Vitamin D status did not related to calcium status in active tuberculosis patients in North Sumatera, Indonesia

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Abstract. Background: Tuberculosis is one of the highest mortality caused in tropical country with abundant sunlight such Indonesia. Vitamin D and calcium plays important roles in tuberculosis pathogenesis. Objective:We sought to determine whether there is an association between vitamin D status and calcium status in tuberculosis patients. Design: We conducted a cross-sectional study of 32 man and women aged 18-60 years with active tuberculosis in North Sumatera, Indonesia. Parameters were 25(OH)D and calcium serum level, body mass index, fat mass, and others lifestyles factors also assessed. The association was analysis using chi-square or fischer test. Results: the mean of study subjects age were 37.2±14.9 years old and BMI were 20.8±4.4 kg/m^2. There were 81.2% subjects categorized into vitamin D deficiency-insufficiency and 18.8% categorized into vitamin D sufficiency. There were 29% subjects categorized into normal calcium level, and 3% were hypocalcemia. Based on food recall analysis, there were found lower vitamin D and calcium intake. There is no association between vitamin D and calcium classification. Conclusions: based on this result, although there is no association between vitamin D and calcium, but there could be altered by lower food intake and tuberculosis progression.
Key word: 25(OH)D, calcium, tuberculosis, association

1. Introduction

Tuberculosis (TB) remains one of the top four causes of death in Indonesia which have many nutritional problems involved, such malnutrition and vitamin D deficiency. Indonesia is a tropical country with abundant sunlight, still have higher rate of vitamin D deficiency in normal people or people with chronic disease [1-3]. In addition to the results of a recent prevalence survey which showed a much higher TB prevalence than previously estimated [4].

The malnutrition host-pathogen interaction and involvement of vitamin D-calcium (Ca) signaling in tuberculosis infection is crucial and plays a significant role in tuberculosis pathogenesis [5,6]. Vitamin D has a potential role in the prevention and treatment of infection, supports induction of pleiotropic antimicrobial responses and immunomodulatory effects [5]. Vitamin D is an essential factor for the intestinal absorption of dietary calcium and skeletal mineralization [4,5].

The aims of this study were to determine whether there is association between 25(OH)D with calcium serum in tuberculosis patient. To our knowledge, this is the first to study to find out the association between 25(OH)D serum with calcium serum in Medan, North Sumatera, Indonesia.

2. Method

We conducted a cross-sectional study of 32 man and women aged 18-60 years in North Sumatera, Indonesia July to September 2016, during the dry season (dry season in Indonesia is between April andOctober, when there was abundant sunlight exposure). The location of recruitment was in Sumatera Island (Medan, North Sumatera, Indonesia) with latitude: 3.57 N and longitude 98.65 E, average temperature: ±32^{o}C (90^{o}F). This study was carried out after ethical approval was obtained from the Health Research Ethics Committee of Sumatera Utara University Medical School (No. 264/TGL/KEPK FK USU-RSUP HAM/2017) and all participants were given written informed consent to the study procedures.

2.1. Study participants

The subjects of this study consisted tuberculosis patients in three community health centers with the higher tuberculosis prevalence in Medan, North Sumatera, Indonesia, man and women with various occupations, and taken purposively, there were 32 subjects The inclusion criteria were tuberculosis patients within the range of 18-60 years old. Exclusion criteria were subjects with history of diabetes mellitus, myocardial infarction, renal orliver dysfunction. In addition to those exclusion criteria, subjects who were pregnant and lactating were also excluded.

2.2. Anthropometry, status body fat, and nutrient intake

Anthropometry included height (to the nearest 0.5 cm), weight (to the nearest 0.1 kg), waist circumference using a standardized measuring tape in centimetres, systolic and diastolic blood pressure measurement, and body mass index (calculated as kg/m^2). Categorized BMI was based on Asia Pacific [6], <18.5 classified as underweight, 18.5-22.9 classified as normoweight, 23-24.9 classified as overweight/at risk, 25-29.9 classified as obese I, and >30 classified as obese II. Assessment of body fat percentage were using Body Composition Monitor with Scale (*HBF-362, KaradaScan-Omron*). Body fat percentage refered to the amount of body fat mass in regards to the total body weight expressed as a percentage, the following classified: normal ≤29.9% and high >30.0% based on Bioelectrical Impedance.Assessment of nutrient intake was based on food recall for two days (one day for weekday and one day for weekend), including energy, protein, fat, carbohydrate, calcium, phosphor, cholesterol, vitamin D, and percentage of fulfilment. Calculation were using *Nutrisurvey*2005, which included Indonesian foods.

2.3. Laboratory analysis

We measured 25(OH)D serum concentration by chemiluminescent immunoassay (CLIA) technology (Diasorin, Stillwater, MN), measures were between 4.0 and 150 ng/mL. The lowest value was 4.0 ng/mL which is based on an inter-assay precision 3.90% CV. Reference range were <20 ng/mL categorized deficiency, 20-30ng/mL (insuficiency), 30-100 ng/mL (suficiency) [7]. To convert ng/mL to nmol/L is multiply with 2.496. Calsium serum was measured by ADVIA Bayer Assayed Chemistry Controls, with principle procedure: calcium ions form a violet complex with *o*-cresolphthaleincomplexone in an alkaline medium. The reaction is measured at 545/658 nm, and normal concentration of calcium was 8.3-10.6mg/dL.

2.4. Statistical analysis

Continuous variables were expressed as continuous variables as means±SDs. Categorical variables were expressed as percentage proportions, using chi-square to expressed significancy difference between two groups, and Fischer test if the data did not met the criterias. The p values <0.05 were considered statistically significant. We used SPSS program (version 11.5; SPSS Inc, Chicago, IL) to perform the analysis.

3. Results and Discussions

The results will be discussed in 4 subsections, they are characteristic of study participants, body mass index, factors to low vitamin D, and bone health.

3.1. Characteristic of study participants

The aim of this study was todetermine whether there is association between 25(OH)D with calcium serum in tuberculosis patients in tropical country, who lived in abundant sunlight exposures area such as Indonesia.

Table 1. Demographic and clinical characteristics

Variables	n(%)
Age classification	
18-30	13 (40.6)
31-40	7 (21.9)
41-50	4 (12.5)
51-60	8 (25)
Gender	
Male	24 (75)
Female	8 (25)
Ethnic	
Javanese	7 (21.9)
Bataknese	19 (59.4)
Minang	3 (9.4)
Others	3 (9.4)
Occupation	
Student	4 (12.5)
Employed	16 (50)
Unemployed	7 (21.8)
Housewife	5 (15.7)
Sputum conversion time (wk), mean±SD	2.1±1.4
Distribution by severity class/TB score	
Class I	30 (93.8)
Class II	2 (6.2)
Class III	-
BCG scar	
Clear	17 (53.1)
No scar	3 (9.4)
Dubious	12 (37.5)

BCG: Bacille Calmette-Guérin

Age in this study showed the age were 37.2±14.8 years, the higher age group was found in 18-30 years old group and the lowest group found in 40-50 age group. A previous study reported that tuberculosis also found in thirties age group, could be the lower immune respon in that age group.

Most of them had sputum conversion in week 2, along with tuberculosis treatment, this result showed faster sputum conversion week than other study that reported 6 weeks after vitamin D supplementation as presented in **Table 1** [8].

3.2. Anthropometry and fat mass

There were 31.3% of study subjects were underweight, 40.6% were normoweight, 9.4% were overweight, and 18.7% were obese. Underweight and tuberculosis (TB) are linked and have a bidirectional relationship. Underweight increases the risk of TB which in turn, can lead to

malnutrition. Undernutrition not only is a risk factor for progression of latent TB infection to active disease, but also increases the risk of drug toxicity, relapse and death once TB develops. The dietary intake of TB patients in the country is inadequate [9,10] as presented in **Table 2**.

Table 2. Anthropometric characteristics

Parameters	Mean±SD
Weight (kg)	54.5±12.3
Height (cm)	161.6±8.2
BMI (kg/m^2)	20.8±4.4
Fat mass (%)	21.2±6.8

BMI: body mass index

3.3. Lifestyle in tuberculosis patient

Based on food recall analysis found lower food intake especially vitamin D and calcium intake, this result similar with normal subjects. Previous studies reported that especially women had lower food sources intake [2] as presented in **Table 3**.

Table 3. Lifestyle variables in tuberculosis patient

Variables	Mean±SD/n(%)
Daily food intake	
Energy (kcal)	1113.2 ± 53.1
Carbohydrates (g)	162.3 ± 80.1
Proteins (g)	34.2 ± 11.4
Fats (g)	26.2 ± 18.5
Cholesterol	138.3 ± 80.7
Fibers	4.8 ± 5.9
Vitamin D intake (µg)	4.2 ± 2.9
Calcium intake	101.3±69.1
Vitamin D intake, n(%)	
Low	26 (81.3)
Moderate	6 (18.7)
Calcium intake, n(%)	
Low	27 (84.4)
Moderate	5 (15.6)
Daily sun ray exposure, n(%)	
≤1 hour	23 (71.8)
>1 hour	9 (28.2)
Physical activity, n(%)	
Low	30 (93.7)
Moderate	2 (6.3)

Continues variable: mean ± SD; categorical variable: n (%);
SD=standard deviation

Lower sunlight exposure was one of the risk factor of lower 25(OH)D serum level, most of them were employed. The tuberculosis patient had a job and all of them had to leave their house to stay at work place as presented in **Table 1** and **3**. They spent all day long in the building which less sunlight exposure. Previous study also report that higher percentage women work indoors than outdoors, other factor was sunscreen application that lower the exposure [3].

3.4. Vitamin D and Calcium

Activity vitamin D in tuberculosis as an immunomodulator, the pathogenesis is when monocytes, and macrophages exposed to a lipopolysaccharide or to *Mycobacterium tuberculosis*, there is an up-regulate the vitamin D receptor gene and the 25-hydroxyvitamin D-1α-hydroxylase gene. This respons resulting an increased production of 1,25-dihydroxyvitamin D3, increase the synthesis of cathelicidin, a peptide capable of destroying *M. tuberculosis* [9].

In this study found similar results with normal person which have lower vitamin D intake and sunlight exposure [3]. The presence of vitamin D deficiency among tuberculosis patients has led to altered calcium metabolism and resulting worsening disease as presented in **Table 4**, but in this study found normal calcium serum level.

Table 4. Serum levels of 25-hydroxyvitamin D and calcium

Parameters	Mean±SD; n(%)
25-hydroxyvitamin D serum levels (ng/mL)#	21.8±7.6
Vitamin D status, n(%)	
Deficiency	13 (40.6)
Insufficiency	13 (40.6)
Sufficiency	6 (18.8)
Normal in sunny countries	-
Percentile values for vitamin D (ng/mL)	
Minimum	7.3
5% Percentile	8.3
25% Percentile	16.2
50% Percentile	21.6
75% Percentile	25.7
95% Percentile	36.5
Maximum	37.4
Serum calcium (mg/dL)	9.1±0.5
Calcium classification, n(%)	
Low	3 (9.4)
Normal	29 (90.6)

Continues variable: mean ± SD; categorical variable: n (%); SD=standard deviation

Although the calcium and vitamin D metabolism appeared to be altered in tuberculosis, there was no association between vitamin D and calcium categorization in this study. Previous study confirmed that serum calcium is raised in tuberculosis but the effect may be reduced by a low calcium intake and a low parathyroid hormone level as presented in **Table 5** [11].

Table 5. The analysis of vitamin D and calcium status

		Calcium status, n(%)		*p*
		Low	Moderate	
Vitamin D status, n(%)	Deficiency-insuficiency	3 (9.4)	23 (71.9)	0.52*
	Sufficiency	0	6 (18.8)	
Total		3 (9.4)	29 (90.6)	

Using Fisher test, * not significant

Calcium is known as an ubiquitous second messenger that could control multiple processes and is included in cellular activities like division, motility, stress response, and signaling [5]. In previous

study, reports that in tuberculosis patient found hypercalcemia, but could but the effect may be reduced by a low calcium intake and a low parathyroid hormone level [5,6]. Therefore, as a suggestion, nutritional supplementation in patients with TB is associated with better cure and treatment completion rates as well as better performance status. Limitation of this study that we did not assed parathyroid hormon and albumin serum that affect vitamin D and calcium metabolism.

4. Conclusion

Based on this result, although there is no association between vitamin D and calcium, but there could be altered by lower food intake, sunlight exposure, and tuberculosis progression.

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