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2. Negative correlation between interleukin-2 (IL-2) serum with bacterial index in leprosy



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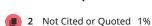
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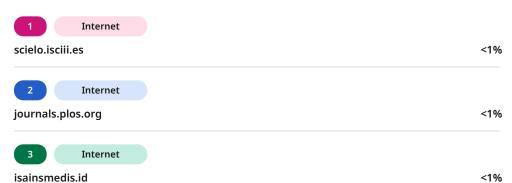
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Negative correlation between interleukin-2 (IL-2) serum with bacterial index in leprosy



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ABSTRACT

Background: Leprosy is strongly influenced by the host immunity response. One of the cytokines that play a role in cellular immunity is IL-2. IL-2 will stimulate macrophages to activate phagocytosis and form granulomas, and this occurs in the leprosy tuberculoid type. Aim of this study was to verify the IL-2 serum levels are negatively correlated or not with bacterial index in leprosy.

Methods: This study was a cross-sectional observational analytic study involving 50 leprosy subjects (41 MB and 9 PB types). Study was conducted at Dermatolovenerology Polyclinic of Sanglah General Hospital from December 2019 to March 2020. Ridley's scale was used to determine bacterial index (BI). IL-2 serum levels were measured by ELISA.

Result: The mean of IL-2 serum in PB was 75.53±10.32 pg/mL

and in MB was 37.59±17.13 pg/mL. Strong negative correlation (r= -0.67; p<0.001) between serum IL-2 levels with bacterial index (CI 95% = 25.96 - 49.93; p<0.001) was found. Every 1 pg/mL increase in IL-2 serum levels will be accompanied by a decrease in the bacterial index of 0.039 and vice versa. Determinant coefficient shows that 42.2% of the BI is affected by the IL-2 serum levels. Low IL-2 serum levels were the risk factor of high BI 2.8 times (PR= 2.8; CI 95% = 1.444 - 5.519; p<0.001).

Conclusion: IL-2 serum levels were found to be higher in PB than MB. There was a negative correlation between IL-2 serum levels with bacterial index. Low IL-2 serum levels were the risk factor of a high bacterial index in leprosy patients.

Keywords: Leprosy, IL-2 serum, bacterial index

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INTRODUCTION

Leprosy is an infectious disease that still receives special attention from the World Health Organization (WHO), especially in developing countries, including Indonesia. Transmission of leprosy is still a significant health problem in the community. Patients who have not received MDT with a high number of bacteria have a high risk of transmitting to others and at risk for leprosy reaction, leading to disability.

Based on WHO data in 2017, the incidence of leprosy throughout the world as many as 210.617 cases. The three countries with the highest burdens India, Brazil, and Indonesia, accounted for 80.2% of the new caseload globally in 2017.1

People with leprosy show various clinical types. The immunological response mounted by the host dictates the clinical phenotype that develops. Experimentally, the polar forms of the disease are said to conform to an immunological paradigm.2 Tuberculoid leprosy (TT) is characterized by minimal nerve damage, low bacterial index, and high cellular immunity, where predominantly Th-1 secrete IFN-y and IL-2 cytokines, which will

form tuberculoid granulomas and associated with destructive M. leprae. In contrast to lepromatous leprosy (LL), which is characterized by the appearance of lesions in the skin, high bacterial index, and nerve damage, where an increase in the production of Th-2 cytokines (IL-4, IL-5, and IL-10).3,4 This study aimed to confirm the correlation between IL-2 and BI in leprosy.

MATERIALS AND METHODS

This study was analytical cross-sectional research conducted in Dematovenereology Polyclinic (Sanglah General Hospital, Bali) from December 2019 until March 2020. Subjects were classified into MB and PB types, which are taken using consecutive sampling. All samples which fulfill the inclusion and exclusion criteria will be performed anamnesis, physical examination, and bacteriological examination. The inclusion criteria were all leprosy patients on multidrug therapy, Indonesian citizenship, aged 18 until 65 years old. Leprosy patients who have finished multidrug therapy (MDT) or release from treatment (RFT), suffering leprosy reactions, systemic disease, Submission ID trn:oid:::3618:114866346

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pregnancy, breastfeeding, receiving corticosteroid, non-steroidal anti-inflammatory treatment, in last month were excluded. Blood samples were taken and performed with ELISA to detect IL-2 cytokine. The bacterial index evaluated using Ridley's scale. Statistical tests were using SPSS version 25.0.

RESULTS

Fifty subjects that fulfilled the criteria consist of 41 subject MB and nine subjects PB were involved in this study. The characteristics of the subjects are presented in Table 1.

Comparison of IL-2 serum levels by type of leprosy presented in Table 2. There was a difference in IL-2 serum levels between the 2 types of leprosy (95% CI: 29.96-49.93; p<0.001). PB type of leprosy group obtained IL-2 serum levels higher (75.53 ± 10.32 pg/mL) compared to the MB type of leprosy group (37.59 \pm 17.13 pg/mL). The analysis was performed using an unpaired T-test.

Table 1. Subjects of research characteristics

Characteristics	MB	РВ	
Characteristics	n=41 (%)	n=9 (%)	
Age (years)			
18-25	9 (21.95)	4 (44.44)	
26-35	7 (17.07)	2 (22.22)	
36-45	12 (29.27)	1 (11.11)	
46-55	3 (7.32)	1 (11.11)	
56-65	10 (24.39)	1 (11.11)	
Sex			
Male	30 (73.17)	3 (33.33)	
Female	11 (26.83)	6 (66.67)	
Treatment history			
Haven't received treatment	9 (21.95)	5 (55.56)	
MDT 1-3	13 (31.71)	1 (11.11)	
MDT 4-6	9 (21.95)	N/A	
MDT 7-9	8 (1951)	2 (22.22)	
MDT 10-12	2 (4.88)	1 (11.11)	
Type of leprosy			
TT	N/A	N/A	
BT	N/A	9	
BB	5 (12.20)	N/A	
BL	28 (68.29)	N/A	
LL	8 (19.51)	N/A	
Bacterial index			
0	N/A	9	
+1	16 (39.02)	N/A	
+2	12 (29.27)	N/A	
+3	5 (12.02)	N/A	
+4	8 (19.51)	N/A	
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Analysis using One-way ANOVA to evaluate the comparison of IL-2 serum levels by bacterial index presented in Table 3. There were differences in IL-2 serum levels between each bacterial index that were significantly different (p<0.001).

The result of the correlation between the bacterial index and IL-2 was -0.67, with a p<0.001. Linear regression analysis was performed to know the effect of IL-2 serum on the bacterial index and obtained beta coefficient -0.039, coefficient of determination 0.422 and p-value < 0.001.

Table 4 shows that there is a relationship between IL-2 serum and bacterial index, with a prevalence ratio (PR) 2.8. These results indicate that low IL-2 serum levels (<44.42 pg/mL) increased the risk factor for a higher bacterial index 2.8 times (RP=2.8; 95% CI=1.444-5.519) compared with high IL-2 serum levels.

DISCUSSION

This study showed that there were differences in the mean IL-2 serum levels between both types of leprosy were significantly different (p<0.001). The MB type of leprosy group had a lower mean IL-2 serum level (37.59 \pm 17.13 pg/mL) compared to PB $(75.53 \pm 10.32 \text{ pg/mL})$. TT patient (PB) immune response is characterized by a Th-1 cytokine response (IL-2, IFN-γ, TNF-α), vigorous T-cell response to M. leprae bacteria and containment of the bacilli in well form granulomas. In contrast, the LL patients' immune response is characterized by a Th-2 immune profile with the production of IL-4 and IL-10 and activation of T regulatory cells that fail to inhibit M. leprae growth. In LL patient, relatively deficient in CD4+ T cells, but rather have numerous CD8+ T cells and macrophages heavily infected with bacilli that develop a characteristic foamy appearance.5

T-cell response to mycobacteria will activate and cause the proliferation of Th-1 cells and releasing IL-2. IL-2 will stimulate Th-2 and NK cells expansion in the lesions, so it produces an increase of IFN-y production. IFN-y will activate macrophages, thus secreting IL-2. The existence of IL-2 can stimulate SIS against intracellular pathogens in tuberculoid leprosy. The opposite occurs in lepromatous leprosy.4

In this study, mean IL-2 serum levels were found to be significant differences between each bacterial index (p<0.001). The lowest mean IL-2 serum level was found in the group of subjects with a bacterial index of +4 (25.80 \pm 18.08 pg/mL), followed by a bacterial index of +2 (34.29 \pm 17.00 pg/mL), followed by bacterial index +3 (37.71 \pm 12.34 pg/ mL), followed by bacterial index +1 (45.92 \pm 14.83 pg/mL), and the highest was obtained at the bacterial

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Table 2. Comparison of mean IL-2 serum levels based on the type of leprosy

Type of leprosy	Mean IL-2 serum ± SD (pg/mL)	p-value	
MB	37.59 ± 17.13	0.001*	
PB	75.53 ± 10.32	0.001	

^{*}Significant result if p<0.05

Table 3. Comparison of mean IL-2 serum levels based on the bacterial index

Bacterial index	Mean IL-2 serum ± SD (pg/mL)	p-value
0	75.53 ± 10.32	<0.001*
+1	45.92 ± 14.83	
+2	34.29 ± 17.00	
+3	37.71 ± 12.34	
+4	25.80 ± 18.08	

^{*}Significant result if p<0.05

Table 4. Prevalence risk between IL-2 serum levels and bacterial index

IL-2 -	Gre	Group		050/ 61	
	High BI	Low BI	PR	95% CI	p-value
Low <44.42 pg/mL	18 (76%)	6 (24%)	2.8	1.444-5.519	<0.001*
High ≥44.42 pg/mL	7 (26.9%)	19 (73.1%)			

^{*}Significant result if p<0.05

index 0 (75.53 \pm 10.32 pg/mL). The role of IL-2 in the tuberculoid type, which is produced by Th-1, results in a good immune system against *M. leprae*. This correlated with the ability of macrophages to prevent the growth of mycobacterium.⁵

Statistical analysis with the Spearman test gave a strong negative correlation (r=-0.678) between IL-2 serum levels and bacterial index. These results indicate the lower IL-2 serum levels, the higher bacterial index, and vice versa. In tuberculoid present well-formed epithelioid granulomas consisting of T lymphocytes and differentiated macrophages. Bacilli are rarely found. Lepromatous leprosy consist of macrophages containing large of bacteria.⁶

Linear regression analysis was performed to examine the effect of IL-2 serum levels on the bacterial index. The results obtained beta coefficient (β) -0.039, which indicates a significant influence of IL-2 serum levels on the bacterial index. Each decrease 1 pg/mL of IL-2 serum level will be

followed by an increase in the bacterial index 0.039 (p<0.001). The coefficient of determination (R^2) was 0.422 (42.2%), which showed that 42.2% of bacterial index was influenced by IL-2 serum levels and the remaining 57.8% was influenced by other factors.

Besides IL-2, there are several cytokines that also affect leprosy. Sutedja et al., found a positive correlation between IL-10 and bacterial index.7 While Moubasher et al., found a negative correlation between IFN-γ dan TNF-α and bacterial index.8 Moreover, the course of leprae infection is dependent on individual factors that influence the host immunologic response. In its turn, the immune response can be influenced by genetic and environmental factors, including nutritional status.9 The body's immune system, however, needs proteins, vitamins, and minerals to effectively fight off infections. 10 The immune response of leprosy patients is affected by several micronutrients, including retinol (vitamin A), α-tocopherol (vitamin E), ascorbic acid (vitamin C), vitamin D, zinc, selenium, and magnesium.11-13

CONCLUSION

In the PB type of leprosy, the mean IL-2 serum level was higher than the MB type of leprosy. There was a negative correlation of IL-2 serum level with bacterial index in a leprosy patient. Low IL-2 serum levels are risk factors for increasing bacterial index in leprosy.

ETHICAL CLEARANCE

This research was ethically approved by the Research Ethical Commission of Medical Faculty of Universitas Udayana/ Sanglah General Hospital (ethical code: 2494/UN14.2.2.VII.14/LP/2019).

CONFLICT OF INTEREST

There is no conflict of interest declared in this research.

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AUTHORS CONTRIBUTION

All authors contributed to this research.

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