

Vitamin D Deficiency Prevalence and Its Association with Bacterial Load Sputum in Newly Diagnosed TB Patients

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Abstract

Vitamin D plays a role in innate and adaptive immune functions, and its deficiency has become a risk factor for tuberculosis (TB) infection. This study aimed to describe the prevalence of vitamin D deficiency in newly diagnosed TB patients and to examine the relationship between vitamin D levels and sputum smear positivity that reflects bacterial load. This is a cross-sectional study conducted at several primary health care facilities in Medan and Deli Serdang from November 2015 to June 2016. As many as 86 subjects adult TB patients with positive smear sputum who had not received therapy or had at most 1 week on therapy were recruited. Sputum examination were interpreted using International Union Against Tuberculosis and Lung Disease (IUATLD) grading scale. Serum 25(OH) vitamin D level was estimated using enzyme-linked immunosorbent assay (ELISA) and interpreted as deficiency (<20 ng/mL), insufficiency (20–29 ng/mL), or optimum (30–100 ng/mL). The association between vitamin D level and positivity level of AFB sputum was analyzed using Spearman correlation test. The prevalence of VDD in new pulmonary TB patients with positive smear sputum was 17.4%. There was a weak relationship between vitamin D level and positivity level of AFB sputum ($p=0.014$), with a correlation coefficient of (-0.264). Many of newly diagnosed TB patients had vitamin D deficiency despite the fact that Indonesia is an equatorial area with sufficient sunshine throughout the year. This study shows vitamin D indeed affect bacterial load and a low 25(OH) vitamin D level is associated with higher bacterial load.

Key words: Bacterial load, pulmonary tuberculosis, vitamin D deficiency

Prevalensi Defisiensi Vitamin D pada Pasien TB Paru Baru dan Hubungan dengan Derajat Kepositifan Sputum Basil Tahan Asam

Abstrak

Vitamin D berperan dalam imunitas bawaan dan adaptif. Kekurangan vitamin D merupakan faktor risiko terinfeksi tuberkulosis (TB). Penelitian ini bertujuan menggambarkan prevalensi defisiensi vitamin D pada pasien TB baru dan mengetahui hubungan kadar vitamin D dengan derajat kepositifan sputum yang mencerminkan kepadatan bakteri. Penelitian potong lintang ini dilakukan di beberapa Puskesmas di wilayah Medan dan Deli Serdang pada November 2015 hingga Juni 2016. Subjek penelitian sebanyak 86 pasien TB dewasa dengan sputum basil tahan asam (BTA) positif, yang belum atau maksimal 1 minggu mengkonsumsi obat. Pemeriksaan sputum dinilai menggunakan skala *International Union Against Tuberculosis and Lung Disease (IUATLD)*. Kadar vitamin D serum 25(OH) dinilai dengan metode ELISA dan diklasifikasikan menjadi defisiensi (<20 ng/mL), insuffisiensi (20–29 ng/mL), optimum (30–100 ng/mL). Hubungan kadar vitamin D dan tingkat kepositifan sputum BTA dilakukan dengan uji korelasi Spearman. Prevalensi vitamin D pada pasien TB paru baru dengan sputum positif adalah 17,4%. Terdapat hubungan antara kadar vitamin D dan tingkat kepositifan sputum BTA ($p=0,014$), dengan koefisien korelasi (-0,264). Banyak pasien TB yang baru didiagnosis memiliki defisiensi vitamin D meskipun Indonesia berada pada daerah khatulistiwa dengan sinar matahari yang cukup sepanjang tahun. Penelitian ini menunjukkan bahwa vitamin D berhubungan terhadap kepositifan sputum dan rendah kadar vitamin D dengan kepadatan bakteri yang lebih tinggi.

Kata kunci: Defisiensi vitamin D, kepadatan bakteri, TB paru

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Introduction

Tuberculosis (TB) has become the most infectious disease in the world with the highest morbidity and mortality rates after HIV/AIDS. In 2017, Indonesia ranks third among the countries with the highest number of TB sufferers globally after India and China. This situation emphasizes the need for preventive and curative efforts to manage TB cases.¹

Mycobacterium tuberculosis (MTB) infection is mainly transmitted through aerosols. When a person with a positive sputum result for pulmonary TB coughs or sneezes, he or she expels infectious bacillus-contained droplet nuclei. Once the nuclei enter the respiratory tract, they will be engulfed by the lung's alveolar macrophages after attachment through the surface receptors, such as the toll-like receptors (TLR) 2. After binding, the bacteria are internalized and engulfed into phagosomes where they are killed by several mechanisms.² Vitamin D deficiency has become one of the risk factors that facilitate TB infection. Besides its role in the skeletal system, vitamin D also plays a role in the innate and adaptive immune functions.³ Vitamin D deficiency will interfere with the formation of cathelicidin, which is a compound produced by macrophages to lyse bacteria.^{4,5}

Vitamin D status is determined based on serum 25(OH) vitamin D level. Most experts agree that 25(OH) vitamin D level of <20 ng/mL is considered to reflect a deficiency in vitamin D whereas a 25(OH) vitamin D level of 21–29 ng/mL is considered to represent an insufficient level of vitamin D in the body.⁶ Even though Indonesia is a tropical country, vitamin D deficiency is still a common finding. A previous study involving 504 women of childbearing age in Indonesia and Malaysia demonstrated that the prevalence of vitamin D deficiency in the two countries is 63%.⁷

There are not many studies performed in Indonesia that assess the prevalence of vitamin D deficiency in newly diagnosed TB patients. Moreover, controversies regarding the relationship between vitamin D level and TB are still strong. This study aimed to describe the prevalence of vitamin D deficiency in newly diagnosed TB patients and to examine the relationship between the vitamin D level and sputum bacterial load.

Methods

This is a cross-sectional study conducted at several primary health care facilities in Medan and Deli Serdang from November 2015 to June 2016. All subjects had provided informed consent prior to the study. As many as 86 newly diagnosed adult TB patients with positive smear sputum result but had not received any therapy or had only received therapies up to 1 week recruited. Patients with conditions that may affect vitamin D metabolism such as human immunodeficiency virus, diabetes mellitus, pregnancy or lactation, under immunosuppressive treatment, and liver or renal disorders were excluded from the study. Approval to conduct the study was obtained from the Health Research Ethical Committee of the Faculty of Medicine, Universitas Sumatera Utara, through the issuance of the ethical clearance number 653/KOMET/FK USU/2015.

Examination and assessment of the Acid-Fast Bacillus (AFB) smear sputum were carried out by expert officers in each health facility that has been appointed as the Health Facility for TB service programs by the government. Sputum examination results were interpreted using the *International Union Against Tuberculosis and Lung Disease* (IUATLD) grading scale and subjects were divided into 3 groups, namely 1+, 2+, and 3+ groups, based on the number of AFB seen in the fields.

Three milliliters of venous blood samples collected from each subject were placed into the Eppendorf tube. Serum 25(OH) vitamin D level was estimated using the enzyme-linked immunosorbent assay (ELISA) kits from DIA source, Belgium. A level of <20 ng/mL was considered to reflect a vitamin D deficiency whereas a level of 21–29 ng/mL was considered to represent insufficient vitamin D level. The vitamin D level was considered to be optimum when the level was >30 ng/mL.

The normality of the data was analyzed using the Shapiro-Wilk test. Results were expressed as mean±standard deviation. The association between the vitamin D level and the positivity level of AFB sputum was carried out using the Pearson correlation test. Statistical analysis was then performed using SPSS ver 22.0. Statistical significance was considered to be achieved by a 5% level of significance and a p-value <0.05.

Table 1 Baseline Characteristics

Characteristics	Sputum AFB Positivity			Total n (%)	p
	+1	+2	+3		
Age (median, range)	43 (18–69)	41 (18–62)	42 (18–62)	42 (18–62)	0.633
Sex					0.208
Male	22 (39.28%)	22 (39.28%)	12 (21.42%)	56 (65.11%)	
Female	7 (23.33%)	12 (40%)	11 (36.6%)	30 (34.88%)	

Results

This study involved 86 subjects, comprising of 56 males and 30 females. The median age of the subjects was 42 years. There was no significant difference between the 3 groups in terms of age and sex (Table 1).

This study used a classification of vitamin D status based on Holick, with a 25(OH) vitamin D level of >3 ng/mL considered adequate. A 25(OH) vitamin D level of <20 ng/mL and 20–29 ng/mL were considered deficient and insufficient, respectively (Table 2).⁶ Using these criteria, the prevalence of vitamin D deficiency of the new pulmonary TB patients with positive smear sputum in this study was calculated as 17.4%.

The average vitamin D level decreased with the increasing degree of AFB sputum positivity (Table 3). On average, the subjects in +3 and +2 groups had an insufficient vitamin D level and subjects in the +1 group had, on average, an optimum vitamin D level.

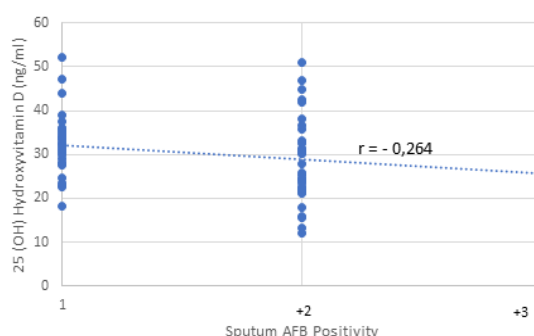


Figure Vitamin D Level and Sputum AFB Positivity

Discussion

This study involved 86 subjects whose ages ranged from 18 to 69 years with predominantly male sex. There is no difference in vitamin D requirements between men and women and within the age range of 18 to 69 years.⁸ Most experts agree that in determining the status of

Table 2 Comparison of Vitamin D Status by Sputum AFB Positivity

Vitamin D Status	Sputum AFB Positivity			Total n (%)
	+1 (n=29)	+2 (n=34)	+3 (n=23)	
<20 ng/mL (deficiency)	1	6	8	15 (17.4%)
20–30 ng/mL (insufficiency)	9	14	7	30 (34.9%)
>30 ng/mL (optimum)	19	14	8	41 (47.7%)

Table 3 Average Value of Vitamin D level by Sputum AFB Positivity

Parameter	Sputum AFB Positivity		
	+1	+2	+3
25(OH) Vitamin D level (ng/mL)	32,48±7.22	28,29±9.66	26,21±8.78

Based on the two-way Spearman statistical test, vitamin D level and sputum AFB positivity level were related (p=0.014) with a correlation coefficient of (-0.264).

vitamin D the minimum adequacy of 25(OH) vitamin D level is >30 ng/mL with <20 ng/mL and (20–29 ng/mL) considered deficient and insufficient, respectively, regardless of age and gender.⁶

Forty-five subjects (52.3%) in this study had a vitamin D level below the optimum level with 17.4% experienced deficiency and 34.9% experienced insufficiency. Several studies in Europe, Asia, and Africa^{9,10,11} revealed the variable numbers in vitamin D deficiency in TB patients of 82.3%, 92%, and 95.4%, respectively. Compared to the findings of a study evaluating vitamin D level in TB patients in Sabah, Malaysia, our deficiency rate is lower (17.4% vs 27.5%). This may be due to the fact that this Malaysian study did not consider the insufficient vitamin D 25(OH) level (20-29 ng/mL) as a vitamin D deficiency. However, the average vitamin D level between the two studies was not too different (29.14 ng/mL vs 25.56 ng/mL). Vitamin D level is closely related to the geographical location and sun exposure. The farther a country from the equator is, the less sun exposure it gets. This probably explains why the vitamin D deficiency rates in TB patients are lower in Malaysia and Indonesia.

This study did not compare the subjects' vitamin D levels to those of healthy people. However, several studies had suggested that vitamin D deficiency is also common among healthy people.^{7,10,12} A study performed on 504 non-pregnant women in Malaysia and Indonesia⁷ found that more than 60% of women experienced vitamin D insufficiency. A similar study comparing vitamin D levels in subjects with pulmonary TB and healthy people in Medan¹³ found that the mean serum vitamin D level was nearly similar in both groups (25.21 ± 7.93 ng/mL vs 21.50 ± 9.37 ng/mL). With these findings in mind, it seems that vitamin D deficiency may be overlooked in Asian countries. The assumption that vitamin D deficiency is unlikely to occur in regions with adequate exposure to sunlight has been disproved.

This study shows that subjects with lower vitamin D levels had a higher sputum positivity degree ($r = -0.264$, $p = 0.014$). This is comparable to the study in India which also found a negative correlation between sputum AFB positivity and serum 25(OH) vitamin D level ($r = -0.34$, $p = 0.005$).¹⁴

Vitamin D has already been proven to have an antimicrobial effect on *Mycobacterium tuberculosis*. Once the MTB enters the respiratory tract, they will be engulfed by the lung's alveolar

macrophages after attachment through surface receptors, such as the toll-like receptors (TLR) 2. After binding, the bacteria are internalized and engulfed into phagosomes where they can be killed by several mechanisms, one of which is through autophagy and phagolysosomal processes. Calcitriol, the active metabolite of vitamin D, inhibits the multiplication of MTB in the macrophages by inducing the antimicrobial peptide cathelicidin.^{2-5,15}

During TB infection, the formation of cathelicidin by vitamin D will increase but this process will occur only if vitamin D 25(OH) is adequate in the body.^{4,5} Thus, in deficiency the antimicrobial activity will be reduced that the bacterial multiplication inhibition will be minimum. Differences in vitamin 25(OH) D levels are also suggested to affect the speed of sputum conversion. Patients with a sufficient vitamin D level will achieve sputum conversion faster than patients with deficient vitamin D level (Spearman's coefficient -0.24 , $p = 0.02$).¹⁰

Nevertheless, vitamin D level is not the only factor affecting sputum positivity. This study revealed that the average vitamin D levels in the +3 and +2 groups were in the category of insufficient whereas the average level in the +1 group was in the optimum category. The disease severity in TB seems to also be influenced by other host factors and MTB virulence. Another factor that might contribute to this is the vitamin D bioavailability. The bioavailability of vitamin D may not be fully reflected by the total serum 25(OH) D level alone. There are also other factors such as the genotypes of the vitamin D carrier protein, vitamin D binding protein, and other genetic variants of the vitamin D system that will influence the bioavailability.¹⁶ Vitamin D receptor gene polymorphism could affect the synthesis and functionality of its protein that, in turn, will affect cathelicidin.¹⁷ Various possible factors above may explain the reason why despite the proven association between vitamin D level and sputum positivity degree, the association is weak ($r = -0.264$).

The strength of this study is that only new TB pulmonary patients were included and that those who had conditions that could affect vitamin D levels, such as the presence of HIV (+) or DM (+), pregnancy or lactation, under immunosuppressive treatment, and liver and kidney disorders were excluded. Therefore, it can be assured that the results of this study only illustrate vitamin D deficiency with regards to TB and not to other conditions.

To better comprehend the relationship

between vitamin and sputum positivity, a large cohort is needed. It may also be necessary to measure the nutritional status, cathelicidin levels, and type of VDR polymorphism in future studies.

This study seems to be the first study to investigate the association between vitamin D levels and bacterial load in newly diagnosed TB patients in Indonesia. In summary, the findings of this study revealed that many newly diagnosed TB patient experienced vitamin D deficiency despite the abundant sunshine throughout the year in Indonesia, which is a country that is located in the equatorial area.

This study suggests that a low 25(OH) vitamin D level is associated with a higher bacterial load and that vitamin D indeed affects the bacterial load level.

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