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Original Article

Analysis of vitamin D receptor levels in serum of leprosy patients of multibacillary type, erythema nodosum leprosum and normal people

Aznamry, Safruddin Amin, Khairuddin Djawad, Faridha Ilyas

Department of Dermatology and Venereology, Faculty of Medicine, University of Hasanuddin, Makassar, Indonesia.

Abstract

Objective Erythema Nodosum Leprosum (ENL) is an acute inflammatory reaction that occurs only in multibacillary (MB) leprosy patients. Serum Vitamin D Receptor (VDR) has been widely used as a biomarker to determine the progress of therapy in various diseases. This study aims to determine the difference between serum VDR in MB type leprosy patients who experience ENL reactions and those who do not experience ENL reactions and healthy people.

Methods The research was conducted using an analytic observational study with a cross sectional method. The total number of samples is 36 people, where each group consists of 12 people.

Results Mean serum VDR of multibacillary leprosy patients with an ENL reaction was 10.41 (4.34±18.12), whereas in the group without ENL it was 25.92 (23.32±29.42) and in the healthy group it was 39.51 (34.54±42.67).

Conclusion Based on statistical tests, it was found that serum VDR was significantly different between multibacillary leprosy patients who experienced ENL reactions and patients who did not experience ENL reactions and healthy people.

Key words
Erythema Nodosum Leprosum (ENL); Leprosy; Vitamin D Receptor (VDR).

Introduction

Leprosy is a disease caused by the bacterium Mycobacterium leprae (M. leprae) and can cause physical defects (dermatoneurological lesions) which cause negative stigmatization in society.[1,2,3] The number of leprosy cases in Indonesia in 2016 was reported 16,826 cases with the number of new cases being discovered 6.5 per 100,000 population. Among these new cases, 83% are cases of MB (Multi Basiler), this indicates that transmission is still ongoing and there is still a high rate of delay in finding new cases.[1,4] As many as 30-40% of patients with leprosy experience episodes of immune-mediated inflammation such as Reaction Type 1 and Erythema Nodosum Leprosum (ENL or Reaction Type 2).[5]

Currently, vitamin D is thought to have other roles that show non-classical effects (extra-skeletal effects) through discovery of the expression of the CYP27B1 enzyme and Vitamin D Receptor (VDR) in other cells or extra-tissues.[6] Non-classical effects (extra effects-skeletal) vitamin D which acts as an immunomodulator, can induce antimicrobial activity and regulate host innate immunity through binding to VDR.[7-9] M. leprae is known

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Address for correspondence
Dr. Khairuddin Djawad
Dermatology and Venereology Department
Faculty of Medicine, University of Hasanuddin,
Makassar, Indonesia.
Email: duddindj@gmail.com

to inhibit VDR activity through downregulation of CYP27B1 in monocytes.[8-9] Level 1 High,25(OH)2D is required in chronic infections to modulate cathelicidin antimicrobial peptide (cAMP). Decreased serum 25(OH)D levels as a result of vitamin D deficiency can lead to impaired innate immune response to infection.[10,11] This study was conducted to analyze VDR levels in the serum of leprosy patients with type MB, ENL, and healthy people.

Methods

This study was a cross-sectional study by determining serum levels of vitamin D receptor (VDR) in the serum of multibacillary (MB) leprosy patients and ENL. As a comparison are healthy people.

The research sample was the serum of MB leprosy patients with ENL and without ENL who were diagnosed based on clinical symptoms and confirmed by laboratory tests (slit skin smear). ENL was selected based on anamnesis and confirmed with medical records and Bacterial Index (BI) and ELISA IgM NGL-1. All samples used in this study were taken from the sample bank at the Immunology Molecular Biology Laboratory, Faculty of Medicine, UNHAS and began to be collected from January 2004-2014. The comparison subject was the serum of healthy people taken from UTD Makassar which was stored in the sample bank of the FK UNHAS Molecular Biology Laboratory which was used as a sample bank. All research samples were then prepared for bacterial index (BI) examination and Vitamin D Receptor (VDR) level assessment using the ELISA method.

Data analysis used SPSS version 23.0. The analytical method used was the Wilcoxon Sign Rank Test and the Mann Whitney nonparametric statistical test.

Results

Based on **Table 1**, it shows that the gender characteristics with the male category in the diagnosis of ENL were 5 (41.7%) patients, and women were 7 (58.3%) patients. Meanwhile, in the diagnosis of MB Leprosy, there were 9 (75.0%) male patients and 3 (25.0%) female patients.

In terms of characteristics, 9 (75.0%) patients received MDT in the ENL group and 3 (25.0%) did not receive MDT, whereas in the MB Leprosy group, no patients received MDT and did not receive MDT. as many as 12 (100.0%).

In the RFT characteristics, there were 2 (75.0%) patients in the yes category and 10 (83.3%) no categories in the ENL group, while in the MB Leprosy group, there were 0 (0.00%) patients in the yes category and RFT category not as much as 12 (100.0%).

Based on **Table 2**, it shows that the age characteristics showed that the mean age in the ENL group was 32.58 years, while in the MB Leprosy group it was 26.83 years. In the characteristics of the lesions, a mean value of 17.0±11.55 was obtained in the ENL group, while in the MB Leprosy group, the mean value of lesions was 18.42±8.53.

Table 1 Characteristics of respondents based on ENL and MB diagnoses.

Character-istics	*Diagnosis*		
	ENL n(%)	*MB n(%)*	*Total n(%)*
Gender			
Male	5 (41.7)	9 (75.0)	14 (58.3)
Female	7 (58.3)	3 (25.0)	10 (41.7)
MDT			
Yes	9 (75.0)	0	9 (37.5)
No	3 (25.0)	12 (100)	15 (62.5)
RFT			
Yes	2 (16.7)	0	2 (8.3)
No	10 (83.3)	12 (100)	22 (91.7)
Total	12 (100)	12 (100)	24 (100)

Table 2 Average characteristics, BI, and MI based on ENL and MB diagnoses.

Diagnosis	*ENL*				*MB*				*p value*
	Mean	*SD*	*Min*	*Max*	*Mean*	*SD*	*Min*	*Max*	
Age	32.58	12.92	17.00	52.00	26.83	12.06	16.00	52.00	0.183**
Lesions	20.40	9.28	9.00	36.00	18.42	8.53	6.00	31.00	0.692**
BI	3.33	0.89	2.00	5.00	2.67	0.65	2.00	4.00	0.055**
MI	59.62	24.55	27.90	95.30	81.89	8.00	64.30	92.50	0.053**
Illness duration	6.50	2.75	2.00	11.00	6.25	3.47	1.00	12.00	0.847*
MDT duration	8.33	8.33	0.00	24.00	0.00	0.00	0.00	0.00	0.000**

* Independent t test; ** The Mann Whitney test.

Table 3 Comparison of VDR levels in the ENL, MB and healthy people groups.

Group		*VDR*					*p value*
		Mean	*SD*	*Median*	*Minimum*	*Maximum*	
ENL vs. MB	ENL	10.41	4.07	10.70	4.34	18.12	0.000
	MB	25.92	2.13	25.24	23.32	29.42	
ENL vs. HP	ENL	10.41	4.07	10.70	4.34	18.12	0.000
	HP	39.51	2.82	40.04	34.54	42.67	
MB vs. HP	MB	25.92	2.13	25.24	23.32	29.42	0.000
	HP	39.51	2.82	40.04	34.54	42.67	

In the bacterial index characteristics (BI) the mean value was 3.33±0.89 in the ENL group, while in the MB Leprosy group the mean ENL value was 2.67±0.65. In morphological index characteristics (MI) the mean value was 59.62±24.55 in the ENL group, while in the MB Leprosy group the mean MI was 81.90±8.0. In the category of duration of illness in the ENL group, the average duration was 16.25±11.91 months, while the duration of illness in the MB Leprosy group was found to be an average duration of 6.25±33.47 months. In the MDT duration category in the ENL group, the average duration was 8.33±8.33 months, while the duration of illness in the MB Leprosy group was 0 months.

Based on **Table 3**, it is known that the average VDR level in the ENL patient group is 10.41. In the MB group it was 25.92 and in the healthy group it was 39.51. Based on these results it was known that the VDR level in the ENL group was lower than that in the MB group and healthy people. From the statistical test results, it was found that the value of p <0.05 concluded that there was a significant difference in the value of VDR levels for the ENL, MB and healthy people groups.

Based on the results of the analysis carried out using the Anova test, it was concluded that there were differences in VDR levels between groups of patients with ENL, Leprosy (MB), and healthy (normal) people with a p value <0.05 **(Figure 1)**.

Based on **Table 4**, it is known that the Bacterial Index (BI) comparison in the ENL and MB Leprosy patient groups, obtained a mean value of 3.33±0.888 in the ENL group, while the mean BI value was 2.67±0.651 in the MB Leprosy group. Based on the results of the comparative analysis, it was found that the p value <0.05, which means that there is a significant difference in the BI value between ENL and MB Leprosy patients.

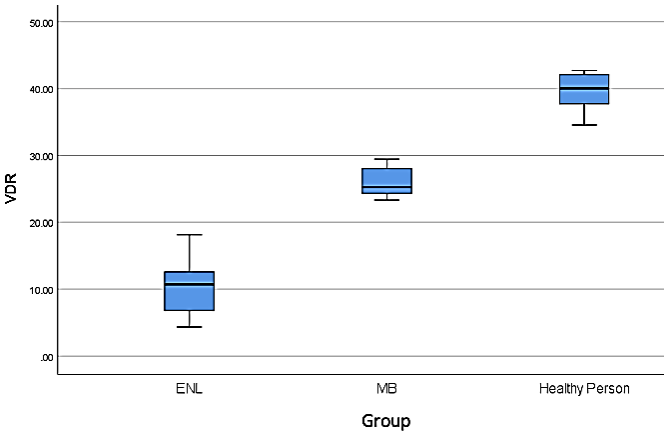

Figure 1 Comparison of VDR levels in the ENL, MB and healthy people groups.

Table 4 Comparison of Bacterial Index (BI) between ENL and MB

Variable	*Group (mean ± SD)*	*p value*
BI	ENL (3.33±0.888) MB (2.67±0.651)	0.048

Discussion

Vitamin D in leprosy is known to have a role as an immunomodulator through the anti-microbial pathway mediated by VDR.[12] Activation of the vitamin D intracrine system in leprosy is in accordance with the host cellular immune response. In tuberculoid leprosy, the vitamin D intracrine system is not disturbed so that the antibacterial pathway mediated by vitamin D in macrophages can function optimally as indicated by a low bacterial index (BI) value. This is the opposite for lepromatous leprosy with disturbed intracrine vitamin D systems, causing a decrease in the ability of macrophages to kill mycobacteria, characterized by a high BI.[13]

This study also explained that the majority of patients diagnosed with lesions were in the ENL group, furthermore the average BI value was 3.33±0.89 in the ENL group, while in the MB group the mean BI value was 2.67±0.65. In the literature study, it is explained that ENL is an inflammatory complication that occurs in 50% of leprosy patients and 5-10% of borderline lepromatous patients. Significantly, the morbidity and mortality in leprosy patients is caused by ENL.[13]

Vitamin D receptors are the result of enzyme metabolism of vitamin D which is found in various types of cells, including immune cells such as antigen-presenting cells (APC), T cells, B cells, and monocytes which are important targets in polymorphism studies so that they can modulate the immune response to pathogens.[14] Among the immunomodulatory roles, vitamin D is an inhibitor of MHC class II, CD40, CD80, and CD86 which causes Th1 responses and activation of regulatory T cells to be disrupted.[15] The core of VDR is an intracellular polypeptide that binds to the active metabolite of vitamin D, namely 1,25-Dihydroxyvitamin D3 and then interacts with chromatin, resulting in various genomic effects, such as pleiotropic regulation of human physiology, protection of the cardiac system, cancer prevention, and modulation of the immune system.[16] Research conducted by Mandal *et al*. found lower plasma 25-OHD levels in leprosy patients, namely 27.47±4.17ng/ml, while in healthy individuals it was 33±3.76ng/ml.[17]

Many previous studies have shown a deficiency of Vitamin D in mycobacterial disease.[17] Vitamin D deficiency was found to have a strong correlation with an increase in proinflammatory cytokines, such as TNF-α. As previously observed, there was a significant increase in TNF-α which acts as a modulator of inflammation in skin lesions of leprosy patients experiencing type II reactions. In addition, higher levels of TNF-α can cause direct damage to the nerve myelin sheath, stimulation of bone reabsorption and inhibition of bone collagen synthesis. The role of these cytokines provides a possible explanation for clinical symptoms such as nervous disorders, disability and deformities that often occur in leprosy patients.[13,17]

Conclusion

Based on the results of the research conducted, it can be concluded that there are differences in VDR levels between groups of ENL, MB, and healthy people. Where the lowest VDR level was found in the ENL group, then MB, and the highest was in the healthy group.

Declaration of patient consent The authors certify that they have obtained all appropriate patient consent.

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Authors' contribution

A, KD: Conception, design of the work, drafting the work, final approval of the version to be published.

SA, FI: Acquisition, analysis, critically review, final approval of the version to be published.

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