

# The Correlation Between Subclinical Stage of Leprosy and Antibody Level of IDALLE L-ESAT 6 on Household Contact in Endemic Leprosy Area

Moch Irfan Hadi<sup>1</sup>, Soejajadi Keman<sup>2</sup>, Indropo Agusni<sup>3</sup>, Chatarina Umbul Wahyuni<sup>4</sup>

<sup>1,2,&4</sup> Faculty of Public Health, Airlangga University, Indonesia

<sup>3</sup> Faculty of Medicine, Airlangga University, Indonesia

## Abstract

Leprosy is an infectious disease caused by *M. leprae*. One of a high-risk group of leprosy is people who live at home together with the lepers. Since a long ago, it had been known that there was a certain subclinical stage in leprosy. In this case, the leper doesn't show the clinical symptoms of leprosy, but it can be found the antibody against the leprosy bacillus, especially Phenolic Glycolipid-1 in his/her blood serum. Moreover, this research aimed at analyzing the correlation between the occurrence of subclinical stage of leprosy and the antibody against protective epitopes of IDALLE L-ESAT 6, which was discovered by Kurdi et al. (2010) in a group of healthy nurse who had treated the lepers more than 10 years. This observational research utilized cross sectional approach. The total sample was 95 people of household contact in a house with the lepers at Brondong sub-district, Lamongan district, East Java, Indonesia. Subclinical stage of leprosy was measured based on titer of IgM anti PGL-1 by utilizing ELISA method. In addition, a level of protective antibody of IgG anti IDALLE L-ESAT 6 was also measured by utilizing ELISA method. The results showed that 48 samples were seropositive household contact PGL-1 ( $1286.6 \pm 756.428$  U/ml) and 47 samples were seronegative household contact PGL-1 ( $287, 06 \pm 152,084$  U/ml). The mean of IgG anti IDALLE L-ESAT 6 at PGL-1 seropositive household contact was  $344.80$  U/ml, whereas, PGL-1 seronegative household contact was  $508.09$  U/ml. However, it was found that cut off of antibody anti IDALLE L-ESAT 6 was in  $348$  U/ml with 50% percentile calculation. By applying simple logistic regression analysis, it was known that there was a correlation between the occurrence of subclinical stage of leprosy and the level of IDALLE L-ESAT 6, the higher antibody level toward the IDALLE L-ESAT 6, in household contact the higher protection against *M. leprae*. Therefore, it would not grow to be subclinical stage of leprosy.

**Keywords:** household contact, leprosy, IDALLE L-ESAT 6, Phenolic Glycolipid-1

## I. INTRODUCTION

Leprosy is an infectious disease that is caused by *Mycobacterium leprae*, in which the transmission of this disease proceeds chronically. In a term of leprosy, it has known 'household contact'. It is a condition which the people had high risk for being infected by leprosy disease due to interaction frequency/ having contact intensively with germ sources. The lepers, particularly multibacillary (MB) lepers and is also a sufferer who has not gotten regiment of medical treatment of Multi Drug Therapy (MDT) from WHO which is a major germ source in environment. The main requirement of leprosy transmission is the long, close, and continuous relationship among the lepers, particularly multibacillary (MB leprosy) with prospective patients. Recently, the condition that became an attention from the development of leprosy disease was a sufferer who had subclinical symptom subclinical stage of leprosy was a condition which *M. leprae* germ had entered into the body and caused immune response that resulted a specific antibody toward *M. leprae*. The specific antigen of *M. leprae* that was known and agreed upon by the experts was Phenolic glycolipid 1 (PGL-1). This PGL-1 was produced by life germ and would be more less if the germ was dead for example due to anti leprosy treatment. This antigen of PGL-1 could cause humoral specific of the immune respond, but it could stimulate cellular immune response. Thus, it would not cause the immune system. Cellular immunity was an appropriate immunity to eliminate *M. leprae* which had an obligate intracellular characteristic.

Subclinical stage of leprosy was an episode in leprosy that it would be passed someone before clinical leprosy. In East Java, subclinical stage of leprosy was if in serology test to the household contact was obtained that value of antibody of IgM anti PGL-1 was above  $605$  U/ml (Agusni, 2002). In epidemiology, 85% people who lived in endemic areas were resistant toward the bacillus of *Mycobacterium leprae*. The others (15%) was a susceptible group or seropositive group of leprosy that underwent subclinical infection from *Mycobacterium leprae*, then, this subclinical group which 15% of the population was perhaps only in 5% would change to be manifest leprosy. Moreover, it had been suspected in subclinical stage of leprosy (KSS) that innate immunity phase had been passed because in the subclinical case, the leper had formed specific antibody against *M. leprae* which was antibody of IgM anti PGL-1.

Until recently, the question of how the transmission of leprosy was still debated among the experts. However, it was believed that leprosy could be transmitted from the leper of multibacillary (MB) type to other people by direct

transmission. The way of transmission still had not been known, but most of the experts were said that leprosy could be transmitted through respiratory tract and skin (Job et.al, 2008; Melo et.al, 2009; Naves et.al, 2013).

The immune response of leprosy was begun from innate immunity in which macrophage had a role to catch and phagocyte *M.leprae*. In this phase, IL-12 cytokine was produced by macrophage, then IL-12 stimulated Natural Killer (NK) cell and lymphocyte T produced IFN- $\gamma$ . After that, IFN- $\gamma$  would activate macrophage in order to kill *M.leprae*. If the macrophage was failed to kill *M.leprae*, the macrophage would become antigen presenter cell (APC) and it began to enter in the phase of adaptive immune response. In this specific phase, IL-2 acted. In T cell, IL-2 had a role in the process of proliferation in terms of increasing the synthesis of cytokines and regulating the growth of T cell. In NK cell, IL-2 gave an activation effect and proliferation of NK cell. Next, macrophage served strange antigen to naive T cell and it was occurred a contact and lymphocyte T cell would produce IFN- $\gamma$  to activate macrophage as the destruction of antigen. Macrophage that had caught and served antigen would activate lymphocyte T of CD<sub>4</sub> and CD<sub>8</sub>. Thus, it underwent the proliferation and differentiation to be some types of an active lymphocyte. Moreover, it was formed some types of Tc cell from CD<sub>4</sub> and CD<sub>8</sub> that would attack the macrophage which contained known antigen. Besides, leprosy was considered as immunologic disease because of its huge role of cellular immunity toward the patients. The degree of cellular immunity response would determine a spectrum of its leprosy. In a type of paucibacillary (PB) leprosy, the leper's cellular immunity was still good, while, in type of multibacillary (MB) leprosy, cellular immunity had been decreased that caused anergy. This was a condition which humoral immunity would increase (Abbas *et.al.*, 2007).

Besides PGL-1, *M.leprae* had several types of antigen from protein class that had been identified. This antigen included antigens of 12 kDa, 18 kDa, 28 kDa, 36 kDa, 55 kDa and 65 kDa. Protein class antigen was also owned by *M.leprae* and its nearest relative. *M.tuberculosis* was an early secreted antigenic target 6 kDa (ESAT-6). *M.leprae* of ESAT-6 was known as L-ESAT 6. It was a protein that was secreted by extracellular and done by *M.leprae* and having a molecule weight of 6 kilo Dalton (kDa). Spencer, *et.al* (2002) found that sequencing result of amino acid on *M.leprae* of ESAT-6 showed that only 36% of amino acid was homologous with what was on *M.tuberculosis*. Anti L- ESAT-6 of polyclonal, monoclonal and hybridoma T cell reacted only within a homolog protein. In addition, it could identify B and T cell epitopes.

Based on the facts above, L-ESAT-6 could be considered as specific antigen to diagnose leprosy. Geluk, *at.al* (2002) identified the characteristics of L-ESAT-6 that was homolog with *M.tuberculosis* of ESAT-6 (T-ESAT-6). *M.tuberculosis* showed 11 genes (Rv3867 up to Rv3877), which were needed to secret ESAT-6. Rv3870 and Rv3871 was AAA+ class AT Pases, meanwhile, Rv3877 was a huge trans membrane protein. Those 11 genes, except Rv3872, the rest of 10 genes were in *M.leprae* genome in similar genetic structure. On this basis, it was believed that *M.leprae* produced ESAT-6, such as *M.tuberculosis*. In addition, it was proved that the transcripts for L-ESAT-6 was with reverse transcription-PCR on nu/nu mouse-derived *M.leprae*, by using Thai strains of 53 and 4089 in biopsies of leprosy patients. Although the function of protein was not an obvious, both those things gave response of cellular mediation immune during infection (Cole, 2001).

A conducted study by Kurdi *et al.* (2010) who found that epitope of B cell of L-ESAT-6 was in several clinical spectrums of the lepers. By applying epitope scanning technique, it had been known and found that later on, epitope could be used as biologist marker to any spectrums of the clinical lepers. It had conducted the tracking of epitope from L-ESAT-6 antigen toward antibody in the sera of leprosy patient's subject in type of Lepromatous (LL), Borderline (BB) and Tuberculoid (TT) that was compared to serum-free leprosy and healthy nurse who had cared for leprosy patients in the leprosy hospital at least in 10 years. Furthermore, it had been discovered 3 kinds epitope of N- region Terminus L-ESAT-6 as follows:

1. An epitope of leprosy marker was LEQCQES (28-34) which was types of LL and BB leprosy.
2. An epitope VNELQG (14-19) was an epitope of leprosy marker type of TT.
3. An epitope IDALLE (24-29) was the only reactive within the antibody in the sera of healthy nurse group.

In this case, an interesting thing was not all three epitopes which were found were in primary structure of the T-ESAT-6 (*M. tuberculosis*), hence, it was very specific. Besides, the research findings also showed that the leper in type of LL, TT and healthy nurse without clinical symptoms showed reactivity to L-ESAT 6 at amino acid/AA 11-36: QGAVNELQGSQSRIDALLEQCQESLT, while, the serum of healthy people in endemic area of leprosy was not obtained reactivity against epitopes L-ESAT-6.

Regarding to the conducted study by Kurdi *et al.* (2010), he stated that IDALLE L-ESAT 6 was protective epitope which was found in leprosy serum of healthy nurse who had worked and cared the lepers in hospital, minimally over 10 years. If there was a household contact in a house with the lepers, those people would be healthy. In addition, they also would not be infected by the lepers, although they had long contact with them. However, it

was because an expression of antibody of IgG anti IDALLE L-ESAT 6 that had an important role toward these. Further, this research aimed at knowing the distribution and influence on antibody of IgG anti IDALLE L-ESAT 6 toward the lepers in subclinical stage of leprosy (seropositive toward PGL-1) on household contact in leprosy endemic area.

## II. METHODS

The type of this research was an epidemiology research in analytical observational immunology-based which in this research, the researcher conducted an observation, and then, analyzed the correlation between the occurrence of subclinical stage of leprosy on household contact in a house with multibacillary lepers. It was along with protective factor in antibody of IgG anti IDALLE L-ESAT 6. In addition, this research utilized cross-sectional design.

This research was conducted at Brondong sub-district, Lamongan district, East Java, Indonesia from January 2014 - March 2016. The population consisted of all people who lived in a house with multibacillary lepers who had not healed yet (in the last 3 years), minimally during 6 months. Based on the data of Public Health Center at Brondong sub-district, Lamongan district, there were 52 lepers who had not healed yet. From these lepers, there were 156 people of household contact. The sample of the research was obtained by using simple random sampling formula for 112 people. However, among those samples, there were 95 people who had a willing to participate for this research. Then, these samples would be divided into two groups which were seropositive household contact (the level of IgM anti PGL-1 > 605 U/ml) and seronegative household contact (the level of IgM anti PGL-1 < 605 U/ml). Technique of indirect ELISA test was used to know the antibody level of IgM anti PGL-1 and IgG anti IDALLE L-ESAT 6 in serum of a household contact who lived together in a house with the lepers in endemic area.

### A. The Substance for IgM Serology anti PGL-1 Test

The substances which were needed for IgM serology anti PGL-1 test were *coating Buffer* (pH=9,6), NTP BSA *solution*, *washing buffer*, *blocking buffer*, secondary antibody, *substrate solution*, sample serum, and *stopping solution*.

### B. Procedure of ELISA test for IgM Anti PGL-1 Detection

Firstly, entered 50 µL of *coating buffer* and antigen of NTP-BSA *working solution* into *micro plate* that had been divided appropriately with the schema and it was incubated for 1 hour in 37°C. Secondly, the *micro plate* was washed in 3 times by using *washing buffer* (liquid of PBST/phosphate buffered saline+ 0,05% tween 20). Thirdly, entered the *blocking buffer* 200 µL into the *micro plate*, and it was incubated for 1 hour in 37°C. Fourthly, washed the *blocking buffer*, then, entered 50 µL of serum that had been diluted with *dilution buffer* (1:300) into the *micro plate* and it was incubated again for 1 hour in 37°C. Fifthly, washed the *micro plate* with *washing buffer* in 3 times, then, entered 50 µL of second antibody (IgM which was located appropriately with the schema) into the *micro plate* and it was incubated again for 1 hour in 37°C (IgM was diluted with *dilution buffer* in 1:2000). After that, washed *micro plate* again with *washing buffer* in 3 times, *Substrate solution* was given in 100µL into *micro plate* until it changed to be yellow color or orange one. Coloring reaction was stopped after 10-20 minutes by adding 100 µL of *stopping solution*. Finally, it was counted the optical density (OD) by utilizing ELISA reader, then, the data was processed by certain software such as by *Biolise*.

### C. Materials and Test Device of IgG anti IDALLE ESAT 6

Some materials and devices which were used for testing antibody of IgG anti IDALLE L-ESAT 6 were micro titter plate that had been layered by streptavidin (Maxisorp, Nucn), IDALLE *biotinylated synthetic peptides*, *streptavidin coated plates*, which were layered before by BSA, *phosphate buffered saline*, bovine serum albumin, sodium azide PBS/Tween 20 (0,1% tween 20 in PBS), PBS/BSA/azide (0,1% BSA and 0,1% sodium azide in PBS), 2% BSA/PBS (PBS contained 2% BSA), conjugate goat *antihuman* IgG labeled HRP, *micro ELISA* reader.

### D. Procedures of ELISA test for Testing IgG Anti IDALLE ESAT 6

Peptide IDALLE ESAT 6 was synthesized by PT. Genetika Science Indonesia that cooperated with 1<sup>st</sup> BASE Peptide-Asia and located in Singapore. It was produced in dry powder and it was conjugated by biotin in *Micronics* tube. For its use, this *peptide* must be reconstituted in 200 micro liter of pure liquid of *dimethyl sulfoxide* or solvent/*water mixture*. Moreover, this Peptide must be diluted before as soon as possible before it was used as a work concentration of 1/1000 in PBS/BSA/azide, which was a PBS that contained 0,1% BSA and 0,1% *sodium azide*. Afterwards, it was diluted again until the concentration of 1/5000.

In the first stage was to make first emulsion with concentration of 1/100 that was made by utilizing *polypropylene* tube of 1 ml, and it was taken on shelf 8x12 by utilizing *multichannel* pipet. Then, moved the 10µL of aliquot

from peptide emulsion into each tube, and added 1 ml of PBS/BSA/azide. After that, the tubes were closed tightly and shaken (using vortex) so that the mixture became homogenous. The peptide that had been diluted could be saved in several days in 4°C and it could be saved longer if it was frozen in -80°C. Before, it was conducted a test, all reagents and plates which were layered by *streptavidin* were put in a room until the temperature was as similar as the room temperature. Peptide emulsion that labeled biotin 100 µL was poured into micro titter wells that had been layered by *streptavidin*. Afterwards, the plate was put on *shaker* for 1 hour in 20°C, hence, the peptide IDALLE that labeled biotin was bounded tightly on the wall of the well that was layered by streptavidin. Conducted a washing toward the plate of phosphate buffered saline (PBS 0,01 M sodium phosphate in 0,15 M sodium chloride, pH 7,2) that contained 0,1% (v/v) Tween 20 (PBS/Tween 20). For wasting residue of the liquid, put the plate in inverted position on filter paper. This stage was repeated in 4 times. The micro titter plate must be dried in 37°C before being saved in 4°C if it was not used as soon as possible. Then, diluted the serum that would be tested by using 2% BSA in PBS which was depended on the availability of antibody level, then, it was added 100 µL from serum liquid to every wells that had bounded the peptide IDALLE. Micro titter plate was put on shaker and incubated for a night in 4°C. The washing was repeated such as in the previous process. For detecting the antibody in bounded sample, it was added the 100 µL of conjugate emulsion that was consisted of goat anti-human IgG in label of HRP, then, it was incubated for 1 hour in 20°C. Conducted the washing again as the previous procedure, after that, the plate was washed twice only by using PBS in order to loss the residue of Tween. For next step, it was added 100 µL/ substrate well of H<sub>2</sub>O<sub>2</sub> 0,03% and chromogen of ABTS. Then, it was incubated for 45 minutes in 20°C in dark place. A reading was conducted by *micro titter* plate in ELISA reader with the wavelength of 405 nm or 492 nm for determining how big the absorbents that had been obtained.

### III. RESULTS AND DISCUSSION

#### A. Level of IgM Anti PGL-1 and Subclinical Stage of Leprosy on Household Contact

By utilizing Elisa test toward blood serum from the respondents of the research, it could be known that 48 (50,5%) respondents were group of subclinical stage of leprosy (seropositive toward PGL-1), meanwhile, there were 47 (49,5%) respondents who were seronegative toward PGL-1. Status of respondents' seropositivity was determined by the level of IgM anti PGL-1 that was tested. If the antibody value was more than equal to 605 U/ml, it would be categorized as seropositive (leprosy in subclinical stadium). This was based on *cut off* that had been found by Agusni (2002) particular for East Java area, Indonesia.

Table 1 Test Result of Antibody of IgM Anti PGL-1 on Household Contact with Leper at Brondong sub-district, Lamongan district in 2016

The Level of IgM anti PGL-1 (U/ml)	
subclinical stage of leprosy / seropositive toward PGL-1 (n=48)	Seronegative toward PGL-1 (n=47)
$\bar{x} \pm SD = 1286,600 \pm 756,428$	$\bar{x} \pm SD = 287,100 \pm 152,084$
<i>cut off</i> value of leprosy seropositivity $\geq 605$ U/ml (Agusni, 2002)	

Some researches regarding seropositivity of household contact from leper were such as conducted research by Tunggal *et al.* (2005) at Sampang, Madura, Indonesia which was known that there were 34 (49,3%) from 69 respondents were stated as subclinical stage (seropositive toward PGL-1). They were consisted of 19 persons of household contact who lived with MB leprosy patient and 15 household contacts who lived a house with PB leprosy. Besides, conducted research in Brazil by Pinho *et al.* (2015) toward 808 household contacts obtained that there were 113 (14%) from 808 respondents who were positive leprosy in subclinical stadium. Subclinical stage of leprosy case was not only occurred on adult person (> 15 years old), but there were so many research result of seroepidemiology which were known that subclinical stage of leprosy case also attacked on children age, particularly to children who lived in a house with leper (household contact). Iswahyudi (2012) concluded that there were 28 (29,5%) from 95 primary school children at Watestani Village, Nguling Sub-district, Pasuruan were subclinical stage of leper, and they were measured from titter antibody PGL-1 by utilizing filter paper method. However, the result was not different with conducted research by Sujagat *et al.* (2015) whose research was toward children who lived in a house with leper in area of Public Health Center of Pegirikan Surabaya. Moreover, they obtained that there were 8 (26,7%) from 30 respondents who were positive subclinical stage of leprosy.

#### B. Distribution of Antibody of IgG anti IDALLE L-ESAT 6 and Its Correlation with Subclinical stage of leprosy on Household Contact

Table 2. Distribution of Antibody of IgG Anti IDALLE L-ESAT 6 on Household Contact who lived in a house with Leper at Brondong sub-district, Lamongan district in 2016

<b>The Level of IgG anti IDALLE L-ESAT 6 (U/ml)</b>	
<b>Subclinical Stage of Leprosy/seropositive toward PGL-1 (n=48)</b>	<b>Seronegative toward PGL-1 (n=47)</b>
$\bar{x} \pm SD = 344,80 \pm 176,03$ U/ml	$\bar{x} \pm SD = 508,09 \pm 340,15$ U/ml

Based on table 2 above, it could be known that the mean of antibody level of IgG anti IDALLE-ESAT 6 on household contact of subclinical stage of leprosy (seropositive PGL-1) was 344,80 U/ml, meanwhile, on household contact who was seronegative PGL-1 was 508,09 U/ml. By utilizing *Kolmogorov Smirnov* test, it was known that normality test result of antibody level of IgG anti IDALLE L-ESAT 6 in both groups (seropositive and seronegative) was distributed normally. Then, by utilizing *independent t-test*, it was obtained p value that was 0,036 which meant that there was a significant difference of antibody level of IgG anti IDALLE L-ESAT 6 between a group of subclinical stage of leprosy household contact (seropositive toward PGL-1) with seronegative household contact PGL-1, which in seronegative group PGL-1 had antibody level of IgG anti IDALLE L-ESAT 6 that was higher than subclinical stage of leprosy (seropositive toward PGL-1).

Invasion from the *M.leprae* in human would give immune response either in unspecific nature or specific one. In preliminary stage, *M.leprae* would be phagocytosis by macrophage. However, in this stage the macrophage could not act much because *M.leprae* germ could be out from phagosome and could proliferate in its cytoplasm. The life defense system of *M.leprae* was strengthened by being out virulence factor which was polypeptide L-ESAT-6 that was produced in germ cytoplasm and secreted through wall of germ cell to around (Geluk *et al.*, 2002). This L-ESAT-6 had various types of epitope for either triggering immune response of cellular mediation (CMI=Th<sub>1</sub>) which was epitope of T cell or humoral immune response (Th<sub>2</sub>) which the B cell aroused antibody production of either specific antibody or unspecific antibody and it was depended from epitope type of ESAT 6 that triggered it. If it was reviewed from the length and infection frequency of with *M.leprae*, it could be grouped into two groups which were group of long and often infection and group of minimal infection. Moreover, household contact in a house with leper was included in first group which was group of long and often infection. Theoretically, adaptive response immune was started if epitope from the molecule of L-ESAT-6 was together with molecule of MHC class 2 that was exposed in surface of APC and was known by receptor from lymphocyte T-CD<sub>4</sub> and T-CD<sub>8</sub>. In this immune respond, lymphocyte T-CD<sub>4</sub> would be activated and would change to be lymphocyte Th<sub>0</sub>. However, it was depended on the availability of cytokine environment. The lymphocyte Th<sub>0</sub> would change to be lymphocyte Th<sub>1</sub> or lymphocyte Th<sub>2</sub>. Activity of Th<sub>1</sub> directed to immune response of CMI, meanwhile, activity of Th<sub>2</sub> directed to humoral immune response and specific antibody production. In infection with *M.leprae*, both of these responses were spurred and the final result was depended on immune response which was dominant, besides it was influenced by the individual's HLA, infection degree, and the condition of the environment.

L-ESAT 6 protein would be secreted/ provided by macrophage through Toll receptor (TLR)-2, hence, it stimulated macrophage to produce *nitric oxide* and *reactive oxygen intermediates* that would lyse the *M.leprae* germ and IL-1 $\beta$  and IL-12 would stimulate cellular response of T-CD<sub>4</sub> type 1 (cell T helper 1, Th<sub>1</sub>) such as secretion of IFN- $\gamma$ . Secretion from IFN- $\gamma$  would activate and make macrophage more active. Thus, it was occurred a reaction and inhibition growth and germ multiplication. IFN- $\gamma$  was a cytokine of major macrophage activator and had an important role in specific cellular immunity on infection by micro bacterium such as intracellular *M.leprae* and the function from IFN- $\gamma$  was very important.

Epitope of IDALLE L-ESAT-6 was found by Kurdi *et al.* (2010) on healthy nurse who had nursed leper in more than 10 years. Based on his research, it was stated that this epitope was only reactive with antibody that was in serum of healthy nurse group and there was no (significant smaller frequency) in healthy subject serum (no contact/ no leprosy) or leprosy patient (LL, BB, TT). Regarding this research result and finding, recently the researcher wanted to know how the epitope of IDALLE ESAT-6 that was stated as protective epitope and its distribution in household contact, particularly for household contact. The subclinical stage of leprosy was a condition which actually, the sufferer underwent an immunopathology abnormality without having clinical symptoms. In subclinical stage of leprosy cases, the sufferer could change to be leper in MB type or he/she could heal and did not grow into clinical leprosy disease. Protection factor that was owned by antibody of IgG anti IDALLE L-ESAT 6 perhaps correlated with how strong the cellular activity of Th<sub>1</sub> was. Regarding the IDALLE L-ESAT 6 was a protein. According to its character was if an antigen was from protein class, the formed immune response was perhaps through activity path of lymphocyte T CD<sub>4</sub> by macrophage which would not only induce

the release of cytokine Th<sub>1</sub> or Th<sub>2</sub>, but also would send a signal to B cell so that it also produced specific antibody, hence, it would be resulted antibody from class IgG.

This research focused on humoral immune response, hence, the cytokine expression levels that correlated with *Cell Mediated Immunity* (CMI) which either IL-2 or IFN- $\gamma$  were not accurate. Among all literature reviews by researcher, it was not found other researchers who researched about antibody of IgG anti IDALLE L-ESAT 6. According to research result, it was known that the average of antibody level of IgG anti IDALLE L-ESAT-6 on household contact of seropositive PGL-1 (subclinical stage of leprosy) was in 344,80 U/ml, meanwhile, on household contact of seronegative PGL-1 was in 508,09U/ml. By utilizing t-test of two independent samples, it was obtained that p value was  $0,036 < 0,05$  which meant that there was a significant difference of antibody level of IgG anti IDALLE L-ESAT 6 between seropositive household contact and seronegative household contact toward PGL-1, which the level of seronegative household contact group was higher than seropositive household contact group. Based on this result, the research hypothesis had been accepted which there was a significant difference of antibody level of IgG anti IDALLE L-ESAT 6 between seropositive household contact and seronegative household contact toward PGL-1 (subclinical stage of leprosy).

Another obtained information was in this research had been obtained cut off from the level of IgG anti IDALLE L-ESAT-6 on household contact in a house with leper at Brondong sub-district, Lamongan district which was in 348 U/ml. This value was obtained from percentile number of 50% in which the seronegative individual proportion value toward PGL-1 and seropositive individual of PGL-1 (subclinical stage of leprosy) in this research was in 50%. Thus, it could be stated that the household contact in this research that had antibody level of IgG anti IDALLE L-ESAT 6  $> 348$  U/ml had smaller risk (more resistant) to be subclinical stage of leprosy (seropositive PGL-1) if it was compared with a household contact that had antibody level of IgG anti IDALLE L-ESAT 6  $< 348$  U/ml. Regarding that there had not ever had other researches which measured the antibody level of IgG anti IDALLE L-ESAT-6, it could be stated that the cut off value from this research was 348 U/ml and it could be a reference for serology test in the field in assessing the household contact that had probability for staying healthy although had a contact with leprosy sufferer.

Afterwards, in order to know the correlation between antibody level of IgG anti IDALLE L-ESAT 6 and the occurrence of subclinical stage of leprosy (seropositive PGL-1), it was conducted simple logistic regression test which the obtained result was Exp (B) value = 0,998 (less than 1), hence, it could be meant that the correlation had negative characteristic which the higher the value of antibody of IgG anti IDALLE L-ESAT 6, it tended to be seronegative toward PGL-1. The condition that showed higher mean on the level of IgG anti IDALLE L-ESAT 6 on seronegative group toward PGL-1 showed that protective humoral immunity had protected this individual by hindering the multiplication from *M.leprae* germ and this also perhaps correlated with the quite strong activity of cellular immune from the individual regarding the exposure of L-ESAT 6 protein commonly which could arouse protective immune response (Geluk *et.al.*, 2002; Kurdi *et al.*, 2010).

#### IV. CONCLUSION

According to the result of this research, it could be concluded that there was a significant correlation between the occurrence of subclinical stage of leprosy with antibody level of IgG anti IDALLE L-ESAT 6 on household contact in endemic leprosy area. Besides, on household contact with high antibody level of IgG anti IDALLE L-ESAT 6 would be more protective toward *M.leprae*. Hence, it did not develop to subclinical stage of leprosy case (seropositive PGL-1). However, the antibody of IgG anti IDALLE L-ESAT 6 was stated as protective if the level was  $> 348$  U/ml based on the calculation result of *percentile 50%*.

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