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Analysis of Serum Vitamin D Level in Leprosy Patients

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ABSTRACT

Introduction: Leprosy is a chronic granulomatous infection with a variety of clinical spectrum caused by *Mycobacterium leprae (M. leprae)* that is highly influenced by host immune response. In leprosy, vitamin D acts as an immunomodulator through a VDR-mediated antimicrobial pathway that affects the innate immune system to bacterial killing. M. *leprae* inhibits VDR activity through the down-regulation of CYP27B1. Elevated $1.25(OH)_2D$ levels are required to modulate cathelicidin antimicrobial peptide (CAMP) production, which leads to reduced 25(OH)D level. This study aimed to analyze the difference in serum vitamin D level between leprosy patients and healthy people.

Methods: This research was an analytical observational study with a cross-sectional design involving 20 patients with new cases of leprosy and 20 controls. The diagnosis of leprosy was confirmed through physical examination and laboratory examination. We conducted blood sampling and measurement of serum vitamin D (25(OH)D) level in both groups using the chemiluminescence immunoassay (CLIA) method. The collected data were then processed and analyzed statistically using the Mann-Whitney test.

Result: In this study, we found that the mean serum vitamin D level in leprosy patients (22.27 ± 5.418 ng/mL) was lower than controls (33.00 ± 1.913 ng/mL), and the difference was statistically significant ($p < 0.05$). The mean serum vitamin D level in male leprosy patients (23.69 ± 4.034 ng/mL) was higher than females (16.55 ± 7.081 ng/mL), and was highest in patients aged 36 – 45 years (25.314 ± 2.2945 ng/mL).

Conclusion: Serum vitamin D level was significantly lower in leprosy patients than in controls. Serum vitamin D level in leprosy patients was higher in the male group and was highest in patients aged 36 – 45 years.

Keywords: leprosy, vitamin D, 25(OH)D

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INTRODUCTION

Leprosy or morbus hansen is a chronic infectious disease caused by *Mycobacterium leprae (M. leprae)* that mainly affects the skin and peripheral nerves.[1,2] Indonesia has the third highest incidence rate of leprosy, with as many as 17,202 cases reported. Among these cases, 14,545 were multibacillary (MB) cases.[3] Meanwhile, in 2015, 197 new cases of leprosy were reported in the province of North Sumatra, with a prevalence rate of 1.41 per 100,000 population.[4]

The clinical appearance in leprosy patients represent the pathology, which changes depending on the balance between the multiplication of bacilli and the cellular immune response in the host. According to Ridley Jopling, leprosy was classified into tuberculoid (TT), borderline tuberculoid (BT), borderline (B), borderline lepromatous (BL), and lepromatous (LL). However, in 1982, the World Health Organization (WHO) changed the classification to facilitate treatment into paucibacillary (PB) and multibacillary (MB).[2,5]

Among all components of the cellular immune system, macrophages are the principal component, and they respond to the presence of *M. leprae* through several signaling pathways. Four pathways may play a central role in leprosy with their molecular interactions, including the Toll-like receptor-2 and 1 (TLR 2/1), tumor growth factor-α (TNF-α), tumor growth factor-β (TGF-β) and vitamin D receptor (VDR). In leprosy, vitamin D acts as an immunomodulator through a VDR-mediated antibacterial pathway that affects the innate immune system.[6,7] Although no evidence for a deficiency in serum vitamin D in leprosy cases has yet been reported, the indirect evidence of having bone deformities due to low calcium absorption suggests that vitamin D deficiency may play a role in leprosy.[8] Based on that, this study aimed to determine the difference in vitamin D levels between people with leprosy and healthy people.

METHODS

This research was conducted from November 2017 to August 2018. This research was an analytical observational study with a cross-sectional design involving 20 leprosy patients and 20 controls aged 20 - 70 years old in the Leprosy Division of the Department of Dermatology and Venereology Clinic in Haji Adam Malik General Hospital and Dr. Pirngadi Regional Public Hospital, Medan, North Sumatera, Indonesia. Ethical clearance was obtained from the Health Research Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara No. 557/TGL/KEPK FK USU-RSUP HAM/2017. Each subject who had signed the informed consent was included in this study. The exclusion criteria were: consumption of multivitamin supplement which containing vitamin D within the last one month, breastfeeding and pregnancy, and had a reaction to leprosy. Patients suffering from dermatology diseases (psoriasis, systemic lupus erythematosus, vitiligo) and systemic diseases (multiple sclerosis, chronic renal failure, chronic liver disease, cardiovascular disease, diabetes mellitus and infectious diseases, such as tuberculosis and upper respiratory tract, as well as malignancy) were also excluded.

All subjects with leprosy were diagnosed by clinical, dermatological and neurological examinations. Subjects' blood samples were then processed into serum. Serum of vitamin D level (25(OH)D) was measured using CLIA – DiaSorin Liaison®.

Data normality test was performed using the Shapiro Wilk test, and the Mann Whitney test was used to determine the difference between the two subject groups of the study, where the difference between groups was considered significant if $p < 0.05$.

Table 1. Baseline characteristic of subjects.

Characteristic	Leprosy		Control	
	n	%	n	%
Gender				
Male	16	80.0	16	80.0
Female	4	20.0	4	20.0
Total	20	100.0	20	100.0
Age (years old)				
18 – 25	4	20.0	4	20.0
26 – 35	9	45.0	11	55.0
36 – 45	7	35.0	4	20.0
46 – 55	0	0	1	5.0
Total	20	100.0	20	100.0

Table 2. Distribution of leprosy patients based on leprosy type.

Leprosy type	n	%
PB	1	5.0
MB	19	95.0
Total	20	100.0

RESULTS

Baseline characteristics of all subjects in each group are presented in Table 1. In each group, more than half of the subjects were male. The subjects' age ranged from 18 – 46 years old, and the majority of them were in the 26–35 years old age group (45.0%). The majority of leprosy cases in this study was type MB, as shown in Table 2.

The mean serum vitamin D level in leprosy patients was higher in the male group (23.69±4.034 ng/mL) than the female group (16.55±7.081 ng/mL) (Table 3), and the mean level of vitamin D serum was highest in the 36-45-year-old group (25,314±2,2945 ng/mL) (Table 4).

As shown in Table 5, the mean serum vitamin D level in leprosy patients (22.27 ± 5.418 ng/mL) was lower than controls (33.00 ± 1.913 ng/mL), and the difference was statistically significant ($p < 0.05$).

DISCUSSION

For many years, vitamin D has been believed to play an essential role in the development of bone mineralization, known as the classic effects (skeletal effects). However, vitamin D has also been suggested to have other roles.[9,10] Recently, vitamin D is known to display non-classical effects (extra-skeletal effects) through the discovery of the expression of CYP27B1 enzyme and VDR in other cells or extra-renal tissues.[11] This revealed extra-skeletal effects of vitamin D including the immune system, endocrine, muscular, cardiovascular, cancer, neurogenerative, renal and pulmonary.[12-14]

Photoproduction of vitamin D begins with the synthesis of the sterol provitamin D molecule 7-dehydrocholesterol. In most vertebrate animals, including humans, it is produced in large quantities in the skin and is incorporated into the plasma membrane lipid bilayers of cells in the dermis and epidermis. When the skin is exposed to sunlight, 7-dehydrocholesterol absorbs ultraviolet B (UVB) radiation in the wavelength range of 290–315 nm. The absorbed energy causes the chemical bonds within the 7-dehydrocholesterol molecule to break and rearrange, resulting in the formation of previtamin D_3. In the skin, previtamin D_3 undergoes rapid, thermally-induced transformation to vitamin D_3. Once formed, previtamin D_3 and

Table 3. Serum vitamin D level based on gender in leprosy patients.

Gender	Serum vitamin D level (ng/mL)					
	n	Mean	Median	SD	Min	Max
Male	16	23.69	24.10	4.034	12	28
Female	4	16.55	13.60	7.081	12	27

Table 4. Serum vitamin D level based on age in leprosy patients

Age (years old)	Serum vitamin D level (ng/mL)					
	n	Mean	Median	SD	Min	Max
18 – 25	4	23.050	22.70	3.1043	19.9	26.9
26 – 35	9	19.544	21.70	6.7374	11.9	28.1
36 – 45	7	25.314	24.80	2.2945	22.6	28.4

Table 5. Difference in serum vitamin D level in leprosy patients and controls.

Group	Serum vitamin D level (ng/mL)						
	n	Mean	Median	SD	Min	Max	p
Leprosy	20	22.27	23.85	5.418	12	28	< 0.001
Control	20	33.00	32.95	1.913	30	28	

*Mann-Whitney test

vitamin D_3 continue to absorb UV radiation in a wide range of wavelengths. This sometimes results in the breakdown of these molecules into biologically inert photoproducts. For this reason, during prolonged exposure to UV radiation, a steady state is reached, in which only 10–15% of cutaneous 7-dehydrocholesterol is converted to pre-vitamin D_3. It has been suggested that this process of photoregulation ensures that toxic levels of vitamin D_3 are not synthesized under conditions of excessive sun exposure.[10,15,16]

Cutaneously synthesized vitamin D_3 is released from the plasma membrane and enters the systemic circulation bound to vitamin D-binding protein (DBP). As a lipid-soluble molecule, vitamin D_3 can be taken up by adipocytes and stored in subcutaneous or omental fat deposits for later use. Circulating vitamin D_3 is metabolized in the liver, by the enzyme vitamin D-25-hydroxylase, to 25(OH) D. This is the major circulating form of vitamin D and the molecule typically measured by clinicians wishing to assess vitamin D status. The serum half-life of 25(OH)D is approximately 15 days. Following cutaneous synthesis or oral consumption, vitamin D bioavailability is dependent on intestinal absorption, fat storage, and metabolism. Dietary vitamin D consists of vitamin D_2 (ergocalciferol) derived from non-vertebrate species (invertebrates, fungi, and plants) and vitamin D_3 (cholecalciferol) derived from vertebrates. Other dietary sources of vitamin D are supplements. Following process the vitamin D2 and vitamin D3 absorbed through the gastrointestinal tract, and transport through the lymphatic system to systemic circulation and bound to DBP also metabolized in the liver to 25(OH)D. Activation requires its conversion to $1,25(OH)_2D_3$ in the kidney and other organs by the enzyme 25(OH) D-1α-hydroxylase. Production of $1,25(OH)_2D_3$ is tightly regulated by a number of factors, the most important of which are serum phosphorus and parathyroid hormone (PTH) levels. However, $1,25(OH)_2D$ is also produced in many other tissues, such as the skin, macrophages, colon, pancreas, blood vessels, etc.[15,16]

Non-classical effects (extra-skeletal effects) of vitamin D acts as an immunomodulator, which induced antimicrobial activity and upregulation of innate immune response host through VDR-binding.[13,14,17] One of the earliest studies to describe a potential mechanism for the beneficial effects of vitamin D on tuberculosis (TB) showed that the active form of vitamin D, 1,25(OH) D, potently suppressed proliferation of the infectious pathogen associated with TB, *Mycobacterium tuberculosis* (*M. tb*), in human monocytes. *M. leprae* and *M. tb* are intracellular bacteria, so the defense mechanisms of the host against the pathogens are similar.[9,11,17] Innate antibacterial activity mediated by vitamin D intracrine pathway (intracrine vitamin D system) was induced following the activation of TLR2/1. As a consequence, TLR2/1-activated cells treated with 25(OH)D showed an increased local synthesis of $1,25(OH)_2D$, which could then bind to and signal via the VDR. This represented a potential vitamin D intracrine system for localized, VDR mediated regulation of gene expression. Amongst the potential targets for VDR in this setting is the gene for cathelicidin (LL37), which encodes a protein known to be involved in promoting intracellular killing of bacteria.[11,17] *M. leprae* is known to inhibit VDR activity through the down-regulation of CYP27B1 in monocytes.[11,18] High levels of $1,25(OH)_2D$ is needed in chronic infections to modulate cathelicidin antimicrobial peptide (CAMP), and 25(OH)D is rapidly metabolized in the process, resulting in a low serum level.[17,18]

In this study we found that the mean serum vitamin D level was lower in leprosy patients (22.27 ± 5.418 ng/mL) than in the controls (33.00 ± 1.913 ng/mL), where there was a significant difference in serum vitamin D level between leprosy patients and the control group (p = 0.0001). This result is consistent with the study of Mandal et al., which *also* found that leprosy patients had a lower mean

serum vitamin D level (27.47 ± 4.17 ng/mL) than the control group (33.40 ± 3.41 ng/mL).[8]

Based on the DiaSorin Liaison® kit (USA, DiaSorin Inc, 310600) was used in this study, serum vitamin D level (25(OH)D) was classified into *deficient* (<10 ng/mL), *insufficient* (10-30 ng/mL), *sufficient* (30-100 ng/mL), and *toxic* (>100 ng/mL). The mean serum vitamin D level in leprosy patients, which was 22,27 ± 5,418 ng/mL, fell into the insufficient category.

The most notable observation from studies of the intracrine induction of monocyte LL-37 is that this response enhanced bacterial killing by simply increasing the levels of the precursor form of vitamin D, 25(OH)D.[11,19] Therefore, vitamin D status could enhance or impair the intracrine response to infection. Decreased availability of serum 25(OH) D as a result of vitamin D insufficiency may lead to impaired innate immune response to infection.[18,19]

The three potential sources of vitamin D are diet, exposure to UVB, and supplements. Vitamin D is available in two distinct forms, namely ergocalciferol (vitamin D_2) dan cholecalciferol (vitamin D_3). Sunlight exposure provides vitamin D in the form of D_3 only, while dietary sources can provide both forms. Multivitamins may contain either vitamin D_2 or vitamin D_3, but most companies are now reformulating their products to contain vitamin D in the D_3 form. Dietary sources of vitamin D include cod liver oil, cheese, egg yolks, sea fish (bloated, salmon, tuna, sardines), shitake mushrooms, beef liver, and fortify foods such as orange juice, milk, yogurt, and cereal with vitamin D. Exposure to sunlight or daylight for 10-15 minutes to a small area of skin, such as the forearm or face, twice a week without sunscreen, supplies all the vitamin D necessary for health. Therefore, these things can be done to enhance vitamin D levels in human.[10,12,15,18]

CONCLUSION

In this study, serum vitamin D level in leprosy patients was significantly lower than the controls. Serum vitamin D level was higher in male patients with leprosy than females and was highest in patients aged 36 – 45 years.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

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AUTHOR'S CONTRIBUTION

NIMD, RDL and NKJ shared equal contribution in the component of this study from concepts; design; literature search; clinical studies; data acquisition and analysis; statistical analysis; and manuscript preparation, editing and review.

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