

Serological Antibody Profile *IgM* and *IgG* of *Mycobacterium leprae* *PGL-1* and *L-ESAT-6* in Patients and Household Contact from Leprosy Endemic Area in East Java Indonesia

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Abstract: Leprosy patients in Indonesia is the third largest in the world. The problem of high transmission is the difficulty of early detection of leprosy. At present, diagnosis leprosy still based on clinical sign. Some of supporting diagnostic tools are developed, such as serological test. The most widely used antigen for diagnostics is *Phenolic-glycolipid-1* (*PGL-1*), but some limitations of the antigen, provide a challenge to find a potential candidate antigen representing specific *Mycobacterium leprae*. Purpose of this research is for studying *Mycobacterium leprae* *L-ESAT-6* (epitope AA11-36) compare to *PGL-1* to leprosy patients and household contacts in leprosy endemic region. Analysis have been conducted to MB and PB leprosy patients, as well as their families with total 173 respondents by testing Indirect ELISA of the *L-ESAT-6* and *PGL-1*. In general, the profile of ELISA test anti *PGL-1* vs *L-ESAT-6* in all the patients don't have significant difference, but in household contacts, with the Pearson correlation test, it can be concluded that there are significant difference between *PGL-1* and *L-ESAT-6* (p -value = 0.049; $p < \alpha = 0.05$). Healthy individuals who are exposed to *M.leprae* found high titers of antibody anti *L-ESAT-6* and lower profile of antibody anti *PGL-1*. It is assumed that the individual is relatively immune to *M.leprae*. So it can be concluded that *L-ESAT-6* (AA11-36) can be used as candidate diagnostic test which is a predictor marker for people living in endemic leprosy areas, for it still need a further research.

1 INTRODUCTION

Leprosy in Indonesia still become a health problem with the discovery of new cases is still increasing from year to year (WHO, 2016). The most important cause of the high incidence rate is the difficulty of early detection which play an important role in the transmission process so that subclinical leprosy stage continues to be manifest (Agusni, 2001). Diagnostic tools to detect leprosy is still limited, based on clinical sign. Laboratory testing based on a serological by the method of ELISA (*Eenzyme Linked Immunosorbent Assay*) and MLPA method (*Mycobacterium leprae particle agglutination*) is used mostly using *PGL-1* (*Phenolic-glycolipid-1*), but the lack of this antigen, provide a challenge for finding a potential biomarker used for early detection of leprosy (Spencer *et al*,

2012). Inspired from the successful use of *Mycobacterium tuberculosis* ESAT-6 (T-ESAT-6) for the detection of *M. tuberculosis* associated specific responses (Parkash *et al*, 2007), we have previously found that the use of ESAT-6 of *M. leprae* was limited. *Mycobacterium leprae* ESAT-6 (L-ESAT-6) is a protein secreted by the extracellular *M.leprae* molecular weight 6kDa and an antigen peptide representing specific epitopes of *M. leprae* potent. L-ESAT-6 encoded by the genes of pathogenic RD1 consists of 95 amino acids that has multiple epitopes of which are located on the N-Terminus (Geluk *et al*, 2002). In previous studies conducted by Kurdi (Kundi *et al*, 2010) on epitope tracking with B cell epitope scanning techniques, has proven that serum leprosy patients type MB, PB and subclinical leprosy reactive against L-ESAT-6 (AA

11-36), while in healthy people in endemic areas no such reactivity. Those studies also have found three types of epitopes of the N-Terminus region of the L-ESAT-6 as follows: epitope markers leprosy of type LL and BB leprosy (MB) is LEQCQES (28-34), epitope VNELQG (14-19) which is an epitope markers of type TT leprosy (PB), epitope IDALLE (24-29) are only reactive with antibodies contained in the sera of healthy household contact (HHC) group. What is interesting in this case, that the three epitopes found is not in the primary structure of the T-ESAT-6 (*Mycobacterium tuberculosis*) so this epitopes are highly specific to *M.leprae* (Spencer *et al*, 2002). *M.leprae* is uncultivable mycobacteria, production of synthetic proteins was conducted on L-ESAT-6 (AA11-36). The purpose of this study was to analyzed the profile of specific antibodies reaction of L-ESAT-6 (AA11-36) compare to PGL-1 against leprosy patients and household contact. The development of tools is important to facilitate diagnosis and provide a more thorough understanding of transmission and the incidence of *M.leprae* infection in high endemic regions, even throughout the country.

2 METHODS

This study was approved by national ministry of health and local ethic commission from Dr.Sutomo Hospital Surabaya and participants were included only after signing written informed consent forms. Patients groups has the following inclusion criteria: newly diagnosed and previously untreated or recently diagnosed and within the first 3 months treatment with WHO-MDT. Household contacts of both MB and PB leprosy patients were recruited as a group at elevated risk of subclinical leprosy. Samples consisted of 3 groups, leprosy patients Multibacillary (MB) type, Paucibacillary (PB) type and household contact (HHC), each of the groups were 42, 36 and 95 respondents respectively and they were taken from a district in Lamongan, one of the endemic areas in East Java Indonesia. The number of samples is calculated based on the formula *stratified random sampling*.

2.1 ELISA (Enzyme Linked Immunosorbent Assay)

A total of 3 mL of blood serum isolated to then proceed with the analysis of indirect ELISA. The antigen is a synthetic antigen : PGL-1 (NT-P-BSA) and synthetic L-ESAT-6 (epitop AA 11-36) include 3 epitope markers for MB, PB and HHC, consists of LEQCQES (28-34), VNELQG (14-19), IDALLE

(24-29)-N-terminus labeled with Biotin. Antigen diluted 1 mg / ml with carbonate buffer, all components coated into 96-wells microtiter plates (Nunc, Maxisorp) for L-ESAT-6, microplate coated with streptavidin. Blocking buffer consisting of 1% skimmed milk / PBS and serum total diluted 1/300 in 0.1% skimmed milk / PBS / Tween-20. Samples were analyzed in duplicate and incubated for 1 hour at room temperature. The wells were washed with PBS-Tween20, and then incubated with horseradish peroxidase (- HRP-) conjugated antibodies (Dako, Denmark) and then diluted to 0.1% skimmed milk/PBS/Tween-20.

After washing, the plates stained with substrate ortho-phenilen-diamine (OPD) and peroxidase 30% (MERCK) in phosphate-citrate buffer and incubated until developed a yellow color and stopped with 1.25M H₂SO₄. Both antibody IgM anti-NT-P-BSA and antibody IgG anti-L-ESAT-6 were measured. The antibody titer was measured by optical density (OD) of all the wells that have been read at a wavelength of 450 nm and a reference wavelength at 492nm. Real OD obtained from reduction of OD in both wavelengths (delta-OD) and converted automatically by the BIOLISE software to unit/mL.

2.2 Evaluation of ELISA Test Results

Interpretation of the ELISA test result is to see the yellow signal above background values, it is recommended to use the plot algorithm. The results of diagnostic tests in the form of quantitative data and for the statistical analysis used Pearson correlation test by SPSS (Statistical Package for the Social Sciences version 16.0).

3 RESULTS

3.1 Distribution of Samples

There were totally 173 inhabitants who participated in this study, consisting of 69 respondents (39.88%) were male and 104 (60.12%) were female. In terms of age, respondents are divided into 3 groups: children (0-21 years), adults (22-45 years) and elderly (more than 45 years) and the adults group is the most frequent 56.7% (98/173).

Table 1. Distribution of the respondents

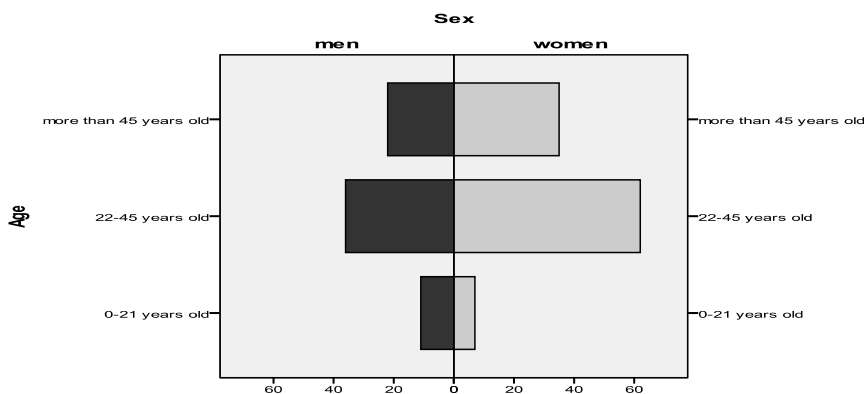


Table 2. Antibody reaction of IgM anti PGL-1 and IgG anti L-ESAT-6

Antibody Titer (unit/mL)	MB patients	PB patients	Household Contacts (HHC)
	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)
IgM anti PGL-1	32136.07±184706.60 (43-126870)	1508.36±1955.23 (219-8240)	774.93±752.79 (31-4332)
IgG anti L-ESAT-6	990.28±1232.37 (48-6300)	575.5±349.38 (234-1650)	404.21±268.43 (108-1512)

3.2 Antibody-specific Responses in PGL-1 and L-ESAT-6

Pearson correlation test, shows the levels of IgM anti PGL-1 with IgG anti L-ESAT-6 epitope LEQCQES, epitope marker for MB patients showed the value of $p = 0.598$, $p > 0.05$. It concluded that there was no correlation between them. Pearson correlation test shows the levels of IgM anti PGL-1 with IgG anti L-ESAT-6-epitope VNELQG, specific epitope for PB patients is obtained $p = 0.962$, $p > 0.05$. It concluded that there was no correlation between the levels of IgM anti PGL-1.

The levels of IgM anti PGL-1 and IgG ESAT-6 epitope IDALLE, specific epitope for healthy household contacts showed the value of $p = 0.049$ ($p < 0.05$). It can be concluded that there is a significant correlation between the levels of IgM anti PGL-1 with IgG anti L-ESAT-6- epitope IDALLE.

The strength of the correlation is 0.584 it can be seen from the Fig.2 that there is a pattern of negative correlation between the levels of IgM anti PGL-1 with IgG anti L-ESAT-6-IDALLE.

4 DISCUSSION

The most extensively evaluated serologic test for leprosy is still based on the detection of IgM anti-PGL-1, because highly titer of anti-PGL-1 serology reflects the bacillary load and has limited application for diagnosis of all clinical forms of leprosy (8,9,10), but according to the profile of IgM anti-PGL-1 compare to IgG ESAT-6, it seems this antigen still recommended as an important adjunct tool for the differentiation of PB and MB leprosy. For IgG anti L-ESAT-6 profile, similar to anti-PGL-1 serology, the presence of IgG antibodies that react to protein antigens reflect to the bacillary load as well. Most MB patients have high IgG titres but few PB patients are responsive. The serological profile antibody anti- L-ESAT-6 with specific epitope of MB and PB patients has not been able to support the lack of PGL-1.

Interesting result shows at the comparison between IgM anti-PGL-1 and IgG anti-L-ESAT-6 with epitope IDALLE specific for healthy household contacts. It has a negative significant correlation with PGL-1 so that it can be said that healthy individuals exposed to *M.leprae* if antibodies are found with high

titers of L-ESAT-6 epitope IDALLE and could suppressed the development of bacterial load in the body which indicated from the titer of IgM anti-PGL-1, then it is certain that the individual is relatively immune against *M.leprae*. This correlation means that household contacts with the greater risk of becoming leprosy has the greater protective antibody against leprosy.

5 CONCLUSION

In the future it might said that antigen L-ESAT-6 can be a candidate for predictor marker in order to develop an alternative tools for detecting leprosy in the early stage. For these purpose still needed further research. The result of IgG anti-L-ESAT-6 in leprosy patients might have a different profile if possible tested in L-ESAT-6 epitope IDALLE that specific for healthy household contact. This can be also as a suggestion for further research.

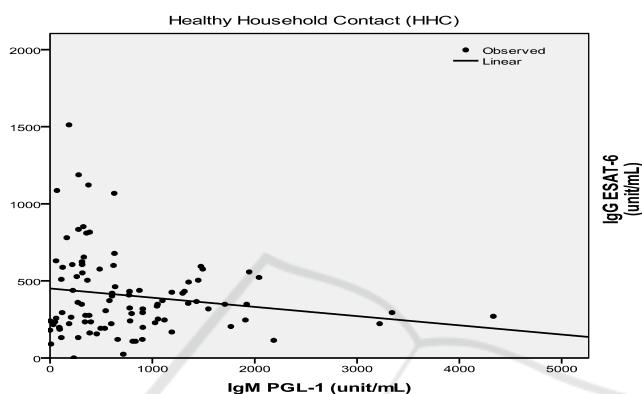


Figure 1. Antibody responses of leprosy patients and household contacts. Multibacillary (MB; n=42), Paucibacillary (PB; n=36), healthy household contacts of confirmed patients (HHC; n=95) were assessed. Results from each individual serum sample against the mean titer in units/mL both from antibodies anti PGL-1 (IgM) (A) and anti ESAT-6 (IgG) (B)

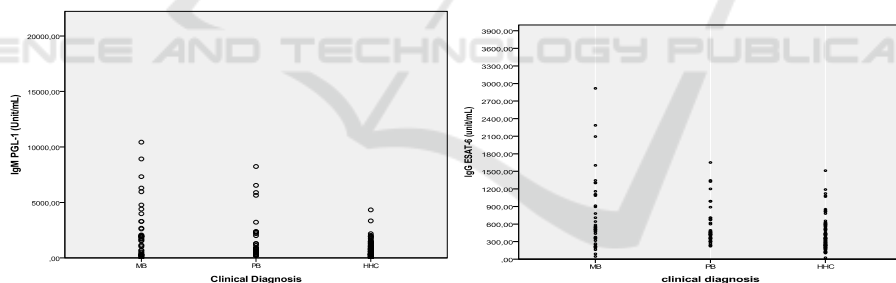


Figure 2. Antibody responses of IgM anti-PGL-1 and IgG anti- *Mycobacterium leprae* protein antigen ESAT-6 epitope IDALLE from household contacts (HHC)

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