DOI : 10.35124/bca.2020.20.S1.2963

Biochem. Cell. Arch. Vol. 20, Supplement 1, pp. 2963-2969, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

ANTI PHENOLIC GLYCOLIPID I (PGLI) OF SALIVARY IgA/IgM FROM *MYCOBACTERIUM LEPROSY* FOR EARLY DETECTION OF SUBCLINICAL LEPROSY

Maria Andisa Mayangsari[1], Muhammad Yoga Wardhana[1], Nastiti Faradilla Ramadhani[2]*, Alexander Patera Nugraha[3] and Rini Devijanti Ridwan[4]

[1]Undergraduate Student, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
[2]Department of Dentomaxillofacial Radiology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
[3]Department of Orthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
[4]Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
*e-mail: nastitifaradillar@gmail.com

(Received 6 February 2020, Revised 15 April 2020, Accepted 21 April 2020)

ABSTRACT : Leprosy is a chronic infectious disease caused by the bacterium *Mycobacterium leprae*. Indonesia is one of the countries with the highest leprosy, 3rd after India and Brazil. In 2013, Indonesia has 16.856 of new leprosy cases. In diagnosing leprosy, physical clinical examination not always able to detect the early phase of the disease. Therefore, we need a diagnostic tool that can detect infection with *Mycobacterium leprae* early so that treatment can be performed optimally. Saliva contains a lot of protein and nucleic acid molecules that reflect physiological and pathological status. Through saliva, anti Phenolic glycolipid 1 (anti-PGL1) dan be examined which is a specific antibody against PGL1 *M. leprae* found in the cell wall. Anti-PGL1is produced as a form of immune response against *M. leprae.* Individuals with a positive anti-PGL1 shown to have six times greater risk of developing leprosy. Serological examination by ELISA for detection of anti-PGL1 is potential to become a tool for early detection of subclinical leprosy (presence of bacilli), these results are useful to monitor and prevent the possibility of leprosy. Journal and other literature study searched with specific keywords. The result shows salivary anti-PGL1 is representative for *M. leprae* infection. Examination of anti-PGL1 in saliva can unlock the potential diagnosis of safe, sensitive and non-invasive to leprosy especially in endemic areas.

Key words : Leprosy, anti-PGL1, neglected disease, *Mycobacterium leprae*, salivary IgA/IgM.

INTRODUCTION

Leprosy is a chronic infectious disease caused by the bacterium *Mycobacterium leprae*. Indonesia until now is one of the countries with the most leprosy, ranked third after India and Brazil. In 2013, Indonesia had a total of 16,856 new leprosy cases. Prevalence is generally more in areas with poor sanitation and the difficulty of availability of clean water.In Indonesia, most cases were found in East Java, which is 25.4% of the total cases of the whole country. The biggest problem due to leprosy is not only the number of cases but also the disability caused (Ministry of Health of Republik Indonesia, 2015).

Vaccination has not yet been found for leprosy, but leprosy can be cured with drugs. Treatment with multidrug therapy (MDT) methods is determined by WHO based on leprosy classification. Indonesia also applies MDT in the management of leprosy cases. The effectiveness of MDT is quite high in curing leprosy, the rate of relapse is reported to be very low, which is less than 0.1% (WHO, 2016).

The emphasis on strategies to eradicate leprosy in addition to treatment is also pursued by the early discovery of new cases. However, physical examination cannot detect the initial phase of the course of the disease because its clinical manifestations are rarely found. Biomarkers are very useful as early diagnostic tools, can improve the prognosis and success of therapy. Besides, early diagnosis is also useful for the prevention of transmission to individuals around patients (Cabral *et al*, 2013).

Saliva is an important fluid produced by the salivary glands and helps maintain oral and systemic health and affects the quality of life (Ongole *et al*, 2012). Proteins and nucleic acid molecules that reflect the physiological status contained in many saliva. This makes saliva a potential biomarker. The process of taking saliva also has many advantages compared to other body fluids such as

blood, which is more non-invasive, easier, cheaper, safer, and faster. Saliva has been used as a biomarker in infectious diseases such as HIV and has the potential to be a biomarker in malignant diseases (Yoshizawa *et al*, 2013).

Phenolic glycolipid (PGL1) is a dominant lipid component in *M. leprae* These lipids also act as antigens that determine the immunological specificity of M. leprae. Although anti-PGL1 IgM in serum has been extensively studied, the results of studies of anti-PGL1 in IgA and IgM in saliva also have the potential to indicate M. leprae infection in the human body (Cabral *et al*, 2009; Cabral *et al*, 2013). Da Rosa *et al* (2013) have investigated that a combination of the bacteriological index, slit-skin smear, and salivary qPCR diagnostic techniques can be a potential diagnostic tool, especially in cases of paucibacillary leprosy.

Current technological developments have supported the creation of an early diagnosis technique for leprosy in a non-invasive and safe way of using saliva. Therefore, this paper is made to open up insights on the use of anti-PGL1 in salivary IgA/IgM as a means of early detection of subclinical leprosy so that later can increase the success of leprosy eradication through the discovery of new cases early.

Leprosy

Leprosy, also known as Hansen's disease is a chronic disease that is an infection caused by *Mycobacterium leprae*. Leprosy throughout its history is associated with stigma, social exclusion, discrimination against people affected by leprosy. Leprosy has existed since BC. Leprosy is mentioned several times in the scriptures. Before modern times, leprosy was known as a curse. It is estimated that leprosy originated in Egypt and surrounding countries since 2400 BC (Hansen, 1895).

Ridley and Jopling (1966) classify leprosy based on the pattern and transition of its clinical form. Clinically, histopathologically, and immunologically, leprosy is identified as being 5; TIubercoloid polar form (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL). For therapeutic purposes, WHO (1982), from this classification, grouped patients into two groups, namely; Paucibacillary (TT, BT) and multibacillary (BB, BL). Then it is recommended that a better classification be based on the number of lesions, less than or up to five for Paucibacillary (PB), and more than 5 for Multibacillary (MB).

Incubation of leprosy for tuberculoid type during 2.9-5.3 years and 9.3-11.6 years in the lepromatous type. IgM antibody titers will appear 1-2 weeks after germ infection and will stay for 2 to 3 months or more. Detection of specific IgM levels against microbes in serum will indicate a moderate or recent infection at the host.After that, the IgG titer will increase 2 to 3 weeks after contact with the antigen and will stay forever.

Epidemiology

The number of new cases of leprosy in the world in 2011 was around 219,075, the most occurring in southeast Asia with 160,132 cases. In 2013, Indonesia was the third highest country with leprosy infection in the world with 20,032 adult cases, after India and Brazil. 12 out of 34 provinces in Indonesia have new cases with an average of 10/100,000 population, while West Papua has cases over 100/100000. Leprosy in Indonesia was recorded in history in 1655, namely the construction of special refugee leprosy made in the Thousand Islands in Jakarta, and the increase in refugee camps in the two decades later as many as 45 refugees. In 1932, isolation of leprosy was stopped, and several national control programs were implemented. In 1969, the State integrated leprosy control into basic health services. In 2010-2014, Indonesia synchronized the Ministry of Health's strategic plan based on Enhanced Global Strategy for Further Reducing the Disease Burden due to Leprosy 2011-2015 as a WHO global effort supported by ILEP (Ministry of Health, 2012), which then continued to global targets in 2016-2020 to reduce level II disability in new cases to less than 1/ 1000,000 population by 2020.

Medicamentosa

Leprosy can be cured with multidrug therapy (MDT) treatment, according to WHO guidelines; MB patients can be given rifampicin 600 mg once a year, clofazimine 300 mg once a month and 50 mg per day, and dapsone 100 mg per day. Whereas for PB patients can be given rifampicin 600 mg per month and dapsone 100 mg per day for 6 months (Lockwood and Britton, 2004).

If the patient cannot receive clofazimine or dapsone, then fluoroquinolone, ofloxacin, moxifloxacin, minocycline and clarithromycin macrolide can also be used as secondary agents for the treatment of M. leprae (Lockwood and Britton, 2004).

Microbiology *M. leprae*

M. leprae is an intracellular obligate gram-positive obligate gram-resistant bacterium that shows tropism for cells of the reticuloendothelial system and peripheral nervous system (especially schwann cells). *M. leprae* is in the form of a curve with a length of 1 to 8 um and a diameter of 0.3 um. M. leprae replicates in a binary way. Replications take 11 to 13 days. Basil leprosy has a predilection for macrophages in gathering intracellular

groups called globi.*M. leprae* tends to infect cold areas in the body such as the skin, nasal mucosa and nerve perfier. *M. leprae* grows optimally in temperatures between 27°C and 30°C. M. leprae has a predilection for schwann cells by specifically binding to the G domain in the laminin-a2 chain, which is specific to the basal lamina of the peripheral nerve. When the pathogen penetrates the cell, replication will occur slowly until the T cells recognize the antigen and then proceed to a chronic inflammatory reaction (Pinheiro *et al*, 2011).

In survival, this pathogen survives with its structural elements, namely capsules and cell walls. The capsule structure is formed by the lipid phthiocerol dimycocerosate and phenolic glucolipid-1 (PG-1), which are the target of the IgM humoral immune response. the cell wall component consists of lipoarabinomannan which is antigenic to macrophages (Pinheiro *et al*, 2011).

Immune Response to Leprosy

There are two kinds of the immune response in leprosy, namely natural and adaptive immune responses. The body's natural immune response to leprosy depends on the human leukocyte antibody (HLA) system. The histocompatibility complex depends on HLA class one (HLA-A, B, C) and second class HLA (HLADp, DQ, and DR). HLA class one is a glycoprotein with heavy chain (BM 44 kDa) and light chain or beta2 microglobulin. Class 2 HLA is present on the surface of immunocompetent cells (B lymphocytes, T lymphocytes, and macrophages). TT/BT leprosy group is associated with HLA Dr-3 while LL/BL type is associated with HLA-DR-2, DQ1 (Indrayanti and Yuanita, 2009).

The adaptive immune response will be active if there is sensitivity from *M. leprae* antigens on CD4 + Th lymphocytes so that they become active and proliferate into CD4+ TH-1 lymphocytes or Th-2 with specific cytokine secretions. Th-1 secretes gamma interferon and IL-2 which then activates cellular immune responses. Th-2 will secrete IL-4 and IL-10 to activate B lymphocytes which then proliferate into plasma cells and produce IgG and IgM (Indrayanti and Yuanita, 2009).

IgG shows an immune response to a chronic disease, whereas IgM is an acute immune response or is suffering from the disease. Carbohydrate and lipid components (PGL-1) will stimulate the immune response, but the immune system is not enough to fight M. leprae, so what happens is PGL-1 in the clinic is used as a diagnosis of subclinical disease. In sub-clinical conditions, there is an increase in IgM levels against PGL-1. The results can be said to be seropositive if the increase in IgM against PGL-1 has a value > 600 iu / ml (Indrayanti and Yuanita, 2009).

In its journey, clinical leprosy depends on the patient's immune status. The role of genetics, associated with locus susceptibility on chromosome 10p13 near the mannose receptor 1 gene. Mannose receptors on the surface of macrophages play an important role in phagocytosis. Some components of the immune system are associated with the clinical phenotype of the disease. Specific, intensified, and organized cellular responses are seen in the tuberculoid pole, compared to the absence of a specific immune response in the tuberculoid pole.

Lepromatous shape affects the skin and peripheral nerves to form annular or ovoid plaque infiltrates with clear boundaries. These lesions are anesthetic and can occur in all parts of the body. On skin biopsy and nerve tissue, granulomas with epithelioid histiocytes, multinucleated giant cells and CD4 + T cells that secrete gamma interferon are seen. Clinical and immunological situations in lepromatous leprosy do not show specific immune responses. The proliferation of bacilli in macrophage tissue and bubbles can be seen and there are several CD4 + and CD8 + T cells. Granulomas usually don't form. On immunohistochemical examination, IL-4 and IL-10 will be seen.

The immune response to *M. leprae* is clinically varied, which is divided into type 1 and type 2 reactions. These reactions are related to changes in the immune system due to anti-leprosy, stress, or pregnancy treatment (Pinheiro *et al*, 2011).

In a type 1 reversal reaction, type IV hypersensitivity occurs. Increased cytokines in the blood, such as increased gamma interferon and TNF and CD4 + activation are activated resulting in edema and pain from inflammation. Reversal reaction is characterized by upgrading from the clinical picture of tuberculoid patterns including reduction of the bacillary (Pinheiro *et al*, 2011).

In type 2 reactions, type III hypersensitivity reactions occur due to the deposition of immune complexes associated with systemic toxicity. An increase in tumor necrosis factor, and neutrophil infiltration and complement to the skin. This type is usually found in borderline leprosy and lepromatous leprosy types (Pinheiro *et al*, 2011).

PGL-1

Phenolic Glycolipid - 1 (PGL-1) in *M. leprae* plays a role in invasion, defense by attacking schwann cells by binding directly to laminin-2 domains on the basal lamina surrounding the schwann cell axons. When a schwann cell is invaded by bacteria, it will be difficult to kill intracellular bacilli and bacteria that proliferate in the cell. In addition, *M. leprae* also induces foamy macrophage

formation from lipids in lesions due to increased Prostaglandins (PGE2) and IL-10 and IL-12 and nitric oxide production in infected Schwann cells (Brennan and Spencer, 2011).

PGL-1 has become a reference method for detecting new leprosy or as an initial diagnosis of subclinical infection. PGL-1 was first reported by Brennan and Barrow in 1980 in *M. leprae* who infected the liver armadillo (Brennan and Barrow, 1980). Several studies show that seropositive PGL-1 has improved the rate of leprosy detection (Baumgart *et al*, 1987).

PGL-1 can represent 3% of the total bacillus leprosy because it is located on the surface of *M. leprae* cells. After the purification method of PGL-1 from infected armadillos then a similar method was tested on biological specimens of leprosy, including skin lesions, serum samples, and urine. Detection of PGL-1 in serum samples is by drying 100 ul of serum on a paper disc filter and extracting lipid material with CHCL3/CH3OH as much as 2: 1 followed by fractionation in the silicic acid column. GL-1 Antigens extracted from serum samples will be ready to be analyzed by dot-blot ELISA using polyclonal rabbit anti-PGL-1 antiserum or monoclonal antibodies. Lepromatous leprosy patients classified as type MB (BL or LL) are positive for serum examination as much as 88% and 96%, however, for PB type only 30-60% is detected, which lacks detectable serum antibody levels. This is due to local immunity in skin lesions. Most TT / BT lesions produce antibodies locally without causing significant B cell proliferation, which is a prerequisite for the formation of a systemic antibody response. Therefore, PGL-1 examination is only sensitive for MB types (Brennan and Spencer, 2011).

In the examination of PGL-1, which is also performed on leprosy patients who have been given MDT therapy, there is a drastic decrease in serum PGL-1 and IgM. The relationship between IgM against anti-PGL-1 and bacterial index shows that the anti-PGL-1 IgM level reflects the total number of bacilli in leprosy sufferers.The presence of serum IgM antibodies for PGL-1 in lepromatous leprosy can help classify patients, monitor cases, identify the risk of recurrence and identify transmission with a high risk of developing the disease (Spencer *et al*, 2012).

Serological tests in people affected by leprosy show a decrease in anti-PGL-1. These serological tests have been used to diagnose subclinical infections and to monitor the effect of leprosy therapeutics although it is still debated about the high correlation of anti-PGL-1 antibodies to active, past and future disease (Spencer *et al*, 2012).

Saliva

Saliva is a liquid that is secreted by three pairs of secretory glands, namely the sublingual, submandibular, and parotid glands which are then channeled into the oral cavity through the duct. Saliva contains electrolytes such as Na+, K+, Cl-, HCo2-, Ca2+, Mg2+, HPO42-, SCN-, and F-. Saliva also contains humoral antibodies in the form of immunoglobulin IgA, IgG, and IgM as an initial defense function in the oral mucosa (Rai, 2007).

In saliva, there are molecules with a broad spectrum that can be useful as clinical diagnostic information. Intact saliva is generally used to diagnose systemic disease because it contains serum of several systemic diseases such as Sjorgen syndrome, multiple sclerosis, heart disease, caries and periodontal disease, which can be detected through saliva (Malathi *et al*, 2016).

Immunoglobulin in saliva

The level of immunoglobulin in total saliva can allow assumptions about the susceptibility of the host in responding to microbial aggression because the immunoglobulin sub-type found in salivary fluid functions as a neutralizer, activation of complement, and phagocytosis. The protective effect of the humoral immune system is mediated with several specific functions (Rai, 2007).

IgA plays a role in neutralizing antigens and macromolecules in general that penetrate the mucosa, inhibit the sensitivity of foreign macromolecules and protect against infectious diseases. IgG, which is mostly derived from Gingival Crevicular Fluid (GCF) derivatives, serves in the secondary immune response, and IgM plays a role in the primary response to microorganisms in general. IgA and IgM can be transported through the epithelium secretor, whereas IgG has a slight role in mucosal protection due to the absence of receptors on secretory epithelial cells to transmit. However, IgG can still be found in intact saliva (Rai, 2007). Cabral *et al* (2013) have also conducted studies on the use of serum IgG and IgM and anti-PGL IgA/IgM in saliva to diagnose leprosy.Most studies choose anti-PGL 1 IgM as the main parameter in the diagnosis of leprosy. However, the rheumatoid factor is a cause of false-positive detection of IgM. Serum anti-PGL 1 IgG can be an important parameter for evaluation.

DISCUSSION

Leprosy is still a global health problem, around 250,000 new cases are reported each year. Leprosy is a chronic infectious disease caused by the bacterium *Mycobacterium leprae*. Indonesia until now is one of the countries with the most leprosy, ranked third after

India and Brazil. Data obtained from the Data and Information Center on Indonesia's Health Profile shows the prevalence of leprosy ranges from 0.79 to 0.96 per 10,000 population. Based on data from 2011-2013, 14 out of 33 provinces in Indonesia have a high leprosy burden. The highest burden of leprosy, especially in children, is found in 13 provinces. The most recent cases of leprosy are found in the provinces of East Java, West Java, Central Java, Papua and South Sulawesi (WHO, 2016; Ministry of Health RI, 2015).

The mechanism of transmission for leprosy is unclear, but the transmission is suspected through prolonged contact with leprosy patients through secretion of nasal fluid and oropharynx (saliva) and or through skin lesions. M. leprae attacks Schwann cells that surround axons from peripheral nerves, causing thermal progressive degeneration, tactile and pain sensitivity and more severe can cause defects in the limbs. In most patients, nerve lesions and manifestations occur first than skin lesions (D' Abreu *et al*, 2000; Margarido and Rivitti, 2005; Pontes *et al*, 2008).

In the era of Millennium Development Goals (MDGs) infectious diseases of tropical infections often did not get attention and were called Neglected Tropical Disease (NTD), even though Indonesia is a tropical country. Leprosy includes NTD caused by bacteria. Poverty as a risk factor for NTD often occurs in rural areas, slums, and marginalized. Other risk factors are limited resources, such as access to clean drinking water, poor sanitation. Leprosy as an NTD disease has an impact on permanent disability limbs so that it can reduce productivity and economic status, even worse will have social consequences and stigmatization. Leprosy can also cause morbidity and mortality. Women are most vulnerable to stigmatization and discrimination after suffering from leprosy. One of the MDG targets is the eradication of poverty and hunger. Although, leprosy is not directly included in the MDG target, poverty issues are very relevant to the case of leprosy, so if the poverty rate is lowered then control of leprosy is expected. Indonesia is still trying to accelerate the elimination of leprosy through early detection of cases, quality leprosy services, dissemination of leprosy, elimination of stigma, empowering individuals who have had leprosy and strengthening their participation in efforts to control leprosy, working together with stakeholders, increasing support for leprosy program, and applying different approaches based on leprosy endemicity.Besides, second-degree disability arising from leprosy is estimated to reach one million cases in 2020, but the prevention of patients with nerve damage to develop disability and deformity is also still a challenge. Education should be given about foot and hand care for patients with anesthesia and muscle weakness. The latest technology to detect leprosy is very much needed, especially to detect subclinical leprosy because in subclinical leprosy clinical signs are not yet visible. (United Nations, 2016; Feasey *et al*, 2015; Rodrigues *et al*, 2011; Nsagha *et al*, 2002; Infodatin, 2015).

Diagnosing leprosy is very challenging because of the complexity of the immune response that leads to various clinical and subclinical forms. Several studies have used nucleic acid amplification tests which are more sensitive than microscopic examination in detecting the presence of bacilli in biological samples such as blood and saliva. This approach is very important in detecting *M. kustae* (Martinez *et al*, 2011; Patrocínio *et al*, 2005; Phetsuksiri and Rudeeaneksin, 2006; Banerjee *et al*, 2011).

Saliva is an important fluid produced by the salivary glands and helps maintain oral and systemic health and affects the quality of life. Proteins and nucleic acid molecules that reflect the physiological status contained in many saliva. This makes saliva a potential biomarker. The salivary retrieval process also has many advantages compared to other body fluids such as blood, which is less invasive, easier, cheaper, safer, and faster (Ongole *et al*, 2012).

Phenolic glycolipid (PGL1) is a dominant lipid component in *M. leprae*. These lipids also act as antigens that determine the immunological specificity of *M. leprae*. PGL1 can be detected with an Enzyme-Linked Immunosorbent Assay (ELISA) and be a biomarker candidate relevant for leprosy activity.The PGL-1 fraction is part of *M. leprae* cell envelope and induces the production of humoral specific responses to PGL-1 detected in the patient's serum. When antibodies are at high levels, an infection can be active, especially during reactional episodes, which are very common complications in leprosy progression. Serum anti-PGL1 IgM has been widely studied, whereas saliva is still not popular.The results of studies on anti-PGL1 in IgA and IgM in saliva also have the potential to indicate *M. leprae* infection in the human body (Cabral *et al*, 2009; Cabral *et al*, 2013; Goulart *et al*, 2002).

Saliva sampling of 5 mL of unstimulated saliva was collected from each subject in a sterile container for 5 minutes. The saliva collected was immediately centrifuged at 4500 rpm for 30 minutes at 4°C, then divided into 500 uL aliquots and stored at -70°C until the time of analysis. Saliva samples were obtained between 8:00 and 10:00:00

in the morning. The patients were instructed not to eat or drink, not smoke or brush their teeth 1 hour before saliva collection to minimize the risk of contamination (Bonfitto *et al*, 2009).

Analysis of antibody titers in saliva, namely anti-PGL1 in saliva, was determined by ELISA using 96-well polystyrene plates (Costar, Cambridge, USA). The plates were coated with antigens (PGL-1) in sodium carbonate buffer (3 mg/10 ml) for 24 hours at 4°C.Saliva from each patient was diluted 1: 4 in PBS-Tween, 100 ìL was added to each with wells and plates incubated for 1.5 hours at room temperature. After washing, using antihuman IgA (goat) and anti-human IgM (rat) conjugate alkaline phosphatase (Sigma, USA) diluted 1: 250 and 1: 100 in PBS-T-0.05%, respectively, added to the well and incubated for 1 hour at room temperature.The plates are washed and incubated for 15 minutes at room temperature with a substrate solution containing p-nitrophenyl phosphate. The reaction was stopped with 3 M NaOH. Standard antigen, saliva, and conjugate concentrations are based on serial dilution to get the ideal concentration for testing. In addition, different incubation times and temperatures, the number of different washing cycles and dilutions of antigens are standardized. Absorbance was determined at 450 nm (Bonfitto *et al*, 2009).

Anti-PGL-1 antibodies in salivary samples can be used as an alternative for determining leprosy exposure so it is useful for detecting high-risk groups of leprosy. Measurement of *M. leprae* in specific saliva and anti PGL-1 IgA antibodies can detect the presence of bacilli, this is an important result because this technique can identify bacillus, which can be used as an easy, non-invasive and relatively inexpensive method of detecting leprosy in a population in the leprosy endemic region. There is good potential to use salivary tests instead of serum tests to detect subclinical leprosy. A multidisciplinary approach is needed, including health professionals such as doctors, nurses, laboratory technicians, dentists who can contribute to the determination of early diagnosis, assessment and rehabilitation of leprosy patients (Silva *et al*, 2007; Oskam *et al*, 2003; Da Rosa *et al*, 2003).

CONCLUSION

PGL 1 of IgA/IgM can be detected in saliva and it can be used as a biomarker in detecting subclinical leprosy.

REFERENCES

Banerjee S, Biswas N, Kanti Das N, Sil A, Ghosh P, Raja A H H, Dasgupta S, Datta P K and Bhattacharya B (2011) Diagnosing leprosy: revisiting the role of the slit-skin smear with critical analysis of the applicability of polymerase chain reaction in diagnosis. *Int. J. Dermatol.* **50**, 1522-1527.

Bonfitto N L B, Motta A C F, Furini R B, Komesu M C, do Nascimento M M P, Figueiredo J F C and Foss N T (2009) Determination of the Salivary Anti-Phenolic Glycolipid-1 Antibody in Leprosy Patients as a Tool to Monitoring Multidrugtherapy. *Am. J. Infectious Diseases* **5**. 2009

Britton W and Lockwood D (2004) Leprosy. *The Lancet* **363**(9416), 1209-1219.

Cabral P B, Alves R A, Medeiros K B, Castelo-Branco E S, Alencar C P and Nagao-Dias A T (2009) Use of serum and salivary anti-PGL1 markers as parameters for occupational exposure to Mycobacterium kustae. *Rev. PanamInfectol.* **11**, 21–26.

Cabral P B, Júnior J E C, Macedo A C, Alves A R, Gonçalves T B, Cabral T C B, Gondim A P S, Pinto M I M, Oseki K T, Camara L M C, Rabenhorst S H B and Nagao-Dias A T (2013) Anti-PGL1 salivary IgA/IgM, serum IgG/IgM and nasal *Mycobacterium kustae* DNA in individuals with household contact with leprosy. *Int. J. Inf. Dis.* **17**, e1005-10.

D'Abreu P C, Durães S M B, Estrela R R, Baltazar M C N P and Rochael M C (2000) Hansenía sevirchowiana diagnosticadaatravés de examehistopatológico de lesão oral empaciente com pênfigofoliáceo. *An. Bras. Dermatol.* **75**, 339-346.

Da Rosa F B, de Souza V C, de Almeida T A, do Nascimento V A, Vásquez F C, Cunha M G S and Naveca F G (2013) Detection of *Mycobacterium kustae* in saliva and the evaluation of oral sensitivity in patients with leprosy. *Mem. Int. Oswaldo. Cruz* **108**(5), 573-577.

Hansen G (1895) *Leprosy, in its Clinical & Pathological Aspects*. Bristol: John Wright & Co.

Indrayanti E S and Yuanita D U (2009) Overview of IgG and IgM Anti Phenolic Glycolipid-1 Mycobacterium leprosy in Islamic Boarding School Students WringinJajar Village Mranggen District Demak Regency. Media MedikaIndonesiana. *Semarang: Fakultas Kedokteran Universitas Diponegoro*. **43**(5), Hal: 220-223.

Infodatin, Pusat Data danInformasi Kementerian Kesehatan RI. Kusta. (2015). Jakarta:Kementerian Kesehatan RI.

Kementerian Kesehatan Republik Indonesia (KEMENKES RI) (2015) Menkes canangkanresolusi Jakarta gunahilangkan stigma dandiskriminasikusta. Di akses 19 Januari 2020 dari: *http://www.depkes.go.id/article/view/15012700001/menkes-canangkan-resolusi-jakarta-guna-hilangkan-stigma-dan-diskriminasi-kusta.html*

Malathi M, Rajesh E, Babu N and Jimson S (2016) Saliva as A Diagnostic Tool. *Biomed. Pharmacol. J.* **9**(2), 867-870.

Margarido L C and Rivitti E A (2005) Hanseníase. In : Focaccia R (sci. ed.). Tratado de infectologia, 3rd ed., Atheneu, São Paulo, p. 939-972.

Martinez T S, Figueira M M N R, Costa A V, Gonçalves M A, Goulart L R and Goulart I M B (2011) Oral mucosa as a source for M. kustae infection and transmission and implications of bacterial DNA detection and the immunological status. *Clin Microbiol Infect.* **17**, 1653-1658.

Feasey N, Wansbrough-Jones M, Mabey D C W and Solomon A W (2010) Neglected tropical diseases. *British Medical Bull.* **93**(1), 179–200.

Nsagha D S, Bamgboye E A, Assob J C N, Njunda A L, Kamga H L F and Bissek A Z (2009) Elimination of leprosy as a public health

problem by 2000 AD: an epidemiological perspective. *Pan. Afr. Med. J*. **9**, 4.

Ongole R, Ravikiran and Praveen B N (2012) *Textbook of Oral Medicine, Oral Diagnosis, and Oral Radiology*. New Delhi: Elsevier, p. 265.

Oskam L, Slim E and Buhrer-Sekula S (2003) Serology: recent developments,strengths, limitations and prospects: a state of the art overview. *Lepr. Rev*. **74**, 196-205. *http://www.ncbi.nlm.nih.gov/14577464*

Patrocínio L G, Goulart I M, Goulart L R, Patrocínio J A, Ferreira F R and Fleury R N (2005) Detection of *Mycobacterium kustae* in nasal mucosa biopsies by the polymerase chain reaction. *FEMS Immunol. Med. Microbiol*. **44**, 311-316.

Phetsuksiri B and Rudeeaneksin J (2006) A simplified reverse transcriptase PCR for rapid detection of *Mycobacterium kustae* in skin specimens. *FEMS Immunol.Med. Microbiol.* **48**, 319-328.

Pinheiro R, de Souza Salles J, Sarno E and Sampaio E (2011) Mycobacterium kustae –host-cell interactions and genetic determinants in leprosy: an overview. *Future Microbiology* **6**(2), 217-230.

Pontes A R B, Almeida M G C, Xavier M B, Quaresma J A S and Yassui E A (2008) Detecção do DNA de Mycobacterium kustaeemsecreção nasal. *Rev. Bras. Enferm*. **61**, 734-737.

Rai B (2007) Oral Fluid in Toxicology. *The Internet J. Toxicol.* **3**(2).

Ridley D and Jopling W (1962) A Classification of Leprosy for Research Purposes. *Leprosy Review* **33**(2).

Rodrigues L C and Lockwood D N J (2011) Leprosy now: epidemiology, progress, challenges and research gaps. *Lancet Infect. Dis*. **11**, 464–470.

Silva E A, Iyer A and Ura S (2007) Utility of measuring serum levels of anti-PGL-I antibody, neopterin and C-reactive protein in monitoring leprosy patients during multi-drug treatment and reactions. *Trop. Med. Int. Health* **12**, 1450-1458. DOI: 10.1111/j.1365-3156.2007.01951.x.

Spencer J S and Brennan P J (2011) The Role of Mycobacterium kustae Phenolic Glycolipid I (PGL-I) in Serodiagnosis and in the Pathogenesis of Leprosy. *Lepr. Rev*. **82**(4), 344-357.

Spencer J, Duthie M, Geluk A, Balagon M, Kim H, Wheat W, Chatterjee D, Jackson M, Li W, Kurihara J, Maghanoy A, Mallari I, Saunderson P, Brennan P and Dockrell H (2012) Identification of serological biomarkers of infection, disease progression and treatment efficacy for leprosy. *Mem. Inst. Oswaldo Cruz* **107**, 79-89.

United Nation (2015) *The Millennium Development Goals Report 2015*, UN, New York, NY, USA, 2015.

Yoshizawa J C, Schafer C A, Schafer J J, Farrell J J, Paster B J and Wong D T W (2013) Salivary biomarkers: toward future clinical and diagnostic utilities. *Clin. Microbil. Rev.* **26**(4), 781-91.

World Health Organization (WHO) (2016) Effectiveness of MDT: FAQ. Diakses 20 Januari 2020dari*http://www.who.int/lep/mdt/effectiveness/en/*