ORIGINAL ARTICLE

Vitamin D Deficiency in Patients with Tuberculosis

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ABSTRACT

Objective: To determine the frequency and association of Vitamin D deficiency in patients with tuberculosis.
Study Design: Case control study.
Place and Duration of Study: Medical Department, Combined Military Hospital, Kharian, from July 2010 to June 2012.
Methodology: One hundred and five outdoor patients of tuberculosis were selected with 255 gender matched controls. Tuberculosis was diagnosed by presence of acid fast bacilli in sputum smears, positive culture for *Mycobacterium tuberculosis* or demonstration of chronic caseating granulomatous inflammation in tissue specimens. Controls were drawn randomly from general population. Serum 25 hydroxyvitamin D [25 (OH) D3] levels < 25 ng/ml was considered Vitamin D deficiency. The results were analyzed on SPSS version 17.
Results: Mean Vitamin D levels were 23.23 ± 6.81 ng/ml in cases, 29.27 ± 8.89 ng/ml in controls ($p < 0.0001$). Vitamin D deficiency was found in 57% of cases and 33% controls ($p < 0.0001$). Mean Vitamin D levels were significantly lower in females with tuberculosis (20.84 ng/ml) as compared to males (25.03 ng/ml, $p = 0.002$). Mean BMI in patients of tuberculosis with Vitamin D deficiency were 19.51 ± 1.77 kg/m^2 and in patients with normal Vitamin D were 21.65 ± 1.79 kg/m^2 ($p < 0.0001$). Mean Vitamin D levels in patients with multi-drug resistant tuberculosis was lower to a mean of 15.41 ± 4.67 ng/ml ($p < 0.0001$).
Conclusion: There is significant deficiency of Vitamin D in patients with tuberculosis as compared to controls. This deficiency is more pronounced in females, individuals with low BMI, extra pulmonary and MDR tuberculosis.

Key Words: *Tuberculosis. Vitamin D. Female. Body mass index.*

INTRODUCTION

More than two billion people across the world (about one-third of the world population) are estimated to be infected with *Mycobacterium tuberculosis.*[1] Approximately 95% of tuberculosis (TB) cases occur in developing countries with highest rates (100/100,000 or higher) observed in sub-Saharan Africa, India, China, and the islands of Southeast Asia. Pakistan ranks sixth globally among the 22 high tuberculosis risk countries, contributing 43% of the disease towards the Eastern-Mediterranean region, according to the World Health Organization (WHO).[2]

Vitamin D (Vit D) is believed to have an important role in macrophage activation and the subsequent restriction of MTB growth.[3-4] Low levels of Vitamin D (< 30 ng/dL) is a common finding world over, specially prevalent in developing countries and varies depending on the food fortification policies, demographic features, geographic location and season. Vitamin D deficiency has been implicated as a risk factor for tuberculosis (TB).[5] An association between 25 hydroxyvitamin D (25[OH] D) levels and TB has been described in few studies including one on Chinese patients with pulmonary TB.[6] Asymptomatic Vit D deficiency is common in Pakistan which is evident by a cross-sectional study being carried out in Karachi.[7-8] Pakistan was included in first five countries with maximum number of incidents of TB cases in 2011.[9] Despite this high burden of TB, no study is yet carried out in Pakistan to determine association of Vit D deficiency and tuberculosis.

This study was conducted to determine the association of Vitamin D deficiency in patients with tuberculosis.

METHODOLOGY

This study was carried out at the Department of Medicine, Combined Military Hospital, Kharian, from July 2010 to June 2012. Informed written consent was taken and permission was sought from hospital ethical committee. One hundred and five newly diagnosed patients of tuberculosis between 35 to 65 years of age were selected along with 255 gender matched individuals taken as control. Sample size was calculated using Raosoft sample size calculator taking 5% margin of error and 95% confidence level. Study was conducted throughout the year. Cases were newly diagnosed patients of tuberculosis who were yet to commence treatment. These were diagnosed by the identification of acid fast bacilli in sputum smears, isolation of *Mycobacterium tuberculosis* on culture or demonstration of chronic caseating granulomatous inflammation in tissue specimens. Drug susceptible testing was done on all patients of tuberculosis to find out presence of drug

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Received: January 21, 2013; Accepted: July 04, 2013.

resistance. Control group were age and sex matched and randomly selected from individuals reporting in our hospital outpatient department for routine medical examination. Both cases and controls were asked to fill a proforma relating to clinical history, anthropometry, physical activity, job description, smoking status, dietary pattern and excluded from study if they had any renal impairment, previous bone disease, recent surgery. Body mass index was recorded as (kg/m^2). Venous blood samples were collected for 25 (OH) D3 levels. We collected 5 ml of venous blood in plastic serum tubes. Samples were placed in ice boxes and sent immediately to laboratory. Serum was separated by centrifugation and 25 (OH) D3 levels measured using chemiluminescence assay using Roche diagnosis Elesys. All the results were duly verified by pathologist. For the purpose of study, a patient with vitamin D level less than 25 ng/ml was considered to be Vitamin D deficient.

Statistical interpretation of data was performed using Statistical Package for Social Sciences (SPSS) version 17. Results were expressed as mean, standard deviation (±SD) for all continuous variables and frequency and percentage for categorical data. We used t-test and chi-square test as appropriate to the nature and distribution of the variables. A p-value < 0.05 was considered statistically significant.

RESULTS

There were 105 patients (60 men and 45 women) with tuberculosis and 255 patients (145 men and 110 women) as control. Mean age of cases was 48.62 ± 8.84 years while that of controls was 48.20 ± 8.87 years. Using the criteria of Vitamin D deficiency less than 25 ng/ml, 60 patients of tuberculosis out of 105 (57 %) were deficient in Vitamin D as compared to 33% control (85 out of 255) with (p < 0.0001). Mean Vitamin D levels were lower in cases as compared to controls (Figure 1). Mean Vitamin D levels were 23.23 ± 6.81 ng/ml in cases and 29.27 ± 8.89 ng/ml in controls (p < 0.0001, Table I). Vitamin D deficiency was higher among women in both cases and controls. Out of 45 women with tuberculosis,

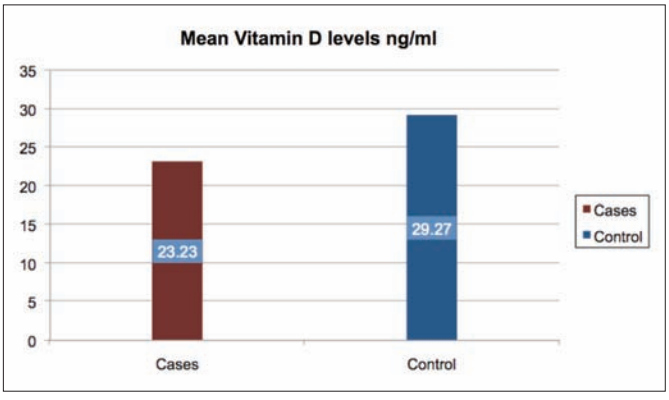

Figure 1: Mean Vitamin D levels in cases and control.

Table I: Clinical and demographic characteristics of study group.

Demographics	Cases n =105	Controls n =255	p-value
Age (years) mean [SD]	48.62 (±8.84)	48.20 (±8.87)	0.687
Males n (%)	60 (57%)	145 (57%)	0.961
Females n (%)	45 (43%)	110 (43%)	0.961
BMI (kg/m^2) mean [SD]	20.43 (±2.06)	23.62 (± 2.35)	<0.0001
Vitamin D3 (ng/ml) mean [SD]	23.23 (±6.81)	29.27 (±8.89)	<0.0001
Vitamin D 3 deficiency (n[%])	60 (57%)	85 (33%)	<0.0001

Table II: Clinical characteristics of patients with tuberculosis.

Variables	Vitamin D levels		p-value
	Deficient < 25 ng/ml	Normal > 25 ng/ml	
Number of patients n (%)	60 (57%)	45 (43%)	< 0.001
BMI(kg/m^2) mean [SD]	19.51 (± 1.77)	21.65 (± 1.79)	< 0.001
Vitamin D3(ng/ml) mean [SD]	18.55 (± 3.70)	29.48 (± 4.62)	<0.0001
Pulmonary tuberculosis (n [%])	40 (49%)	42 (51%)	0.001
Extra-pulmonary tuberculosis (n [%])	20 (87%)	3 (13%)	0.001
Sensitive (n[%])	49 (53%)	44 (47%)	< 0.01
Multidrug resistant (n[%])	11(92%)	1 (8%)	< 0.01

35 (77%) were deficient in Vitamin D. Female patients suffering from TB had lower Vitamin D levels than male patients with TB (p = 0.002). Similarly, 41.8% (46 out of 110) women in control group had Vitamin D deficiency (p = 0.01). Among cases, 82 were patients of pulmonary tuberculosis (78%) and 23 were extrapulmonary tuberculosis (22%) [Table II]. Among patients of pulmonary tuberculosis 48.7% (40 out of 82) had Vitamin D deficiency while in extrapulmonary tuberculosis Vitamin D deficiency was found in 86.9% (20 out of 23) cases (p < 0.001). Out of 105 patients of tuberculosis, 12 (11.4%) were found to have multi-drug resistant (MDR) tuberculosis. Mean Vitamin D levels in patients with multi-drug resistant tuberculosis was lower to a mean of 15.41 ± 4.67 ng/ml (p-value < 0.0001). Mean BMI levels were 20.43 ± 2.06 in cases and 23.62 ± 2.35 among controls (p < 0.0001, Table I). Mean BMI levels in patients of tuberculosis with Vitamin D deficiency were 19.51± 1.77 kg/m^2 and in patients with normal Vitamin D were 21.65 ± 1.79 kg/m^2 (p < 0.0001, Table II). As per Odd ratio (OR 2.667, 95% confidence interval 1.67 – 4.25), it was found that persons with tuberculosis are 2.667 times more likely to be Vitamin D deficient as compared to normal person.

DISCUSSION

Vitamin D (Vit D) plays an important role in macrophage activation and restriction of mycobacterial growth. Several biological studies to detect effects of Vit D on immune system of the body show that Vit D has a definitive role in suppression of proliferation of *Mycobacterium TB* and generalized inflammatory response produced secondary to it.[10,11] 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.[12] However, the *in-vivo* association between Vitamin D status and tuberculosis is still a debatable issue. In this study, we found that Vitamin D insufficiency, as assessed by 25 (OHD) level, was high in patients with TB, both in men and women. 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 more than 20 years ago,[13] but subsequent studies have yielded conflicting findings. A number of studies in Gujarati Indian,[14] African residents in London,[15] African immigrants living in Australia[16] and people of West Africa[17] all have shown that tuberculosis had lower levels of 25 (OH) D 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,[18] 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 absorption is impaired directly by smoking.

This study also showed no significant relationship between BMI and change in Vit D level. As most of the patients with TB have low BMI, which is further associated with Vit D deficiency, thus low BMI is important confounder for association of the low Vitamin D tuberculosis. Low Vit 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).[19] 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.[20] The incidence of tuberculosis is high in CKD patients partly as a result of impaired cell-mediated immunity but if low serum Vitamin D levels also predisposed to tuberculosis, the growing population of people with 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 Vit 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 Vit D deficiency was much lower than what was found in another study conducted in Karachi.[8] Similarly, in this study, prevalence of Vit D deficiency in asymptomatic females were much lower than in premenopausal women bone health survey carried out in Karachi in year 2010 by Mansoor *et al*. where 82.8% women were found Vit D deficient.[21] The smaller size, different cut off limit of Vit D deficiency level, darker skin pigmentation in women of Karachi and betal chewing,[22] habit may explain the disparity between results.

The present study postulates that Vit D may be effective as adjuvant therapy in patients with tuberculosis. This observation is supported by the work of Martineau *et al*. in which a single dose of Vitamin D improved immunity to Mycobacteria *in vitro* in contacts to patients with TB.[23] In this study, authors also compared Vit D deficiency in pulmonary versus extra pulmonary TB and it was found that extrapulmonary TB is associated more frequently with Vit D deficiency (72%) than Pulmonary TB (Prevalence 52%).

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

CONCLUSION

Patients with tuberculosis are significantly Vitamin D deficient as compared to normal people. This deficiency is more marked in females, those with low BMI, extrapulmonary and MDR tuberculosis. The present finding also warrants further studies to determine whether Vitamin D supplementation can have a role in the prevention and treatment of tuberculosis in developing countries like Pakistan or not.

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