Serum Vitamin D Levels in Diabetes Mellitus Patients with Pulmonary Tuberculosis

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Abstract

Purpose: The association between Pulmonary Tuberculosis (PTB) and Diabetes Mellitus (DM) has attracted much attention. They coincide with micronutrient deficiencies among which Vitamin D plays a major role. Our aim was to investigate serum 25 [OH] Vitamin D levels in DM patients with TB (TBDM).

Methods: Total of 249 subjects were studied including TBDM patients, PTB patients, DM patients and Healthy Controls (HC).

Results: There was a significant difference in age of the TBDM patients (p<0.001), PTB females (p<0.03) and DM males (p<0.001) when compared to HC. BMI was found to be significantly low in TBDM males (p<0.007), females (p<0.026) and PTB patients (p<0.0001). TBDM & DM patients with high blood sugar levels & DM duration with <5 years was associated with insufficient vitamin D levels. Vitamin D levels were found to be low in TBDM & DM (p<0.005) patients and in PTB patients (p<0.0005). DM patients with insufficient vitamin D levels have shown significance at p<0.03.

Conclusion: Severe hypovitaminosis D is more in DM patients with high blood sugar levels raising the risk of TB. Vitamin D may thus be considered as the missing link between emerging epidemic of PTB and DM.

Keywords: Tuberculosis; Diabetes mellitus; Vitamin D levels; Blood sugar levels

Introduction

Diabetes Mellitus (DM) and Tuberculosis (TB) are two diverse epidemics of different etiopathogenesis that have grown exponentially and merged into one another in developing countries [1]. India has the highest TB burden and the second highest DM burden in the world. Annual TB incidence was found to be 2.8 per million cases (range 2.0 to 2.7 million) and an estimated 15.3% have DM [2,3]. The frequency of DM in pulmonary tuberculosis patients is reported to be about 10% to 15%, and the prevalence of this infectious disease is 2-3 times higher in diabetic patients than in non-diabetic controls [4]. The burden of TB and DM represent a huge health threat, making the treatment failure more frequent and thereby results in more community acquired TB infection. Diabetes alters immunity to TB, which leads to higher mycobacterial burden and longer culture conversion time with treatment, and even may result in relapse [5].

Although both TB and DM are of different etiology there are many promising linking features and Vitamin D Deficiency (VDD) is one such link between the two [6]. Vitamin D, a unique member of class of vitamins, which is synthesized in the body, seems to influence glucose homeostasis from mild to moderate, insufficiency of which has been proposed as a risk factor for DM [7]. It is known to be involved in biological functions like cell differentiation, inhibition of cell growth and immunomodulation [8]. The key action of vitamin D on macrophages was believed to be its ability to stimulate differentiation of precursor monocytes to more mature phagocytic macrophages [9]. This concept was supported by observations showing differential expression of VDR and 1α-hydroxylase during the differentiation of human monocytes/macrophages [10]. Early studies have shown that normal human macrophages were able to synthesize 1,25(OH)2D3 when stimulated with Interferon gamma (IFNg) [11]. Localized activation of vitamin D, coupled with expression of endogenous VDR was strongly suggestive of an autocrine or intracrine system for vitamin D action in normal monocytes/macrophages. Vitamin-D is an important effector of macrophage functions and thus limits growth or survival of intracellular pathogen Mycobacterium tuberculosis which helps in...
prevent several malignancies [12]. Vitamin D has several known and unknown actions, modifies gene expression in the tissues where it acts by binding to specific receptors (VDR) [13].

VDD appears to be related to the development of DM [14]. Both in vitro and in vivo studies showed that vitamin D might prevent the destruction of pancreatic beta-cells and thereby reducing the incidence of autoimmune DM, possibly secondary to inhibition of pro-inflammatory cytokines, like Tumor Necrosis Factor (TNF-α) [15]. Vitamin D appears to play a major role as an immunomodulator of the innate immune response by inducing anti-mycobacterial activity [16]. Deficiency of serum 25 (OH) vitamin D has long been implicated as a risk factor in the activation of TB [17]. Recent studies have shown that 1,25-dihydroxyvitamin D induced the expression of the antimicrobial peptide, cathelicidin, that restricts the growth of Mycobacterium tuberculosis in monocytes under in vitro culture conditions [18]. We therefore hypothesized that vitamin D deficiency which may be widespread in T2DM patients with pulmonary tuberculosis, because an imbalance in the glycemic control leading to mycobacterial immunity.

The present study was designed to evaluate the serum levels of 25-hydroxy vitamin D [25(OH)D] and the relationship between glycemic control and 25(OH)D3 levels in DM patients with pulmonary tuberculosis.

**Materials and Methods**

**Subjects**

A case-control study was carried out including a total 249 subjects, out of which 61 were DM patients with pulmonary Tuberculosis (TBDM), 55 Pulmonary Tuberculosis patients (PTB), 51 DM patients; and 81 Healthy Controls (HC). TBDM & PTB patients were selected from those who attended free chest clinic by PPM-DOTS at Bhagwan Mahavir Medical Research Centre at Hyderbad, India. Diabetes was confirmed based on the blood sugar levels & body mass index of the studied subjects were on vitamin D supplementation.

**Measurement of serum vitamin D**

Blood (3 ml) was drawn from all the subjects and allowed to stand for around 2 h to 3 h for the separation of serum. Serum concentration of 25(OH) D was measured by Enzyme Linked Immunosorbent Assay (ELISA) by using the IDS 25-hydroxyvitamin D EIA kit (Demeditec diagnostics Kiel, Germany). The procedure was followed according to the manufacturer’s instructions wherein each test was run in duplicate, with mean absorbance computed from the average for two wells normalized to a zero calibrator well. The absorbance was read at a dual wavelength of 570- nm and 650- nm reference filter. Levels of vitamin D were expressed as ng/ml. The vitamin D status was assessed according to the following criteria: Sufficiency (VDS), 75-250; Insufficiency (VDI), 25-75; and Deficiency (VDD), <25 ng/ml. A cutoff point of <25 ng/ml of 25(OH) D was used to classify patients as on low vitamin D status.

**Statistical analysis**

The data was analyzed using the Statistical Package for Social Sciences (SPSS), version 20.0 and Prism GraphPad. Independent t-test was used to assess differences in serum among groups. The statistical significance (p<0.05), direction and strength of linear correlation between 2 quantitative variables were measured using Pearson’s correlation coefficient test. Categorical variables were compared by Chi-square test and quantitative data was reported as mean ± standard deviation.

**Results**

The study included a total of 249 subjects which were divided into four groups to determine the serum 25 [OH] vitamin D statuses. The data for clinical investigations was shown in Tables 1 and 2. The data was analyzed according to gender, age, BMI, blood sugar levels & vitamin D levels in TBDM, PTB & DM patients compared to healthy controls.

The study included 61 TBDM patients (39 males & 22 females), 55 PTB patients (26 males & 29 females), 51 DM patients (27 males & 24 females) and 81 HC (43 males & 38 females). There was a significant difference in age of the TBDM patients (p<0.005), PTB females (p<0.03) and DM males (p<0.001) when compared to that of the HC. BMI was found to be significantly low in TBDM males (p<0.007), females (p<0.026) and in PTB patients (p<0.0001) compared to that of HC. BMI of the DM patients was not significant compared to HC. Most of the TBDM & PTB patients were identified TST+ (Table 1).

<table>
<thead>
<tr>
<th>Demographic feature</th>
<th>TBDM N=61</th>
<th>PTB N=55</th>
<th>DM N=51</th>
<th>HC N=81</th>
</tr>
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<tr>
<td>Gender</td>
<td>M/F N (%)</td>
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<td></td>
<td></td>
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<tr>
<td>p value</td>
<td>ns</td>
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<tr>
<td>Age</td>
<td>Mean ± SD</td>
<td>p value</td>
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<tr>
<td>BMI</td>
<td>Mean ± SD</td>
<td>p value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TST+</td>
<td>35(57);20(33)</td>
<td>23 (41);27 (48)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TST-</td>
<td>4 (7); 2(3)</td>
<td>4 (7); 2 (4)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*p<0.05 statistically significant; ns: Not Significant

TBDM vs. HC, PTB vs. HC, DM vs. HC

**Table 1: Clinical characteristics of TBDM, PTB & DM patients.**
Vitamin D levels of TBDM & DM patients were categorized based on age, gender, BMI, Blood sugar levels & Diabetes duration in Table 2. Insufficient levels of vitamin D were found in TBDM & DM patients of >35 years age group. Significant difference was reported in TBDM patients <35 years (p<0.05) & DM patients >35 years age (<0.02) when compared to the HC. Gender wise comparison had shown both males and females with insufficient Vitamin D levels. Females were predominant in the TBDM (<0.0004) while males & females were equally significant (<0.03 & <0.01) in the Diabetics. Most of the TBDM & DM patients with normal weight & Insufficient vitamin D levels have shown significance at p<0.003 & p<0.002 respectively. TBDM & DM patients with high blood sugar levels have shown insufficient vitamin D levels. Diabetes duration with <5 years correlated well in patients with insufficient vitamin D levels shown in Table 2.

Sub group analysis of the severity of vitamin D levels between the groups was carried out (Table 2). There was no significant difference in the PTB group with respect to HC. Most of the subjects were found to have insufficient levels of vitamin D. Healthy subjects had a higher mean vitamin D level than the other groups. However, there was no difference in PTB patients with respect to vitamin D levels. TBDM & DM patients having sufficient vitamin D levels have shown significant difference at p<0.002. DM patients with insufficient vitamin D levels have shown significance at p<0.03. Vitamin D insufficiency was 25% among healthy individuals, 49% among TBDM patients, 55% among patients with only diabetes, 45% among PTB patients. Interestingly, we also found 16% of TBDM patients, 12% diabetics, 23% with only PTB and 10% healthy subjects were having severe VDD defined as vitamin D level below 25 ng/ml suggesting patients having TB & DM disease dual burden were more severely affected (Table 3).

When vitamin D levels were demonstrated on the whole in all the subjects, we observed it to be comparatively low in TBDM & DM patients with respect to HC at p<0.005. Vitamin D levels were significantly low in PTB patients when compared to the healthy controls at p<0.0005 (Figure 1).

**Discussion**

Vitamin D which induces immune tolerance [19] is related to
glucose metabolism and the development of T2DM and the metabolic syndrome [20]. It has been shown that Macrophages and other immune cells can express 1α-hydroxylase, the enzyme that converts circulating 25-hydroxyvitamin D3 into 1,25-dihydroxyvitamin D3, the active form of vitamin D [21]. Vitamin D receptors have been found in pancreatic beta cells, which even express the enzyme 1α-hydroxylase. It helps in the secretion of insulin from pancreatic beta cells, thereby appears to regulate insulin secretion [22].

In this study when data was analyzed in TBDM & PTB patients between 25-OH-vitD and other studied parameters, BMI was significantly lower when compared to DM patients & HC. In agreement to these results, Leung [23] & Nansera et al. [24] demonstrated that low body weight was associated with risk of TB, and that BMI below 18.5 increases the risk by 2 to 3 fold. The finding of decreasing levels of serum 25-OH-vit D with increasing BMI was in accordance with previous reports, establishing that obese individuals as a group have decreased levels of 25-OH-vit D [25]. However, these findings cannot be considered conclusive since the association may be confounded by important variables, such as smoking and sunlight exposure, which were not accounted in the analysis.

We observed that 49% of TBDM patients have insufficient vitamin D levels similar to a study in West Bengal where 46% patients were having vitamin D level <10 ng/ml, which may suggest that patients with DM and severe vitamin D deficiency were more susceptible to develop tubercular infection than those having normal or low vitamin D status [1]. In the present study, lower vitamin D levels were observed in TBDM patients than in healthy controls which was similar to a cross-sectional analysis of Eastern Finland population, where an inverse association was observed between 25(OH)D levels and fasting glucose tolerance test results [26], implying that low serum 25(OH)D3 may be associated with impaired glucose metabolism. In a prospective study in high risk Asian subjects, 25(OH)D deficiency was associated with a higher risk for the development of T2DM. In a large cohort study of older adults involving 7791 subjects who were diabetes-free in the beginning, serum 25(OH) D levels were inversely associated with incident diabetes in women but not in men [27] unlike our study where adult males were found to show insufficiency. In patients with T2DM, normal levels of vitamin D in the blood may facilitate glucose control and insulin resistance [28].

The possible association between vitamin D and TB was first reported more than 20 years ago [29]. In the current study, we demonstrated a high 25(OH)D deficiency among TB patients when compared to healthy controls which was similar to several other studies Japan (10% to 42%) involving non-HIV TB patients, in China 45% Gujarati Indian [30], Indian, Pakistani, Afghan, Somali, Sri Lankan and African residents in London [31], and African immigrants living in Australia [32]. Previous studies have reported an in-vivo association between vitamin D status and TB, demonstrated through significantly lower vitamin D levels in TB patients compared to controls in India, Europe, Africa, Asia, Kenya [33] and West Africa [34]. Asians have been reported to have lower vitamin D levels than Europeans. In a recent case report, very low serum vitamin D levels were implicated in primary infections as well as reactivation infections with M. tb. Thus, our results are consistent with the results of most previous studies. In a study performed in Vietnam, hypovitaminosis was more frequent only among males with TB, compared with control subjects. Sufficient 25(OH)D levels protect against Tuberculin Skin Test (TST) conversion, therefore supporting the hypothesis that vitamin D status may be a TB risk factor [35].

On the other hand, some studies have reported higher [36] or similar [16] vitamin D levels in TB patients compared to control subjects. The results from studies conducted in Asia have been variable. Discrepancies between the results of Asian and African studies may be due to the prevalence of VDD among Asians. There was no significant difference in 25(OH)D levels between TB patients and healthy controls in Indonesia [37] and Hong Kong [38]. Moreover, such inconsistencies between studies may be caused by differences in the characteristics of subjects, methodologies used for detecting serum vitamin D levels and the criteria applied for VDD. From our data it appears that patients with both DM & TB from similar ethnic and social backgrounds and with comparable sun exposure have lower serum 25-hydroxy vitamin D concentrations than their healthy controls. This indicates that other factors such as aging, diet intake, medications, and malnutrition contribute to vitamin D deficiency. Further studies in large number of samples are required to investigate vitamin D intake, metabolism and storage to unravel the relationship between Vitamin D and disease.

Conclusion

In conclusion, we found that a low serum 25(OH)D concentration is significantly associated with a high risk of TB in DM patients with poor glycemic control. In considering its potential role in treatment of TB and as an immune modulator; we can suggest that, vitamin D supplementation in DM patients may help in glycemic control thereby protecting them from developing TB. Vitamin D supplementation in TB patients under treatment might help in their early recovery and prevents them from turning into relapse.

Acknowledgement

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References


