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Correlation of Serum Vitamin D Receptor Level with Bacterial Index in Multibacillary Leprosy Patients at Sanglah General Hospital, Bali-Indonesia

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Leprosy cases were still a common problem in Indonesia. Even though Bali was not considered as a high epidemic region in Indonesia, new cases of multibacillary leprosy continuously appeared. Vitamin D and its receptor, Vitamin D Receptor (VDR) has a role in modulation of immune system against *M. leprae*. This study aimed to find correlation between blood VDR serum level with bacterial index of multibacillary leprosy patients. Study design using cross-sectional model conducted in Sanglah General Hospital involving 47 multibacillary leprosy patients taken consecutively from July-October 2017. The level of VDR was examined by ELISA method. Characteristic of study participant is 29 (61.7%) male patients and 18 (38.3%) female patients. The mean age of the patient was 38.83 years. The mean VDR level was 27.80 pg/dl. Spearman correlation test found that there is a strong negative correlation ($r = -0.954$; $p < 0.001$) between plasma level of VDR with bacterial index in multibacillary patients. This result suggests that the lower the VDR level, the higher bacterial index in leprosy patients. This study emphasizes more the role of Vitamin D and its receptor in immunomodulation especially in leprosy infection.

Keywords: leprosy, multibacillary, VDR, bacterial index.

Leprosy that caused by *Mycobacterium leprae* is still a common problem in Indonesia because Indonesia has the third most multibacillary cases in the world after India and Brazil with 14.213 cases. It has more burden also because there is still stigmatization in the society against leprosy patients.[1]

The course of leprosy is not merely caused by the infection of organism, but also complicated by host factors such as genetic and immunity. This interaction has given a variety of clinical appearance and disease spectrum and generally divided this disease into paucibacillary and multibacillary classification. Host immune system that acts as the first barrier in *M.leprae* infection is cellular immunity especially macrophage.[2, 3] Macrophage will respond to infection by several different ways, and one of the pathways is mediated by vitamin D and vitamin D receptor (VDR).[3, 4] In Leprosy, vitamin D, through its interaction with VDR, were thought to act as immunomodulator that influences macrophage in killing the pathogen by increasing the expression of antimicrobial peptide cathelicidin.[1,5] For the immunological activity of vitamin D to happen, it needs to interact with its receptor, the VDR. This study aimed to find

correlation between the level of VDR, examine in blood plasma, with bacterial index in multibacillary leprosy patients.

MATERIAL AND METHOD

This is an observational analytic study with cross-sectional design to correlate the plasma level of VDR with bacterial index in multibacillary leprosy patients. This study was conducted in Sanglah General Hospital from July until October 2017. This study involved 47 patients of multibacillary leprosy patients aged five until 65 years old that came to Morbus Hansen Subdivision Dermatology Polyclinic that came in period of the study and taken consecutively following the inclusion and exclusion criteria. The inclusion criteria include all multibacillary leprosy cases, aged 5 to 75 years old with slit skin smear examination based on Ridley's scales of bacterial index, with good general condition, and willing to participate in the study, whereas the exclusion criteria include patients that already released from treatment (RFT), had hormonal imbalance condition such as thyroid and parathyroid disease, pregnancy, lactating, menstruation, and ovarian tumor, had systemic condition such as chronic kidney disease, chronic liver disease, multiple sclerosis, and cardiovascular disease, had chronic systemic infection such as tuberculosis, had autoimmune condition such as diabetes mellitus, arthritis rheumatoid, systemic lupus erythematosus, psoriasis, and vitiligo, and had history of taking anti-inflammatory drugs in the past 2 weeks (any systemic condition that affect VDR plasma directly). The bacterial index was determined by slit skin smear with Ziehl-Nielsen staining following logarithm of Ridley's bacterial scales. Specimens for examination of VDR plasma level were taken from blood drawn from venous vein in fossa cubiti. Level of VDR was determined by quantitative method with enzyme link immune sorbent assay (ELISA).

Statistical analysis in this study using software SPSS version 16.0 for windows. Spearman correlation test was used to determine the correlation between serum VDR levels and bacterial index. This research has been approved by the ethics committee of the Faculty of Medicine, Udayana University / Sanglah General Hospital Denpasar. All participant in this study has signed an inform consent of every procedural done in this study.

Table 1. Descriptive Characteristic of Sample

Characteristics	MB Leprosy (n= 47)	Percentage (%)
Gender		
Male	29	61.7
Female	18	38.3
Age (years old)		
5 – 15	1	2.1
16 – 25	7	14.9
26 – 35	9	19.1
36 – 45	10	21.3
46 – 55	6	12.8
56 – 65	8	17
≥ 66	6	12.8
Bacterial Index		
0	7	14.9
1	11	23.4
2	6	12.8
3	12	25.5
4	10	21.3
5	1	2.1
VDR Plasma (Mean ± SD)	27.80 ± 2.24	

RESULTS

This study involved 47 patients with multibacillary leprosy, consist of 29 (61,7%) male patients and 18 (38,3%) female patients. The mean age of the sample was 38,83 years old, with the youngest was 11 years old and the oldest was 75 years old. Based on bacterial index, most patients had bacterial index of +3. Table 1 showed the descriptive characteristic of the sample.

Mean level of VDR among multibacillary patients in this study was 27.80 pg/ml, with the lowest level was 19.51 pg/ml and the highest was 34.56 pg/ml. For the correlation of plasma VDR

Table 2. Correlation between plasma VDR level and bacterial index

		Bacterial Index
Plasma level of VDR	r	-0.954
	p	<0.001
	n	47

level and the bacterial index, using Spearman correlation, there was strong negative correlation between plasma VDR level and bacterial index ($r = - 0.954$; $p < 0.001$). This result suggests that the lower plasma VDR level, the higher the index bacterial of the leprosy patients (Table 2).

DISCUSSION

Mycobacterium leprae as the causative organism of leprosy has a low virulence and very slow doubling time, but yet this organism is capable of provoking an intricate immune response that influences the clinical outcome and course of the disease. The first response against *M.leprae* infection was macrophage from innate immune response and then followed by response of adaptive immunity.[2,6] One of macrophage activation pathway involved the role of vitamin D and its receptor.[3,4,7] Recently, there was plenty study that elaborates the role of vitamin D in infectious disease and suggests that deficiency of vitamin D will result in deterioration of the infection. Mechanism of vitamin D as immunomodulator in mycobacterial infection is not entirely understood yet, but some theory has been proposed.[8,9] In leprosy, vitamin D through its interaction with VDR were thought to influence macrophage capability in killing the pathogen by increasing expression of antimicrobial peptide cathelicidin.[7,9] Other study suggest a more comprehensive role of vitamin D as an anti-inflammatory and regulator of immune system.[8,12]

Macrophage was found to produce 1á,25 hydroxyvitamin D3 (1,25(OH)2D3) and VDR was expressed by varies of immune cells such as CD4+, CD8+, T-cells, B-cells, neutrophils, *antigen presenting cell* (APC), and dendritic.[8,10] Interaction between 1,25(OH)2D3 and VDR will provoke phagocytosis, chemotaxis, and proliferation B cells, also the production of immunoglobulin.[11] Previous study by Mandal *et al*.[10] in 2015 found level of vitamin D in leprosy patients was significantly lower than normal control. Another study by Goulart *et al*.[13] found a significant relationship between VDR polymorphism against bacilloscopic index, this illustrates that VDR acts as a susceptibility factor for leprosy infection. Study by Mandal *et al*.[10] also found that VDR expression levels may determine the complexity and severity of the progression of leprosy. The VDR mRNA was found significantly lower in leprosy patients compared to healthy controls. Furthermore, patients with very low VDR mRNA were more likely to be associated with neuritis, leprosy reaction and high bacterial index.[10,12] This result had shown more of the role of Vitamin D and its receptor in leprosy infection and lead to reconsideration in using vitamin D or VDR therapy in treating individuals with leprosy.

Through these findings a recommendation can be given, in addition to using multidrug therapy, vitamin D supplementation is also needed to increase the expression of plasma VDR which will then help mediate the immune system.

The limitation of this study is that the number of samples used in this study is still very small so that this situation tends to be less representative of the actual state of the population

CONCLUSION

From this study we found that plasma level of VDR had a significantly strong negative correlation with bacterial index in multibacillary leprosy patients.

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