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Research Article

RELATIONSHIP BETWEEN SERUM LEVELS OF IMMUNOGLOBULIN M-ANTI PHENOLIC GLYCOLIPID 1 AND IMMUNOGLOBULIN G-ANTI PHENOLIC GLYCOLIPID 1 WITH BACTERIA INDEX VALUES IN MULTIBACILLARY LEPROSY

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ABSTRACT

Objective: This study aims to determine the relationship of serum levels of anti-phenolic glycolipid (PGL-1), immunoglobulin M (IgM), and anti-PGL-1 immunoglobulin G (IgG) with bacterial index (BI) values in multibacillary (MB) leprosy.

Methods: This study was an observational study with a cross-sectional design. A total of 30 study samples were examined for a Slit Skin Smear and examined serum levels of IgM and IgG Anti PGL-1 antibodies by ELISA method.

Results: The results showed the higher the BI value, the serum levels of IgM anti-PGL-1 and IgG anti-PGL-1 increased.

Conclusion: There was a significant relationship between IgM anti-PGL-1 with BI value in MB leprosy ($p<0.05$), but there was no significant relationship between IgG anti-PGL-1 with BI value in MB leprosy ($p>0.05$). IgM anti-PGL-1 has more role in increasing the value of BI in MB leprosy.

Keywords: Immunoglobulin M-anti-phenolic glycolipid 1, Immunoglobulin G-anti-phenolic glycolipid, Bacterial index, Slit skin smear.

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INTRODUCTION

Leprosy is still a public health problem in various countries [1]. These microbial infections are endemic in more than 15 countries and around 250,000 new cases of leprosy are reported each year from around the world [2,3]. During 2012 new cases of leprosy were reported as many as 232,857 cases and are expected to reach 4 million cases by 2020 [1,4]. Although have completed therapy many people develop leprosy reactions, disability and neurological dysfunction due to peripheral nerve damage that is irreversible [4]. This disease is also closely related to myths about stigma, death, and mutilation. This perception raises prejudice, discrimination, and social exclusion so that sufferers are not only physically ill but also mentally ill and affect their personal and professional lives [5].

Indonesian in 2013 ranks third in the world after India and Brazil with the number of cases leprosy as many as 16,856 cases and the number of second level of disability among new patients as much as 9.86% [6]. New cases of leprosy in 2015 were reported as many as 17,202 cases with 84.5% of which is a type of Multibacillary (MB). Level 2 disability rate in 2015 was 6.60 per 1 million population. North Sulawesi Province ranks highest with a disability rate of 2 per 1,000,000 population in 2015 of 21.14% followed by other regions such as West Papua (19.51%) and Gorontalo (18.53%) [7]. In 2014 in Jambi Province, there were nine reported new cases of Pausibasilar (PB) and 68 Multibasilar (MB) types. The district with the highest number of new leprosy cases is Tanjung Jabung Timur district with 34 cases with a prevalence of 4.48 cases per 10,000 population [8]. The number of leprosy patients visiting the Skin and Sex Polyclinic of Raden Mattaher Jambi Hospital from 2014 to 2016 was 29 people with 18 male patients and 11 female patients.

Leprosy is a chronic granulomatous infectious disease caused by the obligate intracellular bacillus *Mycobacterium leprae*, mainly affecting the skin and peripheral nerves and can lead to disability and obvious physical damage [3,5]. The criteria for leprosy diagnosis according to the World Health Organization (WHO) are based on three cardinal symptoms, namely macular erythema or numb hypopigmentation, thickening of peripheral nerves and smears of acid-resistant bacilli or positive skin biopsy [3]. Slit Skin Smear (SSS) is still a laboratory method for confirmation and classification of the clinical form of leprosy. The WHO classifies all leprosy cases with positive SSS results regardless of the number of lesions as MB leprosy. Therefore, the results of a papalbacillary (PB) leprosy smear will always be negative [9].

MB leprosy arises in patients with a low cellular immune response to *M. leprae*, so it has a high bacillary burden and is a source of infection. MB leprosy sufferers are primarily responsible for the transmission of *M. leprae* in endemic areas [10]. *M. leprae* cannot be cultured *in vitro*; thus finding smear with a microscope remains a standard diagnostic technique. The number of bacilli in each microscope field or bacterial index (BI) was calculated according to the Ridley index for skin smears (ear lobes and skin lesions). Classification according to the WHO (1988) included all lepers with positive BI in the MB group. In lepromatous leprosy, BI results are always positive >2 if accompanied by globus on microscopic examination [11].

Slit-Skin Smear examination is an essential screening procedure for all sufferers suspected of having leprosy. In addition to establishing the diagnosis, classification of diseases (papalbacillary and MB) and the continued observation of therapeutic response, SSS is also very helpful in studying the distribution of *M. leprae* to the skin and ascertain the degree of infectious and severity of the disease. *M. leprae* is usually found in large quantities in the dermis of MB leprosy sufferers. Because it requires at least 104 bacilli/g tissue to give positive SSS results, SSS usually gives negative results on papillary bacilli leprosy which only has a small amount of bacilli [12].

Phenolic glycolipid-1 (PGL-1) M. leprae has high sugar content immunogenicity and is a very important virulence factor. Infection by *M. leprae* in MB leprosy results in the emergence of cell-mediated

humoral responses and the production of non-protective antibodies. The presence of PGL-1 antigens will stimulate the formation of antibodies both immunoglobulin M (IgM) and immunoglobulin G (IgG). IgG antibodies show an immune response to chronic diseases, meaning that even if the patient is not sick, they have been exposed to *M. leprae* antigens. IgM is the opposite, IgM against anti-PGL-I shows the patient has an acute immune response or is suffering from leprosy [13].

According to Manzel *et al*., (1987) with this PGL-1 antigen, it is unlikely that a cross reaction with other mycobacteria will occur. It has been observed that IgM antibodies with PGL-1 correlate well with clinical activity. Positive results of serum IgM antibodies in someone without a clinical picture suggest the possibility of a subclinical infection. The results of the anti-PGL-1 IgM ELISA examination have a threshold value for anti-PGL-1 IgM antibodies that is ≥605 u/ml [14] whereas the anti PGL-1 IgG antibody titer has a cutoff value, which is about nilai 630 u/ml [15].

MB leprosy arises in patients with a low cellular immune response to *M. leprae*, so it has a high bacillary burden and is a source of infection [10]. Skin smear examination results give positive results according to the number of bacilli accompanied by elevated levels of *M. leprae*-specific IgM antibodies [16].

Based on the description above it is important to determine the relationship of serum levels of IgM-anti-PGL 1 and IgG anti-PGL 1 with BI values in MB leprosy.

MATERIAL AND METHODS

This research was an observational cross sectional design study. This research was conducted in February to November 2019.

Patients

The research subjects were MB leprosy patients of new or old cases who came for treatment at Leprosy Hospital Dr. Rivai Abdullah Palembang and Puskesmas Sukajadi, Banyuasin District, South Sumatra Province in the study period that met the inclusion and exclusion criteria. MB leprosy sufferers do history taking, physical examination, supporting examination in the form of smears of skin incisions to diagnose, determine the type of leprosy, and know the value of the BI.

Samples

Serum examined blood levels of IgM and IgG antibodies anti-PGL-1 ELISA and using a kit made by Leprosy Laboratory Institute of Tropical Disease, Airlangga University, Surabaya.

ELISA methods

The ELISA examination procedure to determine the PGL-1 antibody level is as follows 50 µl of coating buffer and NT-P-BSA working solution were inserted into the microcaps that had been divided according to the scheme and incubated for 1 h 37°C. The microcrafts were washed 3× with washing buffer (PBST solution). Blocking buffer 200 µl was inserted into the microplatograph, incubated for 1 h 37°C. Blocking buffer was discarded, 50 µl serums were diluted with dilution buffer (1: 300), into the microplatograph, and reincubated for 1 h 37°C. Wash microplate with washing buffer 3 times. 50 µl second antibodies (IgG/IgM are placed according to the scheme) into the microplate, incubated for 1 h 37°C (IgG/IgM diluted with dilution buffer as much as 1: 2000). Microplate was washed again with washing buffer 3 times. Substrate solution is given as much as 100 µl into the microplate to yellow/orange. The staining reaction is stopped after±10–30 min by adding 100 µl stopping solution. Absorption prices (OD) with Elisa Reader are calculated.

RESULTS

The study was conducted at Leprosy Hospital Dr. Rivai Abdullah and Puskesmas Sukajadi, Banyuasin Regency, South Sumatra Province. Patients obtained from outpatient polyclinics in hospitals and health centers. During the period December 2017 to March 2018, there were 30 subjects with MB leprosy. A total of 17 MB leprosy (MB) subjects were obtained from Dr. Leprosy Hospital Rivai Abdullah and 13 subjects from the Sukajadi Community Health Center in Banyuasin District. The data on the characteristics of these respondents can be explained in the following Table 1.

Based on Table 1, it is found that the most age group of leprosy patients is the age group of 31–40 years, namely, ten people (33.3%). More male sex was found, 26 people (86.7%). Research subjects with more elementary school education levels were 17 people (56.7%). Farmers are the most work, ten people (33.3%). In this study, BI values were divided into two groups as shown in Table 2.

Table 2 shows that as many as 13 people (43.3%) of MB leprosy patients had BI values <3 and 17 people (56.7%) BI values ≥3.

The results showed that the relationship of serum levels of anti-PGL-1 IgM with BI values is shown in Table 3 below.

Table 3 shows the results of the Independent Sample Test statistics obtained p=0.036 (p<0.05), meaning that statistically there are significant differences between serum levels of anti-PGL-1 IgM with BI values in MB leprosy. The results showed that the relationship of serum levels of IgG anti-PGL-1 with the index value of bacteria in MB leprosy is shown in Table 4 below.

Table 4 shows the results of the Independent Sample Test statistical test obtained p=0.954 (p>0.05), meaning that there is no statistically significant difference between serum levels of anti-PGL-1 IgG with BI values in MB leprosy. The results obtained from the Logistic

Table 1: Characteristics of research subjects

Characteristics research subject	Research subject	
	F	%
Age		
20 years old	4	13.3
21–30 years	8	26.7
31–40 years old	10	33.3
41–50 years	6	20.0
>50 years old	2	6.7
Total	30	100.0
Gender		
Male	26	86.7
Girl	4	13.3
Total	30	100.0
Level of education		
Elementary school	17	56.7
Middle school	8	26.7
High school	5	16.7
Total	30	100.0
Occupation		
Does not work	4	13.3
Housewife	1	3.3
Laborer	9	30.0
Farmers	10	33.3
Private	5	16.7
Student	1	3.3
Total	30	100.0

Table 2: Bacteria index values in research subjects

Bacteria index value (ib)	Research subject	
	F	%
<3	13	43.3
≥3	17	56.7
Total	30	100

Table 3: Relationship between serum levels of IgM anti-Phenolic Glycolipid-1 with values BI in MB leprosy

Variable	Group	Amount	Average	Elementary school	p value
IgM anti PGL-1 (u/ml)	BI value<3	13	12794.96	24471.28	
	BI value≥3	17	31849.38	40093.11	0.036

Table 4: Relationship between serum levels of IgG anti-Phenolic Glycolipid-1 with values BI in MB leprosy

Variable	Group	Amount	Average	Elementary school	p value
IgM anti PGL-1 (u/ml)	BI value<3	13	19410.23	25868.87	
	BI value≥3	17	22968.50	28515.04	0.954

Regression test are p value IgM anti-PGL-1=0.013 ($p<0.05$) and IgG anti-PGL-1=0.56, meaning IgM anti-PGL-1 has more role in increasing BI value in MB leprosy.

DISCUSSION

Data on the characteristics of the research subjects showed more male sexes namely 26 people (86.7%). This study is similar to research in Brazil, where the distribution of male sex in MB leprosy patients was 51.28% and women were 48.71% [9]. This can be explained because men interact more in social activities outside the home so that they have a higher risk of exposure to leprosy. Whereas women, for the most part, did not interact much with the community, were more silent, and were unable to raise their health problems to surrounding communities. This is mainly due to social stigma, low dependency on economic and social status, personal stigma, and gender insensitivity [17]. MB leprosy cases were found in the age range 31–40 years, 10 people (33.3%) and 21–30 years as many as eight people (26.7%). Silva (2017) reports similar results, that new leprosy cases average age 40 years with an age range of 15–44 years as much as 51.28%. Leprosy mainly concerns individuals in the economically active period of the 20–60 year age range [9]. Martin (2016) reports that 54% of people with leprosy are in that age range. The tendency of people with leprosy in old adulthood may be caused by reduced exposure to *M. leprae* in children and the incubation period for MB leprosy for a long time. In addition, there is a role for age-related immune changes and increased susceptibility to infectious diseases in the form of decreased monocyte and neutrophil function, phagocyte capacity, antigen presentation, and cytokine profile changes from Th-1 to Th-2 [10].

Research subjects with more elementary school education levels were 17 people (56.7%). The characteristics of the level of education of this study are consistent with those obtained in other studies, where many people affected by leprosy suffered by individuals with low education, weak socio economic and poor basic health conditions [18]. Farmers constituted the most work in the study subjects, ten people (33.3%). In fulfilling the needs of farmers, working is much heavier and has high mobility so that low immunity is consequently susceptible to MB type leprosy [13].

M. leprae cannot be cultured *in vitro*; thus finding smear with a microscope remains a standard diagnostic technique. The number of bacilli in each microscope field or BI was calculated according to the Ridley index for skin smears (ear lobes and skin lesions). Classification according to the WHO (1988) included all lepers with positive BI in the MB group. In lepromatous leprosy, BI results are always positive > 2 if accompanied by globus on microscopic examination. 11 Slit-Skin Smear examinations is an essential screening procedure for all sufferers suspected of having leprosy. In addition to establishing the diagnosis, classification of diseases (papalbacillary and MB) and the continued observation of therapeutic response, SSS is also very helpful in studying the distribution of *M. leprae* to the skin and ascertain the degree of infectious and severity of the disease. *M. leprae* is usually found in large quantities in the dermis of MB leprosy sufferers. Because it requires at least 10 4 bacilli/gr tissue to give positive SSS results, SSS usually gives negative results on papillary bacilli leprosy which only has a small amount of bacilli [12].

PGL-1 is able to suppress host immune responses by direct inhibition of Toll-like receptors or bind to lectin receptors (CR-3) to facilitate the entry of mycobacteria into host cells and inhibit TNF-α secretion by macrophages. The PGL-1 saccharide content 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 its ability to induce T cell responses [19]. Adaptive immune response will arise if there is a race of M leprae antigens on CD4+Th lymphocytes. In MB type leprosy, Th-2 CD4 + will secrete IL-4 and IL-10 which then activates B lymphocytes. Active B lymphocytes will proliferate and produce immunoglobulin (Ig) either IgM or IgG. IgM against anti-PGL-1 indicates that the patient has an acute immune response or is suffering from leprosy and IgG shows an immune response to a chronic disease or even if the patient is not sick, but has been exposed to *M. leprae* antigen. Serological examination of leprosy is limited in use to the lepromatous/MB spectrum, because humoral immune responses play a role. The PGL-1 component can stimulate an immune response, but the immune response that occurs is not strong enough to eradicate *M. leprae* [13]. The high antibody titer is closely related to the BI, and the titer will decrease following the anti-leprosy treatment given [20].

In this study, from 30 sample subjects, an increase in mean serum IgM PGL-1 levels was accompanied by an increase in the number of bacilli in patients who were shown by BI values. The mean serum level of leprosy patients with BI value <3 was 12794.96 u/ml and leprosy patients with BI value ≥3 was 31849.38 u/ml. Likewise, the mean serum IgG PGL-1 level with an average value of 19410.23 u/ml for BI values <3 and 22968.50 u/ml for BI values ≥3. The presence of antibodies to PGL-1 is related to the number of bacteria (BI) *M. leprae* in lepers [13]. Moura *et al.* (2008) reported in their study that there was a higher PGL-1 antibody titer in the lepromatous type compared to the tuberculoid type, and that the titer tended to decrease with effective treatment, statistically there was a significant association between the number of bacteria and antibody titer. PGL-1 antigens can cause specific humoral immune responses, so they can be used in sero-diagnostic examinations of leprosy, but cannot stimulate cellular immune responses so they cannot cause immunity [21].

The results of this study indicate that the higher the BI value, the serum levels of IgM and IgG anti-PGL-1 will increase. Skin smear examination results give positive results in accordance with the number of bacilli accompanied by increased levels of *M. leprae* specific antibodies [16]. From the statistical test results obtained $p=0.036$ ($p<0.05$), meaning that there is a significant difference between serum levels of anti-PGL-1 IgM with leprosy severity based on BI values. However, although there was an increase in serum IgG levels to an increase in BI values the statistical results showed no significant difference with a value of $p=0.954$ ($p>0.05$).

The results of this study are similar to studies conducted by Schuring (2006) and Fabri (2015) which state that there is a positive correlation between antibody titers and the index of leprosy bacteria. Schuring (2006) reported that seropositive levels increased according to the value of BI, that is, Adjusted Odds Ratio (aOR) of patients with BI ≤2 was 6.33 (95% CI 2.42–16.5) and the aOR of patients with IB> 2 was 59, 0 (95% CI 25.0–139) compared with BI patients who were negative [16].

Research by Rawlinson *et al.* states that there is an increase in levels of anti-PGL-1 IgG in MB leprosy compared to papillaryasila. In MB leprosy, there is a defect in T lymphocytes (T helper and T suppressor). T cells control B cell proliferation, so that uncontrolled B cell proliferation causes an increase in IgG levels in MB leprosy [22].

Interpretation of anti-PGL-1 IgG results is needed in the examination of anti-PGL IgM 1. PGL-1 antigens can trigger IgM antibodies obtained in tissues infected with M leprae and can last for a very long time even though the *M. leprae* bacillus is dead. The antibody response in particular the IgM class shows that IgM does not depend on the response of lymphocyte T cells to this glycolipid antigen. In contrast to the IgG response which is dominated by LAM major carbohydrate antigens [15].

The results obtained from the Logistic Regression test are p value of anti-PGL-1 IgM=0.013 and anti-PGL-1 IgG=0.56, meaning that anti-PGL-1 IgM has more role in increasing BI value in MB leprosy. This can be explained by the fact that IgM antibodies appear 1–2 weeks after the onset of an antigen or germ attack, and will stay 2–3 months more depending on the presence of germ persistence. Detection of serum IgM with high levels specific to certain microbes is consistent with ongoing or recent infection in the host. Conversely, IgG titers will increase 2–3 weeks after infection and will be detected throughout life. High IgG titers indicate that the patient has been exposed to germ antigens and contact with leprosy patients has occurred in a long time, possibly having months or annual contact with lepers [23].

CONCLUSION

The BI value shows the severity of leprosy. Serum IgM levels of anti- PGL-1 are significantly related to BI values, where the higher the BI value, the serum levels of anti-PGL-1 IgM will increase. The higher the BI value, the serum levels of IgG anti- PGL-1 will increase, but this relationship is not statistically significant. The most important factor in increasing the value of BI is IgM anti- PGL-1.

CONFLICT OF INTEREST

None.

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