



RESEARCH ARTICLE

The Difference in Immunoglobulin M Anti-Phenolic Glycolipid-1 Antibody Levels in Leprosy, Household Contact and Control

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Abstract

Background: Leprosy is a disease caused by *M. leprae* infection, may affect the peripheral nervous system, skin and other tissues. Until recently, leprosy is still a health problem in Indonesia. Phenolic Glycolipid-1 (PGL-1) is a stable and unique component of *M. leprae*, and an antibody examination against this antigen may play a role in assisting early detection of subclinical stage leprosy (SSL) and treatment monitoring. Objective: This study aims were to define the level of immunoglobulin M (IgM) anti-PGL-1 antibody in leprosy patient, household contact and control and their serology status. Methods: The examination of IgM anti-PGL-1 antibody was carried out on 52 leprosy patients, 95 household contacts and 95 healthy controls. The data from subjects were then analyzed and tested using Kruskal-Wallis statistical test and a p-value <0,001 was obtained. Results: It was obtained that the levels of IgM anti-PGL-1 antibody significantly differed between leprosy patient, household contact and control groups (p<0.05). Most of the leprosy patients had seropositive status; contrary to dominant seronegative that were found in household contact and control groups. In subgroup analysis, the IgM titer levels did not significantly differ between household contact and control group (p=0,122). Conclusion: the IgM anti-PGL-1 antibody differed between leprosy patient, household contact and control. IgM antibody titer may also depict a person's exposure status to *M. leprae* bacilli.

Keywords: *M. leprae*, Leprosy, IgM anti-PGL-1 antibody, Household contact.

Introduction

Leprosy is caused by *Mycobacterium leprae* (*M. leprae*) infection. It affects the peripheral nervous system, skin, and other tissues such as the reticuloendothelial system, bones, joints, mucous membranes, eyes, testes, muscles, and adrenal glands [1, 2]. The transmission is suggested occurs through inhalation, direct contact, in-uterine transmission, gastrointestinal tract, and post-traumatic inoculation [3].

Negative stigma of leprosy contributes to the significant delay in early diagnosis and treatment [4]. Leprosy is still one of the health problems in Indonesia. Data reported by WHO in 2016 showed that Indonesia has been the third-largest contributor to new

leprosy cases in the world with a prevalence of 0.70 per 10,000 population in 2017 [5]. The new cases of leprosy in both adults and children are continuously reported. The 2017 Data from the Ministry of Health of the Republic of Indonesia showed that 10 out of 34 provinces in Indonesia have not achieved leprosy elimination yet [6]. Phenolic Glycolipid-1 (PGL-1) is a stable and unique component to *M. leprae* and can be found abundantly on the surface of the bacillus [7].

The body responds to PGL-1 antigen can be in the form of IgM or immunoglobulin G (IgG) antibodies production and these antibodies titer can be measured by the Enzyme-Linked method Immunosorbent

Assay (ELISA) [8]. High titer level of anti-PGL-1 antibodies correlates with high numbers of antigens or *M. leprae* bacilli in a person's body. In the condition when clinical not well-develop yet, the presence of high IgM anti-PGL-1 antibody titer may assist in determining person status to leprosy infection. It may help in early detection of subclinical stage leprosy (SSL) as well as treatment monitoring [9, 10].

Methods

This study was an analytical, with a case-control design to compare IgM anti-PGL-1 antibody levels in leprosy patient, household contact and control. This was a multicenter studies conducted at Leprosy Division, Dermatology and Venerology Department of H. Adam Malik General Hospital Medan, Dr. Pirngadi Medan General Hospital, and several Puskesmas located in Medan Municipality, Binjai Municipality, Deli Serdang Regency, Tebing Tinggi Municipality, Simalung Regency, based on data of leprosy patient from Dinas Kesehatan Tk I dan II of North Sumatra Province.

The study has been approved by Research Ethical Commission of Faculty of Medicine Universitas Sumatera Utara under the ethical clearance number: 454/ TGL/KEPK FK USU-RSUP HAM/2018. Informed consent was obtained from all subjects who were enrolled in this study. The venous blood sampling was performed by drawing 5 cc of blood from the sample and subsequently

examined by ELISA method at Tropical Disease Diagnostic Center (TDCC) a.k.a Leprosy Laboratory LPT Building, Kampus C Mulyorejo street, Universitas Airlangga (UNAIR) Surabaya for IgM anti-PGL-1 antibody titer measurement. The antibody titer level was categorized as seronegative and seropositive. A titer level of less than 605 µ/ml was defined as seronegative, meanwhile seropositive was further classified into two, low seropositive if antibody titer level felt between 605 to 1000 µ/ml and high seropositive if the level exceeded 1000 µ/ml.

The characteristic of the sample, according to anamnesis, physical examination, and laboratory test results, were described and analyzed using cross-tabulation. Bivariate analysis was performed by Kruskal Wallis and Mann-U Whitney statistical tests. The results were significant if the p-value <0.05 with 95% confidence interval (CI).

Results

A total of 52 people affected by leprosy, 95 household contacts, and 95 controls were included in this study. Most of the household contact and control had anti-PGL-1 IgM antibody levels of less than 605µ/ml and therefore were categorized as seronegative. Meanwhile, in leprosy patient, the majority of the samples had IgM anti-PGL-1 antibody levels of more than 1000 µ /ml and classified as a high seropositive titer. Table 1 shows the comparison in IgM anti-PGL-1 antibody levels between leprosy patient, household contact, and control.

Table 1: The difference of IgM anti-PGL-1 antibody level between leprosy patient, household contact, and control

Sample Groups	Mean ± SD	p-value
Leprosy patient	12408.82 ± 19514.33	< 0.001
Household contact	5899.56 ± 19857.71	
Control	7900.31 ± 22269.50	

*Kruskal-Wallis statistical test

Table 1 suggested that there was a significant mean difference in anti-PGL-1 IgM antibody titer levels between leprosy patient, household contact, and control (p<0.001). The leprosy group, among others, showed the highest level of IgM anti-PGL-1 antibody titer with mean ± SD of 12408.82 ± 19514.33

Table 2: The comparison of IgM anti-PGL-1 antibody titer levels between groups

Sample Groups	p-value
Leprosy patient vs household contact	< 0.001
Leprosy patient vs control	< 0.001
Household contact vs control	0.122

*Mann-U Whitney statistic test

The statistical test using Mann-U Whitney was conducted to analyze the difference in IgM anti-PGL-1 antibody titer levels between groups. There were significant differences in almost all group comparison except between household contact and control (p=0.122)

Discussion

Leprosy poses a very complex problem not only for the patients' life, but also for the surrounding community, in almost all aspect, including health service, social, and politic [11].

A significant delay in early diagnosis and management due to negative stigma may contribute to leprosy transmission [12]. Leprosy transmission is also thought to be mediated by individuals with subclinical stage leprosy.

Individuals with SSL are clinically healthy, but a high level of specific antibodies against *M. leprae* obtained from a serologic examination may already be found in this group of people [13, 15]. PGL-1 is a unique and specific component on the surface of *M. leprae* bacilli which contributes to the resistance development to intracellular death in macrophages, immunomodulatory activity, serological analysis and complement fixation [7, 16].

Previous research shows that PGL-1 plays a role in the pathogenesis of leprosy as it can interact with laminin from Schwann cells to allow entry of bacteria to the nerve cell [16, 17]. This component is highly antigenic and is present in large quantities and can stimulate the formation of IgG and IgM antibodies against it [18]. The result of anti-PGL-1 IgM antibody may further divide into two categories, the low titers with antibody level of 605-1000 u/ml and high titers with antibody level > 1000 u/ml [9, 10].

This study involved leprosy patient, household contact, and healthy control of 52 people, 95 people and 95 people, respectively, which were recruited from various health care services in North Sumatra. Most of the household contact and control showed the results of seronegative IgM anti-PGL-1 antibodies. When compared between the two groups, more subjects in the household contact group had seropositive results compared to the control group.

In contrary, in leprosy patient group, it was found that the majority of patients had antibody levels of higher than 1000 μ /ml. Seropositive status poses a higher risk for leprosy development than the negative one. Based on a meta-analysis study by Penna et al, it was found that in healthy contact

person, the risk for leprosy was 3 times higher in subjects with a positive anti-PGL1 [19]. This finding was similar to the result found by Bakker et al., (2006) in Indonesia, which saw an increased risk up to 3.8 times in the household contact with seropositive status to develop leprosy disease [20]. When compared between the three groups, the highest mean of IgM anti-PGL-1 antibody titers was found in leprosy patient.

This finding was consistent with the statement that the IgM anti-PGL-1 antibody titer is related to the number of *M. leprae* bacillus in the host body [10]. The higher level of antibody titer in control compared to household contact was suggested due to the fact that the control included in this study was picked from the health care professional who possibly ever had contact with leprosy patient when working and handling patient. In the household contact, a high antibody titer levels were also obtained.

This study involved household contact of MB leprosy patient who had absolutely a high number of *M. leprae*, with a substantial long and close exposure. It could affect the value of IgM anti-PGL-1 antibody titer in the household contact group. The WHO guidelines recommend the administration of single-dose rifampicin chemoprophylaxis to household contact after being excluded from leprosy and tuberculosis, also when no other contraindications were found [21]. The data from this study was not in a normal distribution; therefore a Kruskal Wallis test was used and seen a significant mean difference between groups ($p < 0.001$) which suggested that the mean of IgM anti-PGL-1 antibody levels significantly different between leprosy patient, household contact, and control group.

Finally, it can be concluded that the titer of IgM anti-PGL-1 antibody may describe the person's exposure status to *M. leprae* bacilli. However, this study did not analyze other possible factors that may influence IgM anti-PGL-1 antibody titers such as the type of leprosy or nutritional factors which may also have a role in immunity. This fact should be considered when interprets the study results. The comparison of mean IgM anti-PGL-1 antibody titers between household contact showed a non-significant difference (p -value > 0.05). Antibody titer is associated with exposure to *M. leprae* in the community;

thus, seropositive result in household contact in high endemic areas may not necessarily be interpreted as a higher level of an antibody that in control. Therefore, in leprosy patient, serology examination of IgM anti-PGL-1 antibody can be useful in determining diagnosis if combined with the clinical presentation. Besides, in leprosy patient, this serological examination can also be helpful in classifying the disease, monitoring the effectiveness of treatment, and predicting the leprosy reaction [10, 22].

Conclusion

The increased in IgM anti-PGL-1 antibody titers indicated an increasing number of bacillus present in a person body. This antibody examination could help in diagnosing SSL. In this study, there were significant differences in IgM anti-PGL-1 antibody titers between the groups of leprosy patient, household contact, and control. In addition, an IgM anti-PGL-1 IgM antibody titer could provide a piece of information about a person's exposure status to *M. leprae* bacilli.

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