Research Article

The role of immunoglobulin M anti-phenolic glycolipid-1 and matrix metalloproteinase-9 levels in blood serum in peripheral nerve damage of multibacillary leprosy

Fitriyanti[1]*, Yanwirasti[1], Jamsari[2], Raden Pamudji[3]

ABSTRACT

Background: Schwann cells can also induce upregulation of matrix metalloproteinase (MMP)-9 productions, which is thought to be one of the cellular mediators of nerve damage from leprosy, especially in paucibacillary (PB) leprosy. **Objective:** This study aimed to analyze the roles of immunoglobulin M (IgM) anti-phenolic-glycolipid 1 (PGL-1) and MMP-9 in peripheral nerve damage in multibacillary (MB) leprosy. **Methods:** The levels of IgM anti-PGL-1 and MMP-9 were examined by ELISA technique, while laboratory examination of the skin incision was done to determine the bacterial index (BI) and the morphological index (MI) after Ziehl–Neelsen staining. **Results:** Serum IgM anti-PGL-1 and MMP-9 levels were significantly different between leprosy patients and household contacts ($P < 0.005$). There was a significant difference in serum IgM anti-PGL-1 levels between leprosy patients with BI values <3 and ≥3 ($P < 0.005$), but no significant difference was found in serum MMP-9 levels between leprosy patients with BI scores of <3 and ≥3. IgM anti-PGL-1 ($P = 0.006$) was statistically significant as a variable that was more responsible for peripheral nerve damage in MB leprosy than MMP-9 ($P = 0.042$). **Conclusion:** IgM PGL-1 was more important than MMP-9 in causing peripheral nerve damage in MB leprosy.

KEY WORDS: Matrix metalloproteinase, Multibacillary leprosy, *Mycobacterium leprae*, Peripheral nerves

INTRODUCTION

Leprosy is a chronic granulomatous infectious disease caused by an intracellular obligate bacilli *Mycobacterium leprae*, primarily affecting the skin and peripheral nerves, causing disability, and physical damage.[1,2] Leprosy remains a public health problem in many countries. This microbial infection is endemic in more than 15 countries, and about 250,000 new leprosy cases are reported each year from around the world.[1,3] In 2015, 17,202 new cases of leprosy were reported, with 84.5% of cases the MB type. In 2015, the rate of those with a disability. Grade 2 amounted to 6.60 per 1 million population.[4]

The diagnostic criteria of leprosy according to The World Health Organization (WHO) are based on three cardinal symptoms: macular erythema or hypopigmentation with numbness, peripheral nerve thickening, and a smear of acid-tolerant bacillus or positive skin biopsy. The WHO classified the clinical condition of leprosy as either MB leprosy, which is characterized by more than five skin lesions, and PB leprosy, with less than five skin lesions.[1] Early identification of leprosy cases remains a top priority in controlling the disease and as a strategy to break the chain of transmission and prevent physical disability.[3,5] *M. leprae* cannot be cultured *in vitro*; therefore, the identification of acid-resistant bacillus with a microscope is a standard diagnostic technique.[6] However, the techniques require at least 10^4 organisms per gram of tissue to be detected.[7]

Phenolic Glycolipid-1 is known to be an important virulence factor. The biosynthesis of PGL involves the addition of three primary sugars: rhamnose, rhamnose, and glucose, which are catalyzed by the methyltransferase enzyme.[8] PGL-1 can modulate the initial immune response of hosts mediated by trisaccharide domains through mechanisms which are not yet fully known.[9] The presence of PGL-1 antigens will stimulate the formation of the anti-PGL-1 IgM

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

[1]Department of Doctoral Program Biomedical Science, Faculty of Medicine, Andalas University, Padang, Indonesia, [2]Department of Biotechnology, Faculty of Agriculture, Andalas University, Padang, Indonesia, [3]Department of Dermatology and Venereology, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia

***Corresponding author:** Fitriyanti, Department of Doctoral Program Biomedical Science, Faculty of Medicine, Andalas University, Perintis Kemerdekaan Street No 94 Jati, Padang, West Sumatera, 25127 Indonesia. Tel.: +62 8117452096 E-mail: pipit_ikkk@yahoo.co.id

Received on: 05-02-2018; Revised on: 07-03-2019; Accepted on: 09-04-2019

antibody, and the antibody will react with the newly formed PGL-1 antigen. IgM anti-PGL-1 is associated with a large number of *M. leprae* bacteria found in patients and may be useful as a determinant of leprosy diagnosis.[10]

Peripheral nerve damage is a typical sign of progression of *M. leprae* infection and can be found in all clinical forms of leprosy. The primary factor determining neuronal damage is the ability of *M. leprae* to bind and infect Schwann cells. PGL-1 interacts with laminin-2 receptors in the Schwann cell membrane and mediates the entry of *M. leprae* into intercellular Schwann cells.[11] Furthermore, *in vitro* data show that due to interaction with *M. leprae*, Schwann cells (with or without Tumor necrosis factor-α [TNF-α]) may induce the upregulation of MMP-9 production, which is thought to be one of the cellular mediators of nerve damage in leprosy, especially in PB leprosy.[12]

MMP-9 is produced by keratinocyte, monocyte, and macrophage and serve as a marker of functioning macrophage, epitheloid granuloma formations, and potential damage in peripheral nerve of tuberculoid leprosy.[13,14] Initially, the enzyme is known as the primary enzyme to degrade extracellular matrix, but now it is well known for its immunomodulating properties.[15] MMP-9 is responsible for human neurological damage by degrading the neurovascular barrier and facilitate the immune cells migration and demielinasi.[12,13] MMP-9 expression is also linked to the TNF gene expression in pure neural leprosy patient biopsy, suggesting the involvement of the same enzyme to the pathogenesis of neural damage in leprosy patients.[12] Based on the above arguments, this study aimed to analyze the association of IgM anti-PGL-1 with levels of MMP-9 and peripheral nerve damage in MB leprosy.

METHODS

Research Subject

The study was conducted at Dr. Rivai Abdullah Leper Hospital and Sukajadi Health Center of Banyuasin Regency of South Sumatra Province for 4 months (December 2017–March 2018). Samples were taken from the venous blood of MB leprosy patients and household contacts who had lived in the same house as a patient for more than 5 years. Patients whose blood was drawn met the diagnosis of leprosy patients according to the WHO criteria, and family members who had lived at least 5 years with the patient were the comparison. Patients aged 18–60 years and willing to be followed in the study signed an informed consent document after being explained the research procedure. Patients were not severely ill, especially related to inflammation disease or infection, not pregnant or breastfeeding, and had no history of asthma, tuberculosis, chronic obstructive pulmonary disease, lupus erythematosus, or diabetes mellitus. The cohort of 64 patients was analyzed. In this study, we enrolled 32 subjects with MB leprosy and 32 subjects who had household contact with the patient. A total of 17 MB leprosy subjects were obtained from Dr. Rivai Abdullah Leper Hospital and 15 subjects from the Sukajadi Health Center of Banyuasin regency, as well as the subject of household contact.

Nerve Damage Assessment

The nerve damage assessment was based on the WHOs criteria for the leprosy severity level: (1) disability grade 0: no observable nerve damage; (2) Disability grade : lost of sensation or anesthetic in hands or feets; (3) disability grade 2: nerve damage signs are clearly visible, such as clawing hand, saddle nose, and ulcus plantaris.[16]

Blood Sample Collection

The blood sampling technique followed the operational standards of applicable hospital laboratory procedures. Examination of MMP-9 levels was performed using the Human MMP 9 ELISA kit produced by Bioassay Technology Laboratory. The anti-PGL-1, IgM antibody kit was made by the Leprosy Tropical Disease Institute of Airlangga University. Laboratory analysis of the skin incision was required to examine the presence of acid-resistant bacteria to diagnose leprosy based on the WHO criteria. The examination was performed on two sites in leprosy patients, skin lesions, and ear lobes, and then the average numbers of bacteria were used. In the household contact, the sample was drawn from venous blood. The excised tissue was examined to determine the BI and MI through Ziehl–Neelsen staining. BI was calculated semi-quantitatively to assess the density of acid-tolerant bacteria without differentiating solid or non-solid bacteria formed on the specimen with 0–6 values according to the Ridley scale. The assessment, according to Ridley's logarithm scale is shown in Table 1.

The MI is a presentation of live bacteria, characterized by a solid form compared to the total number of bacteria in both solid and split forms. MI is useful for determining the transmission of bacteria, the potential for transmission, and helps to determine drug resistance.

Table 1: Bacterial index according to the Ridley's logarithm scale

n	Scale	Meaning
1	0	No acid tolerant bacteria in 100 view field
2	1+	1–10 acid tolerant bacteria in 100 view field
3	2+	1–10 acid tolerant bacteria in 10 view field
4	3+	1–10 acid tolerant bacteria in 1 view field
5	4+	11–100 acid tolerant bacteria in 1 view field
6	5+	101–1000 acid tolerant bacteria in 1 view field
7	6+	>1000 acid tolerant bacteria in 1 view field

Table 2: Research subject Characteristic

Subject characteristic	Household contact f(%)	Disability grade 1 f(%)	Disability grade 2 f(%)	Total f(%)
Age				
<20	1 (3.1)	3 (16.7)	2 (14.3)	6 (9.4)
21-30	7 (21.9)	4 (22.2)	5 (35.7)	16 (25.0)
31-40	8 (25.0)	5 (27.8)	5 (35.7)	18 (28.1)
41-50	9 (28.1)	5 (27.8)	1 (7.1)	15 (23.4)
>50	7 (21.9)	1 (5.6)	1 (7.1)	9 (14.1)
Marital status				
Married	28 (87.5)	9 (50.0)	7 (50.0)	44 (68.8)
Single	4 (12.5)	9 (50.0)	7 (50.0)	20 (31.2)
Gender				
Male	10 (31.3)	16 (88.9)	11 (78.6)	37 (57.8)
Female	22 (68.8)	2 (11.1)	3 (21.4)	27 (42.2)
Education level				
Primary	25 (78.1)	8 (44.4)	8 (57.1)	41 (64.1)
Junior high	1 (3.1)	6 (33.3)	2 (22.2)	9 (14.0)
Senior high	6 (18.8)	4 (28.6)	4 (28.6)	14 (21.9)
Occupation				
No occupation	1 (3.1)	2 (11.1)	2 (14.3)	5 (7.8)
Housewife	14 (43.8)	0 (0.0)	1 (7.1)	15 (23.4)
Laborer	3 (9.4)	7 (38.9)	2 (14.3)	12 (18.8)
Farmer	9 (28.1)	5 (27.8)	6 (42.9)	20 (31.3)
Private sector	4 (12.5)	3 (16.7)	3 (21.4)	10 (15.6)
Student	1 (3.1)	1 (5.6)	0 (0.0)	2 (3.1)
Contact status				
Husband/wife	14 (43.8)	0 (0.0)	0 (0.0)	14 (21.9)
Parent/child	5 (15.6)	4 (22.2)	3 (21.4)	12 (18.6)
Other	13 (40.6)	14 (77.8)	11 (78.6)	38 (59.5)

Table 3: Bactericidal index characteristics of research subject

BI	Severity			Total
	Household contact (%)	Disability grade 1 (%)	Disability grade 2 (%)	
0	32 (100.0)	0 (0.0)	0 (0.0)	32 (100.0)
<3	0 (0.0)	10 (55.6)	4 (28.6)	14 (43.8)
≥3	0 (0.0)	8 (44.4)	10 (55.6)	18 (56.2)
Total	32 (50.0)	18 (28.1)	14 (21.9)	64 (100.0)

BI: Bacterial index

Data Analysis

Statistical analysis was conducted using SPSS version 23. Data that were not normal were transformed using Log 10. The results of standard data were analyzed with a parametric test, while non-normal data were analyzed using a non-parametric test. Bivariate analysis was conducted by an independent *t*-test, while multivariate analysis was done by a logistic regression test.

RESULTS

Characteristics of Multibacillary Leprosy Patients and Household Contacts based on Anamnesis

The subjects of the study were partially distributed normally ($P > 0.05$), and some were not normally distributed ($P < 0.005$). The subjects of this research were 32 MB leprosy patients and 32 household contacts who were divided into several characteristics to include age, gender, education level, and occupation. The most common age of leprosy patients was between 31 and 40 years (31.3%). Married status was mostly found in the household contact group, with 28 people (87.5%). Male gender was more common in the patient group, 26 people (81.3%); while in the group of household contacts females was more common, 22 persons (68.8%) [Table 2].

Subjects with primary/elementary education were common in both groups, 25 (78.1%) and 16 (50.0%), in leprosy patients and household contacts, respectively. In the patient group, 11 people (34.4%) worked as farmers, whereas in the household contact group, the most common occupation was a housewife, at 14 people (43.8%). The most common type of household contact was either a husband or wife of the patient, at 14 people (43.8%). Other types of contacts included 13 people (40.6%) [Table 2].

Characteristics based on Examination

In this study, characterization of the BI was conducted on both the household contact and the MB leprosy patients. The characteristics of respondents appear in Table 3. Fourteen patients (43.8%) had a BI score of <3, and 18 people (56.2%) had level ≤ 3. Most of the patients with BI score >3 had level 2 leprosy. None of the household contacts had leprosy, indicated by 0 BI score [Table 3].

The normality Test of the Variables Studied

Before testing the influence of independent variables on the dependent variable, it is necessary to conduct a normality test to determine the distribution normality of the data. Based on the normality test in Table 3, *P* values of IgM anti-PGL-1 and MMP-9 were <0.05; thus, the distribution was not normal, so both data were transformed. After data were transformed, IgM anti-PGL-1 data had normal distributions, with $P > 0.05$, while the MMP-9 data were still not normal. Therefore, a non-parametric test was used without any *post hoc* test.

The Differences In Immunoglobulin M Anti-Phenolic-Glycolipid-1 and Matrix Metalloproteinase-9 Levels between Leprosy Patients and Household Contacts

Leprosy patients had mean levels of IgM anti-PGL-1 (8,209.31 ± 18,941.00 μ/ml) that were significantly higher than those of household contacts (249.03 ± 295.26 μ/mL) ($P < 0.05$). The high IgM level was corresponding to the severity of leprosy, which level 2 leprosy was significantly higher IgM level than level 1 ($P < 0.05$). The MMP-9 level in leprosy patient was observed to be significantly lower than the household contact ($P < 0.05$). Level 1 leprosy patients had mean levels of MMP-9 (2060.62 ± 775.39 μ/mL), while level 2 had 2.180,64 + 1.015,46 μ/mL compared to those of household contacts (2744.34 ± 1092.83 μ/mL) [Table 4].

The Difference of Immunoglobulin M Anti-Phenolic-Glycolipid-1 and Matrix Metalloproteinase-9 Levels Among Leprosy Patients with Bacterial Index Values <3 and ≥3

Leprosy patients with BI values < 3 had mean IgM anti PGL-1 values (1482.82 ± 1,641.91 μg/dL) significantly lower than leprosy patients with BI values ≥3 (15832 ± 25963 μg/dl) ($P < 0.05$). Leprosy patients with BI values less than 3 had mean MMP-9 levels (2240.52 ± 946.52 μg/dL) insignificantly higher than leprosy patients with BI ≥3, which was 1856.73 ± 472.80 μg/dL ($P = 0.062$; $P > 0.05$) [Table 5].

The Role Of Immunoglobulin M Anti-Phenolic-Glycolipid-1 with Matrix Metalloproteinase-9 on peripheral Nerve Damage in MB Leprosy

The results obtained from the logistic regression test as shown in Table 6 revealed *P* values of IgM anti PGL-1 = 0.006 ($P < 0.005$) and MMP-9 = 0.042 ($P < 0.05$), showing that IgM anti PGL-1 plays the most important role in MB leprosy peripheral nerve damage compared to MMP-9 with Exp (B) value >1.

Table 4: Differences in immunoglobulin G anti-phenolic-glycolipid 1 levels and matrix metalloproteinase-9 between leprosy patients and household contacts

Variable	Group	*n*	Mean±SD	*p*
IgM anti-PGL-1 (μ/mL)	Household contact	32	249.03±295.26	0.000
	Level 1 leprosy	18	8209.31±18941.00	
	Level 2 leprosy	14	17.42678±26.28808	
MMP-9 (μ/mL)	Household contact	32	2744.34±1092.83	0.016
	Level 1 leprosy	18	2060.62±775.39	
	Level 2 leprosy	14	2.18064±1.01546	

PGL-1: Phenolic-glycolipid 1, MMP-9: Matrix metalloproteinase-9, IgM: Immunoglobulin M, SD: Standard deviation

Table 5: Differences in immunoglobulin M anti-phenolic-glycolipid 1 and matrix metalloproteinase-9 levels between leprosy patients with bacterial index values <3 and ≥3

Variable	Group	Mean±SD	*p*
IgM anti-PGL-1 (μg/dL)	BI <3	1482.82±1641.91	0.000
	BI ≥3	15,832.67±25,963.66	
MMP-9 (μg/dL)	BI <3	2240.52±946.53	0.062
	BI ≥3	1856.73±472.80	

PGL-1: Phenolic-glycolipid 1, MMP-9: Matrix metalloproteinase-9, IgM: Immunoglobulin M, SD: Standard deviation, BI: Bacterial index

Table 6: Logistic regression test result

Variable	*p*	Exponentiation of *B* coefficient (Exp (B))
IgM anti-(PGL-1)	0.006	1.004
MMP-9	0.042	0.999

PGL-1: Phenolic-glycolipid 1, MMP-9: Matrix metalloproteinase-9, IgM: Immunoglobulin M

DISCUSSION

The patient group consisted of 27 males (84,4%) and 5 females (15,6%) while the household contact consisted of 10 males (31,3%) and 22 females (68,8%). The result of this study is similar to a study in Brazil, where the distribution of male gender in MB leprosy was 51.28%, and the female was 48.71%. As for the household contact group, the female distribution was 83.3%, much higher compared to men 16.7%.[17] This is mainly due to social stigma, the low dependence of economic and social-culture, personal stigma, and gender insensitivity.[18]

The MB leprosy cases were found mostly in the age range of 31–40 years with 10 people (31.3%) and 41–50 years with 9 people (28.1%). Alison *et al*. also reported similar results, in which the new leprosy patients had an average age of 40 years, with a range of 15–44 years, as many as 51.28% and 44.4% household contact.[17]

Few results were correspond with other studies. The educational level of most patients and a household contact group was in elementary school. This result is consistent with those obtained in other studies, where leprosy is suffered primarily by poor people

with low education and socioeconomic status.[10,19] The heavy work and high mobility of farmers lead to a depressed immunity system. Consequently, they are easily affected by leprosy type MB.[10] Other studies in Brazil have also reported that close contacts with MB leprosy patients have the highest prevalence of seropositive results due to high-exposure intensity in patients with a high bacillary load.[20]

PGL-1 suppresses the host immune response by direct inhibition of Toll-like receptor or binding to the lectin receptor (CR-3) to facilitate the entry of mycobacteria into host cells and inhibition of TNF-α secretion by macrophages.[9] The adaptive immune response will be active when there is an *M. leprae* antigen in lymphocytes Th CD4+. In MB leprosy, Th2 CD4+ will secrete interleukin (IL)-4 and IL-10, which then activates B lymphocytes. The activated B lymphocytes proliferate and produce IgM or IgG Ig. The binding of IgM against anti-PGL-1 indicates that the patient has an acute immune response or is suffering from leprosy.[10]

This suggests that MB leprosy arises in patients with low cellular immune responses to *M. leprae*, and thus, having a high bacillary load, become a source of infection.[8] The smear of skin test results gave a positive result according to the number of bacilli accompanied by elevated levels of specific IgM antibodies *M. leprae*.[21]

M. leprae is an intracellular obligate pathogen with specialized tropism to Schwann cells in the peripheral nervous system and macrophage. The specify of *M. leprae* to Schwann cells is closely related to the Laminin-2 expression, specific to this type of cell.[22] PGL-1 in *M. leprae* can infect the Schwann cells if the PGL-1 binds to laminin-2 and the complex then interact with α-dystroglycan, a laminin-2 receptor in Schwann cells. *M. leprae* infected macrophage can phase through the blood-brain barrier, enter the endoneurial space, and then inflict axonal damage. In MB type leprosy, the peripheral nervous is filled with *M. leprae*-engulfed macrophage and increase the deposit of collagen in between neuron, leading to nervous hardening. In tissue level, immune cells and interstitial fluid influx inside a hard neuron membrane can lead to nervous damage due to compression and ischemia. In the cellular level, the immunological damage is considered as the main mechanism of nervous system damage.[22,23]

In multibacillary type of leprosy, the cellular immunity system is not functioning properly; thus, the immune response by Th^{-2} cell is upregulated. Th^{-2} will secrete IL-4 and IL-10 which then activate the B lymphocyte activated B lymphocyte will produce Ig. As the excessive bacillar capacity due to *M. leprae* infection, the IgM and IgG level will soar but will not enough in eliminating the pathogen and opens the possibilities for other infection.[24] High IgM level to PGL-1 is the symptom to the acute immune response in leprosy.[10]

In the skin, MMP-9 is produced by keratinocytes, monocytes, and macrophages. In a healthy immune response to infection, successful eradication of infection by the host requires effector cells, elimination of pathogens, inflammatory resolution, and ultimately matrix remodeling. The process of migrating dendritic cells and T-cells is known to be dependent on MMP-9.[25] MMP-9 secretion is an overview of mycobacterial infections and high MMP-9 levels in serum may be an indicator of disease activity.[25] In accordance with other study, low serum MMP-9 levels in MB leprosy patients may be attributed to multiple diffusion of basil-filled macrophage infiltrates and no specific cellular immune response to *M. leprae*.[21]

The results of this study were similar to those of Teles *et al*., who reported that serum MMP-9 levels in lepromatous leprosy were lower than in healthy controls and tuberculoid leprosy, which was 169 ± 29.3 ng/ml in lepromatous leprosy; 205 ± 29 ng/ml in tuberculoid leprosy; and 389 ± 84 ng/ml in healthy control.[25] These findings indicate that MMP-9 is a marker for many functioning macrophages, epitheloid granuloma formation, and potential destructive effects of peripheral nerves in tuberculoid type (PB) patients.[14]

In this research, leprosy patients with BI values <3 had a mean of IgM anti-PGL-1 lower than leprosy patients. The results of this study were similar to another study, which suggested that there was a positive correlation between antibody titers with leprosy BI.[21] Seropositive levels increased compared to BI-negative patients.

PGL-1 interacts with the laminin-2 receptor in the Schwann cell membrane and mediated the infection of *M. leprae*.[11] *In vitro,* data showed that the result of the interaction of *M. leprae* with Schwann cell (with or without TNF α) can upregulates the production of MMP-9, which is known as one of cellular mediator of nerve damage in leprosy.[12] MMP-9 activity, which is inducted by TNF α, can damage the blood nerve barrier and pull the macrophage into the endoneurium.[16] This MMP-9's activity is cannot be detected in normal condition but can escalate quickly in the first stages of nerve damage.[13]

Interestingly, low serum MMP-9 levels in MB leprosy patients may be attributed to the diffusion of multiple macrophages infiltrates which are filled with basil and have no specific cellular immune response to *M. leprae*.[21] Furthermore, the increased MMP-9 expression has been observed in functioning macrophage, epithelial cells polar leprosy tuberculoid and immunoreactivity will decrease toward polar lepromatosa (MB) characterized by damage

functioning of foamy histiocytes.[25] Lower IB values show fewer bacilli, so the titer of IgM PGL-1 antibody is also low. However, serum levels of MMP-9 will increase as it demonstrates a better cellular immune system, and vice versa.

This research has shown that the higher the value of BI accompanied by elevated IgM anti-PGL-I levels and decreased levels of MMP-9, reflects the severity of clinical leprosy. However, this study has some limitations, such as a limited number of MB leprosy patients, and no PB leprosy patients, who tend to avoid treatment because they think the disease is not vital.

CONCLUSION

In this study, serum IgM anti-PGL-1 levels were higher in leprosy patients compared with household contacts, while serum MMP-9 levels of leprosy patients were lower than those of household contacts. Leprosy patients with BI values <3 had lower serum IgM anti-PGL-1 levels than leprosy patients with BI scores ≥3; whereas leprosy subjects with BI scores <3 had higher serum MMP-9 levels than leprosy patients with BI scores ≥3. The more important variable in the occurrence of peripheral nerve damage in MB leprosy disease is IgM PGL-1.

ACKNOWLEDGMENTS

The authors would like to thank all who contributed to this study; the patients who donated their blood and provided all the information for this study; all medical staff at Dr. Riva Abdullah Hospital and Sukajadi Health Center of Banyuasin Regency for choosing samples to taking and managing blood sample. This study was conducted using personal funds without any conflicts of interest.

REFERENCES

1. Eichelmann K, González González SE, Salas-Alanis JC, Ocampo-Candiani J. Leprosy. An update: Definition, pathogenesis, classification, diagnosis, and treatment. Actas Dermosifiliogr 2013;104:554-63.
2. Garbin CA, Garbin AJ, Carloni ME, Rovida TA, Martins RJ. The stigma and prejudice of leprosy: Influence on the human condition. Rev Soc Bras Med Trop 2015;48:194-201.
3. Brito e Cabral P, Júnior JE, de Macedo AC, Alves AR, Gonçalves TB, Brito e Cabral TC, *et al.* Anti-PGL1 salivary igA/IgM, serum igG/IgM, and nasal *Mycobacterium leprae* DNA in individuals with household contact with leprosy. Int J Infect Dis 2013;17:e1005-10.
4. Indonesian Ministry of Health. Profil Kesehatan Indonesia Tahun 2015 (in English: Indonesian Health Profile in 2015). Jakarta: Indonesian Ministry of Health; 2016. p. 175-8.
5. Franco-Paredes C, Rodriguez-Morales AJ. Unsolved matters in leprosy: A descriptive review and call for further research. Ann Clin Microbiol Antimicrob 2016;15:33.
6. Reibel F, Cambau E, Aubry A. Update on the epidemiology, diagnosis, and treatment of leprosy. Med Mal Infect 2015;45:383-93.
7. Turankar RP, Pandey S, Lavania M, Singh I, Nigam A, Darlong J, *et al.* Comparative evaluation of PCR amplification of RLEP, 16S rRNA, rpoT and sod A gene targets for detection of *M. leprae* DNA from clinical and environmental samples. Int J Mycobacteriol 2015;4:54-9.
8. Berg S, Kaur D, Jackson M, Brennan PJ. The glycosyltransferases of mycobacterium tuberculosis roles in the synthesis of arabinogalactan, lipoarabinomannan, and other glycoconjugates. Glycobiology 2007;17:35-56R.
9. Arbues A, Lugo-Villarino G, Neyrolles O, Guilhot C, Astarie-Dequeker C. Playing hide-and-seek with host macrophages through the use of mycobacterial cell envelope phthiocerol dimycocerosates and phenolic glycolipids. Front Cell Infect Microbiol 2014;4:173.
10. Syafei MH. Association Between Transforming Growth Factor-β with Repeating Leprosum Nodosum Erytema Based on Immunoglobulin-M Anti Phenolic Glycolipid. Master Thesis. Padang: Postgraduate Program Andalas University; 2016.
11. Fonseca AB, Simon MD, Cazzaniga RA, de Moura TR, de Almeida RP, Duthie MS, *et al.* The influence of innate and adaptative immune responses on the differential clinical outcomes of leprosy. Infect Dis Poverty 2017;6:5.
12. Oliveira AL, Antunes SL, Teles RM, da Silva AC, Silva TP, Teles RB, *et al.* Schwann cells producing matrix metalloproteinases under *Mycobacterium leprae* stimulation may play a role in the outcome of leprous neuropathy. J Neuropathol Exp Neurol 2010;69:27-39.
13. Shubayev VI, Angert M, Dolkas J, Campana WM, Palenscar K, Myers RR, *et al.* TNFalpha-induced MMP-9 promotes macrophage recruitment into injured peripheral nerve. Mol Cell Neurosci 2006;31:407-15.
14. Sahar SY, Enas AS, Nahla MA, Ghada M. Expression of matrix metalloproteinases (MMP-3 and MMP-9) in skin lesions of leprosy patients. J Egypt Women Dermatol Soc 2009;6:80-7.
15. Khoka R, Murthy A, Weiss A. Metalloproteinases and their natural inhibitors in inflammation and immunity. Nat Rev Immunol 2013;13:649-65.
16. Van Brakel WH, Sihombing B, Djarir H, Beise K, Kusumawardhani L, Yulihane R, *et al.* Disability in people affected by leprosy: The role of impairment, activity, social participation, stigma and discrimination. Glob Health Action 2012; 5:1-11.
17. Alison SR, Marcos F, Ishikaw Y, Edna A. Evaluation of agreement between tests for the diagnosis of leprosy. J Bras Patol Med Labor 2017;53:100-7.
18. Price VG. Factors preventing early case detection for women affected by leprosy: A review of the literature. Glob Health Action 2017;10:1360550.
19. Prasad PV, Kaviarasan PK. Leprosy therapy, past and present: Can we hope to eliminate it? Indian J Dermatol 2010;55:316-24.
20. Carvalho AP, Fabri AC, Oliveira RC, Lana FC. Factors associated with anti-phenolic glycolipid-I seropositivity among the household contacts of leprosy cases. BMC Infect Dis 2015; 219:1-8.
21. Schuring RP, Moet FJ, Pahan D, Richardus JH, Oskam L. Association between anti-pGL-I igM and clinical and demographic parameters in leprosy. Lepr Rev 2006;77:343-55.
22. Misch EA, Berrington WR, Vary JC Jr., Hawn TR. Leprosy and human genome. Microbiol Mol Biol Rev 2010;74:589-620.
23. Bokhary M, Phung TL. Molecular pathogenesis of leprosy. Curr Trop Med Rep 2016;3:127-30.
24. Nobre ML, Illarramendi X, Dupnik KM, Hacker MA, Nery JA, Jeronimo SM, *et al.* Multibacillary leprosy by population groups in Brazil: Lessons from an observational study. PLoS Neglect Trop Dis 2017;11:1-14.
25. Teles RM, Teles RB, Amadeu T, Moura DF. High matrix metalloproteinase production correlates with immune activation and leukocyte migration in leprosy reactional lesions. Infect Immun 2010;78:1012-21.

Source of support: Nil; Conflict of interest: None Declared