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Vitamin D Deficiency in Patients with Type 2 Diabetes Mellitus with and without Pulmonary Tuberculosis

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ABSTRACT

Background: Vitamin D has a role in the body's calcium balance and immunodeficiency effects, and stimulates dendric cells, monocytes, T cells, macrophages in pathogenic microbes' elimination. The variation occurrence as polymorphism on VDR might cause problem in production, transportation, and activity of vitamin D. The gap between the occurrence findings in previous studies shows that there are indication factors of VDR levels that probably associate with the T2DM range in comorbid with pulmonary tuberculosis. To determine vitamin D levels and factors associated with vitamin D deficiency in Type 2 Diabetes Mellitus (T2DM) patients with and without pulmonary Tuberculosis (TB).

Method: The design of this study was cross-sectional. T2DM subjects with pulmonary TB were 45 people, and 43 people without pulmonary TB were 43 people. Sampling was done by consecutive sampling from October to December 2017. We collected data through interviews and examination of plasma vitamin D (25-OH) levels by ELISA. Data analysis using the Mann-Whitney test, and chi-square test with a confidence level of 95%.

Result: There were 88 T2DM patients who met the inclusion criteria, consisting of 43 T2DM patients without pulmonary TB and 45 T2DM patients with pulmonary TB as the study subjects. There were differences in the distribution of T2DM subjects based on sex and BMI measurements. The mean plasma vitamin D levels in T2DM with pulmonary TB and T2DM without pulmonary TB were 20.08 ng/ml (SD ± 0.83 ng/ml) and 20.29 ng/ml (SD ± 0.81 ng/ml), respectively, we found no significant result in statistic ($p = 0.287$). Vitamin D deficiency was not associated with the incidence of pulmonary TB in T2DM, whereas female sex, age> 50 years, BMI ≥ 25.0 kg /m2, and duration of T2DM> 5 years were associated with vitamin D deficiency ($p < 0.05$). This research has contributed in novel findings of the correlation between sex and BMI associated in duration of T2DM impacted the Vitamin D insufficiently.

Conclusion: Sex, age, BMI, and duration of T2DM had a significant correlation with vitamin D deficiency in T2DM patients with and without pulmonary TB.

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INTRODUCTION

Vitamin D consists of 2 active metabolites, calcidiol 25-hydroxyvitamin D (25 [OH]D) and calcitriol (1,25[OH]2D), which have proven skeletal benefits, such as preventing the development of rickets and osteomalacia (1,2). Illnesses such as asthma and upper respiratory tract infections, cancer, autoimmune disorders,

falls, and chronic diseases of adulthood, including osteoporosis and cardiovascular disease, are all linked to low vitamin D level (2).

An estimated 1 billion people in the world are in a condition of vitamin D deficiency. Vitamin D deficiency is a global problem that occurs in many regions. One of the leading causes of vitamin D deficiency is the lack of sun exposure and inadequate food intake sources of natural vitamin D and fortified food ingredients, this condition is found both in children and in adults (3). Vitamin D deficiency can affect many cells and organs. Thus vitamin D deficiency is associated with the risk of various health conditions, including infections such as TB and metabolic diseases such as DM (4).

Previous studies found vitamin D deficiency in Javanese elderly women between 60 and 84, showed that in 73.3% were deficient in vitamin D, and 26.7% were not (5). Recent studies have shown an association between vitamin D deficiency (VDD) and type 2 diabetes mellitus patients (T2DM), a significantly lower vitamin D level was found in diabetic subjects than in non-diabetic subjects (6).

Meanwhile Taiwan, a high-income society, still faces a moderate tuberculosis burden, with an annual TB incidence of 30.1 per 100,000 population in 2021 (7). Other studies also found low serum vitamin D levels in tuberculosis patients in Indonesia, Thailand, China, and Kenya. Research in Taiwan and Ethiophia TB case patients had a significantly higher proportion of vitamin D deficiency compared to the control (8).

There is a two-to four-fold higher risk of active TB in individuals with DM compared to non-diabetic individuals (9). The reported global number of people newly diagnosed with TB was 7.5 million in and an estimated 1.30 million deaths in 2022 (10). Reports from research in several countries state that prevalence of diabetes in tuberculosis patients was found to be 29% (known diabetics - 20.7%, new Diabetes cases - 8.3%) (11), in India, nearly 50% of TB patients had either diabetes or pre-diabetes (12). We estimated that more than a quarter of people with DM have latent TB infections, research in Mexico found a prevalence of 51.3%, and in Indonesia shows 23.4% (13). The role of vitamin D in the regulation of immune response has been shown to increase chemotaxis, antimicrobial, and macrophage differentiation so that the condition of vitamin D deficiency in T2DM is expected to affect the incidence of pulmonary TB in T2DM. Without treatment, the death rate from TB disease is high (about 50%) (14). With treatments currently recommended by WHO (a 4–6 months course of anti-TB drugs), about 85% of people with TB can be cured (10)

This study aims to determine differences in vitamin D levels in T2DM patients with and without pulmonary TB and factors that affect vitamin D deficiency in T2DM subjects with and without pulmonary TB in Indonesia. This study hopefully that contribute into the scientifically found bases to the importance of vitamin d control level in prevented and treatment that is processed in T2DM and Pulmonary TB.

METHOD

This study conducted from October 2017 to December 2017, with a cross-sectional design. The sample was taken using consecutive sampling with a sample size of 88 people consisting of 45 T2DM subjects with pulmonary TB and 43 T2DM subjects without pulmonary TB obtained from two hospitals in Surabaya. Sample inclusion criteria were T2DM patients aged 18-65 years, the physiological function of the liver and kidneys within normal limits, no history of smoking, and no history of consuming alcoholic beverages. Exclusion criteria were T2DM patients with abnormalities of pulmonary malignancies, severe heart or blood vessel disease, severe mental disorders, kidney failure, severe atopic disease, HIV and AIDS, leukemia, malignant lymphoma.

The sampling process in the study utilized a probability method known as consecutive sampling, which is a non-random technique often employed in clinical research. This method involves selecting participants sequentially as they become available, ensuring that each individual meets specific inclusion criteria. In this study, the researchers targeted Type 2 Diabetes Mellitus (T2DM) patients, both with and without pulmonary tuberculosis (TB), from two hospitals in Surabaya, Indonesia. The sample comprised 88 individuals, with 45 diagnosed with pulmonary TB and 43 without.

By employing consecutive sampling, the researchers aimed to gather data efficiently while still adhering to ethical standards and maintaining a focus on the specific health conditions being studied. However, it is important

to note that while this method allows for a clear selection process, it may introduce biases related to the order of participant availability and may not fully represent the broader population of T2DM patients.

T2DM subjects with pulmonary TB are T2DM patients diagnosed with pulmonary TB based on the results of microscopic examination or positive smear Gene Expert sputum and supported by chest X-ray examination with abnormalities in the form of pulmonary lesions. We took 5ml of blood samples of 5 ml from each subject and put it into Ethylene Diamine Tetra Acid (EDTA) anticoagulant tubes, while blood plasma was used to check vitamin D levels.

Examination of vitamin D levels uses total plasma 25 hydroxyvitamin D (25-OH) D ELISA performed according to the procedure of Biochem Canada Inc.CAN-VD-510 DBC Diagnostic. Calculation of vitamin D levels using the program computer immunoassay 4-parameter. In this study, vitamin D levels considered to be deficient if the level is <20 ng / ml.

Data of the subjects were obtained from hospital medical records and measurement of body weight and height to find out body mass index. The disease information interviews, taken from measurements of vitamin D sources in daily food ingredients were asked through interviews using a food frequency questionnaire (FFQ), the frequency of consuming ingredients food sources of natural vitamin D, fortification, and supplements in a day, a week and a month. The assessment related to sun exposure in the subjects obtained data in the form of frequency of exposure, duration of exposure, and use of sun protection and carried out after receiving explanation and approval from the subject through informed consent.

The statistical analysis used in this study was the Mann-Whitney test and the chi-square test at a confidence level of 95%. All data processing uses mathematical product and service solutions (SPSS) computer programs.

This study has obtained ethical feasibility information No. 533 / Panke.KKE / IX / 2017 from the Ethics Committee of the RSUD Dr. Soetomo Surabaya.

RESULTS

There were 88 T2DM patients who met the inclusion criteria, consisting of 43 T2DM patients without pulmonary TB and 45 T2DM patients with pulmonary TB as the study subjects. There were differences in the distribution of T2DM subjects based on sex and BMI measurements. In T2DM subjects with pulmonary TB, the proportion of men was found to be higher compared to women and BMI <25.0 kg/m^2 was found more. In contrast, in T2DM subjects without pulmonary TB, the proportion of women and BMI ≥25.0 kg/m^2 was found more. Based on age groups ≥50 years and <50 years, duration of T2DM >5 years and ≤5 years, HbA1c values >7% and <7% and sources of vitamin D in the form of natural sources, fortified food ingredients, vitamin D supplements, and no sun exposure; there were differences in distribution between T2DM subjects with and without pulmonary TB (Table 1).

Table 1. Characteristics of Subject

Characteristics of Subject	Category	T2DM with Pulmonary TB (%)	T2DM without Pulmonary TB (%)
Sex	Man	21 (80.8)	5 (19.2)
	Women	24 (38.7)	38 (61.3)
Age (years)	> 50	28 (45.2)	34 (54.8)
	≤ 50	17 (65.4)	9 (34.6)
BMI (kg/m^2)	≥ 25.0	12 (30.8)	27 (69.2)
	< 25.0	33 (67.3)	16 (32.7)
Duration of T2DM (years)	> 5	23 (48.9)	24 (51.1)
	≤ 5	22 (53.7)	19 (46.3)
HbA1c levels (%)	≥ 7	42 (51.9)	39 (48.1)
	< 7	3 (42.9)	4 (57.1)
Natural Source of vitamin D	Yes	38 (48.7)	40 (51.3)
	No	7 (70.0)	3 (30.0)
Source of fortified vitamin D	Yes	28 (52.8)	25 (47.2)

	No	17 (48.6)	18 (51.4)
Vitamin D supplement	Yes	0 (0.0)	2 (100.0)
	No	45 (52.3)	41 (47.7)
Sun exposure	Yes	40 (48.8)	42 (51.2)
	No	5 (83.3)	1 (16.7)

T2DM = type 2 diabetes mellitus; TB = Tuberculosis; BMI = body mass index; HbA1c = glycated hemoglobin

Table 2 shows the mean plasma of vitamin D levels was similar in T2DM with and without pulmonary TB, which were 20.08 ng/ml and 20.29, respectively. The results of statistical tests showed no significant differences between the two groups (p = 0.287).

Table 2. Plasma vitamin D levels in T2DM patients

Subject	Direct Paths	Average (SD) Vitamin D levels ng/ml	p-value
T2DM	with pulmonary TB	20.08 (0.83)	0.287
	without pulmonary TB	20.29 (0.81)	

Based on Table 3, the results of the statistical test showed a difference in frequency between Sex, age group, BMI and duration of illness in T2DM with vitamin D deficiency in T2DM subjects with and without pulmonary TB (p <0.05). Female sex showed a higher proportion of deficiencies (40.3%) than male (15.4%), while according to age group, the percentage of age >50 years (40.3%) is higher than those of age ≤50 years (15.4%). Based on the BMI category, 46.2% vitamin D deficiency found in BMI >25.0 kg/m^2 compared to BMI <25.0 kg/m^2 which was only 22.4%. Based on the duration of illness T2DM >5 years showed higher proportion of vitamin D deficiency that is equal to 42.6%, while those with ≤5 years duration of T2DM is 22.0%.

Table 3. Plasma vitamin D levels in T2DM patients

Subject	Category	Vitamin D deficiency (%)	Vitamin D insufficiency (%)	p-value
T2DM	with pulmonary TB	11 (24.4)	34 (75.6)	0.131
	without pulmonary TB	18 (41.9)	25 (58.1)	
Sex	Man	4 (15.4)	22 (84.6)	0.043*
	Women	25 (40.3)	37 (59.7)	
Age (years)	> 50	25 (40.3)	37 (59.7)	0.043*
	≤ 50	4 (15.4)	22 (84.6)	
BMI (kg/m^2)	≥ 7	18 (46.2)	21 (53.8)	0.034*
	< 7	11 (22.4)	38 (77.6)	
Duration of T2DM (years)	Yes	20 (42.6)	27 (57.4)	0.068
	No	9 (22.0)	32 (78.0)	
HbA1c levels (%)	Yes	27 (33.3)	54 (66.7)	1.000
	No	2 (28.6)	5 (71.4)	
Natural source of vitamin D	Yes	26 (33.3)	52 (66.7)	1.000
	No	2 (28.6)	7 (70.0)	
Source of fortified vitamin D	Yes	18 (34.0)	35 (66.0)	0.805
	No	11 (31.4)	24 (68.6)	
Vitamin D supplement	Yes	2 (100.0)	0 (0.0)	0.106
	No	27 (31.4)	59 (68.6)	
Sun exposure	Yes	27 (32.9)	55 (67.1)	1.000
	No	2 (33.3)	4 (66.7)	

*Value significant at <0.05

The T2DM group without pulmonary tuberculosis had a deficiency of 41.9%, while in T2DM with pulmonary TB was 24.4%. The results of the analysis showed no significant differences in the distribution of vitamin D deficiency, HbA1c values, consumption of natural vitamin D sources, fortification, and vitamin D supplements and sun exposure ($p > 0.05$) in T2DM subjects with and without pulmonary TB.

DISCUSSION

Interpretation of Key Findings

Several factors that influence the occurrence of vitamin D deficiency include nutritional status, sun exposure, and sun protection, use of certain drugs such as (antiepileptic, corticosteroid, cimetidine, anti-tuberculosis, theophylline, orlistat, antiretroviral) and the presence of degenerative chronic diseases such as cirrhosis of the liver and chronic kidney disease (15). Age factors, climate, geographical location, and skin pigmentation affect the production of vitamin D by the skin. Cultural behaviour also contributes to the practice of consuming vitamin D sources and how to dress that may reduce D vitamin production by the skin (16). We also found vitamin D deficiency in various groups of people; children, adolescents, elderly, men, women. The factors studied included demographic variables, nutritional status, consumption of vitamin D sources, and sun exposure in T2DM patients.

Age and sex are demographic factors associated with vitamin D deficiency in this study. At the age of >50 years, many people experience deficiencies due to lower activities outside from the house, for that exposure to sunlight also decreases, the risk of some degenerative diseases also increases in old age such as diabetes mellitus, hypertension, heart disease, and rheumatism. Women are more at risk of developing vitamin D deficiency due to factors such as higher levels of sun protection in female by using hijab or tight clothes and lack of outdoor activities exposed to direct sun exposure.

Research in Cipto Mangunkusumo Hospital, Jakarta Indonesia in T2DM patients aged >60 years found vitamin D deficiency of 75.0%, more in female Sex between the ages of 60-75 years, and half of them were T2DM patients with obesity (5).

One of the essential factors in the body's defence against infection is nutritional status, including in determining the incidence of TB. Nutritional deficiencies are associated with impaired immune function. The immune response weakens in a person with inadequate nutrition, thus that the ability to defend itself against infection decreases (17). In this study, there was a significant difference in the distribution of vitamin D deficiency based on BMI categories in T2DM subjects with and without pulmonary TB. Previous studies have found that vitamin D deficiency in the obese group due to increased serum 25 (OH) D absorption by fat tissue, increase of the basal metabolic, a tendency of low activity in the outside and lack of sun exposure. Also, the fat content level affects the decrease in the bioavailability of vitamin D, which causes the serum to be detected low in the blood.

Research in Tanzania with subjects over the age of 18 years discovered TB-DM patients have low vitamin D status reached up to 50.0% with a mean age of 43.5 years and a mean BMI of 20.5 kg/m^2 compared with DM patients with low vitamin D status that only 29.7% with an average age of 43.5 years and an average BMI of 28.5 kg / m 2. Statistical test results showed no correlation between low vitamin D status between the two groups ($p = 0.22$) (18).

This study also obtained an average vitamin D level in both groups of T2DM subjects with and without pulmonary TB that was low. The deficiency was found in T2DM subjects with pulmonary TB was 24.4%, while T2DM without pulmonary TB was 41.9%. The results of this study showed no significant differences between the two groups. The absence of significant differences in vitamin D levels in the two groups showed that many factors determined vitamin D levels. The factors are the intake of vitamin D sources and sun exposure as a source of natural vitamin D. The result showed that there were no significant differences between natural sources of vitamin D intake, fortification, vitamin D supplements and sun exposure between the two groups. Although the source of vitamin D in T2DM subjects with and without pulmonary TB, in general, is available both-natural sources,

fortification results, and sun exposure, but the adequacy of the source of vitamin D is still inadequate according to minimum needs.

Based on the results of interviews using FFQ to the research subjects, we collect information that the consumption of egg yolk was the source of natural vitamin D, the source of fortified vitamin D mainly was obtained from milk consumption while the source of vitamin D was supplements or multivitamins, most of subjects never consumed it. Adequacy of vitamin D consumption was assessed based on the frequency of consumption of vitamin D sources, namely in a day, a week and a month.

Interview results related to sun exposure stated that most of the subjects in both groups claimed to have been exposed to the sun with the frequency of exposure to morning sunlight more than >3 times a week with a total exposure time of 210 minutes a week, and mostly use at least 1 sun protection such as clothes long sleeves, headgear such as hijab, hat or umbrella. Sun exposure is considered sufficient if ≥ 210 minutes a week with also the use of sun protection. Based on these criteria, the majority of subjects were in conditions with less exposure to sunlight in both-groups, 55.6% in the T2DM group with pulmonary TB and 67.4% in the T2DM group without pulmonary TB.

Some studies on vitamin D status in T2DM patients show mixed results, including studies in T2DM patients in Morocco, getting an average vitamin D level of 26.07 ± 13.03 ng/ml. Vitamin D levels showed a significant difference between T2DM patients and controls ($p < 0.001$). Vitamin D levels of T2DM patients who experience deficiencies are 40% (16). The study in Harbin China in patients aged 20 to 74 years found a proportion of 25 (OH) D vitamin D deficiency in patients experiencing T2DM, i.e., 20.14% (19).

Meanwhile, previous studies related to TB patients with DM are as follows: Research in hospitals and TB clinics in urban and rural areas in China received 128 TB patients with DM with a median vitamin D level of 12.1 ng/ml (20). The vitamin D status category of the subjects who experienced deficiency reached 53.9% and severe deficiencies of 29.7%. These results also found a large proportion of deficiency in TB-DM compared to TB patients who had normal fasting blood sugar levels or TB patients with pre-DM (53.9% vs. 41.1% and 41.0%) (21).

Research on 75 DM-TB patients in South India received vitamin D levels on average TB-DM patients at 13 ng/ml in the range (8-20) ng/ml lower than TB patients with new diagnoses of DM and TB patients without DM. The results of the statistical analysis showed that there were significant differences ($p = 0.026$) between the three groups(22).

There are differences in the prevalence of vitamin D status in various regions, indicating that each region has different risk factors(23). Vitamin D deficiency is influenced by various factors such as lifestyle, tradition, and activity so that proper handling of these conditions is needed, especially in patients with potency of having vitamin D deficiency such as people with diabetes mellitus, tuberculosis or having T2DM with TB. The disease becomes effective. In T2DM patients, the risk prevention of pulmonary TB is not a priority for vitamin D supplementation because there is no significant difference between vitamin D levels between T2DM with and without pulmonary TB.

Limitation and Implication

The research on vitamin D deficiency in patients with Type 2 Diabetes Mellitus (T2DM) both with and without pulmonary tuberculosis (TB) revealed several novel findings. The study determined that the mean plasma vitamin D levels were similar in both groups, with T2DM patients with pulmonary TB averaging 20.08 ng/ml and those without TB averaging 20.29 ng/ml, indicating a widespread deficiency in both cohorts. Notably, the study found that vitamin D deficiency was not significantly associated with the incidence of pulmonary TB among T2DM patients. Instead, factors such as female sex, age over 50 years, a body mass index (BMI) of 25 kg/m² or higher, and a duration of T2DM exceeding five years were significantly correlated with vitamin D deficiency.

However, the study faced limitations that could affect the interpretation of its findings. The cross-sectional design restricts the ability to draw causal conclusions regarding the relationship between vitamin D levels and TB incidence. The relatively small sample size of 88 participants may not provide a comprehensive representation of the broader population, limiting the generalizability of the results. Additionally, potential confounding factors such

as dietary intake of vitamin D and varying levels of sun exposure were not thoroughly controlled for, which could influence vitamin D status.

The implications of this research are significant for public health and clinical practice. The high prevalence of vitamin D deficiency among T2DM patients underscores the need for targeted public health initiatives aimed at improving nutritional intake and encouraging safe sun exposure practices, particularly among older adults and women. Clinicians should consider routine screening for vitamin D levels in T2DM patients to identify those at risk for deficiency and implement appropriate interventions. Furthermore, this study opens avenues for future research to explore other immunological factors that may contribute to TB susceptibility in diabetic patients, highlighting the need for longitudinal studies to better understand these relationships over time.

CONCLUSION

All subjects were in vitamin D deficiency/insufficiency. There were no differences in vitamin D levels in T2DM patients with and without pulmonary TB. Vitamin D deficiency was significantly associated with female sex, age> 50 years, and BMI ≥ 25.0 kg / m 2 in T2DM subjects with and without pulmonary TB.

AUTHOR'S CONTRIBUTION STATEMENT

Wahiduddin developed up with the idea for the study, collected the data, carried out the analysis, and composed the manuscript. As the promotor, Agung Pranoto supplied crucial direction and oversight during the investigation. As the co-promotor, Sudjarwo provided invaluable support in honing the research approach and making a substantial contribution to the study's conception and interpretation of its results. The final manuscript was read and approved by all writers.

CONFLICTS OF INTEREST

The author states that there is no conflict of interest with the parties involved in this study.

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All relevant data are within the article

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