The Overview of Anti-PGL-1 and Anti-IDALLE L-ESAT 6 IgG Antibody Titers in Household Contact of Leprosy

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Abstract Household contact is a high risk group for leprosy transmission. Household contact with high titer of Glycolipid-1 phenolic possesses higher risk to develop into leprosy. This study was an observational study. The subject of the study consisted of 95 household contacts living at the house of leprosy patient in Brondong Subdistrict, Lamongan Regency, East Java, Indonesia whom anti-phenolic Glycolipid-1 and anti IDALLE L-ESAT 6 IgG antibody titers were measured. Measurement of anti-PGL-1 and anti-IDALLE L-ESAT 6 IgG levels used the enzyme linked immunosorbent assay (ELISA) technique. The mean levels of anti-PGL-1 IgG in all subjects were 134.54 units / mL (± 295.15) while the levels of anti-IDALLE L-ESAT 6 IgG were 408.61 units / mL. There was a significant positive relationship between levels of anti-PGL-1 IgG with anti-IDALLE L-ESAT 6 IgG ($r = 0.247$, $p = 0.016$) in household contact of leprosy patient.

1 INTRODUCTION

Indonesia is in the third place of the most number of leprosy sufferers in the world after India and Brazil. In ASEAN area, Indonesia is in the top ranking. Myanmar is in second place and the Philipine is in third place (WHO, 2016). One of the problems in control effort of leprosy in Indonesia is lack of the subclinical stage of leprosy control. Leprosy in subclinical stage in does not show any symptoms of leprosy. Meanwhile, in laboratory, it have shown specific antibody against *Mycobacterium leprae*. This antibody is an antibody against one of specific epitopes from *Mycobacterium leprae* that has been known nowadays, such as Phenolic Glycolipid-1 (PGL-1) 36 kDa and 12 kDa proteins, as well as *Mycobacterium leprae* ESAT-6 (L-ESAT 6) (Agusni, 2002; Izumi, 1999).

High antibody titer against PGL-1 (>605 Unit/mL) on healthy population who had a history of having contact with leprosy is an indicator of high risk for becoming leprosy manifest later on.2,7 Detection of leprosy in subclinical stage could be done by serological test of anti PGL-1 through ELISA technique. The examination of IgG and IgM serum of anti PGL-1 have sensitivity value in 30%, however, it had high specificity in 97% (Izumi, 1999; Noordeen, 1994). Household contact with seropositive leprosy against PGL-1 antibody have high risk to suffer leprosy (particularly for multibacillary) rather than seronegative household contact against PGL-1 (Agusni, 2002; Izumi, 1999). In Venezuela, it is stated that the level of anti PGL-1 antibody on household contact of leprosy has high risk of suffering leprosy in 4 years later. Meanwhile, in Philipine, it is stated that household contact of seropositive PGL-1 has risk 24 times greater for suffering leprosy. (Douglas et al., 2004; Richardus, 2004).

IgM antibody appears in 1-2 weeks after M. leprae attack and would reside in 2-3 months or more. Detection of specific IgM level against microbe in serum is consistent with medium infection or ongoing infection to the host. IgG would increase in 2-3 weeks after having contact with antigen or infected germ and it would reside and be detected in lifetime. IgG shows immune respond against chronic disease, which

meant that although the sufferer is not ill, but ever being exposed to M. leprae antigen. IgM would be in contrary, IgM against PGL-I shows the sufferer has chronic immune respond or suffering leprosy. The activity of

immune respond in the body was apparently spurred by various components of lipid sugar antigen, which was PGL-I and protein component of M.leprae. Component of carbohydrate and lipid (PGL-I) could spur the immune respond, however, the immune respond that occurred is not strong enough for eradicating M.leprae. (Noordeen, 1994; Rote, 2006).

One of the antigens from the class of protein by M. leprae is IDALLE L-ESAT 6, which has potency in arousing the antibody that is protective against M.leprae. (Geluk et al., 2002; Kurdi et al., 2010; Spencer et al., 2002). High level of anti IDALLE L-ESAT 6 IgG antibody on household contact who lived in endemic area of leprosy could protect the household contact to develop into leprosy in subclinical stage. This case is perhaps caused by the epitope of IDALLE L-ESAT 6 that does not only stimulate the appearance of the antibody, but also stimulate the appearance of cellular immune (Geluk et al., 2002; Kurdi et al., 2010; Spencer et al., 2002). There is still limitation for study to analyze IgG antibody of anti IDALLE L-ESAT 6 that become valuable information which could be obtained from the result of this study. Therefore, the aim of this study was to analyze the correlation between the levels of anti PGL-1 natural IgG antibody and anti IDALLE L-ESAT 6 protective IgG antibody.

2 METHOD

There were 95 people of household contact in Brondong Subdistrict, Lamongan Regency, East Java, Indonesia, recruited in this study. They had undergone of having contact with leprosy sufferer. Moreover, it was done laboratory check for obtaining the value of IgG and IgM serum of anti PGL-1 and IgG of anti IDALLE L-ESAT 6 by using enzyme linked immunoabsorbent assay (ELISA) technique.

Data normality of IgG level of anti PGL-1 and IgG of anti IDALLE L-ESAT 6 were tested using by Kolmogorov-Smirnov statistical test, which resulted that the data were not normally distributed. Thus, the correlation test between natural antibody of IgG anti PGL-1 level to protective antibody of IgG antibody of anti IDALLE L-ESAT 6 level was using by Spearman-Rank's correlation test.

3 RESULT AND DISCUSSION

There were 95 participants who in average was 40 ± 10,8 years old and who the 31 respondents (32,6%) were male and 64 respondents (67,4%) were female. Among the 95 research subjects were known that there were 48 participants (50,5%) were group of leprosy in subclinical stage which had the level of IgM serum of anti PGL-1 above 605 Unit/mL. The others (47 respondents (49,5%) were in a group that had IgM level of anti PGL-1 under 605 Unit/mL (seronegative). The mean of IgG level of anti PGL-1 in all research subjects was 134.54 ± 295.15 Unit/mL, meanwhile, IgG level of anti IDALLE L-ESAT 6 was 408.61 ± 263.92 Unit/mL (See also Table 1 below).

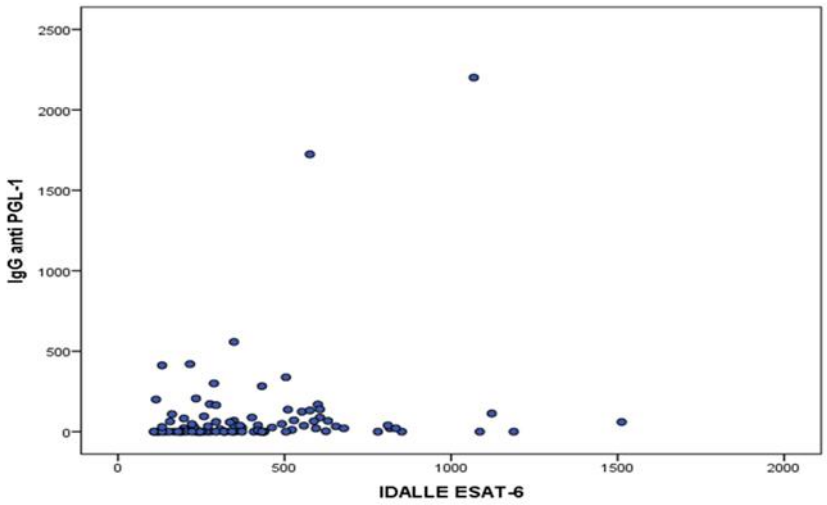
Table 1 Serum Levels of anti PGL-1 Natural IgG Antibody and anti IDALLE L-ESAT 6 Protective IgG Antibody Among Household Contacts in Endemic Leprosy Area of Brondong Subdistrict, Lamongan Regency, East Java, Indonesia.

Variables	Serum Level (min-max)	Mean ± SD
anti PGL-1 IgG (Unit/mL)	3 – 2201	134.54 ± 295.15
anti IDALLE L-ESAT 6 IgG (Unit/mL)	108 – 1512	408.61 ± 263.92

Spearman-Rank's correlation test, p= 0.02, r= 0.25

There was a significant positive correlation between natural IgG antibody of anti PGL-1 and protective IgG antibody of anti IDALLE L-ESAT 6 levels (Spearman-Rank's correlation test, p= 0.02 r= 0.25) among Household Contacts in Endemic Leprosy Area of Brondong Subdistrict, Lamongan Regency, East Java, Indonesia. Support this correllation analysis result, picture 1 bellow explain the relationship between IDALLE L- ESAT 6 and anti PGL-1 IgG antibodies.

In order to construct the diagnosis against leprosy in subclinical stage, the standard test that has used is anti PGL-1 IgM antibody, which is stated that it is positive leprosy in subclinical stage if its the level is > 605 Unit/mL (Agusni, 2002; Izumi, 1999). Serum of IgM antibody is primary respond if in the body is infected by *Mycobacterium leprae*. IgM antibody is formed in relatively short time or even directly when the body was infected by *Mycobacterium leprae*. IgM antibody of anti PGL-1 would arouse in first week when it was infected by *Mycobacterium leprae*. anti PGL-I IgM shows that



Picture 1 Scatterplot between IDALLE L-ESAT 6 IgG and anti PGL-1 IgG

the sufferer has chronic immune respond or has been suffering leprosy. Meanwhile, IgG anti PGL-1 shows immune respond against chronic disease, which means that although the sufferer is not ill, the sufferer has ever exposed by *Mycobacterium leprae* antigen (Noordeen, 1994). If it is detailed further from several previous researches, which in every condition (leprosy in manifest of PB and MB, leprosy in subclinical stage, even healthy person/seronegative). IgM titer of anti PGL-1 would always be higher rather than anti PGL-1 IgG, whereas, in theory concept of either primer immune responds or secondary immune responds, it should be occurred in contrary. The possibility is the occurrence of PGL-1 antigen could not activate the interaction between macrophage and lymphocyte, thus, there is no activation of the B cells to produce IgG. Moreover, it is correlated to PGL-1 form that is included a polysaccharide, not a protein. Hence, it is difficult to be provided by macrophage.(Abulafia and Vignale, 1999). Besides, it is also because antibody respond that is occurred is only primary respond and it is not ever occurred secondary respond. However, it correlated with the number and interval of stimulation by antigen. If antigen that entered the body is only a little by little with short interval, it would be occurred little form of antibody. Nevertheless, if the interval between primary antigen and secondary antigen is quite far and the antigen number that entered in next contact is quite big, it would be formed antibody in great number (Noordeen, 1994).

Meanwhile, protective IgG antibody of anti IDALLE L-ESAT 6 showed that someone has ever exposed by *Mycobacterium leprae* antigen chronically, but the person is still healthy and did not develop into leprosy in subclinical stage. The result of the research in which there was a positive correlation between natural IgG antibody of anti PGL-1 level and protective IgG antibody anti IDALLE L-ESAT 6 level showed that the higher the natural IgG antibody of anti PGL-1 level, it would be followed by the increase of protective IgG antibody of anti IDALLE L-ESAT 6 level. This is in accordance with the theory, which if the antibody is from IgG class, it is a long-term respond from the body against exposure of an antigen. Natural IgG antibody of anti PGL-1 could not give an immune, but it was only as an instruction that the person has been exposed chronically with *Mycobacterium leprae*. However, it is different with protective IgG antibody of anti IDALLE L-ESAT 6 that could give an immune against *Mycobacterium leprae* (Geluk et al., 2002; Hadi et al.,2017.; Kurdi et al., 2010; Spencer et al., 2002).

Both antigens from these Mycobacterium lepraewere from 2 different kinds. PGL-1 is a class of polysaccharide, meanwhile, IDALLE L-ESAT 6 is a class of protein. As what has been known that if an antigen is from polysaccharide such as PGL-1, the antigen could induce directly to the B cell. Hence, it influences plasma cell for producing antibody. However, the produced antibody is as an impact of the respond against PGL-1 which is known to have no role in the immune against Mycobacterium leprae because it could not arouse the respond of cellular immune. It is different from IDALLE L-ESAT 6, which this antigen is a protein

class. If an antigen is from protein class, the immune respond that is formed it is possible through activation path of lymphocyte T CD4 by macrophage in which besides inducing the release of cytokine Th1 or Th2, it also would send the signal to the B cell so that it also produces specific antibody. Thus, it would be produced antibody from IgG class.

The polypeptide of IDALLE L-ESAT 6 would be provided by macrophage through Toll receptor (TLR)-2, hence, it stimulates macrophage to produce nitric oxide and reactive oxygen intermediates that would lyse M. leprae and IL-1β and IL-12 which would stimulate cellular respond of T-CD4 type 1 (Th1) in form of IFN-γ secretion. The secretion from IFN-γ later would activate and make macrophage in more active. Thus, it would be occurred reaction series, growth obstacle, and germ multiplication (Brycesson and Pfaltzgraft, 1990; Cutolo et al., 1998; Rote, 2006).

3 CONCLUSIONS

The average level of natural serum IgG antibody of anti PGL-1 on 95 research subjects was 134.54 ± 295.15 Unit/mL, meanwhile, the level of protective serum IgG antibody of anti IDALLE L-ESAT 6 was 408.61 ± 263.92 Unit/mL. There was a significant positive correlation between natural IgG antibody of anti PGL-1 level and protective IgG antibody of anti IDALLE L-ESAT 6 level. It means household contacts with the greater risk of becoming leprosy has the greater protective antibody against leprosy.

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