin D and would therefore add unnecessary variables to the results.)[29].

Severe Vitamin D deficiency is generally defined by a serum concentration of 5-10 ng/ml[30]. For the purposes of this study, we defined severe deficiency as a serum concentration of 10 ng/ml or less, deficiency as > 10 to 20 ng/ml and adequate concentrations as 30–80 ng/ml.

Statistical analysis

Data are reported as mean (SE). Unpaired Student t tests were used to compare serum vitamin D, calcium and phosphorus concentration. Statistical significance was defined as $p \leq 0.05$ and 95% confidence intervals (CI) are given for comparisons between means.

RESULTS

Table-1: Demographic Data of the patients

S.no	Parameter	Demographic data
1.	Age range	15-70yrs (mean 40.7±16.3)
2	Sex	Male: 70, female:30
3	religion	Hindu:42, Muslim: 58
4	Smoking status	Smoker:72, non smoker:28
5	Skin colour	Dark:28, Mid:44, light:28
6	Sun exposure	Adequate :69, inadequate:31
7	Eating habit	Vegetarian:32 , non vegetarian: 68
8	Sputum AFB	Sputum positive:19, sputum negative:81
9	Serum creatinine (mg/dL)	1.05±0.31
10	Serum bilirubin (mg/dL)	.61±0.5
11	Random blood sugar (mg/dL)	133.4±36.3

The mean age of the patients was 40.7 ± 16.3 years (range 15-70 years). Male: female ratio was 70:30. Muslims were more in number (58). Majority were smokers (72%) and non-vegetarians (68%). Sputum was positive for AFB in 19 cases. Of the cases taken 57 were newly diagnosed and 43 were on treatment or those who have completed the treatment.

Table-2: Vitamin D, Serum Calcium and Serum Phosphorus comparison among PTB cases and controls

Parameter	Patients	Control
Vitamin D (ng/ml)	14.4±5.6*	29.5±6.5
Serum calcium (mg/dl)	9.7±1.5	9.7±0.4
Serum phosphorus (mg/dl)	5.2±1.3*	4.1±0.4

*significant w.r.t. controls

The value of Vitamin D in the study subjects ranged from 5.7-30 ng/ml with a mean value of 14.4± 5.6 ng/ml. The control group had Vitamin D levels ranging from 21.5-39.4 ng/ml and a mean value of 29.5 ± 6.5 ng/ml, and the difference was statistically significant ($p<0.005$) as shown in table 2. In the study, mean serum vitamin D level in newly diagnosed patients was 13.9±5.8 ng/ml while that in patients on therapy or completion of therapy was 13.6±5.1 ng/ml and in healthy control was 29.5±6.5 ng/ml respectively. Serum vitamin D levels were significantly different in newly diagnosed and PTB patients on treatment or after completion of treatment as compared to healthy controls. The results are shown in table 3 and figure 1.

Sunlight exposure was adequate in 69% of the cases, only 31 % had, if at all, inadequate exposure to sunlight. The inadequacy of sun exposure was not the primary cause for Vitamin D deficiency.

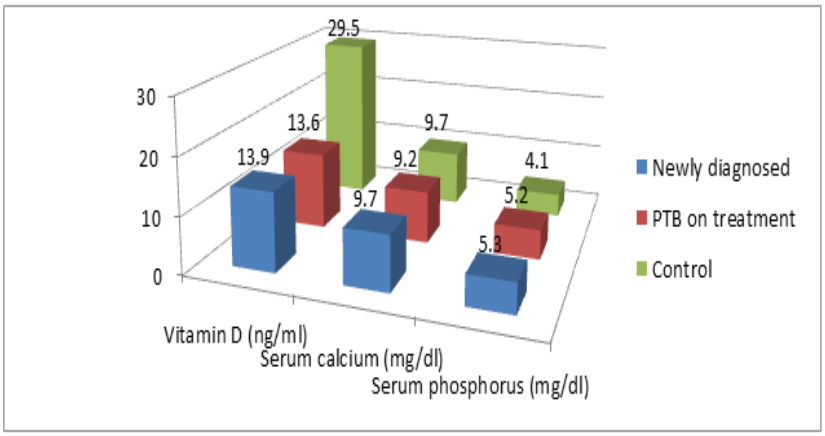

Fig-1: Vitamin D, Serum Calcium and serum phosphorus comparison among newly diagnosed PTB, PTB on treatment, completed treatment and controls

Table -3: Vitamin D, Serum Calcium and serum phosphorus comparison among PTB-0, PTB-Rx and controls

Parameter	Newly diagnosed (PTB-0)	PTB on treatment (PTB-Rx)	Control
Vitamin D (ng/ml)	13.9±5.8*	13.6±5.1*	29.5±6.5
Serum calcium (mg/dl)	9.7±1.5	9.2±1.3	9.7±0.4
Serum phosphorus (mg/dl)	5.3±1.2*	5.2±1.7	4.1±0.4

In the study, mean serum calcium level in newly diagnosed patients was 9.7±1.5 mg/dl while that in patients on therapy or completion of therapy was 9.2 ± 1.3 mg/dl and in healthy control was 9.7 ± 0.4 mg/dl respectively. In the study, mean serum phosphorous level in newly diagnosed patients was 5.3 ± 1.2 mg/dl. The values were estimated to be 5.2 ± 1.7 mg/dl in patients on therapy or completion of therapy and 4.1 ± 0.4 mg/dl in healthy controls respectively. No significant difference was observed in mean serum calcium in different groups and phosphorous levels was significantly in newly diagnosed PTB patients as compared to control. The results are shown in table 3 and figure 1.

Range vise distribution of cases and controls is shown in table 4.

Table -4: Distribution of cases and controls according to different levels of serum Vitamin D

Serum Vitamin D levels	Cases	Controls
5-10 ng/ml	33	-
>10-20 ng/ml	53	-
>20-30 ng/ml	14	67
30-80 ng/ml	-	33

DISCUSSION

This study confirms previous studies[30]. results that patients presenting with TB have significantly lower mean concentrations of serum vitamin D than controls. There was no evidence that the vitamin D assay used was affected by hypergammaglobulinaemia which can sometimes be associated with active TB.

Our study also suggests that this association is independent of skin colour. A previous large study looking specifically at skin type, sun exposure and serum vitamin D concentrations concluded that vitamin D concentrations were not linked with phototype but with sun exposure behaviour[32]. Mewat is a sunshine rich region predominantly occupied by meo population. Population is rural, of low socio economic status and majority people lives on farming and are non-vegetarians. So vitamin D deficiency is not expected in this population due to diet and sun exposure.

An explanation of low vitamin D concentrations could be resulting in reactivation of TB or it could be the result of TB-induced nutritional deficiencies. Insufficiency of vitamin D has been linked with progression of TB disease[33,34]. Vitamin D enhances the manufacture of antimicrobial peptide cathelicidin which help in assassination of MTB.[34] Vitamin D deficiency has long been accepted to be associated with impaired immunity and increased risk of TB[35]. Many types of immune cells including monocyte, macrophage, and T-lymphocyte have been proven to play a role in MTB resistance[36,37]. It was shown that that Vitamin D could induce interleukin-

1beta secretion and further modulated paracrine signaling, which reinforced the role of macrophage in innate immune regulation[38]. Another study noted that Vitamin D could improve the coordinated response to MTB of monocytes and T-lymphocytes in frequent MTB exposure but not in active TB patients[35]. Indeed, most of the studies that investigated the Vitamin D treatment for TB indicated that administration of Vitamin D could not improve clinical outcome among patients with TB[39,40]. It appears that Vitamin D might primarily play a role in preventing a MTB infection from progressing into active TB and but not curing active TB. Although Vitamin D has been shown to be immunoregulatory, clinical trials of vitamin D treatment in patients with active tuberculosis have got largely negative results[41,42]. A large prospective study would be needed to find out whether vitamin D deficiency precedes the development of PTB. Despite generally higher concentrations of serum vitamin D in the control group, there was no difference in dietary intake.

33% of cases had vitamin D in the range 5-10 ng/ml (severe deficiency), 53% had in the range 10-20 ng/ml (deficient) and 14% in suboptimal range of 20-30 ng/ml. 67% of controls were in suboptimal range while 33% were in normal range of 30-80 ng/ml. thus 86% of the PTB cases were in deficient in vitamin D. From our data it appears that patients with PTB from similar ethnic and social backgrounds and with comparable pattern of diet intake and sun exposure have lower serum vitamin D concentrations than their healthy contacts. This indicates that other factors contribute to vitamin D deficiency in the TB group.

This observation is particularly relevant since, even relatively affluent section of society do not consume a balanced diet which contains adequate protein, vegetables and fruits, but consume all kinds of fast foods, junk foods and many of them are exposed to overeating, sedentary habits, alcohol and tobacco smoking. It is also interesting to note the recently recognized role of Vitamin D in decreasing the risk of many chronic illnesses, including common cancers, autoimmune diseases, other infectious diseases, and cardiovascular disease.[43] Clinicians in India are witnessing an increase in lifestyle disorders, autoimmune disorders and malignancies. This could be because already malnourished people are now getting exposed to wrong lifestyle habits (thrown open by promotion of consumerism. The role of vitamin D in cancer prevention is becoming more obvious and needs special mention[44,45]. There could thus be a need for nationally coordinated action to substantially increase intake of vitamin D.

In this study, we observed no significant increase in serum calcium in PTB cases when compared to normal subjects. This is in contrast to findings reported by Okogun Godwin A in 2010 and also Burtis and Ashwood in 2001[46]. Vitamin D (25 hydroxycholecalciferol and 1, 25 dihydroxycholecalciferol) act in pathogenesis by activation of cell mediated immunity in pulmonary tuberculosis, this may alter calcium levels[47-49].

In present study, out of 100 PTB patients, 57 were newly diagnosed tuberculosis patients and of these 14 were found to have serum calcium > 10 mg/dl and rest were normocalcemic. We found that the serum calcium levels were comparable in newly diagnosed TB patients as compared to the control group and with those on completion of treatment. 50% of the patients on treatment or having completed the treatment have serum calcium levels >10.0 mg/dl. This finding is inconsistent with the earlier studies from Pakistan[22], Japan[19], Egypt[20,50] and Nigeria[21]. Though controversial reports of changes in serum calcium levels in tuberculosis patients have been reported. L. Lind *et al*.[51] reported that out of 67 patients with pulmonary tuberculosis 25% were hypercalcemic before the initiation of therapy. The patients suffering from TB had shown that calcium signalling is altered in macrophages infected by mycobacteria. The justification given for our finding can be as according to most reported cases the elevation of calcium ions that normally accompanies phagocytosis of other bacteria or inanimate particles was reduced or absent when mycobacteria were the target[52].

The serum phosphorous level was significantly high ($P<0.001$) in newly diagnosed TB patients as compared to the control. There was a no difference in the mean phosphorous level in patients on treatment or after anti-tubercular treatment and control. The mean phosphorous level in patients after the completion of anti-tubercular therapy or on treatment was comparable with newly diagnosed PTB patients. Similar observations were reported by Well H.G. *et al.* [23], Sweany H.C. *et al.* [24] and Sharma *et al*.[25]. This finding could be due to the distribution of the intracellular phosphate which is liberated due to the destruction of the cells. This result is in contrast to the observation made by Okogun GA who observed that there was no significant statistical difference ($P >0.05$) in the inorganic phosphate level between tuberculosis patients and control group as well as in newly diagnosed tuberculosis patients, when compared to tuberculosis patients on treatment[53].

A lot of previous studies have reported an increase in serum phosphate in tuberculosis patients. Although more attention has previously focused on calcium than on phosphorus in tuberculosis, studies by Kardjito *et al*. suggests that the latter is much more affected than the former in this disease[54]. Unlike

these previous reports, serum phosphorus levels innewly diagnosed (PTB-0) group in our study was significantly more than that of normal controls though the mean value was still within the accepted normal range of serum phosphate in humans (2.4–4.7 mg/dl) and hence our finding is one of normophosphatemia rather than hyperphosphatemia. This is similar to the findings of Hafiez *et al*. who have reported normophosphatemia in tuberculosis patients[50].

CONCLUSION

Randomized controlled studies are not feasible for establishing the link between all aspects of nutrition and diseases like TB. The observations suggest that Tuberculosis control programme (RNTCP), to succeed, may need to incorporate dietary intervention and education of the people on the need for balanced diet. Vitamin D deficiency was observed in all patients with PTB, thus there is a definite relationship between Vitamin D deficiency and TB. Vitamin D deficiency could be a cause of TB rather than the effect of it. Even apparently healthy people have vitamin D deficiency. The lower levels of vitamin D are due to reduced intake and not due to reduced sun exposure. Vitamin D deficiency occurs without any symptoms, if at all any symptoms are present it indicates severe deficiency (<10ng/ml). Serum calcium and phosphate are not sensitive to screen for deficiency. The drawbacks of the study were the small sample size as compared to high prevalence.

Hypovitaminosis D was significantly associated with TB infection in our study. Considering the role of Vitamin D in Cell Mediated Immunity and the protective effect of it in several other diseases, the article highlights the importance of improving the nutrition and if required even supplementing Vitamin D by food fortification, rather than hoping to get vitamin D from sunlight exposure alone. Further studies are needed to evaluate a possible role of vitamin D in the treatment and prevention of PTB.

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