

## Comparison of Serum Interleukin 10 Levels between Leprosy and Non-Leprosy Population

*Perbandingan Kadar Interleukin 10 pada Penderita Kusta bukan Penderita Kusta*

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**Abstract:** Leprosy is a chronic granulomatous infectious disease that attacks the peripheral nerves, skin, and other organs. The cytokine IL-10 can deactivate macrophages, inhibit IL-12 production, which also inhibits IFN- $\gamma$  production. IL-10 directly inhibits CD4+T cells and antigen-presenting cell (APC) function in cells infected with *M. leprae*. The purpose of this study was to determine the differences in IL-10 levels in leprosy patients and non-patients at dr. Mohammad Hoesin Palembang Hospital. This study used a case-control design for comparing 2 groups. Respondents in this study were 80 people. The case samples were all leprosy patients who were treated at the dr. Mohammad Hoesin Hospital, Palembang for the period of January 16 - February 16, 2019, while the control group was medical personnel and paramedics who worked at the same hospital. The examination was carried out using the ELISA Sandwich method. The results showed that there was a significant difference in IL-10 levels between leprosy patients and non-leprosy leprosy ( $p=0.000$ ). The average IL-10 level in leprosy patients (13.24 pg/ml) was lower than that in non-patients (40.15 pg/ml). It is concluded that there is a difference in IL-10 levels between leprosy patients and non-leprosy patients in leprosy sufferers are lower than non-leprosy persons.

**Keywords:** leprosy; interleukin 10 (IL-10)

**Abstrak:** Kusta merupakan penyakit infeksi granulomatosa kronis yang menyerang saraf tepi, kulit dan organ lain. Sitokin IL-10 memiliki kemampuan menonaktifkan makrofag, menghambat produksi IL-12 dan IFN- $\gamma$ . IL-10 berfungsi menghambat CD4 + sel T, fungsi antigen presenting cells (APC) dalam sel yang terinfeksi *M. leprae*. Tujuan penelitian mengetahui perbedaan kadar IL-10 penderita kusta dan bukan penderita di RSUP dr. Mohammad Hoesin Palembang. Penelitian ini menggunakan desain case control untuk membandingkan 2 kelompok. Responden penelitian sebanyak 80 orang. Sampel adalah semua penderita kusta yang berobat di RSUP dr Mohammad Hoesin Palembang periode 16 Januari-16 Febuari 2019, sedangkan kelompok kontrol adalah tenaga medis dan paramedis di RSUP dr. Mohammad Hoesin Palembang. Pemeriksaan menggunakan metode ELISA Sandwich. Hasil menunjukkan ada perbedaan signifikan kadar IL-10 penderita kusta dan bukan penderita kusta ( $p=0.000$ ). Rata-rata kadar IL-10 pada penderita kusta (13.24 pg/ml) lebih rendah dibandingkan dengan bukan penderita (40,15 pg/ml). Disimpulkan terdapat perbedaan kadar IL-10 antara penderita kusta dengan penderita bukan penderita kusta, yaitu pada penderita kusta lebih rendah dibandingkan bukan penderita kusta.

**Kata Kunci:** kusta; interleukin, interleukin 10

## INTRODUCTION

Morbus Hansen (Leprosy) is a chronic infection that attacks the peripheral nerves, skin and other organs.<sup>1</sup> This disease, if not treated promptly, can also trigger psychosocial disorders due to negative stigma developed in the community. The causative agent of leprosy spread through droplets.<sup>2</sup>

The etiology of leprosy, the *Mycobacterium leprae*, is an acid-resistant, gram-positive, rod-shaped, obligate-aerobic bacteria which cannot be cultured on artificial intra cellular media. This microorganism was first discovered by Gerhard Armauer Hansen in 1873.<sup>3</sup>

According to WHO data (2016), leprosy cases have increased from 211.973 in 2015 to 214.783 in 2016. As many as 94% of leprosy cases reported in only 14 countries with >1000 new cases annually. Southeast Asia is the region with the third highest leprosy burden in the world in 2015 with a total of 156.118 cases. In Indonesia, leprosy cases were reported to be 17.025 and 17.202 in 2014 and 2015, respectively.

Interleukin 10 (IL-10) is a cytokine that has an opposite role to IL-17.<sup>4</sup> These cytokines have the ability to deactivate macrophages, inhibit IL-12 as well as IFN- $\gamma$  production. In addition, IL-10 directly inhibits CD4<sup>+</sup> T cells and antigen presenting cells (APC) function in the *M. leprae*-infected cells.<sup>5</sup> In lepromatous leprosy, TH2 Treg lymphocytes participate in the anti-inflammatory response and produce a number of cytokines, including IL 10, which inhibit macrophage activation and facilitate the survival of *M. leprae*.

According to Ministry of Health data in 2012, categorization of leprosy types is divided based on the Ridley Jopling and WHO classification system.<sup>6</sup> Ridley and Jopling divided leprosy into several types, namely tuberculoid type (TT), borderline tuberculoid type (BT), mid-borderline type (BB), borderline lepromatous type (BL), and lepromatous type (LL).<sup>7</sup> Meanwhile, the WHO classification system simplified this categorization into paucibacillary (PB) and multibacillary (MB) type.<sup>8</sup> The statement also provided similar statement leprosy is classified according to WHO and Ridley-Jopling classification system.<sup>1</sup> A study conducted at Kriplani Sucheta Hospital, showed that IL-10 levels among leprosy patients were higher (59.48 pg/ml) compared to the control group (15.90 pg/ml).<sup>9</sup> A similar statement was also expressed, where IL-10 levels in leprosy patients were significantly higher (median of 10 pg/ml) compared to healthy controls (median of 2.5 pg/ml) with ( $p < 0.001$ ).<sup>10</sup> A study entitled "Correlation of Interlukin-10 Levels with Bacterial Index in Leprosy Patients who Have Received Multidrug Therapy (MDT) Treatment performed demonstrated that IL-10 levels were significantly higher among patients diagnosed with MB leprosy (median of 17.5 pg/ml) compared with PB leprosy (median of 13.3 pg/ml).<sup>11</sup>

The purpose of this study was to investigate the difference in serum IL-10 levels between leprosy and healthy population. Individuals who met the inclusion criteria for both case and control groups were recruited consecutively.

## MATERIAL AND METHODS

This observational analytical study was performed using a case-control design which compared the cases group with its matched control group. Blood samples were collected from all participants for laboratory assessment. This study included leprosy patients who visited Dermatovenereology Clinic of dr. Mohammad Hoesin Hospital Palembang and healthy medical personnel and families as the case and the control group, respectively. All the participants who met the inclusion criteria were recruited in a consecutive manner. The study was carried out for two months between January-February 2019. The minimum sample size was 80 subjects.

Mann Whitney U test was performed as bivariate analysis to determine the difference between dependent and independent variables with the measurement scale being the ordinal and nominal difference test.

This study was conducted at a government hospital, after obtaining a research permit from the Department of Research and Development of related institutions from January 16 to February 16, 2019. The minimum 80 samples were achieved by the end of the study period.

All patients who met the inclusion criteria underwent comprehensive clinical interview and provided their written informed consent that has previously been approved by Ethics Committee of Sriwijaya University with reference no. 298/ kepkrsmhfkunsri/2018 prior participation in this study. All participants who met the eligibility criteria underwent blood sample collection procedure for further IL-10 levels examination.

The serum was derived from blood taken from the cubital vein using a disposable 3-cc syringe. The blood sample was then put in a red-capped vacutainer tube and was left at room temperature for  $\pm$  10 minutes. Then the blood sample was transferred to the Biomolecular Laboratory of Sriwijaya University. Immediately following clot formation, the blood sample was centrifuged at 2000 rpm for 5 minutes. Then the plasma was transferred to an Eppendorf tube (RNA Nuclease Free). The plasma was stored at -200C, until the required number of samples was obtained, IL-10 examination was carried out using the ELISA Sandwich method at the Biomolecular Laboratory of Sriwijaya University.

## RESULTS

### Bivariate Analysis

A bivariate analysis was performed to determine the difference in serum IL-10 levels between the case and the control group. For this regard, independent student T test or Mann Whitney U test was performed accordingly.

### Characteristics of the Study Population

The data showed that the mean age of leprosy and healthy subjects was 35 and 36.5 years, respectively. Among leprosy patients, the proportion of males (n=27) was slightly higher than women (n=24). In details of the characteristic of respondents can be seen in Table 1.

Table 1. Characteristics of the Study Population

Characteristics	Leprosy Group (40)	Control Group (40)	<i>P</i> value
<b>Age</b>			
Mean (years)	35	36.5	0.584 *
<b>Age group</b>			
18-40	24	26	0.890 **
40-60	14	12	
> 60	2	2	
<b>Sex</b>			
Male	27	16	0.014 **
Female	13	24	
<b>Ethnicity</b>			
Malay	32	19	0.029 ***
Bataknese	1	2	
Javanese	6	15	
Bugis	0	2	
Chinese	1	2	
<b>Education</b>			
Uneducated	0	1	0.001 ***
Primary school	12	6	
Middle school	3	3	
High school	21	8	

College	1	2	
Undergraduate school	3	18	
Post-graduate school	0	2	
<b>Occupation</b>			
Unemployed	4	0	
Teacher	1	0	
Employee	16	2	
Housewife	11	9	
Farmer	1	5	0.001 ***
Driver	2	0	
Laborer	5	2	
Physician	0	18	
Paramedic	0	4	
<b>Duration of illness</b>			
0 - 5 years	35		
6- 10 years	4		
> 10 years	1		
<b>Duration of MDT course</b>			
No treatment	2		
PB 0 - 6 months	3		
PB>6 months	0		
MB 0 - 12 months	19		
MB> 12 months	9		
ROM	7		

\* = Independent T Test

\*\* = Pearson Chi Square Test

\*\*\* = Kolmogrov Smirnov Test

Table 2. Comparison in Serum IL-10 Levels between Leprosy and Control Group

Study Group	Median	Min	Max	P-value
Leprosy	13.24	5.59	19.41	0.000 *
Non-leprosy	40.15	29.41	44.71	

\* = Mann Whitney U test

Table 2 shows that to determine the difference in IL-10 levels between leprosy patients and healthy controls, the Mann Whitney test was performed. Table 2 shows that p-value of 0.000 indicating a significant difference in IL-10 levels between leprosy and control group.

The difference in IL-10 levels in leprosy and control groups also demonstrated on the Figure 10. That serum IL-10 levels were lower in leprosy patients (13.24 pg/ml) compared non leprosy (40.15 pg/ml).

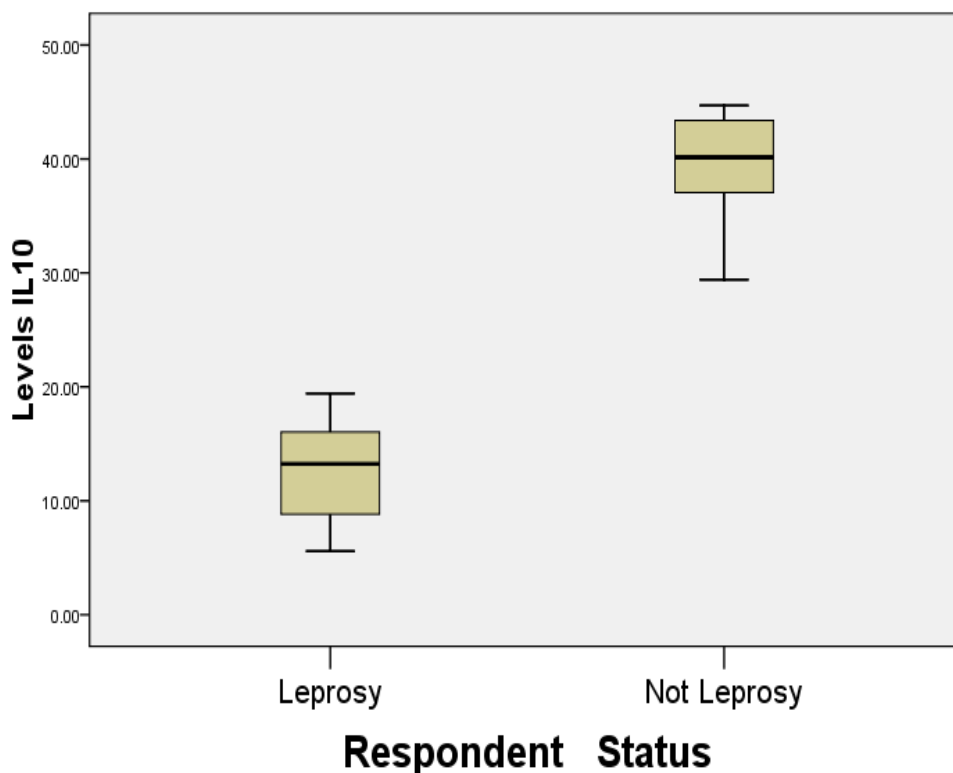


Figure 10. Serum IL-10 levels among leprosy and healthy subject

## DISCUSSION

The results of this case-control study showed that serum IL-10 levels were lower in the case group (13.24 pg/ml) compared to the control group (40.15 pg/ml). Administration of multi drug therapy (MDT) could result in suppression of bacterial index as well as reduced stimulation of specific immune response resulting in reduction of cytokines (including IL-10) production among people with leprosy. This theory is supported by the evidence demonstrating that patients with higher bacterial index show higher IL-10 levels.

The research in 1998 also provided supporting evidence showing a positive correlation between IL-10 and the bacterial index.<sup>12</sup> The study at 2011 showed that, among patients receiving MDT, *M. leprae* will be eradicated within several months in certain types of leprosy.<sup>13</sup>

This study showed contradictive results from the previous studies showing that IL-10 levels were higher among leprosy patients. The research by Madan *et al* in 2011<sup>9</sup> who previously conducted similar study at Kriplani Sucheta Hospital showed that IL-10 levels were found to be higher (59.48 pg/ml) among leprosy patients compared to healthy controls (15.90 pg/ml).

Same with research conducted by Zenha and his collage which states that there is a decrease in bacterial index in leprosy patients who have received MDT therapy for 12-24 months.<sup>14</sup> The study conducted by Negera (2018)<sup>15</sup> stated that the use of prednisolone in people with leprosy who experienced a reaction could reduce the antibody to *M. leprae*. In this case, prednisolone treatment after 1 month decreased productivity from IL-10.

## CONCLUSION

It was concluded that IL-10 in leprosy patients was significantly lower than IL-10 in non-leprosy patients.

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