

Case Report

## EVALUATION OF ANTI PGL-1 ANTIBODY TITER IN A GROUP OF HEALTHY SCHOOL CHILDREN WHO LIVE IN LEPROSY ENDEMIC AREA FROM 2007–2010

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### ABSTRACT

The “Iceberg phenomene” has been used to explain this situation which indicate that these new leprosy cases is originated from Subclinical Leprosy. Fifty eight healthy school children who live in Jeneponto Regency, a leprosy endemic area in South Sulawesi were recruited. The first examination was performed in 2007 and sera samples were kept in deep freeze refrigerator. In 2010 these children were re-examined for clinical leprosy and sera were collected again. ELISA study was performed simultaneously to these 58 pairs of sera (2007 & 2010) for measuring the titer of IgM anti PGL-1 antibody (ELISA) and the level 605u/ml was regarded as cut off value. After three years evaluation, none of these children showed any clinical signs of leprosy, but 20 of 22 ( 90.9%) children were remained sero-positive and only 2 (9.1%) became sero-negatives. In other sites, 5 children that previously sero-negatives became sero-positives after 3 years. Eight of 10 (80%) children who showed sero (+) with high titer (>1.000u/ml) in 2007, were also remained in high titer. The mean titer of 2007 was 627.8 u/ml, and after 3 years became 723.9 u/ml ( $p < 0.05$ ). Although there is no progression from Subclinical to Manifest Leprosy cases among these children, the number of sero (+) cases were increased and the mean titer of IgM anti PGL-1 antibody was significantly increased. The majority who previously showed high anti PGL-1 antibody titer, remained in high level. This study support the “iceberg phenomene” theory in Leprosy.

**Key words:** Leprosy, serology, anti PGL-1 antibody

### INTRODUCTION

Leprosy is a chronic infectious disease, which is primarily involve the peripheral nerve and secondarily to the skin and other organs. It is often cause disabilities and psycho-social problems.<sup>[1]</sup> Although the prevalence of the disease has been decreasing all over the world, after WHO Multi-drugs regiment in 1980s, and the incidence of new leprosy cases is not decreasing especially in leprosy endemic areas.<sup>[2]</sup> Some countries has reduced the leprosy prevalence and achieved the “Elimination status” which means the total leprosy prevalence in the country is less than 1/10.000 population.<sup>[3]</sup> But the decreasing of the prevalence is not followed by reducing the New Case Detection Rate (NCDR) and the majority of these new cases (around 80%) are the Multibacillary (MB) type of leprosy. Many theories has been used to explain this phenomenon, but it seems the “iceberg” theory is more suitable for this situation.<sup>[4]</sup>

Recent development in serological aspects of leprosy using the specific antigen of *Mycobacterium leprae*, the Phenolic Glycolipid-1 (PGL-1), has been used widely for the detection of specific antibody in leprosy.<sup>[5]</sup> This specific antibody is correlated with the antigen load (*M.leprae* bacilli) in the body of leprosy patients. The MB leprosy cases shows a high level of anti PGL-1 antibody.<sup>[6,7]</sup> Interestingly, many healthy individuals in endemic area also positive for serological test for leprosy.<sup>[8,9]</sup> These sero-positive individuals, especially with high antibody titers were called the “Subclinical Leprosy” (SL), since they do not show the clinical sign of leprosy. The SL group are potential to progress toward the “Manifest (Overt) Leprosy” after several years.<sup>[10,11]</sup>

A cohort study on serological pattern of leprosy in a group of inhabitants who live in endemic area will be useful to study the progression of SL cases toward the manifest leprosy. The aim of the study is to observe the change of serological pattern of leprosy in a group of inhabitants who live in leprosy endemic area.

## METHOD

A group of 58 healthy school children (23 male and 35 female, age 7–10 years old) who live in Jeneponto Regency, a leprosy endemic area in South Sulawesi were recruited. The first survey was conducted in 2007.<sup>[12]</sup> by clinical examination to exclude leprosy, followed by collecting 3 ml of venous blood. After separating from blood cells, the sera were kept in deep freezer (–40° C). The second survey was conducted three years later in 2010, by re-examined clinically and collecting sera from these school children. Fifty eight pairs of sera from the first survey (2007) and second survey (2010) were examined together (simultaneously) at the same time, using indirect ELISA technique to measure the level of IgM anti PGL-1.<sup>[13]</sup> The results were converted from OD to unit/ml by BIOLISE software in the computer and the titer of 605 u/ml was regarded as cut off value.

## RESULTS

Clinical examination in the second survey (August 2010), after three years observation, revealed no clinical signs of leprosy among these children.

The serological examination result on 58 pairs of sera using ELISA technique are:

**Table 1.** Number of sero-positive cases after 3 years observation among 58 children

Year	Sero + ( > 605 u/ml )	Sero – ( < 605 u/ml )	Total
2007	23 (41.3%)	35 (58.7%)	58 (100%)
2010	28 (46.5 )	30 (53.5%)	58 (100%)

Changes of serological status after three years observation (2007 to 2010) were as follows:

Mean (average) titer value of IgM anti PGL-titer in 2007 was 627.8 u/ml and became 723.9 u/ml in 2010. (statistically  $p < 0.05$ ).

Ten children in 2007 showed high titer of IgM anti PGL-1 (> 1.000 u/ml) and after three years the number of high anti PGL-1 titer became 17 children in 2010.

**Table 2.** Frequency of serological status changes after three years observation

Serological status	Frequency
2007 sero (+) ---- 2010 sero (+)	22
2007 sero (+) ----2010 sero (–)	2
2007 sero (–) ----- 2010 sero (+)	5
2007 sero (–) ----- 2010 sero (–)	29
Total	58

Based on the serological level, the antibody titers were divided into 4 categories:

**Table 3.** Frequency distribution of 4 categories based on the antibody level

IgM anti PGL-1 level	2007	200
Sero +++ (> 2.000u/ml)	1 (1.7%)	2 (3.4%)
Sero ++ (> 1.000–2.000 u/ml)	9 (5.5%)	15 (25.9%)
Sero + (605-1.000 u/ml)	13 ( 22.4%)	11 (19.0%)
Sero - (< 605 u/ml)	35 (70.4%)	30 (51.7%)
<b>Total</b>	<b>58 (100.0%)</b>	<b>58 (100.0%)</b>

## DISCUSSION

This study was conducted in the area where leprosy is endemic, which means the inhabitants are routinely exposed to *M.leprae*, whether it came from leprosy patients or environment. These antigen exposure will induce the immune response, especially the humoral response and the specific antibody to *M.leprae* is anti PGL-1 antibody which is widely accepted as a marker of *M.leprae* infection.<sup>[14]</sup>

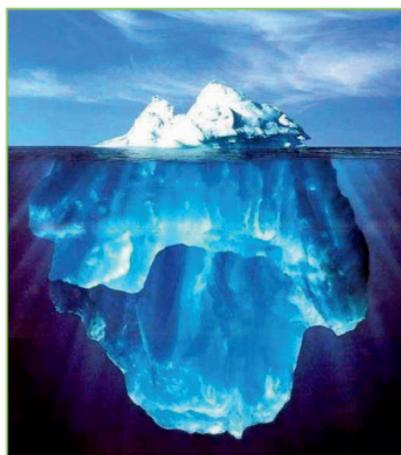
In the “iceberg theory” in leprosy, the population in leprosy endemic area is divided into sero-negative layer, as the bottom of the iceberg, followed by sero-positive layer with low titer, and high titer at the upper layers.<sup>[4]</sup> The sub-clinical leprosy (SL) are healthy people without any clinical signs of leprosy, but showed a high IgM anti PGL-1 antibody level, which means that the antigen load (*M.leprae*) has been achieved a big amount in the body. This group is the candidate of new MB leprosy case in the future, since the humoral response is already dominant and the cellular immunity (CMI) which is needed for eliminate leprosy bacilli became weak.<sup>[15]</sup> The Multi-drug Therapy (MDT) as recommended by WHO is only indicated for the manifest leprosy which is already showed the clinical signs of leprosy. Theoretically these upper part of iceberg will be cut by MDT, but soon it will be replaced by the SL layer that are not treated yet by MDT. This hypothesis might explain why the NCDR in leprosy keep stable for several years.

From the result of this study, total number of sero (+) cases were increased from 23 to 28 cases after 3 years evaluation. Interestingly 22 children remained sero (+) after three years and the majority showed increasing level of the specific antibody to *M.leprae*.

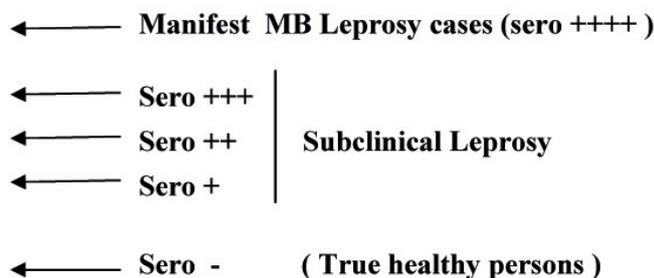
The mean (average) level of IgM anti PGL-1 antibody of this group showed a significant increase from 627.8 u/ml in 2007 to 723.9 u/ml in 2010. This observation has been reported by Agusni in 1997,<sup>[15]</sup> and after 4 years evaluation 1 out of 16 SL cases with high level of anti PGL-1 antibody became manifest leprosy with all clinical signs of leprosy.<sup>[16]</sup>

Although the epidemiological role of subclinical infection in leprosy still debatable,<sup>[17,18]</sup> it is important to know in the field which individuals who have high level of anti PGL-1 antibody. Screening surveys by clinical examination will be costly and time consumed. Using

## THE ICEBERG PHENOMENA IN LEPROSY



**DISTRIBUTION OF GROUPS  
POPULATION IN LEPROSY ENDEMIC  
AREA, BASED ON THE LEVEL OF IgM  
ANTIBODY TO PGL-1**



serological survey, it is clear which individuals need to be monitored and early sign of leprosy might be detected clinically by routine examinations.

Several serological studies have been conducted in East Java Province and the results showed a range of sero-positivity between 34% to 48.5% in leprosy endemic areas.<sup>[19,20,21]</sup>

Following the Iceberg phenomena theory, the sero (-) layer in this study was 70.4% in 2007 and reduced to 51.7% in 2010. The low titer level (sero +) group was also reduced from 22.4% (2007) to 19.0% (2010). But the higher level (sero ++ ) were increased from 5.5% to 25.9% and sero +++ became doubled (from 1.7% to 3.4%). Increasing level of leprosy specific antibody after three years observation in the population of leprosy endemic areas, indicates that transmission of the disease still occurred. Those who have high antibody titer are potential to progress to MB leprosy. Recent global reports on leprosy showed 80% of new case detection rate of (NCDR) are MB cases. It means that these new leprosy cases could be detected earlier by serological examination and this study support the iceberg phenomenon theory.

## CONCLUSION

In leprosy endemic area, the level of specific antibody to *M.leprae* among inhabitants is increasing continuously toward the Subclinical Leprosy which is potential to progress to Manifest Leprosy. The results of this study support the “iceberg phenomena” in leprosy.

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