

Original research article

## The Relationship between Serum IgM Anti Phenolic Glycolipid-1 Levels and Bacterial Index in Multibacillary Leprosy Patients

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### ABSTRACT

**Background:** Leprosy remains a public health concern in various countries, with approximately 250,000 new cases reported annually worldwide. Slit-Skin Smear results show the distribution of *M. leprae* in the skin and determine the degree of infectiousness and severity of multibacillary leprosy. PGL-1 antigens stimulate IgM antibody formation. Anti-PGL-I IgM indicates that the patient has an acute immune response or is actively suffering from leprosy. This study aims to determine the relationship between serum levels of anti-PGL-1 IgM and bacterial index (BI) values in multibacillary leprosy.

**Methods:** This observational study employed a cross-sectional design. The research was conducted at Dr. Rivai Abdullah Leprosy Hospital and Sukajadi Public Health Center, Banyuasin District, South Sumatra Province. During February-November 2019, 30 study samples underwent Slit Skin Smear examination and serum level testing of IgM Anti PGL-1 antibodies using the ELISA method. **Results:** The findings showed that zero persons (0%) had anti-PGL-1 IgM values  $<605 \mu\text{mL}$ , and 30 people (100%) had anti-PGL-1 IgM values  $\geq 605 \mu\text{mL}$ . BI scores  $<3$  were found in 13 people (43.3%) and  $\geq 3$  in 17 people (56.7%). The study results demonstrated a significant correlation between anti-PGL-1 IgM levels and BI values in Multibacillary leprosy patients with  $p=0.036$  ( $p<0.05$ ). **Conclusion:** Higher bacterial index values correlate with increased serum levels of anti-PGL-1 IgM.

**Keywords:** Anti PGL-1 IgM; Bacterial index; Slit skin smear

### INTRODUCTION

Leprosy remains a public health concern in various countries.<sup>1</sup> This microbial infection is endemic in more than 15 countries, with approximately 250,000 new leprosy cases reported annually

worldwide.<sup>2,3</sup> During 2012, 232,857 new leprosy cases were reported, and this number is estimated to reach 4 million cases by 2020.<sup>1,4</sup>

In 2013, Indonesia ranked third in the world after India and Brazil, with 16,856

new leprosy cases and a grade 2 disability rate of 9.86% among new patients.<sup>5</sup> In 2015, 17,202 new leprosy cases were reported, with 84.5% being Multibacillary (MB) type. The grade 2 disability rate in 2015 was 6.60 per 1 million population. North Sulawesi Province had the highest grade 2 disability rate per 1,000,000 population in 2015 at 21.14%, followed by other regions such as West Papua (19.51%) and Gorontalo (18.53%).<sup>6</sup> In 2014, 9 new Paucibacillary (PB) type cases and 68 Multibacillary (MB) type cases were reported in Jambi Province. The district with the highest number of new leprosy cases was East Tanjung Jabung district, with 34 cases and a case prevalence of 4.48 per 10,000 people.<sup>7</sup> The number of leprosy patients who visited the Dermatology and Venereology Clinic at Raden Mattaher Jambi General Hospital from 2014 to 2016 was 29, with 18 male and 11 female patients.

Even after completing therapy, many patients experience leprosy reactions, disability, and neurological dysfunction due to irreversible peripheral nerve damage.<sup>4</sup> This disease is also closely associated with myths regarding stigma, death, and mutilation. These perceptions lead to prejudice, discrimination, and social exclusion, causing patients to suffer not only physically but also mentally, affecting their personal and professional lives.<sup>8</sup>

Slit Skin Smear (SSS) is an essential screening procedure using laboratory methods for confirming and classifying

clinical forms of leprosy. SSS is also beneficial in studying the distribution of *M. leprae* in the skin and determining the degree of infectiousness and disease severity. WHO (1988) classifies all leprosy cases with positive SSS results as multibacillary (MB) leprosy, regardless of the number of lesions. Therefore, skin smear results for paucibacillary (PB) leprosy will always be negative. The bacterial index (BI) in multibacillary leprosy is always positive > 2 when accompanied by globi in neurological examination.<sup>9</sup>

Multibacillary Leprosy occurs in patients with low cellular immune response to *M. leprae*, resulting in a high bacterial load and becoming a source of infection.<sup>10</sup> The Phenolic glycolipid-1 of *M. leprae* has high sugar content immunogenicity and is a crucial virulence factor. Infection by *M. leprae* in MB leprosy results in cell-mediated humoral response and production of non-protective antibodies. The presence of PGL-1 antigen stimulates IgM antibody formation. Anti-PGL-1 IgM antibodies indicate that the patient has an acute immune response or is actively suffering from Leprosy.<sup>11</sup>

According to Manzel et al. (1987), with this PGL-1 antigen, there is little possibility of cross-reaction with other mycobacteria. IgM antibodies with PGL-1 have been observed to correlate well with clinical activity. ELISA IgM anti-PGL-1 test results have a threshold value for anti-PGL-1 IgM antibodies of  $\geq 605$  U/mL (seropositive).<sup>12</sup> Skin smear examination

results show positive results corresponding to the number of bacilli accompanied by increased levels of *M. leprae*-specific IgM antibodies.<sup>13</sup>

Research on IgM anti-PGL-1 serological examination has never been conducted in Jambi before. Additionally, the relationship between these antibody levels and the bacterial index, which indicates the magnitude of the bacterial burden, is also significant in determining the severity and progression of multibacillary leprosy. Based on this background, the researchers want to investigate further the relationship between serum IgM anti-Phenolic Glycolipid-1 levels and bacterial index in multibacillary leprosy patients.

## METHODS

This research is an observational study with a cross-sectional design. The study was conducted from February to November 2019. The research subjects were new or existing multibacillary leprosy patients who sought treatment at Dr. Rivai Abdullah Leprosy Hospital Palembang and Sukajadi Public Health Center, Banyuasin District, South Sumatra Province during the research period who met the inclusion and exclusion criteria.

Multibacillary leprosy patients underwent anamnesis, physical examination, and supporting examinations in the form of skin incision smears to diagnose, determine the type of leprosy, and determine the bacterial index value.

Blood serum was examined for IgM PGL-1 antibody levels using the ELISA method and a Kit by the Leprosy Laboratory of the Tropical Disease Institute, Airlangga University, Surabaya. The ELISA procedure for determining PGL-1 antibody levels is as follows: Add 50 µl of coating buffer and NT-P-BSA working solution to the microplate wells divided according to the scheme and incubate for 1 hour at 37°C. Wash the microplate 3 times with washing buffer (PBST solution). Add 200 µl blocking buffer to the microplate and incubate for 1 hour at 37°C. Discard blocking buffer, add 50µl of serum diluted with dilution buffer (1:300) into the microplate and incubate again for 1 hour at 37°C. Wash the microplate with washing buffer 3 times. Add 50µl of 2nd IgM Antibody (placed according to the scheme) into the microplate and incubate for 1 hour at 37°C (IgM diluted with dilution buffer 1:2000). Rewash the microplate with washing buffer 3 times. Add 100 µl substrate solution to the microplate until a yellow/orange colour appears. Stop the colour reaction after ±10-30 minutes by adding 100 µl stopping solution. Calculate the absorption value (OD) using ELISA Reader.

## RESULTS

The research was conducted at Dr. Rivai Abdullah Leprosy Hospital and Sukajadi Public Health Center, Banyuasin District, South Sumatra Province. Patients were obtained from the outpatient clinic at

the hospital and health centre. During February-November 2019, 30 multibacillary leprosy subjects were obtained. Seventeen multibacillary leprosy (MB) subjects were from Dr. Rivai Abdullah

Leprosy Hospital, and 13 were from Sukajadi Public Health Center, Banyuasin. The characteristics of these respondents can be explained in the following table:

**Table 1.** Research Subject Characteristics (n=30)

Characteristics	Subject n	Percentage %
Age		
< 20 years	4	13.3
21 – 30 years	8	26.7
31 – 40 years	10	33.3
41 – 50 years	6	20.0
> 50 years	2	6.7
Gender		
Male	26	86.7
Female	4	13.3
Education Level		
Elementary School	17	56.7
Junior High School	8	26.7
Senior High School	5	16.7
Occupation		
Unemployed	4	13.3
Housewife	1	3.3
Labourer	9	30.0
Farmer	10	33.3
Private Sector	5	16.7
Student	1	3.3

Based on **Table 1**, the largest age group of leprosy patients was 31-40 years old, with 10 people (33.3%). Males were found more frequently with 26 people (86.7%). Research subjects with elementary school education were more

prevalent with 17 people (56.7%). Farmers constituted the largest occupational group, with 10 people (33.3%). In this study, IgM Phenolic Glycolipid-1 levels in research subjects were divided into two groups, as shown in **Table 2**:

**Table 2.** IgM anti-Phenolic Glycolipid-1 Values

Severity Level	Subject n	Percentage %
< 605 $\mu$ /mL	0	0
$\geq$ 605 $\mu$ /mL	30	100

Based on **Table 2**, none of the multibacillary leprosy patients (0%) had IgM Anti PGL-1 values < 605  $\mu$ /mL, while

30 people (100%) had IgM Anti PGL-1 values  $\geq$  605  $\mu$ /mL. This study divided the BI values into two groups (**Table 3**).

**Table 3.** Bacterial Index Values in Research Subjects

Severity Level	Subject n	Percentage %
< 3	13	43.3
≥ 3	17	56.7

**Table 3** shows that 13 people (43.3%) with multibacillary leprosy have a BI value < 3, and 17 people (56.7%) have a BI value ≥ 3. The research results show

the relationship between serum IgM anti-Phenolic Glycolipid-1 levels and bacterial index values, as seen in **Table 4**.

**Table 4.** Relationship Between Serum IgM Anti Phenolic Glycolipid-1 Levels and Bacterial Index Values in Multibacillary Leprosy Patients

Variable	Group	Total	Mean	SD	p-value
IgM anti PGL-1 (µ/mL)	BI value <3	13	12,794.96	24,471.28	0.036
	BI value ≥3	17	31,849.38	40,093.11	

**Table 4** shows the results of the *Independent Sample Test* statistical test where  $p = 0.036$  ( $p < 0.05$ ), meaning that statistically, there is a significant difference between serum anti-PGL-1 IgM levels and BI values in multibacillary leprosy patients.

## DISCUSSION

### Characteristics of Research Subjects

The study's demographic data showed a predominance of male subjects, 26 people (86.7%). This finding is similar to research in Brazil, where the gender distribution of MB leprosy patients was 51.28% male and 48.71% female.<sup>9</sup> This can be explained by men having more social interactions outside the home, resulting in a higher risk of exposure to leprosy. In contrast, women generally have less interaction with society, tend to stay at home more, and are often unable to express their health concerns to the surrounding community. This finding is

primarily due to social stigma, economic and social status dependency, personal stigma, and gender insensitivity.<sup>14</sup> Research conducted by Wijayanti (2017) reported that males were more affected by leprosy than females at 64.7%. Men typically pay less attention to personal hygiene than women, as evidenced by the average male respondent bathing ≤ 2 times daily.

This poor bathing habit can increase the risk of leprosy transmission.<sup>15</sup> Varkevisser (2009) found that leprosy patients in Asian regions were predominantly male.<sup>16</sup> This was further confirmed by Bhat (2013), who reported male patients (54.35%) outnumbered female patients (45.65%).<sup>17</sup> Similarly, Tiwow (2014) reported a male percentage of 64.3%, with a male-to-female ratio of 1.8:1.<sup>18</sup>

In his research, Varkevisser (2009) also included countries outside Asia, such

as Nigeria and Brazil. Research results in these two countries showed opposite results, with women suffering from leprosy more than men. The male-to-female ratio in Nigeria was 0.87; in Brazil, it was 0.99.<sup>16</sup> Research by Maia and colleagues (2013) showed that the male percentage in Brazil was 80.9% (higher).<sup>19</sup> Maia's research in Brazil does not align with Varkevisser's research. Genetic factors, location, and research time may cause differences in male and female ratios.<sup>20</sup> The inequality in these comparisons can also be caused by the community's cultural, social, and educational factors, as seen in Nepal's society.<sup>16</sup> Sociocultural factors are believed to influence decision-making processes within families, for example, in seeking treatment. This is relevant to Indonesian society, which generally adheres to a patriarchal culture in the family. Balinese society also adheres to the same culture, prioritizing men over women.<sup>20</sup>

Age distribution analysis showed that MB leprosy cases were most common in the 31-40 year age group (10 people, 33.3%) and 21-30 year group (8 people, 26.7%). Silva reported similar results, with new leprosy cases averaging around 40 years of age, with 51.28% in the 15-44 year range.<sup>9</sup> Leprosy primarily affects individuals during their economically active period, between 20-60 years. Martin (2016) reported that 54% of leprosy patients fell within this age range. The tendency for leprosy to affect older adults may be due to reduced *M. leprae* exposure in children and

the long incubation period of MB leprosy. Additionally, age-related immune changes and increased susceptibility to infectious diseases play a role, including decreased monocyte and neutrophil function, phagocytic capacity, antigen presentation, and a shift in cytokine profile from Th-1 to Th-2.<sup>21</sup>

In his research, Bhat (2013) concluded that most leprosy patients were 36-50 years, while the author's research concluded that most leprosy patients were 31-40 years and 21-30 years.<sup>17</sup> Leprosy cases were least found in patients above 50 years old based on the author's data, different from research by Nuari and Darmada (2014) in the 0-15 year age group.<sup>17</sup> This may be due to research being conducted in different places - Bhat in India, Nuari and Darmada in Bali, and the author in Palembang - which allows for differences in genetic factors.

Educational background analysis showed that most subjects had elementary school education (17 people, 56.7%). This characteristic aligns with other studies showing that leprosy predominantly affects individuals with low education levels, weak socioeconomic status, and poor basic health conditions.<sup>21</sup> Fikria (2015) reported that low knowledge levels about leprosy make it difficult for individuals to implement prevention measures against the disease.<sup>22</sup> With low knowledge levels, people struggle to obtain information about leprosy, its transmission methods, and factors influencing its occurrence. Nuari

and Darmada (2015) concluded that lower education levels in communities correlate with reduced disease awareness. Health education is crucial in increasing community awareness and encouraging leprosy patients to seek treatment.<sup>20</sup>

Regarding occupation, farmers constituted the largest group (10 people, 33.3%). Farmers perform heavy labour with high mobility to fulfil their needs, leading to lowered immunity and increased susceptibility to MB leprosy.<sup>11</sup> Wijayanti's research (2017) found that 50% of patients worked as farm labourers, requiring significant energy expenditure.<sup>15</sup> In contrast, Nabila (2010) reported that private sector workers (traders and factory workers) comprised the largest group at 43.33%.<sup>23</sup> Geographical factors and urban development progress may influence this difference. Kediri, located on Java island, has more advanced urban development than other islands. The advancement of urban development and population density in Java has likely resulted in reduced agricultural land, leading to fewer farming occupations than on other islands.

### **IgM anti-Phenolic Glycolipid-1 Levels**

Leprosy serological examination is now widely performed due to its numerous benefits, particularly in seroepidemiological aspects of leprosy in endemic areas. The serological examination process is based on antibody formation in hosts infected by *M. leprae*. One specific antibody against *M. leprae* is the anti-Phenolic Glycolipid-1

(PGL-1) antibody. IgM anti-PGL-1 antibodies circulate in the blood and form complexes with *M. leprae* antigens for phagocytosis. One method of serological examination is the Enzyme-Linked Immuno-Sorbent Assay (ELISA). Besides diagnosis and determining leprosy type, PGL-1 antibody detection is also helpful for monitoring treatment. Examination based on specific antibody detection in serum is attractive due to its simple technique, quick results, low cost, and easy implementation under common conditions found in developing countries.<sup>12</sup>

In this study, the MB leprosy patients' bacterial index (BI) was divided into two groups: BI < 3 (13 patients) and BI ≥ 3 (17 patients). For IgM anti-PGL-1 values, all 30 patients (100%) showed levels ≥ 605 μ/mL, with none below this threshold. According to Manzel et al. (1987), using the PGL-1 antigen minimizes the possibility of cross-reactions with other mycobacteria. IgM antibodies with PGL-1 have been observed to correlate well with clinical activity.

The ELISA IgM anti-PGL-1 test has a threshold value of ≥ 605 μ/mL (seropositive).<sup>12</sup> Gunawan (2014) reported that IgM anti-PGL-1 level testing aims to detect antibody levels against PGL-1 antigen in serum using the ELISA method, with a sensitivity of 69.1% and specificity of 95.1%.<sup>24</sup>

## Bacterial Index Value in Research Subjects

*Mycobacterium leprae* cannot be cultured *in vitro*; therefore, finding AFB (Acid-Fast Bacilli) through microscopy remains the standard diagnostic technique. The number of bacilli in each microscopic field or bacterial index is calculated according to the Ridley index for skin smears (*ear lobe* and skin lesions). WHO classification (1988) includes all leprosy patients with positive bacterial index in the Multibacillary group. In lepromatous leprosy, the BI result is always positive > 2 if accompanied by globi on microscopic examination.<sup>25</sup>

*Slit-Skin Smear* examination is an essential *screening* procedure for all patients suspected of having leprosy. Besides establishing the diagnosis, disease classification (paucibacillary and multibacillary) and monitoring therapy response, SSS is also beneficial in studying the distribution of *M. leprae* in the skin and determining the degree of infectiousness and disease severity. *Mycobacterium leprae* is usually present in large numbers in the dermis of MB leprosy patients. Since it requires at least 10<sup>4</sup> bacilli/g of tissue to give a positive SSS result, SSS usually gives negative results in paucibacillary leprosy, which only has a small number of bacilli.<sup>26</sup>

In this study, the BI value of MB leprosy patients was divided into two groups: BI value <3 In 13 people (43.3%) and leprosy patients with BI value ≥3 in 17

people (56.7%). Reibel (2015) reported that tuberculoid leprosy usually results in negative BI results from ear lobe tissue fluid and negative or +1 results from skin lesions. In lepromatous Leprosy, BI results are always positive > 2, accompanied by globi (bacilli forming groups).<sup>25</sup> High BI values (≥+4) have high transmission risk and relapse rates. WHO (1988) classifies all leprosy cases with positive SSS results, regardless of the number of lesions, as multibacillary Leprosy (MB). Therefore, paucibacillary (PB) leprosy skin smear results will always be negative. BI results in multibacillary leprosy are always positive >2 if accompanied by globi on neurological examination.<sup>27</sup>

## The Relationship Between IgM Anti Phenolic Glycolipid-1 Serum Levels and Bacterial Index in Multibacillary Leprosy Patients

*Phenolic Glycolipid-1 (PGL-1)* is capable of suppressing host immune response through direct inhibition of *Toll-like receptor* or binding to lectin receptor (CR-3) to facilitate mycobacterium entry into host cells and inhibit TNF-α secretion by macrophages. The saccharide content of PGL-1 also suppresses anti-inflammatory processes by reducing the production of other cytokines induced by TLR-2, namely IL-6, IL-1β, and CCL2, and causes low activation and maturation of dendritic cells and reduces their ability to induce T cell response.<sup>28</sup> Adaptive immune response will arise when *M. leprae* antigen

stimulation is performed on CD4+ Th lymphocytes. In multibacillary Leprosy, CD4+ Th-2 will secrete IL-4 and IL-10, which will then activate B lymphocytes. Active B lymphocytes will proliferate and produce Immunoglobulin (Ig). IgM against anti-PGL-1 indicates that the patient has an acute immune response or is suffering from leprosy. The high titer of these antibodies is closely related to the bacterial index (BI), and the titer will decrease following the anti-leprosy treatment given.<sup>19</sup>

In this study of 30 research subject samples, the bacterial index value showed an increase in mean serum IgM PGL-1 levels and an increase in the number of bacilli in patients. The mean serum levels of leprosy patients with BI value < 3 was 12,794.96  $\mu$ /mL, and leprosy patients with BI value  $\geq$  3 was 31,849.38  $\mu$ /mL.

The presence of antibodies against PGL-1 is related to the number of *M. leprae* bacteria (BI) in leprosy patients.<sup>11</sup> Moura et al. (2008) reported in their study that PGL-1 antibody titers in lepromatous type were higher compared to tuberculoid type, and these titers tend to decrease with effective treatment; statistically, there was a significant relationship between the number of bacteria and antibody titers. PGL-1 antigen can induce a specific humoral immune response so that it can be used in the serodiagnostic examination of leprosy, but it cannot stimulate a cellular immune response, so it cannot cause body immunity.<sup>29</sup>

The results of this study show that the higher the bacterial index value, the higher the serum levels of IgM anti-PGL-1 will be. Skin smear examination results are positive according to the number of bacilli and an increase in specific *M. leprae* antibody levels.<sup>13</sup> The statistical test results obtained a p-value = 0.036 ( $p < 0.05$ ), meaning there is a significant difference between serum IgM anti-PGL-1 levels and leprosy severity based on bacterial index values.

The results of this study are similar to research conducted by Schuring<sup>13</sup> and Fabri (2015), which stated that there is a positive correlation between antibody titers and the bacterial index of leprosy patients.<sup>30</sup> Schuring (2006) reported that seropositive levels increased according to BI values, with *Adjusted Odds Ratio* (aOR) for patients with BI  $\leq$  2 being 6.33 (95% CI 2.42-16.5) and aOR for patients with BI > 2 being 59.0 (95% CI 25.0-139) compared to patients with negative BI.<sup>13</sup> Ardharia (2013) stated that BI examination is still a fundamental part in establishing the diagnosis of Leprosy; PGL-1 is a specific *M. leprae* antigen, so the presence of antigen in serum indicates active *M. leprae* infection in the body.<sup>31</sup> Gunawan (2014) reported that in MB-type leprosy, there is an increase in *bacterial load* and antibody levels due to the failure of cellular immune response in eliminating *M. leprae* bacteria.<sup>24</sup> *Phenolic Glycolipid-1* (PGL-1) is a specific antigen in *M. leprae*, so Immunoglobulin M (IgM) anti-PGL-1 levels

can indicate the presence of *M. leprae* infection. Buttlin (2007) found a relationship between antibody levels and increased severity of Leprosy and BI in untreated patients. There was also a decrease in anti-PGL-1 antibody levels of about 50% per year in patients receiving *multidrug therapy* (MDT). The decrease in antibody levels was associated with a decrease in BI after the same period. These patients had never received MDT treatment.<sup>32</sup>

## CONCLUSION

The most common characteristics of research subjects were found in the age group of 31-40 years with 10 people (33.3%), male gender with 26 people

(86.7%), subjects with elementary education level with 17 people (56.7%), and farmers with 10 people (33.3%). Regarding IgM anti-PGL-1 levels in research subjects, zero people (0%) had IgM Anti PGL-1 values < 605 µ/mL, and 30 people (100%) had IgM Anti PGL-1 values ≥ 605 µ/mL.

Bacterial index values through *Slit Skin Smear* examination showed that 13 people (43.3%) had BI values < 3, and 17 people (56.7%) had BI values ≥ 3. Serum levels of IgM anti-*Phenolic Glycolipid-1* are significantly related to bacterial index values, where higher bacterial index values correspond to increased serum levels of IgM anti-*Phenolic Glycolipid-1*.

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