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Relationship Between Serum Vitamin D Levels and Bacterial Load Sputum in Tuberculosis Patients in Simalungun, North Sumatra, Indonesia

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Abstract: ***Vitamin D is involved in innate and adaptive immune function and its deficiency is a risk factor for tuberculosis (TB) infection. The aim of this study was to describe the prevalence of vitamin D deficiency in newly diagnosed tuberculosis patients and to examine the relationship between vitamin D levels and sputum swab positivity, which reflects bacterial load. This is a cross-sectional study conducted from June 2022 to August 2022 at multiple primary health care facilities at Buntu Turunan Public Health Center and Perdagangan Regional General Hospital, Simalungun Regency. Up to 101 adult tuberculosis patients who were sputum smear-positive, untreated, on treatment, or had a treatment duration of 1 week or less to her were recruited. Sputum examination was scored using the International Union for Tuberculosis and Lung Disease (IUATLD) rating 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 levels and positive levels of AFB sputum was analyzed using Spearman's correlation test. The prevalence of vitamin D deficiency in new pulmonary tuberculosis patients with positive sputum smear was 20.79%. There was a weak relationship between vitamin D levels and AFB sputum positive levels (p=0.017), with a correlation coefficient of (-0.278). Despite Indonesia being an equatorial region and having plenty of sunshine throughout the year, many of the newly diagnosed tuberculosis patients were vitamin D deficient. This study shows that vitamin D does influence bacterial load and that low levels of 25(OH)-vitamin D are associated with higher bacterial load.***

Keywords: Bacterial load, pulmonary tuberculosis, vitamin D deficiency

I. INTRODUCTION

Tuberculosis (TB) is the second most contagious disease in the world, after HIV/AIDS, and has the highest morbidity and mortality. In 2017, Indonesia ranked him third in the countries with the highest incidence of tuberculosis cases in the world, after India and China. This situation highlights the need for preventive and therapeutic efforts to treat tuberculosis cases.[1]

Mycobacterium tuberculosis (MTB) infection is primarily transmitted by aerosol. When a sputum-positive person with pulmonary tuberculosis coughs or sneezes, droplet nuclei containing infectious bacteria are expelled. Once the nuclei enter the airways, they are bound to surface receptors such as toll-like receptor (TLR) 2 before being taken up by alveolar macrophages in the lung. After binding, bacteria are taken into the body and taken up by phagosomes, where they are killed by multiple mechanisms.[2] Vitamin D deficiency is one of the risk factors promoting tuberculosis infection. In addition to its role in the skeletal system, vitamin D also plays a role in innate and adaptive immune function.[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] Although Indonesia is a tropical country, vitamin D deficiency is still common. A previous study of 504 women of childbearing age in Indonesia and Malaysia showed a prevalence of vitamin D deficiency of 63% in both countries.[7]

II. METHODS

This is a cross-sectional study conducted at multiple primary health care facilities at Buntu Turunan Public Health Center and Perdagangan Regional General Hospital, Simalungun Regency, North Sumatra, Indonesia from from June 2022 to August 2022. All subjects had provided informed consent prior to the study. As many as 101 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.

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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 Fluorescence Immunoassay (FIA) kits from Ichroma Boditech device. 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 computer software. Statistical significance was considered to be achieved by a 5% level of significance and a p-value <0.05.

III. RESULTS

This study involved 101 subjects, comprising of 61 males and 40 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).

Table 1 Baseline Characteristics

Characteristics	Sputum AFB Positivity			Total	
	+1	+2	+3	n (%)	p
Age (median, range)	43 (18-69)	41 (18-62)	42 (18-62)	42 (18-62)	0.621
Sex					0.230
Male	23 (37.70%)	23 (37.70%)	15 (24.59%)	61 (60.39%)	
Female	10 (25%)	15 (37.50%)	15 (37.50%)	40 (39.60%)	

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). 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 20.79%.

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.

Table 2 Comparison of Vitamin D Status by Sputum AFB Positivity

Vitamin D Status	Sputum AFB Positivity			Total
	+1	+2	+3	
	(n=34)	(n=39)	(n=28)	(n=101)
<20 ng/mL (deficiency)	3	8	10	21 (20.79%)
20-30 ng/mL (insufficiency)	11	16	9	36 (35.64%)
>30 ng/mL (optimum)	20	15	9	44 (43.56%)

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

Parameter	Sputum AFB Positivity			p	r
	1	2	3		
25(OH) Vitamin D level (ng/mL)	32,48±7.22	28,29±9.66	26,21±8.78	0.017	-0.278

IV. DISCUSSION

101 volunteers between the ages of 18 and 69 participated in this study. There is no difference in vitamin D requirements between men and women, age 18 and age 69.[8] Most experts agree that in determining the status of 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]

Fifty-seven subjects (56.34%) in this study had suboptimal vitamin D levels, 20.79% were deficient, and 35.64% were deficient. Several studies in Europe, Asia and Africa showed that the prevalence of vitamin D deficiency in tuberculosis patients was 82.3%, 92% and 95.4% respectively. Deficiency rates are lower (17.4% vs. 27.5%) compared to results from a study evaluating vitamin D levels in tuberculosis patients in Sabah, Malaysia. This may be because this Malaysian study did not consider insufficient vitamin D 25(OH) levels (20-29 ng/mL) as vitamin D deficiency. However, there was no significant difference in mean vitamin D levels between the two studies (29.14 ng/mL vs. 25.56 ng/mL). Vitamin D levels are closely related to geographic location and sun exposure. Countries farther from the equator receive less solar radiation. This may explain the low rates of vitamin D deficiency in tuberculosis patients in Malaysia and Indonesia.[9,10,11]

In this study, the vitamin D levels of the subjects were not compared to healthy people.However, some studies suggest that vitamin D deficiency is common in healthy people as well.Malaysia and Indonesia A study of 504 non-pregnant women found that more than 60% of women had vitamin D deficiency. A similar study comparing vitamin D levels in pulmonary tuberculosis patients and healthy people in Medan found that mean serum vitamin D levels were similar in both groups (25.21±7.93 ng/mL vs. .21.50±9.37 ng/mL). Given these results, it seems likely that vitamin D deficiency is being overlooked in Asian countries. The hypothesis that vitamin D deficiency is unlikely in areas with sufficient sun exposure has been rejected.[7,10,12]

This study shows that subjects with lower vitamin D levels had a higher sputum positivity degree (r=-0. 278, p=0.017). 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]

The antibacterial effect of vitamin D against Mycobacterium tuberculosis has already been demonstrated. Once MTB enters the airways, it is bound through surface receptors such as Toll-like receptors (TLRs) and then engulfed by alveolar macrophages in the lungs 2 . After binding, bacteria can be internalized, taken up by phagosomes, and killed by multiple mechanisms. One of them is due to autophagy and phagolysosomal processes. Calcitriol, an active metabolite of vitamin D, inhibits replication of her MTB in 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]

Still, vitamin D levels are not the only factor affecting sputum positivity. The study found that the mean vitamin D levels of the +3 and +2 groups were in the "poor" category, while the mean vitamin D levels of the +1 group were in the optimal category. Disease severity in TB appears to be influenced by other host factors besides MTB virulence. Vitamin D bioavailability may not be fully reflected by total serum 25(OH)D levels alone. There are also other factors such as vitamin D carrier protein genotype, vitamin D binding protein genotype, and other genetic variants in the vitamin D system that affect bioavailability. , which may affect the synthesis and function of that protein, which in turn may affect cathelicidin. Despite the proven association between vitamin D levels and positive sputum levels, the weak association (r=-0.278) can be explained by several factors mentioned above.[16, 17]

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.

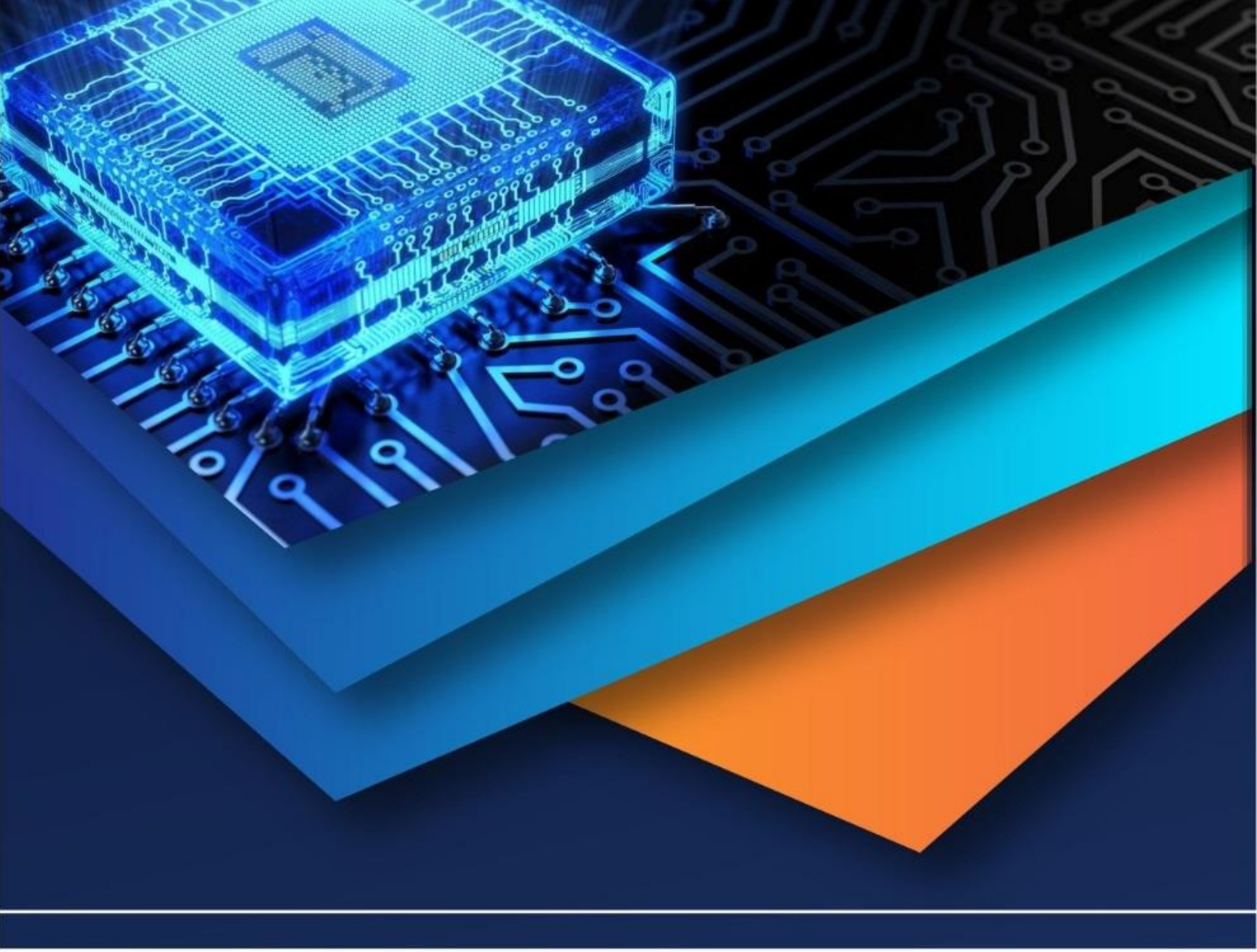
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