Clinics in Oncology

Research Article
Published: 02 Jul, 2020

Serum Vitamin D Levels in Diabetes Mellitus Patients with Pulmonary Tuberculosis

*Meenakshi Ponnana[1] and Sumanlatha Gaddam[1,2]**

[1]Bhagwan Mahavir Medical Research Center, India

[2]Deparment of Genetics, Osmania University, India

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

OPEN ACCESS

Correspondence:
Sumanlatha Gaddam, Department of Genetics, Osmania University, Hyderabad, Telangana-500004, India, Tel: 9848967110; E-mail: sumanlathag@yahoo.com

Received Date: *26 May 2020*
Accepted Date: *30 Jun 2020*
Published Date: *02 Jul 2020*

Citation:
Ponnana M, Gaddam S. Serum Vitamin D Levels in Diabetes Mellitus Patients with Pulmonary Tuberculosis. Clin Oncol. 2020; 5: 1716.

Introduction

Diabetes Mellitus (DM) and Tuberculosis (TB) are two diverse epidemics of different etio-pathogenesis 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

Table 1: Clinical characteristics of TBDM, PTB & DM patients.

Demographic feature	TBDM N=61	PTB N=56	DM N-51	HC N=81
Gender				
M/F N (%)	39 (64); 22 (36)	27 (46); 29 (54)	27 (53); 24 (47)	43 (53); 38 (47)
p value	ns	ns	ns	
Age				
Mean ± SD p value	44.41 ± 12.52; ns 44.1 ± 12.78; **p<0.001**	35.85 ± 16.48; **p<0.05** 24.28 ± 9.45; **p<0.03**	41.3 ± 16.5; **p<0.001** 36.75 ± 17.0; ns	28.8 ± 10.1; 30.26 ± 13.03
BMI				
Mean ± SD p value	20.9 ± 4.89; **p<0.007** 21.1 ± 4.2; **P<0.026**	18.16 ± 4.4; **p<0.0001** 18.3 ± 5.0; **p<0.0001**	23.32 ± 5.65; ns 21.12 ± 6.32; ns	23.95 ± 5.14; 23.69 ± 4.25
TST+	35(57);20(33)	23 (41);27 (48)	-	-
TST-	4 (7) ; 2(3)	4 (7); 2 (4)	-	-

p<0.05 statistically significant; ns: Not Significant
TBDM *vs.* HC, PTB *vs.* HC, DM *vs.* HC

preventing 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 Hyderabad, India. Diabetes was confirmed based on the blood sugar levels of the patients. PTB was diagnosed based on their radiographic examination and sputum culture for Acid-Fast Bacilli (AFB). Tuberculin Skin Test (TST) was assessed in TBDM and PTB patients by administering 5 Tuberculin Units intradermally on the left arm. An induration >10 mm was considered positive. Selection of cases and healthy controls was carried out by using a systematic random sampling technique. Patients with acute illnesses, a history of chronic liver or kidney disease were excluded from the study. Body Mass Index (BMI) was calculated for each subject. Institutional Ethical Clearance and an informed written consent were obtained from all the patients. None 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 ($p<0.001$), 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 2: Serum Vitamin D and clinical characteristics.

Characteristic	TBDM (N=61)			P value	DM (N=51)			p value
Age(yrs)	**Deficient**	**Insufficient**	**Sufficient**		**Deficient**	**Insufficient**	**Sufficient**	
<35 (%)	3 (5)	7(11)	6 (10)	**<0.05***	2 (4)	7(14)	12(3)	ns
>35 (%)	7 (11)	23 (38)	15 (25)	ns	4(8)	21(41)	5(10)	**<0.02***
Gender								
Male (%)	7 (10)	15 (25)	17 (28)	ns	4 (8)	15 (29)	8 (16)	**<0.03***
Female (%)	3 (5)	15 (25)	4 (7)	**<0.0004***	2 (4)	13 (25)	9 (18)	**<0.01***
BMI								
Underweight (%)	5 (8)	8 (13)	6 (10)	ns	2 (4)	5 (10)	7 (14)	ns
Normal weight (%)	5 (8)	22 (36)	15 (25)	**<0.003***	4 (8)	23 (45)	10 (19)	**<0.002***
Random Blood sugar								
Normal (%)	1 (2)	5 (8)	0	-	1 (2)	3 (6)	2 (4)	-
High (%)	9 (15)	25 (41)	21 (34)	-	5 (10)	25 (49)	15 (29)	-
Diabetes duration								
<5years	7 (11)	17(28)	8 (13)	-	2 (4)	15 (30)	7 (14)	-
>5years	3 (5)	13 (21)	13 (21)	-	4 (8)	13 (25)	10 (19)	-

p<0.05 statistically significant; ns: Not Significant
TBDM *vs.* HC, DM *vs.* HC

Table 3: Comparison of vitamin D levels in different groups of patients.

Vitamin D levels	PTB patients	p value	TBDM patients	p value	DM patients	p value	HC
	N (%)		N (%)		N (%)		N (%)
Deficient	13 (23)	ns	10 (16)	ns	6 (12)	ns	8 (10)
Insufficient	25 (45)	ns	30 (49)	ns	28 (55)	**<0.03**	20 (25)
Sufficient	18 (32)	ns	21 (35)	**<0.002**	17 (33)	**<0.002**	53 (65)
Total	56		61		51		81

p<0.05 statistically significant; ns: Not Significant
TBDM *vs.* HC, PTB *vs.* HC, DM *vs.* HC

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).

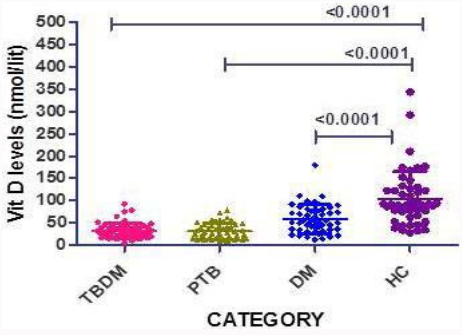

Figure 1: Serum vitamin D levels of TBDM, PTB & DM patients.
TBDM: Tuberculosis with Diabetes Mellitus; PTB: Pulmonary Tuberculosis; DM: Diabetes Mellitus; HC: Healthy Controls

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-hydoxylase, 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 1-α-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 T2DM 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

We thank staff of the free Chest Clinic, Mahavir PPM-DOTS, Tuberculosis Unit (T.U.), Bhagwan Mahavir Trust. Financial support was provided by Department of Science & Technology (DST) (Sanction order no: SR/FT/LS-013/2009, dated: 12/10/2009).

References

1. Chaudhary S, Thukral A, Tiwari S, Pratyush DD, Singh SK. Vitamin D status of patients with type 2 diabetes and sputum positive pulmonary tuberculosis. Indian J Endocrinol Metab. 2013;17(Suppl 3):S670-3.
2. World Health Organization. Global tuberculosis report 2019.
3. Noubiap JJ, Nansseu JR, Nyaga UF, Nkeck JR, Endomba FT, Kaze AD, et al. Global prevalence of diabetes in active tuberculosis: A systematic review and meta-analysis of data from 2.3 million patients with tuberculosis. Lancet Glob Health. 2019;7(4):e448-60.
4. Saeide S. The appearance of tuberculosis in diabetic patients. Biomedical Research. 2019;30(6):224-8.
5. Wang Q, Ma A, Bygbjerg IC, Han X, Liu Y, Zhao S, et al. Rationale and design of a randomized controlled trial of the effect of retinol and vitamin D supplementation on treatment in active pulmonary tuberculosis patients with diabetes. BMC Infect Dis. 2013;13:104.
6. Handel AE, Ramagopalan SV. Tuberculosis and diabetes mellitus: Is vitamin D the missing link? Lancet Infect Dis. 2010;10(9):596.
7. Shymanskyi I, Lisakovska O, Mazanova A, Veliky M. Vitamin D deficiency and diabetes mellitus. 2019; Julia Fedotova, IntechOpen.
8. Sasidharan PK, Rajeev E, Vijayakumari V. Tuberculosis and vitamin D deficiency. J Assoc Physicians India. 2002;50:554-8.
9. Szymczak I, Pawliczak R. The active metabolite of vitamin D3 as a potential immunomodulator. Scand J Immunol. 2016;83(2):83-91.

10. Nurminen V, Seuter S, Carlberg C. Primary vitamin D target genes of human monocytes. Front Physiol. 2019;10:194.

11. Boodhoo N, Sharif S, Behboudi S. 1α,25$(OH)_2$ vitamin D3 modulates avian T lymphocyte functions without inducing CTL unresponsiveness. PLoS One. 2016;11(2):e0150134.

12. Medrano M, Carrillo-Cruz E, Montero I, Perez-Simon JA. Vitamin D: Effect on haematopoiesis and immune system and clinical applications. Int J Mol Sci. 2018;19(9):2663.

13. Stechschulte SA, Kirsner RS, Federman DG. Vitamin D: Bone and beyond, rationale and recommendations for supplementation. Am J Med. 2009;122(9):793-802.

14. Lim S, Kim MJ, Choi SH, Shin CS, Park KS, Jang HC, et al. Association of vitamin D deficiency with incidence of type 2 diabetes in high-risk Asian subjects. Am J Clin Nutr. 2013;97(3):524-30.

15. Zittermann A, Gummert JF. Nonclassical vitamin D action. Nutrients. 2010;2(4):408-25.

16. Koo HK, Lee JS, Jeong YJ, Choi SM, Kang HJ, Lim HJ, et al. Vitamin D deficiency and changes in serum vitamin D levels with treatment among tuberculosis patients in South Korea. Respirology. 2012;17(5):808-13.

17. Talat N, Perry S, Parsonnet J, Dawood G, Hussain R. Vitamin D deficiency and tuberculosis progression. Emerg Infect Dis. 2010;16(5):853-5.

18. Coussens AK, Martineau AR, Wilkinson RJ. Anti-Inflammatory and antimicrobial actions of vitamin D in combating TB/HIV. Scientifica (Cairo). 2014;2014:903680.

19. Weiss ST. Bacterial components plus vitamin D: The ultimate solution to the asthma (autoimmune disease) epidemic? J Allergy Clin Immunol. 2011;127(5):1128-30.

20. Kayaniyil S, Harris SB, Retnakaran R, Vieth R, Knight JA, Gerstein HC, et al. Prospective association of 25(OH)D with metabolic syndrome. Clin Endocrinol (Oxf). 2014;80(4):502-7.

21. Bikle D, Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, et al. Vitamin D: Production, metabolism, and mechanisms of action. 2017; Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.

22. Infante M, Ricordi C, Sanchez J, Clare-Salzler MJ, Padilla N, Fuenmayor V, et al. Influence of vitamin D on Islet autoimmunity and beta-cell function in type 1 diabetes. Nutrients. 2019;11(9):2185.

23. Leung CC, Lam TH, Chan WM, Yew WW, Ho KS, Leung G, et al. Lower risk of tuberculosis in obesity. Arch Intern Med. 2007;167(12):1297-304.

24. Nansera D, Graziano FM, Friedman DJ, Bobbs MK, Jones AN, Hansen KE. Vitamin D and calcium levels in Ugandan adults with human immunodeficiency virus and tuberculosis. Int J Tuberc Lung Dis. 2011;15(11):1522-7.

25. Ho-Pham LT, Nguyen ND, Nguyen TT, Nguyen DH, Bui PK, Nguyen VN, et al. Association between vitamin D insufficiency and tuberculosis in a vietnamese population. BMC Infect Dis. 2010;10:306.

26. Heaney RP, French CB, Nguyen S, Ferreira M, Baggerly LL, Brunel L, et al. A novel approach localizes the association of vitamin D status with insulin resistance to one region of the 25-hydroxyvitamin D continuum. Adv Nutr. 2013;4(3):303-10.

27. Schöttker B, Herder C, Rothenbacher D, Perna L, Müller H, Brenner H. Serum 25-hydroxyvitamin D levels and incident diabetes mellitus type 2: A competing risk analysis in a large population-based cohort of older adults. Eur J Epidemiol. 2013;28(3):267-75.

28. Talaei A, Mohamadi M, Adgi Z. The effect of vitamin D on insulin resistance in patients with type 2 diabetes. Diabetol Metab Syndr. 2013;5(1):8.

29. Davies PD, Brown RC, Woodhead JS. Serum concentrations of vitamin D metabolites in untreated tuberculosis. Thorax. 1985;40(3):187-90.

30. Wilkinson RJ, Llewelyn M, Toossi Z, Patel P, Pasvol G, Lalvani A, et al. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: A case-control study. Lancet. 2000;355(9204):618-21.

31. Ustianowski A, Shaffer R, Collin S, Wilkinson RJ, Davidson RN. Prevalence and associations of vitamin D deficiency in foreign-born persons with tuberculosis in London. J Infect. 2005;50(5):432-7.

32. Gibney KB, MacGregor L, Leder K, Torresi J, Marshall C, Ebeling PR, et al. Vitamin D deficiency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. Clin Infect Dis. 2008;46(3):443-6.

33. Davies PD, Church HA, Brown RC, Woodhead JS. Raised serum calcium in tuberculosis patients in Africa. Eur J Respir Dis. 1987;71(5):341-4.

34. Wejse C, Olesen R, Rabna P, Kaestel P, Gustafson P, Aaby P, et al. Serum 25-hydroxyvitamin D in a West African population of tuberculosis patients and unmatched healthy controls. Am J Clin Nutr. 2007;86(5):1376-83.

35. Maceda EB, Gonçalves CCM, Andrews JR, Ko AI, Yeckel CW, Croda J. Serum vitamin D levels and risk of prevalent tuberculosis, incident tuberculosis and tuberculin skin test conversion among prisoners. Sci Rep. 2018;8(1):997.

36. Selvaraj P, Anand SP, Harishankar M, Alagarasu K. Plasma 1,25 dihydroxy vitamin D3 level and expression of vitamin D receptor and cathelicidin in pulmonary tuberculosis. J Clin Immunol. 2009;29(4):470-8.

37. Grange JM, Davies PD, Brown RC, Woodhead JS, Kardjito T. A study of vitamin D levels in Indonesian patients with untreated pulmonary tuberculosis. Tubercle. 1985;66(3):187-91.

38. Chan TY. Differences in vitamin D status and calcium intake: possible explanations for the regional variations in the prevalence of hypercalcemia in tuberculosis. Calcif Tissue Int. 1997;60(1):91-3.