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Relationship between 25-Hydroxy Vitamin D Levels and Type of Morbus Hansen

Theresia L. Toruan[1], Mutia Devi[1], Theodorus[2], Allin Marlina Rivai[3*]

[1]Departement of Dermatology and Venereology, Faculty of Medicine, Universitas Sriwijaya/ Mohammad Hoesin General Hospital Palembang, Indonesia
[2]Medical Research Unit, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[3]Fellow Students, Department of Dermatology and Venereology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

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***Corresponding author:**
Allin Marlina Rivai

E-mail address:
allin.rivai@gmail.com

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ABSTRACT

Background: Morbus Hansen is a chronic granulomatous infection of Mycobacterium leprae (M. leprae) which is characterized by cardinal signs in the form of numbness of the skin, thickening of peripheral nerves and acid-fast bacteria (AFB) were found on bacterioscopic examination. WHO has classified MH into paucibasilar MH (MH-PB) and multibasilar MH (MH-MB) based on the number of skin lesions or bacterial index (IB). Vitamin D in MH is known to act as an immunomodulator. This study aims to determine the relationship between serum 25(OH)D levels with Morbus Hansen type at RSUP Dr. Mohammad Hoesin and Leprosy Hospital Dr.Rivai Abdullah Palembang. **Methods:** A laboratory observational study with a case series design was undertaken at the Dermatology and Venereology (DV) Polyclinic of Infectious Dermatology (DI) Mohammad Hoesin Hospital Palembang since December 2019 to January 2020. A sample of 33 patient MH met the inclusion criteria, consisting of 22 patients with Morbus Hansen (MH) type MB and 11 patients with Morbus Hansen (MH) type PB. Comparison of serum 25(OH)D levels between MB and PB type MH patients was analyzed using the Independent T Test, the relationship between serum 25(OH)D levels and MH type was analyzed using the Fisher Exact Test and the correlation between serum 25(OH)D levels with type of MH was analyzed using Spearman Rho's test. Data analysis using SPSS version 22.0. **Results:** In this study, there were no differences in demographic characteristics of gender, age, age category, body mass index, education and occupation between MB and PB type MH patients ($p > 0.05$). There were no difference in duration of therapy ($p = 0.155$), ENL reaction ($p = 0.276$) and patient status ($p = 0.304$) between MB and PB type MH patients, but there were differences in bacterial index ($p = 0.000$) and clinical spectrum ($p = 0.000$) between MB and PB type MH patients. There is a difference in the mean level of 25(OH)D between MB and PB type MH patients ($p = 0.006$), there is a significant relationship between 25 (OH)D levels and MH type patients, (OR = 9.643 ; $p = 0.010$) and there is a significant moderate positive correlation between levels of 25 (OH)D and the type of MH ($r = 0.467$; $p = 0.006$). **Conclusion:** It can be concluded that there are differences in serum 25(OH)D levels among Morbus Hansen type. In addition, there is a significant relationship between serum 25(OH)D levels with Morbus Hansen type.

1. Introduction

Morbus Hansen (MH) or leprosy is a chronic granulomatous infection caused by Mycobacteriun leprae (M. leprae) characterized by cardinal signs in the form of numbness of the skin, thickening of the peripheral nerves and acid-fast bacteria (AFB) were found on bacterioscopic examination.[1-3] This disease attacks the peripheral nerves, skin, eyes, mucosa of the upper respiratory tract, muscles, bones and testes.[1]

Based on data from the World Health Organization (WHO) in 2017, the number of MH cases in the world is around 210,617 cases.[4,5] The number of cases in Indonesia in 2017 was 15,910 cases.[6] According to statistical data from the Dermatology and Venereology (DV) polyclinic, Dr. Mohammad Hoesin Hospital Palembang during the last three years 2016-2018 the number of visits by MH patients was 1,157 cases.[7]

WHO in 1997 established a classification of MH to facilitate treatment, namely, paucibasilar MH (MH-PB) and multibasilar MH (MH-MB) based on the number of skin lesions or bacterial index (IB).[1,2] In the chronic phase of MH, there can be an increase in disease activity, namely reactions. The reaction can be a type I

reaction (reversal reaction/RR), or type II (erythema nodosum leprosum/ENL). The reversal reaction shows a shift to the Th1 pole of MH-PB and can result in irreversible nerve damage (neuritis). ENL reactions occur in MH-MB patients and show increased CMI and monocyte/macrophage responses.[9-11]

Vitamin D in MH is known to act as an immunomodulator.[12] In MH-T, the intracrine vitamin D system is not impaired, causing the vitamin D-mediated anti-bacterial pathway in macrophages to function optimally, characterized by low IB values. On the other hand, MH-L with impaired intracrine vitamin D system causes the ability of macrophages to kill mycobacteria to decrease, characterized by high IB.[13,14]

Research on vitamin D levels in MH patients has been done before, but the number is still limited. Based on this background description, a study was conducted to determine whether there was a relationship between serum 25(OH)D levels in MH-PB and MH-MB as well as the relationship with the bacterial index.

2. Methods

Laboratory observational study with a case series design has been carried out at the DV Polyclinic of the Infectious Dermatology Division (DI) Mohammad Hoesin Hospital Palembang. Laboratory examinations were carried out at Prodia Health Laboratories in Palembang and Jakarta since December 3, 2019 to January 2020.

The number of lesions is one or two to five are classified as MH-PB, while the number of lesions more than five is MH-MB.[1,5] If a positive IB is found on the slit-skin smear (SSS) examination, then it is classified as MH-MB regardless of the number of skin lesions.[3]

Comparison of serum 25(OH)D levels between MB and PB type MH patients was analyzed using the Independent T Test, the relationship between serum 25(OH)D levels and MH type was analyzed using the Fisher Exact Test and the correlation between serum 25(OH)D levels with type of MH was analyzed using Spearman Rho's test. Data analysis using SPSS version 22.0.

3. Results

In this study, there were no differences in demographic characteristics of gender (p = 0.163), age (p = 0.385), age category (p = 0.078), body mass index (p = 0.445), education (p = 0.093) and occupation (p = 0,568) between MB and PB type MH patients. (Table 1)

On clinical characteristics, there were no difference in duration of therapy (p = 0.155), ENL reaction (p = 0.276) and patient status (p = 0.304) between MB and PB type MH patients, but there were differences in bacterial index (p = 0.000) and clinical spectrum (p = 0.000) between MB and PB type MH patients (Table 2).

Table 3 shows the levels of 25(OH)D in the MH-PB group and the MH-MB group. The mean levels of 25(OH)D in the MH-PB group were 19.15 ± 3.25. The mean level of 25(OH)D in the MH-MB group was 14.85 ± 4.26. Through statistical analysis, it was stated that there was a difference in the mean 25(OH)D levels between the MH-PB group and the MH-MB group (p = 0.006). The 25(OH)D level of MH-MB patients was significantly lower than the 25(OH)D level of MH-PB patients and none of the MH-PB patients had vitamin D deficiency.

In table 4, it can be seen that the study using receiver operating characteristic (ROC) curve analysis for deficiency and insufficiency found the cut-off point for 25(OH) D levels of 17.55. With the Fisher Exact test, it was reported that there was a significant relationship between levels of 25(OH) D and the type of patient with MH. Levels of 25 (OH) D 17.55 had a 9.6 times risk of suffering from MH-MB compared to patients with levels of 25 (OH) D > 17.55 (OR = 9.643 ; p = 0.010).

Table 1. General characteristics of research subjects (n = 33)

Variable	MH-PB (n = 11)	MH-MB (n= 22)	*P-value*
Sex, n (%)			
• Male	4 (36.4)	14 (63.6)	0.163[b]
• Female	7 (63.6)	8 (36.4)	
Age, n (%)			
• 17- 25 years old	0 (0)	7 (31.8)	0.078[b]
• 26-35 years old	4 (36.4)	2 (9.1)	
• 36-45 years old	2 (18.2)	6 (27.3)	
• 46-55 years old	3 (27.3)	3 (13.6)	
• 56-65 years old	1 (9.1)	4 (18.2)	
• > 65 years old	1 (9.1)	0 (0)	
Age (years), mean ± SD	43.00 ± 14.46	38.23 ± 14.75	0.385[c]
Education, n (%)			
• Elementary School	2 (18.2)	1 (4.5)	
• Junior High School	2 (18.2)	7 (31.8)	0.093[b]
• Senior High School	5 (45.4)	14 (63.6)	
• Bachelor	2 (18.2)	0 (0)	
Occupation, n (%)			
• Low Risk	2 (18.2)	2 (9.1)	0.586[a]
• High Risk	9 (81.8)	20 (90.9)	
BMI (kg/m²), rerata ± SD	22.37 ± 3.24	21.53 ± 3.19	0.445[d]
BMI, n (%)			
• *Underweight*	1 (9.1)	3 (13.6)	
• *Normoweight*	6 (54.5)	16 (72.7)	0.321[b]
• Obese	4 (36.4)	3 (13.6)	

[a]*Fisher Exact Test, p* = 0.05,[b]*Pearson Chi Square, p* = 0.05, [c]*Independent T Test, p* = 0.05 & [d]*Mann Whitney Test,*

Analysis with Spearman Rho's test, it was found that there was a moderately significant positive correlation between levels of 25(OH) D and the type of MH (r = 0.467; p = 0.006). The lower the 25(OH) D level, the more likely the patient has the MB type, which can be seen in table 5 and graph 1.

With statistical tests, it was found that there was a weak and insignificant negative correlation between levels of 25(OH)D and age (r = 0.282; p = 0.112). Very weak positive correlation was not significant between levels of 25(OH)D with BMI (r = 0.172 ; p = 0.340). Very weak negative correlation was not significant between levels of 25(OH)D and duration of therapy (r = 0.172 ; p = 0.340) and weak negative correlation was not significant between levels of 25 (OH) D and IB (r = -0.235 ; p = 0.189) can be seen in table 6.

Table 2. Clinical characteristics of study subjects (n = 33)

Variable	MH-PB (n = 11)	MH-MB (n= 22)	*P-value*
Duration of therapy (years), mean± SD	6.73 ± 6.66	9.95 ± 7.29	0.155[a]
Therapy, n (%)			
• MDT-MB	0 (0.0)	19 (86.4)	
• MDT-PB	9 (81.8)	0 (0)	-
• ROM	2 (18.2)	3 (13.6)	
Clinical spectrum			
• MH - BL	0 (0)	20 (90.9)	
• MH - BT	9 (81.8)	0 (0)	
• MH - LL	0 (0)	2 (9.1)	0.000[b]
• MH - TT	2 (18.2)	0 (0)	
Bacterial Index			
• Negative	11 (100.0)	0 (0.0)	
• Positive 1	0 (0)	3 (13.6)	
• Positive 2	0 (0)	3 (13.6)	0.000[b]
• Positive 3	0 (0)	11 (50.0)	
• Positive 4	0 (0)	3 (13.6)	
• Positive 5	0 (0)	2 (9.1)	
ENL Reaction, n (%)			
• Yes	0 (0)	4 (18.2)	0.276[c]
• No	11 (100.0)	18 (81.8)	
Patient Status, n (%)			
• Old Patient	8 (72.7)	20 (90.9)	0.304[c]
• New Patient	3 (27.3)	2 (9.1)	

[a]*Mann Whitney Test, p* = 0.05, [b]*Pearson Chi Square, p* = 0.05 & [c]*Fisher Exact Test, p* = 0.05

Table 3. Comparison of 25 (OH)D levels in the MH-PB and the MH-MB group

Parameter	MH-PB (n = 11)	MH-MB (n= 22)	*P-value*
25(OH)D Levels, mean ± SD	19.15 ± 3.25	14.85 ± 4.26	0.006[a]
Classification			
• Deficiency	0 (0)	2 (9.1)	0.542[b]
• Insuficiency	11 (100)	20 (90.9)	

[a]*Independent T Test*, p = 0.05, [b]*Fisher Exact Test*, p = 0.05

Table 4. The relationship between 25 (OH) D levels in the MH-PB and the MH-MB group

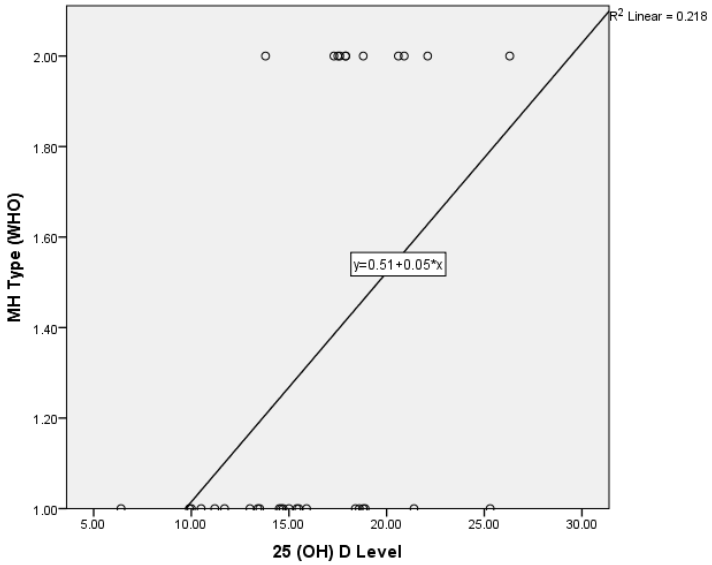
Variable	MH-PB (n = 11)	MH-MB (n= 22)	OR (CI 95%)	*P-value*
25(OH) D Levels				
• ≤ 17.55	2 (18.2)	15 (68.2)	9.643	0.010
• > 17.55	9 (81.8)	7 (31.8)	(1.633 – 56.925)	

Fisher Exact Test, p = 0.05

Table 5. The relationship between 25 (OH) D levels and type of MH

Dependent Variable	Independent Variable		
		r	*P-value*
25 (OH) D Levels	Type • PB • MB	0.467	0.006

Spearman Rho's, p = 0.05



Graph 1. The relationship between 25 (OH) D levels and type of MH

Table 6. Relationship of 25(OH)D levels with general and clinical characteristics of MH patients

Dependent Variable	Independent Variable		
		R	*P-value*
25 (OH) D Levels	Age	0.282	0.112[a]
	BMI	0.172	0.340[b]
	Duration of Therapy	-0.025	0.892[b]
	Bacterial Index	-0.235	0.189[b]

[a] *Pearson correlation,* p = 0.05, [b]*Spearman's rho*, p = 0.05

In this study there were no difference in 25(OH) D levels between male and female patients (p = 0.411). There was no difference between age (p = 0.073), education (p = 0.452), occupation (p = 0.202), BMI (p = 0.259). However, there was a difference in 25(OH) D levels between patients with and without ENL reactions (p = 0.003), 25(OH) D levels in patients with ENL reactions were lower than patients without ENL reactions, which can be seen in table 7.

Table 7. Relationship of 25(OH)D levels with general and clinical characteristics of MH patients

Variable	**25 (OH) D Serum Levels** Mean ± SD	*P-value*
Sex, n (%)		
• Male	15.69 ± 4.56	0.411[a]
• Female	16.99 ± 4.28	
Age, n (%)		
• 17- 25 years old	16.47 ± 3.34	0.073[b]
• 26-35 years old	19.68 ± 4.04	
• 36-45 years old	15.85 ± 5.24	
• 46-55 years old	16.93 ± 3.19	
• 56-65 years old	11.58 ± 3.39	
• > 65 years old	17.60 ± 0.00	
Education, n (%)		
• Elementary School	17.63 ± 3.11	
• Junior High School	14.82 ± 6.13	0.452[b]
• Senior High School	16.36 ± 3.09	
• Bachelor	20.05 ± 8.84	
Occupation, n (%)		
• Low Risk	18.95 ± 5.71	0.202[c]
• High Risk	15.91 ± 4.19	
BMI, n (%)		
• Underweight	16.78 ± 1.58	
• Normoweight	15.45 ± 4.99	0.259[b]
• Obese	18.60 ± 2.56	
ENL Reaction, n (%)		
• Yes	10.43 ± 3.72	0.003[c]
• No	17.09 ± 3.89	

[a] *Independent T Test, p* = 0.05, [b]*One Way ANOVA, p* = 0.05 & [c]*Mann Whitney Test, p*

4. Discussion

In this study, statistical results proved that there were no differences in gender, age, BMI, occupation and education between the MH-PB and the MH-MB group, also analyzed the duration of therapy, ENL reactions and new or old patients. This means that vitamin D levels in both groups are not influenced by characteristic factors so that the two groups deserve to be compared.

However, there were a significant difference in IB between the MH-PB and the MH-MB group (p = 0.000). The initial mechanism for the invasion of M.leprae occurs through innate immune activity. Pathogenic bacteria (M. leprae) are phagocytized by monocyte cells. After phagocytosis, intracellular replication occurs. This replication threatens the host cell. However, the cells have pathogen sensors such as TLR2/1, which

increases the expression of the CYP27B1 enzyme. The CYP27B1 enzyme induces a vitamin D component causing the conversion of 25(OH)D to 1.25(OH) 2D. 1.25(OH) 2D binding occurs with the VDR and the expression of catelicidin and -defensin 2. Both function as AMP. Vitamin D induces catelicidin, an antibacterial protein. This induction occurs due to the enzyme CYP27B1 which activates vitamin D. The bioavailability of 25(OH) D for this enzyme depends on the serum level of 25(OH) D. In MH-PB many bacteria died or phagocytosis, whereas in MH-MB only a few bacteria died or phagolysosomes.[15,16]

This study proved that there was a significant difference in the mean 25(OH) D levels between the MH-PB group and the MH-MB group (p = 0.006), there was a significant relationship between the levels of 25 (OH) D and the MH type, (OR = 9.643; p = 0.010) and there was a moderately significant positive correlation between levels of 25 (OH) D and type MH (r = 0.467; p = 0.006). The results of this study are in line with the case-control study conducted by Rusyati et al.[17] It is proven that the levels of Vitamin D in MH-MB patients are lower than the levels of Vitamin D in MH-PB patients with a mean difference of 4.2784 (2.82-5.73). It is proven that there is a significant relationship between Vitamin D levels and the type of MH patient with an increased risk of 12,667 times for the MH-MB type.

The serum 25(OH) D level in the blood is the best method for determining vitamin D status because of its ease of measurement and its long circulating half-life (range 2 or 3 weeks). Although 1,25(OH)2D is the biologically active form of vitamin D, this test does not provide information on vitamin D status because of its short half-life of 15 hours and is often normal or even elevated in conditions of vitamin D deficiency. In this study refers to the scale of the manufacturer of the CLIA analyzer that has been approved by the FDA, divided into sufficiency, insufficiency, deficiency, and toxicity. So in this study, the insufficiency value was 31 patients and deficiency was 2 patients.

The design of this study was a case series with 33 study participants. The case series design was chosen because of the low prevalence of MH (< 10%). More population studies and prospective cohort studies based on predetermined cutoff points are needed.

5. Conclussion

In this study, it was proven that the levels of Vitamin D in MH-MB patients are lower than MH-PB patients and there is a significant relationship between serum 25(OH)D levels with Morbus Hansen type.

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