Indonesian Journal of
Tropical and Infectious Disease
Vol. 4. No. 4 October–December 2013

Research Report

COMPARATIVE STUDY ON THE INTENSITY OF *Mycobacterium leprae* EXPOSURE TO CHILDREN WHO LIVE IN LOW AND HIGH ALTITUDE IN LOW LEPROSY ENDEMIC AREA OF SOUTH SULAWESI

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ABSTRACT

Background: The intensity of Mycobacterium leprae exposure to people who live in leprosy endemic area could be measured by serological study and detection of the bacilli in the nose cavity. Different geographical altitude might have some influences to this exposure since the bacilli prefer to live in warm areas. Aim: A combined serological and PCR study of leprosy was conducted in Selayar island, South Sulawesi to 80 school children (40 from low land and 40 from highland altitudes) in order to compare the exposure intensity between the two areas. Method: Anti PGL-1 IgM antibody (ELISA) and PCR study to detect M.leprae in the nasal cavity were performed simultaneously from each person. Result: Seropositive cases were found in 23/40 children from low land compared to 16/40 children from high land, but statistically no significant difference (p>0.05). PCR positive for M.leprae in the nasal cavity only found in 1/40 children, both in low and high altitude. Conclusion: It is concluded that although the existence of M.leprae in nasal cavity is minimal, the intensity of exposure to this bacilli still high as indicated by serological study.

***Key words**: leprosy, serology, PCR, children, low and high land*

ABSTRAK

Latar belakang: Intensitas paparan M.leprae terhadap penduduk yang tinggal di daerah endemik kusta dapat diukur dengan uji serologi dan deteksi kuman M.leprae di mukosa hidung. Tujuan: Perbedaan ketinggian geografis dapat memberikan pengaruh terhadap proses tersebut karena kuman kusta lebih menyukai daerah yang lebih hangat. Metode: Telah dilakukan studi serologi dan PCR di Pulau Selayar, Sulawesi Selatan terhadap 80 murid sekolah (40 anak dari dataran rendah dan 40 anak dari dataran tinggi), dengan tujuan untuk membandingkan intensitas paparan diantara kedua daerah. Hasil: Antibodi IGM anti PGL-1 (ELISA) dan studi PCR untuk mendeteksi M.leprae di mukosa hidung diambil secara bersamaan dari tiap-tiap anak. Ditemukan hasil sero positif pada 23/40 anak dari dataran rendah dibandingkan 16/40 anak dari dataran tinggi, namun tidak ada perbedaan bermakna diantara keduanya (p > 0,05). PCR positif terhadap M.leprae di mukosa hidung hanya ditemukan 1/40 anak baik dari dataran rendah maupun dataran tinggi. Kesimpulan: Hal ini berarti walaupun eksistensi M.leprae di mukosa hidung sedikit, intensitas paparan kuman M.leprae tinggi ditunjukkan dari hasil serologi.

***Kata kunci:** kusta, serologi, PCR, anak-anak, dataran rendah dan dataran tinggi.*

INTRODUCTION

Leprosy is still a public health problem in South East Asia, including Indonesia. Although the elimination target of leprosy in Indonesia has been reached in 2001, some pocket areas of leprosy still exist up till now.[1] The Selayar island district in South Sulawesi is an area with a lower prevalence of leprosy, compared to the surrounding areas which have high prevalence of leprosy.[2] Based on the assumption that this area might has a low transmission of leprosy, a study of leprosy exposure to inhabitants of such area will show a low level. Since *M.leprae* is known as a bacilli who prefer to live in relatively colder area of the body, a different geographical altitude might have also influence the exposure of the bacilli. After the *M.leprae* enter the body, an immunologic response will be developed and specific antibody to *M.leprae* will be produced (anti PGL-1 antibody). The level of this antibody is reflected the antigenic load of the bacilli.[3]. The leprosy bacilli enter the body via respiration tract and the detection of *M.leprae* in the nasal cavity could be performed by PCR study.[4] The level of seropositivity and the presence of *M.leprae* in nasal cavity could be used as an indicator for leprosy exposure intensity in endemic area. The aim of this study is compare the exposure of *M.leprae* to inhabitants who live in low and high lands of Pulau Selayar Area of South Sulawesi, using specific serological indicators for leprosy and the presence of *M.leprae* in the nasal cavity by PCR method.

MATERIAL AND METHODS

Fourty healthy school children from Kahu-Kahu village, Selayar island, who live in low land and another 40 healthy school children from Lembang Matene village (high land) aged 9–12 years old were involved in the study (figure 1). Leprosy contact history was taken to exclude contact individuals and clinical examination was conducted to exclude leprosy patients. Blood and nose swab samples from these children were collected simultaneously.

Serological study

From each children 100ul capillary blood was obtained by a needle puncture to finger tip, dried on a small filter paper and sent to Leprosy lab of Institute of Tropical Disease, Airlangga University, Surabaya. Dried blood on filter papers were dissolved in 1 ml of BSA buffer and 10ul of the solution were taken for indirect ELISA test to measure the level of anti PGL-1 antibody. Using N dioctyl-BSA as an antigen, the level of IgM anti PGL-1 antibody were measured follows the ELISA procedure recommended by Patil.[5] The ELISA results in Optical Density (OD) were converted to unit/ml using the BIOLISE computer software. Three ml of peripheral blood were also collected from cubitous venes in 10 children, to measure the real blood level of anti PGL-1. After conversion to serum level, using cut off level 605 u/ml, sero-positive case was detected.[6]

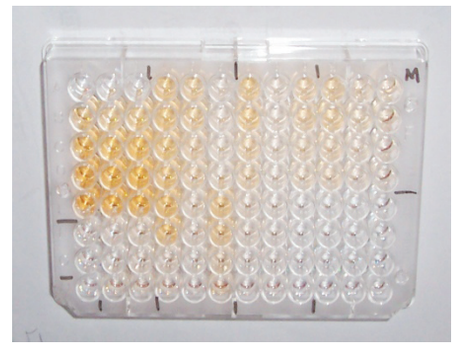

Figure 2. Indirect ELISA

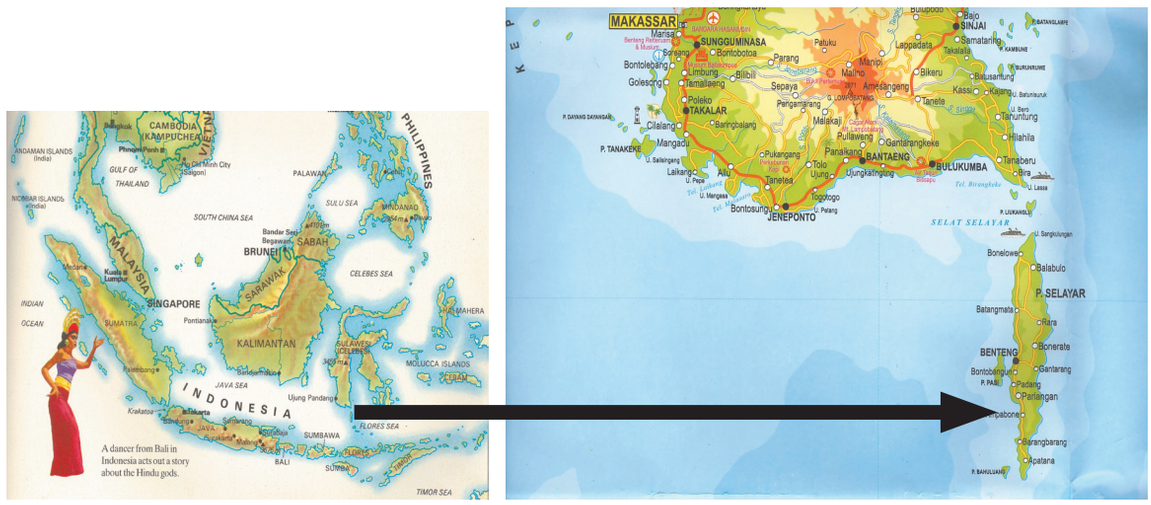

Figure 1. Geographical area of Selayar Island, South Sulawesi.

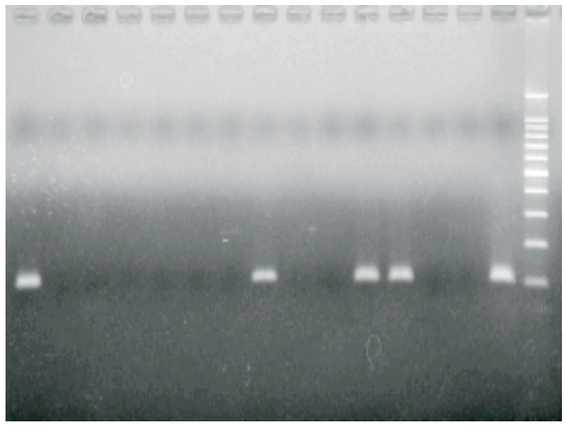

Figure 3. PCR results for detection of *M.leprae* DNA using Lp1 – Lp-4 nested primers
Sample no. : 1 2 3 4 5 6 7 NC PC Ladder (100bp)

PCR study from nose swab samples

Nose swab sample was also taken simultaneously from the same children after collecting finger tips blood for serological sudy. The nose swab samples were kept in freeze condition until ready for PCR study. DNA extraction was performed using the Miniprep Qiagen kit. Using the Lp1-Lp4 nested primers, the *M.leprae* DNA was detected by PCR, following the procedure recommended by Plikaytis.[7] Positive PCR results is indicated by a band of 99 bp in agarose gel field, as pointed by positive control (figure 3)

RESULTS

Clinical examination of all children revealed no sign of leprosy. Based on the cut off value 605 u/ml for IgM anti PGL-1, sero-positive cases was observed in 23 out of 40 (57,5%) children from low land compared to 16 out of 40 (40%) children from highland. Statistically no significant difference between the two groups ($p > 0.05$). After PCR study from the nasal swabs samples, only one out of 40 samples from low land group show PCR positive, similar with the results of nose swabs samples from highland group (1 out of 40 samples or 2.5%). No statistical difference in the PCR results between the two groups.

DISCUSSION

This study was conducted in an area wich is reported as a "low endemic of leprosy" and start with an assumption that the transmission of the disease mainly from the environment. The humoral response to *M.leprae* is represented by specific antibody to cell wall of the bacilli which contains Phenolic Glycolipid-1 (PGL-1). This antibody is not protective to leprosy, but can be used as a marker of immune response to the presence of the bacilli.[9] The level of this specific antibody is corresponded with the amount of antigen or bacilli in the body, which means high level of antibody indicates many bacilli inside the body.[10] In this study, almost half of the school children have already showed sero-positive for leprosy. In this case, the cut off value (> 605 u/ml) used in the study was based on the previous serological study in East Java.[11] A new calculation for measuring the cut off value for South Sulawesi is needed. Although the Selayar island is relatively low endemic for leprosy, it seems that the children who live in this area still exposed to leprosy bacilli quite often. The source of the bacilli might be from leprosy cases who are still not found by surveillance ("the back-log cases") or from non- human resource of *M.leprae* in the environment.[12,13] After tracing the sero-positive children by analyzed the history of contact with leprosy cases, it revealed that most of the children have no contact history with leprosy patients. High percentage of seropositivity to leprosy in South Sulawesi area seems to be common in many parts of this province. Since the island has a long coastal area, it would be possible also that many inhabitants use to go to other surrounding area in South Sulawesi as a sailor and contact with many leprosy patients from other area. Nasal swabs examination have been used to detect the port of entry of the lepra bacilli from inside or from outside or of the body.[14,15] The PCR study for detection of *M.leprae* in the nasal cavity only showed positive in single case from both groups. This minimal results could be correlated with the season during collecting nose swab samples. The study was conducted during rainy season, probably this cause many lepra bacilli were cleaned up from the air and less bacilli aspirated by these children. This reason might explains why the nasal swabs mostly negative in the PCR study, beside the technical eror during sample collection or laboratory work. It is recommended to repeat the nasal swabs collection during the dry season, when the air might be more contaminated with lepra bacilli. The percentage of PCR positivity from nose swabs samples from this study was 2.5%, lower than previous study in other area of South Sulawesi.[16] The serological results of this survey shows that transmission of *M.leprae* is still intense, but PCR results from nose swabs does not support the hypothesis that transmission is occurred via the nasal cavity. Why the percentage of leprosy sero-positivity among these children were high is still a question and more environmental investigation are needed.

CONCLUSION

Low endemic of leprosy does not means less intensity of exposure of *M.leprae* and need more environmental study of leprosy to find out the source of the transmission of the disease in this area.

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