

Vitamin D₃ in Newly Diagnosed Pulmonary Tuberculosis Patients: A Comparative Case Control Study

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ABSTRACT

OBJECTIVE: To evaluate Vitamin D in Pulmonary tuberculosis and normal healthy adults.

STUDY DESIGN: Case control study.

PLACE AND DURATION: Department of Medicine, Isra University Hospital Hyderabad, from January 2013- June 2014.

SUBJECTS & METHODS: A sample of 209 diagnosed pulmonary tuberculosis patients was selected through non-probability purposive sampling according to inclusion and exclusion criteria. After taking informed written consent from the participants, Vitamin D was measured by ARCHITECT I 1000 system for estimation of 25-OH- D₃. The data was recorded on a pre-structured proforma, and analyzed on SPSS version 21.0. The significant p-value was taken at ≤ 0.05 .

RESULTS: The mean \pm S.D serum level of 25-hydroxyvitamin D₃ in cases and controls were 27.1 ± 9.7 and 36.8 ± 8.1 (ngdl⁻¹) respectively ($p=0.0001$). The 25-hydroxyvitamin D₃ levels as low as 6 ng/dl were observed in pulmonary tuberculosis subjects. The normal, insufficiency and deficiency of 25-hydroxyvitamin D₃ were observed in 37(33%), 16 (14.2%) and 59 (52.6%) in cases respectively compared to controls as 57 (58.7%), 21(21.6%) and 19 (19.5%) respectively.

CONCLUSION: Patients with pulmonary tuberculosis are significantly Vitamin D deficient. Vitamin D supplements may be prescribed, however further studies are warranted.

KEY WORDS: Vitamin D₃, Pulmonary tuberculosis, Sindh.

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INTRODUCTION

Worldwide prevalence of vitamin D deficiency is 50% in elderly and 2- 30% in the European adults.¹ Serum Vitamin D level is a sensitive measure of vitamin D status of an individual² and the prevalence of inadequate 25-hydroxyvitamin D is around 30% to 50% in the general population.³

Though vitamin D belongs to the class of vitamins, it is unique as it is activated in the body and functions as a hormone. It plays a crucial role in calcium homeostasis and bone mineralization. It is known to participate in biological phenomena like cell differentiation and cell growth. Vitamin D is reported of having potential role in immunomodulation.⁴⁻⁸ Vitamin-D is an important effector of macrophage functions and thus could be having an important role in limiting growth or survival of intracellular pathogens like Mycobacterium Tuberculosis, Salmonella and all viruses.⁶⁻⁹ The role of Vitamin D in preventing several malignancies is increasingly being recognized. Recent evidences link vitamin D deficiency to diseases like Diabetes, Hypertension, infections, autoimmune disorders and cancer.^{10,11} Vitamin D modifies gene expression in the tissues where it acts by binding to specific receptors

vitamin D binding receptors (VDR) and has several known actions and several more hitherto unknown to us.⁶⁻⁸

Vitamin D deficiency has been implicated as a risk factor for tuberculosis (TB).¹²⁻¹³ An association between Vitamin D and TB has been described in few studies including one from Chinese patients with pulmonary TB.¹⁴ Asymptomatic Vitamin D deficiency is common in Pakistan as reported by previous studies.¹⁵⁻¹⁶ Pakistan was included in first five countries with maximum number of incidents of TB cases in 2011.¹⁷ Despite high burden of pulmonary tuberculosis, only a few studies have been conducted to highlight the issue. The present study was conducted to evaluate circulating levels of 25-hydroxyvitamin D₃ in newly diagnosed pulmonary tuberculosis in rural population of Sindh.

SUBJECTS AND METHODS

A prospective case control study was conducted at Department of Medicine, Isra University Hospital Hyderabad, from January 2013 - June 2014. A sample of 209 subjects (112 pulmonary tuberculosis and 97 controls) was selected through non-probability purposive sampling according to inclusion and exclusion criteria.

Subjects who fulfilled the following criteria: i) clinical history suggestive of tuberculosis, ii) Sputa +ve for acid fast bacilli, iii) Roentgenography findings consistent with the pulmonary tuberculosis like cavity formation in lung apices. Informed written consent was taken from the participants. Patients with past and present history of anti tuberculous drugs who have discontinued the drugs because of unknown reason, chronic cases of pulmonary tuberculosis, drug defaulter, and extra pulmonary tuberculosis were excluded from the study. General physical and chest examination was performed for the presence of cavitations, consolidation, fibrosis, pneumothorax and pleural effusion.

5 ml of fasting venous blood sample was drawn from ante-cubital vein. The blood was centrifuged at 4000 rpm for ten minutes and serum obtained was frozen at -20°C. The serum was used for estimation of blood 25-hydroxyvitamin D₃. The vitamin D₃ was measured by ARCHITECT I 1000 system for estimation of 25-OH-D₃ tem from blood sera. The normal, insufficiency and deficiency of Vitamin D₃ were defined as; normal levels (>30ng/dl), vitamin D₃ insufficiency (20-30 ng/dl) and vitamin D₃ deficiency (<20ng/dl). Study was approved by the ethics committee of the institute. The data was recorded on a pre-structured proforma.

Data was analyzed on SPSS version 21.0. Continuous and categorical variables were analyzed by student's t-test and chi square test respectively. The significant p-value was taken at ≤ 0.05.

RESULTS

The baseline characteristics of study population are shown in table I. Mean ±S.D age of cases and control were 39±6.7 and 40±9.1 years respectively. The gender distribution, obesity, BMI, smoking habit and chest x ray showing cavitations are shown in table I. significant differences were observed for the obesity and smoking p-value 0.045 and 0.043 respectively. Chest x ray revealed cavitory in 39 (34.8%) of cases. Hemoglobin, RBC counts and Platelet counts revealed statistically significant difference between cases and controls. Low normal hemoglobin values were observed in most of study subjects in general and pulmonary tuberculosis patients in particular. Serum alkaline phosphatase, serum calcium and serum phosphate showed significant differences as shown in table I.

The mean± S.D of 25-hydroxyvitamin D₃ in cases and controls were 27.1±9.7 and 36.8±8.1 (ngdl⁻¹) respectively. (p=0.0001) (Table II).

The 25-hydroxyvitamin D₃ levels as low as 6 ng/dl were observed in pulmonary tuberculosis subjects. The normal, insufficiency and deficiency of 25-hydroxyvitamin D₃ were observed in 37(33%), 16 (14.2%) and 59 (52.6%) in cases respectively com-

pared to controls as 57 (58.7%), 21(21.6%) and 19 (19.5%) respectively. All comparisons were found statically significant as shown in table III.

TABLE I: BASELINE CHARACTERISTICS STUDY POPULATION (n=209)

| | Case (n=112) | Control (n=97) | p-value* |
|---|--------------|----------------|----------|
| Age (years) | 39±6.7 | 40±9.1 | 0.08 |
| Male | 67 (59.8%) | 58 (59.7%) | 0.10 |
| Female | 45 (40.1%) | 39 (40.2%) | 0.11 |
| Obesity | 31(%) | 48 (%) | 0.045 |
| BMI (kg/m ²) | 29±3.9 | 29±2.5 | 0.09 |
| Smokers | 47 (41.9%) | 23 (23.7%) | 0.043 |
| Chest X-ray cavity | 39 (34.8%) | - | - |
| Hemoglobin (g/dl) | 11±3.9 | 12±2.9 | 0.031 |
| RBC counts (x10 ⁹ µl ⁻¹) | 3.9±2.1 | 4.1±3.9 | 0.035 |
| WBC counts (µl ⁻¹) | 7693±178 | 7178±156 | 0.10 |
| Platelets (x10 ⁹ µl ⁻¹) | 3.6±1.1 | 3.8±1.3 | 0.09 |
| Erythrocyte sedimentation rate | 47±19 | 6±3.7 | 0.0001 |
| Alkaline phosphatase(iu) | 109.3±7.6 | 96.9±12.3 | 0.01 |
| Serum calcium (mg/dl) | 8.0±1.9 | 9.2±1.7 | 0.01 |
| Serum phosphate (mg/dl) | 2.3±0.5 | 2.5±0.7 | 0.09 |

* Student t test.

TABLE II: 25-HYDROXYVITAMIN D₃ IN CASES AND CONTROLS (n=209)

| | Case (n=112) | Control (n=97) | p-value* |
|-----------------------------|--------------|----------------|----------|
| Normal levels (>30ng/dl) | 33.5±0.7 | 37.5±3.7 | 0.02 |
| Insufficiency (20-30 ng/dl) | 24.1±2.9 | 27.1±2.3 | 0.01 |
| Deficiency (<20ng/dl) | 13.8±5.7 | 17.8±1.7 | 0.001 |
| Total | 27.1±9.7 | 36.8±8.1 | 0.001 |

* Student t test.

TABLE III: FREQUENCY OF 25-HYDROXYVITAMIN D₃ IN CASES AND CONTROLS (n=209)

| | Case n (%) | Control n (%) | p- value** |
|--------------------------------|---------------|------------------|---------------|
| Normal levels (>30ng/dl) | 37(33%) | 57 (58.7%) | 0.01 |
| Insufficiency (20-30 ng/dl) | 16 (14.2%) | 21(21.6%) | 0.02 |
| Deficiency (<20ng/dl) | 59 (52.6%) | 19 (19.5%) | 0.0001 |
| Total | 112(100%) | 97 (100%) | - |

** Chi Square Test

DISCUSSION

Vitamin D plays an important role in macrophage activation to restrict growth of mycobacterium. Several biological studies to detect effects of Vitamin D on immune system of the body show that Vitamin D has a definitive role in suppression of proliferation of Mycobacterium TB and generalized inflammatory response produced secondary to it.^{18,19} Similarly, on triggering of toll-like receptors by molecules of the tubercle bacillus, the production of microbe-killing cathelicidin is impaired in the absence of adequate serum Vitamin D.²⁰ However in-vivo association between Vitamin D and tuberculosis is still a debatable issue. In this study, we found that Vitamin D insufficiency, as assessed by 25[OH]₂ D₃ level, was significant in patients with TB, both in men and women.²¹ As anti tuberculosis chemotherapy can lower serum Vitamin D levels, so only those of tuberculosis patients were included who were yet to commence treatment.

The possible association between Vitamin D and tuberculosis was first reported 20 years ago²² but subsequent studies have yielded conflicting findings. A number of studies in Gujarati Indian,²³ African residents in London,²⁴ African immigrants living in Australia²⁵ and people of West Africa²⁶ all have shown that tuberculosis had lower levels of 25[OH]₂ D₃ and higher prevalence of Vitamin D deficiency than non-TB individuals. Among African immigrants in Australia, for example, individuals with latent or active tuberculosis were observed to have substantially lower serum Vitamin D levels than those without tuberculosis. Although there is good evidence to suggest that a fall in serum Vitamin D levels compromises cell mediated immunity and leads to the activation of latent tuberculosis,²⁷ it is also possible that low serum Vitamin D levels result from tuberculosis itself. Smoking is a risk factor for tuberculosis disease. Although Vitamin D is important for calcium absorption (which is impaired by smoking), there is no evidence to suggest that Vitamin D absorp-

tion is impaired directly by smoking.

This study also showed no significant relationship between BMI and change in Vitamin D level. As most of the patients with TB have low BMI, which is further associated with Vitamin D deficiency, thus low BMI is important confounder for association of the low Vitamin D and tuberculosis. Low Vitamin D level in TB patients needs to be further evaluated as the prevalence of diabetes mellitus (DM) is increasing globally and people with DM are 4-5 times more likely than those without DM to have clinically significant chronic kidney disease (CKD).²⁸ In addition, patients with CKD or those who are dialysis dependent are more likely to have low levels of Vitamin D in comparison to those without kidney disease.²⁹ The incidence of tuberculosis is high in CKD patients partly as a result of impaired cell-mediated immunity, however if low serum Vitamin D levels also predispose to tuberculosis, then growing population of patients of CKD from underlying causes like DM may need early attention to their body Vitamin D levels to mitigate the risk of active tuberculosis. Moreover, in this study, it was noted that Vitamin D deficiency was detected in approximately half of normal female population which is quite significant and does raise a healthcare concern that a sizeable majority of the healthy population is deprived of the proven benefits of Vitamin D. Possible reasons for this female preponderance can be predominantly homebound females, poorer nutritional status than their male counterparts, social stigma associated with TB, which discourages women from seeking early medical care, and Vitamin D deficiency due to poor dietary intake as well as inadequate exposure to sunlight because of poor housing and the culture of wearing hooded cloaks (Burqas). However, prevalence of Vitamin D deficiency was much lower than what was found in another study conducted in Karachi.¹⁶ Similarly, in this study, prevalence of Vitamin D deficiency in asymptomatic females were much lower than in premenopausal women bone health survey of Karachi in 2010 by Mansoor et al²⁹. where 82.8% women were found Vitamin D deficient.³⁰ The smaller size, different cut off limit of Vitamin D deficiency level, darker skin pigmentation in women of Karachi and betel chewing habit may explain the disparity between results. The present study postulates that Vitamin D may be effective as adjuvant therapy in patients with tuberculosis. This observation is supported by the work of Martineau et al³¹ in which a single dose of Vitamin D improved immunity to Mycobacteria in vitro in contacts to patients with TB.

No study is yet carried out to compare this prevalence and our observation needs further verification. In this study, 11.4% of TB patients had Multi drug resistant (MDR) TB on drug susceptibility testing and mean

Vitamin D level was found much lower than other forms of TB. No other study exclusively determined Vitamin D level in MDR TB and this study needs to be verified by future studies upon MDR TB and Vitamin D deficiency associations. There were few limitations of this study. The present study did not exclude smokers whereas smoking indirectly affects Vitamin D metabolism by altering calcium absorption from intestine. Controls were not screened for occult chronic diseases and malignancies which may reduce Vitamin D levels in both control and cases. The patients in this study were from one center so the results cannot be applied to the whole population.

CONCLUSION

In present study, compared to healthy adults, patients of pulmonary tuberculosis were found vitamin D deficient. Vitamin D supplementation is warranted for patients of pulmonary tuberculosis.

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