DETECTION OF *MYCOBACTERIUM LEPRAE* DNA IN BLOOD OF THE SUBCLINICAL LEPROSY

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ABSTRACT

Subclinical Leprosy (SL) is attributed to healthy individuals living in endemic leprosy area, without any clinical signs of leprosy, but showing a high specific antibody level to leprosy bacilli. This laboratory finding indicates that a certain amount of Mycobacterium leprae (M. leprae) have entered the body and induces specific antibody. Due to the potential progress from SL to overt (manifest) leprosy, the management of SL is important in Leprosy Control Program. The aim of this study was to detect M. leprae DNA in the blood of SL cases, using Polymerase Chain Reaction (PCR) technique. Two ml peripheral blood samples from 29 SL cases diagnosed by clinical examination and serological test (ELISA : IgM anti PGL-1 antibody > 600 u/ml) were collected. DNA extraction was performed using Takara technique. Lp1 – Lp4 nested primers was used in PCR to amplify the RLEP sequence (99 bp) that is specific and sensitive for M. leprae. After running in the electrophoresis field, the results were observed by UV transilluminator. Results revealed that from 29 blood samples of SL cases, 6 (20.69 %) showed positive in PCR test. No significant differences the level of anti PGL-1 antibody was observed between positive and negative PCR results. In conclusion, M. leprae DNA can be found in peripheral blood of SL cases. This finding supports the opinion that SL cases need anti-leprosy treatment to prevent progression towards manifest leprosy.

Keywords: *Mycobacterium leprae, DNA, subclinical leprosy*

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INTRODUCTION

Leprosy is a chronic infectious disease caused by *M. leprae* and still a public health problem in many developing countries, including Indonesia. The target of Leprosy Elimination, where every country should reduce the leprosy prevalence < 1 per 10.000 population as stated by World Health Organization (WHO), has been reached by Indonesia. However, the incidence rate of new leprosy is still not declining, indicating that the transmission still going on, although the source of infection (most of MB cases) has been eliminated. Probably there is another source of infection other than leprosy patients. It could be from human or environment. Recent development in serological and molecular biology techniques in Leprosy has made better and accurate results in the diagnostic procedure of the disease. By serological method, it is possible now to detect the Subclinical Leprosy (SL), a term for healthy individuals without any signs of leprosy but showing a high level of specific antibody to leprae bacilli. The existence of SL has been a debatable topic, In one side, there is an opinion that it should be treated due to its potential to progress to overt or clinical leprosy after certain years. Another side argues against this policy since not all SL cases become clinical leprosy. Using Polymerase Chain Reaction, it is also possible now to detect the DNA of *M. leprae* in many tissues, including blood specimens. If the DNA of *M. leprae* could be detected in the blood of SL cases, it may support the first opinion that SL cases need special management to prevent progression to clinical leprosy. Therefore, it will reduce the continuously non-declining new incidence rate of leprosy. The objective of this study was to detect *M. leprae* DNA in the blood of SL cases, using the Polymerase Chain Reaction procedure.

MATERIALS AND METHODS

Twenty-nine SL cases were screened from 122 household contact of leprosy, who lived in two endemic leprosy villages (Kombeng and Poteran) in Talango, Sumenep District, Madura island. The diagnosis of SL was based on negative signs of leprosy in clinical examination, but serological test for leprosy was positive (IgM anti PGL-1 antibody > 600 u/ml). Two ml of venous blood were collected and directly mixed with TaKaRa GenTLE methods for DNA extraction and

followed by PCR using nested primer Lp1-Lp4 from RLEP repetitive sequence (99 bp):

LP_1 490-509 : 5'- TGC ATG TCA TGG CCT TGA GG -3'
LP_2 618-599 : 5'-CAC CGA TAC CAG CGG CAG AA-3'
LP_3 505-522 : 5'-TGA GGT GTC GGC GTGGTC-3'
LP_4 603-586 : 5'-CAG AAA TGG TGC AAG GGA-3'

PCR condition for LP_1-LP_2:
denaturation 1 : 94 ^{0}C for 4 minutes
denaturation 2 : 94 ^{0}C for 30 seconds
annealing : 56 ^{0}C for 30 seconds
extension : 72 ^{0}C for 30 seconds

Denaturation 2 up to extension was repeated 35 cycles, and prolonged extension 72 ^{0}C for 5 minute, and incubation in 4 ^{0}C.

PCR condition for LP_3-LP_4 :
denaturation 1 : 94 ^{0}C in the room temperature
denaturation 2 : 94 ^{0}C for 30 seconds
annealing : 56 ^{0}C for 30 seconds
extension : 72 ^{0}C for 30 seconds

Denaturation 2 up to extension was repeated 30 cycles, and prolonged extension 72 ^{0}C for 5 minutes, and incubation in 4 ^{0}C.

RESULT:

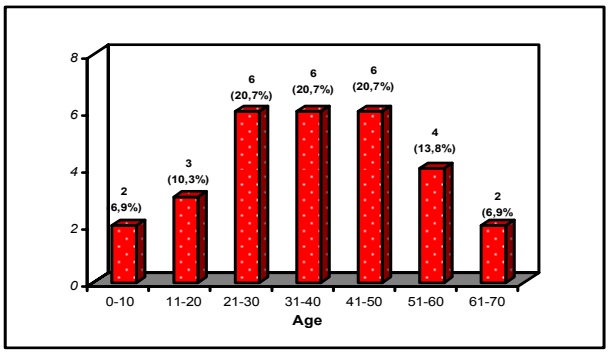

Figure 1. Age-specific distribution of anti PGL-1 Ig M

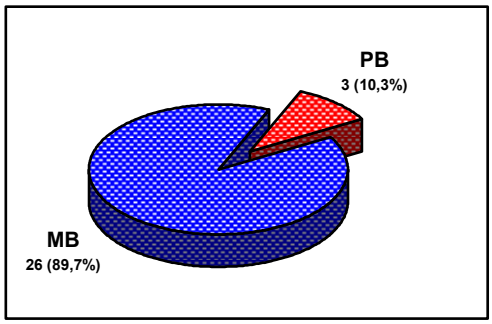

Figure 2. Leprosy type in contact distribution

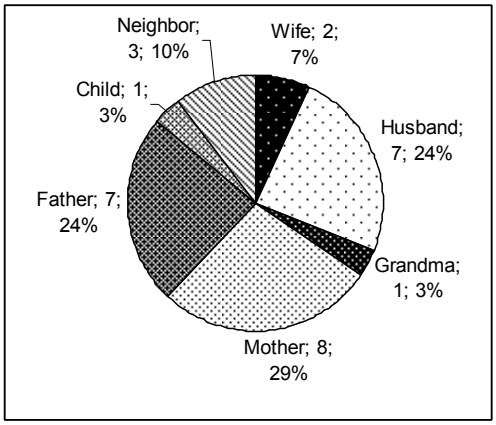

Figure 3. Contact relationship distribution

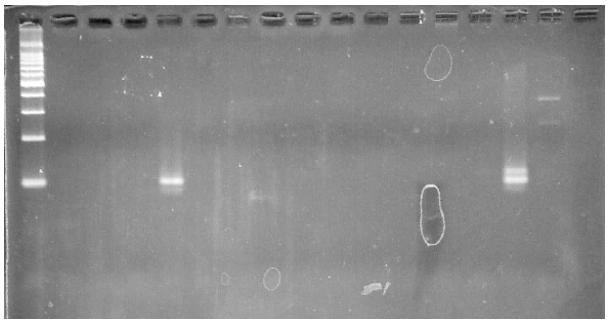

Figure 4. PCR result from 15 samples. Lane 1: base pair ladder; Lane 2: sample 1; Lane 3: sample 2; Lane 4: sample 3; Lane 5: sample 4; Lane 6: sample 5; Lane 7: sample 6; Lane 8: sample 7; Lane 9: sample 8; Lane 10: sample 9; Lane 11: sample 10; Lane 12: sample 11; Lane 13 : sample 12; Lane 14 : sample 13; Lane 15: sample 14; Lane 16: sample 15; Lane 17: negative control.

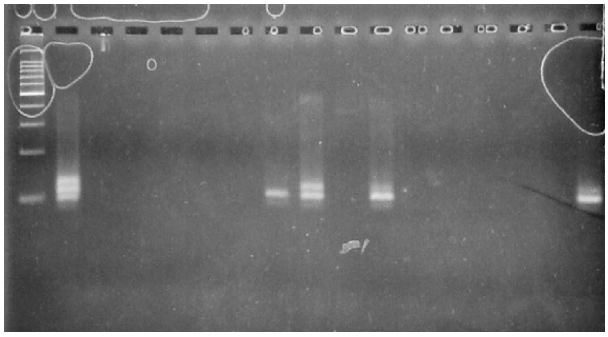

Figure 5. PCR result from 14 samples. Lane 1: base pair ladder; Lane 2: sample 16; Lane 3 : sample 17; Lane4: sample 18; Lane 5: sample 19; Lane 6: sample 20; Lane 7: sample 21; Lane 8: sample 22; Lane 9: sample 23; Lane 10: sample 24; Lane 11: sample 25; Lane 12 : sample 26; Lane 13: sample 27; Lane 14: sample 28; Lane 15: sample 29; Lane 16: negative control; Lane 17: positive control.

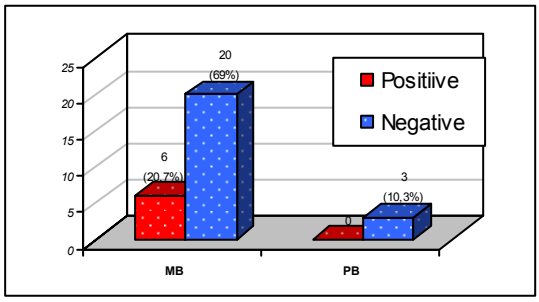

Figure 6. PCR result-type of leprosy contact distribution. Mann Whitney test: No significant differences the titer of Ig M anti PGL-1 between PCR result positive and PCR result negative (p = 0.477 ; ά 0.05).

DISCUSSION

The seropositivity rate of anti PGL-1 Ig M antibodies, which is called subclinical leprosy (SL), was found in 29 of 122 contact leprosy. It was found that the seropositive rate in younger generation are relatively higher compared to that in older generation, suggesting that active transmission of *Mycobacterium leprae* infection was still taking place in this area. Attention should be taken in the younger generation. Theoretically, leprosy transmission needs intimate and long time duration of contact, especially with MB type household contact leprosy, whereas MB type of contact leprosy has 5-10 times possibility compared to PB type (Agusni 2001; Cartel 1990; ILATF 2002). This study shows that 89.7% (26/29) SL have MB type contact, and 10.3 % (3/29) have PB type contact. The causes of transmission could be from direct contact with the sources of infection (human or animal) and also from the environment. The main exposure of *M. leprae* is in the house environment, but other places, such as restaurant, hospital and working area, can also be responsible for exposure (Meima 1999). In this study 89.7 % (26/29) is household contact and only 10.3 % (3/29) is neighbor contact. This fact supports the theory that intimate condition plays a role in the transmission of leprosy.

The seropositive rate of anti PGL-1 Ig M antibody in various endemic areas was intensively studied in the late 1980s. It was found that the seropositive rates were very high in the endemic areas (Izumi 1999). However, the reason for the high positivity rate was not fully analyzed. In this study, we simultaneously conducted a molecular biological study to detect *M. leprae* DNA in blood by PCR technique on the assumption that the infection of leprosy bacillus in subclinical leprosy may play an important role in the transmission of the disease. It was found that 20.69 % of the SL was carrying *M. leprae* DNA in blood. This finding shows that *M. leprae* DNA in blood SL can be caused by resources from the body due to infection of *M. leprae* and spread by haematogen. Besides, in this condition the presence of *M. leprae* DNA in blood can be caused by aerogen and cannot be destroyed by innate immunity, so the bacilli is still in circulating monocyte before reaching the Schwann cells (Trojan horse phenomenon).

From 6 PCR positive are household contacts with MB type of leprosy. This fact support the theory that the main sources of *M. leprae* infection is lepromatous type leprosy, but some studies said that subclinical episode (asymptomatic) could be the more important sources than active cases. In 70% of new cases there was no direct contact with leprosy patients. It showed that there were other sources of transmissions, such as SL, animal or environmental microorganism (Meima 1999; Noorden 1994; Pontes 2006). This hypothesis is supported by several studies that showed positive PCR result from nose swab of subclinical leprosy (Izumi 1999; Meima 1999).

In this study we found no significant difference in the titer of anti PGL-1 IgM antibody between positive and negative PCR result in blood SL, because majority of *M. leprae* live in tissue and only minor spreading of haematogen. One of the reasons in this study was that we used the whole blood. The use of Peripheral Blood Mononuclear Cell (PBMC) that can be extracted from blood could give a higher possibility positive result of PCR due to obligate cellular characteristic of

Mycobacterium leprae that can only survive in macrophage or monocyte.

Detection of *Mycobacterium leprae* DNA by PCR in the blood of leprosy patients has been done (Santos 2001). In this study we used primer Lp1-Lp4 that amplified 99 bp fragment specific with nested methods to increase the sensitivity and specificity (Donoghue 1999; Plykatis 1990). The negative result may show that the gene region was destroyed. More studies are required using several other primers to detect *M. leprae* DNA. DNA examination still have a weak point since it cannot show the viable *M. leprae* case, while the fragmented of dead bacilli still can show the positive result. We need further studies to detect the viable *M. leprae* by RNA examination since RNA was degraded suddenly after the death of bacilli. Usually RNA examination was done to detect the efficacy of treatment, but the use of this method to diagnose early diagnosis of leprosy will provide the possibility to find the sources of transmission.

CONCLUSIONS

This study found 23.77% (29/122) SL among leprosy contact and about 20.69% (6/29) of them had DNA molecules specific to *M. leprae* in the blood. We interpreted the data to indicate the presence of a considerable number of *M. leprae* that infect their body as reservoir and the spread of hematogen. Unfortunately, we could not exclude the possibility of *M. leprae* DNA from the environment which does not induce infection in the body. The finding of SL showed that in endemic area the relatively high transmission risk is not only due to direct contact with leprosy patients, but also due to transmission by animal as a source or environmental organisms. The result of this study is expected to be a consideration in SL patients management, with respect to the potentiality to become manifest leprosy and as a source of transmission. Therefore, we suggest that the new preventive measures, such as chemoprophylaxis, to high risk groups will be urgently needed for the control of leprosy. Our finding also shows that the PCR test is useful as a tool for detecting and early follow-up of possible leprosy cases. It can be used to monitor high-risk populations and also to maintain the achievements of leprosy elimination program in countries where the disease's prevalence has been significantly reduced. But the DNA examination has a weak point due to the incapability of showing that *M. leprae* is still alive. More studies are required to determine the viable *M. leprae* using RNA examination and to decide that these individuals are at a higher potential for leprosy transmission.

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