Chapter 16

Application of Integrated Translational Research as Leprosy Problem Solution in Indonesia

Cita Rosita Sigit Prakoeswa

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67967

Abstract

In Indonesia, leprosy remains a health problem because its elimination has not achieved. This shows the high *Mycobacterium leprae* transmission as a result of difficulties in the early detection, termination of the transmission chain, and management evaluation. Integrated translational research has been carried out as a solution for the problem. Dissemination of the various results of the research is conducted by the educational aspects tiered with a variety of learning methods including a textbook based on research findings, scientific papers at various scientific meetings, and published journals, as well as aspects of community service through electronic media, newspapers, and management and counseling with leprosy patients and their contact person, especially in endemic pockets area.

Keywords: leprosy, *Mycobacterium leprae*, translational research

1. Introduction

Leprosy (Morbus Hansen) is a chronic infectious disease caused by bacteria *Mycobacterium leprae*, which primarily affects the peripheral nerves, and secondary attacks the skin and other organs. World Health Organization (WHO) classified leprosy into two types: paucibacillary (PB), which is relatively not contagious, and multibacillary (MB), which have infectious potential. Complications that occur in leprosy [erythema nodosum leprosum (ENL)] can cause disability in patients resulting in decreased quality of life.

In Indonesia, leprosy remains a health problem. Indonesia has the third-highest number of leprosy patients in the world after India and Brazil, the discovery of new cases is relatively stable from year to year, the dominance of the type of MB (potentially infectious) causes disability (9.9%) and can strike children (7.8%) thus affecting the future of the nation buds. This

shows ongoing leprosy transmissions as a result of difficulties in the **early detection, management evaluation, and termination of the transmission chain**. Integrated translational research is done to resolve leprosy problems both in the community and clinics during the process of medical education.

1.1. Methods featured achievement

The data of leprosy problems in community studies were obtained from Department of Health. In addition, the process of medical education with a unique learning method, not only through lectures but also plunge the management of patients in the clinic (bed side teaching), provides an opportunity to discover a wide range of issues that require to handle leprosy patients.

Research that has been carried out involving all of the Members of the Division of Leprosy, Dermatology and Venereology Department, Faculty of Medicine, Airlangga University—Dr. Soetomo Teaching Hospital Surabaya along with the Leprosy Study Group—Institute of Tropical Disease Airlangga University, students of the Faculty of Medicine, Universitas Airlangga, residents of Dermatology and Venereology Department, Faculty of Medicine, Airlangga University—Dr. Soetomo Teaching Hospital, and postgraduate students, Airlangga University. In addition not only involving the faculty and students of Airlangga University, but also wide range universities such as Indonesia University, Padjadjaran University, Diponegoro University, Hassanudin University, and Sam Ratulangi University to conduct collaboration researches. The cooperation is also carried out by various local and foreign communities, among others Leprosy Research Centre of Tokyo, the Netherlands Leprosy Relief, and the Royal Tropical Institute, Amsterdam.

Working closely with various communities and doing some research setting requires a good strategy. The success of team work was supported by the dedication, discipline, and clear job description. At the time of this research, education and community service aspects are not left behind. By involving medical students, the educational process can still take the time to do some research studies, and to perform community service through health services and counseling.

Broadly speaking, research of **diagnosis**, **management,** and **mode of transmission** has been conducted to overcome the problem of leprosy that includes **early detection**, **management evaluation**, and **termination of the transmission chain**.

2. Review of Research Collaboration by Faculty of Medicine Universitas Airlangga, Dr. Soetomo Teaching Hospital, and Leprosy Study Group, Institute of Tropical Disease, Universitas Airlangga

2.1. Diagnosis of leprosy

Diagnosis of leprosy using cardinal sign only detects the clinical leprosy due to the limitation of this method. Acid-fast bacilli test for detection of *M. leprae* has a limitation in the sensitivity

http://dx.doi.org/10.5772/67967

and specificity. Detecting *M. leprae* by biotechnology based on biomolecular aspect requires laboratories facility and analysts. This method has been performed only in research areas in Indonesia. The advantage of this method is more accurate than the routine method. Its accuracy is important for early detection of leprosy.

2.1.1. Detection of DNA M. leprae using PCR

The presence of *M. leprae* in the blood of patient with subclinical leprosy is still a question mark [1]. The answer should be taken into account by considering the management of subclinical leprosy, because subclinical leprosy has the potency of manifest leprosy and be the source of transmission. *M. leprae* DNA in the blood of subclinical leprosy patient, was investigated using polymerase chain reaction (PCR) test. This study took place in two leprosy endemic villages named Kombang and Poteran in Talango Island of Sumenep District, Madura, East Java, Indonesia. After dermatologic examinations of 122 people with leprosy contact, venous blood was collected to estimate the seropositivity to various mycobacterial antigens. The antiphenolic glycolipid (antiPGL-1) IgM antibody was measured by indirect ELISA.

In those 122 patients with leprosy contact, we found 29 people who had >600 U/ml antiPGL-1 IgM antibody refer to subclinical leprosy (**Figure 1**). From 29 subclinical leprosy patients, we collected 2 ml of venous blood and extracted *M. leprae* DNA using TaKaRa GenTLE methods (**Figure 2**) followed by the PCR test using nested primer Lp1–Lp4 from RLEP repetitive sequence (**Figure 3**).

The result of this study is expected to be important for the management of patients with subclinical leprosy. Considering the potential to manifest into leprosy and become a source of transmission, therefore, we suggest that using new preventive measures such as chemoprophylaxis for high risk groups is important to control the spread of leprosy.

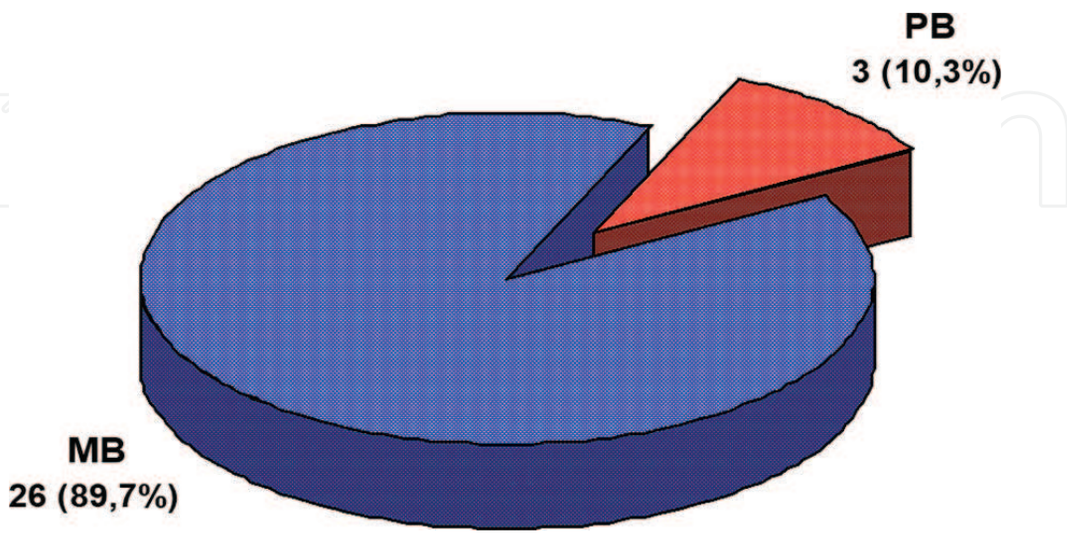

Figure 1. Leprosy type in contact distribution.

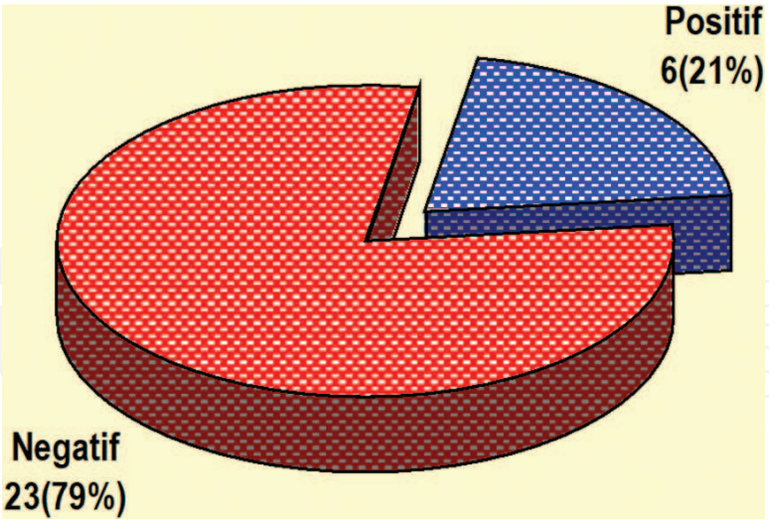

Figure 2. Distribution PCR result: 6 samples positive (21%) and 23 samples negative (79%).

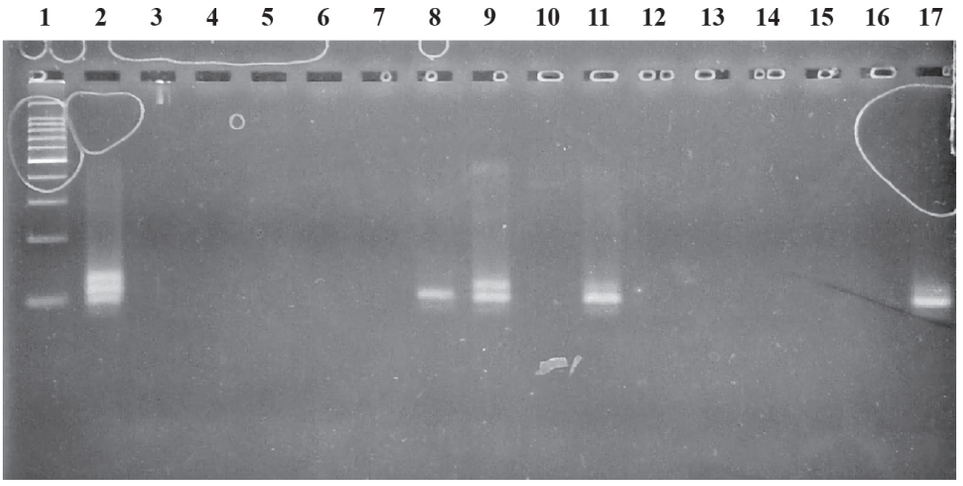

Figure 3. LP1-LP4 PCR product. Lane 1: 100 bp ladder; lanes 2–15: samples; lane 16: NC; lane 17: PC.

2.1.2. Detection of RNA M. leprae (viable) using RT-PCR and quantification of DNA M. leprae using real-time PCR

Detecting viable *M. leprae* is still a problem due to the uncultivable characteristics of the bacilli [2]. A new biomolecular technique of RNA isolation is now used for the detection of viable *M. leprae,* since RNA is rapidly degraded upon cell death. The reverse transcriptase polymerase chain reaction (RT-PCR) method for detecting 16 ribosomal RNA subunit (16S rRNA) *M. leprae* can be used as viable sign of *M. leprae*. 16S rRNA *M. leprae* denoting housekeeping gene containing specific and unique 1170 nucleotide sequence having specific structure variants and were relatively abundant with 1000–10,000 copies in one bacteria with specific characteristic and will be soon degraded after the death of *M. leprae*. Thus, 16S rRNA study by RT-PCR could reflect *M. leprae* viability with high sensitivity and specificity. We detected 16S rRNA *M. leprae* from skin biopsy and blood of new leprosy patients and to

http://dx.doi.org/10.5772/67967

improve the weakness of skin tissue biopsy sample which was invasive and did not take into account the comfortability of the patient. Skin biopsy and peripheral blood mononuclear cells (PBMCs) were obtained from 24 newly diagnosed (14 male and 10 female) untreated leprosy patients in Dr Soetomo Hospital, Surabaya. Diagnosis is based on clinical and AFB examinations. Informed consent was signed beforehand by all of the patients. RNA isolation, cDNA synthesis, conventional PCR, and real-time PCR using primer set P2 (*forward*, 69–91) and P3 (*reverse*, 218–239) were performed in all samples (**Figures 4** and **5**).

In skin biopsy and blood from both MB and PB leprosy, 16S rRNA *M. leprae* can be detected, showing a systemic process that occurred. Reverse transcription methods using conventional PCR and real-time PCR has better sensitivity than AFB staining (**Figure 6**). Detecting viable *M. leprae* by reverse transcription methods may prove to be useful in early detection of leprosy and the potency of transmission, also assessing the efficacy of treatment and potency source of relapse.

2.1.3. The failure of phagolysosome process as a marker viability

Phagolysosome process in macrophage of leprosy patients is important in the early phase of eliminating *M. leprae* invasion [3]. This study was done to clarify the involvement of Rab5, Rab7, and trytophan aspartate-containing coat protein (TACO) from host macrophage and leprae lipoarabinomannan (Lep-LAM) and phenolic glycolipid-1 (PGL-1) from *M. leprae* cell wall as the reflection of phagolysosome process in relation to 16 subunit ribosomal RNA (16S rRNA) *M. leprae* as a marker of viability of *M. leprae*. Skin biopsies were obtained from 47 newly diagnosed and untreated leprosy at Dr Soetomo Hospital, Surabaya, Indonesia and used cross-sectional as the study design (**Figures 7–11**). RNA isolation and complementary DNA synthesis were performed. Samples were divided into two groups of 16S rRNA *M. leprae*-positive and 16S rRNA *M. leprae*-negative. The expressions of Rab5, Rab7, TACO, Lep-LAM, and PGL-1 were assessed with an immunohistochemistry technique. Mann-Whitney U analysis (**Table 1**) showed a significant difference in the expression profile of Rab5, Rab7, Lep-LAM, and PGL-1 ($p < 0.05$), but there was no significant difference of TACO

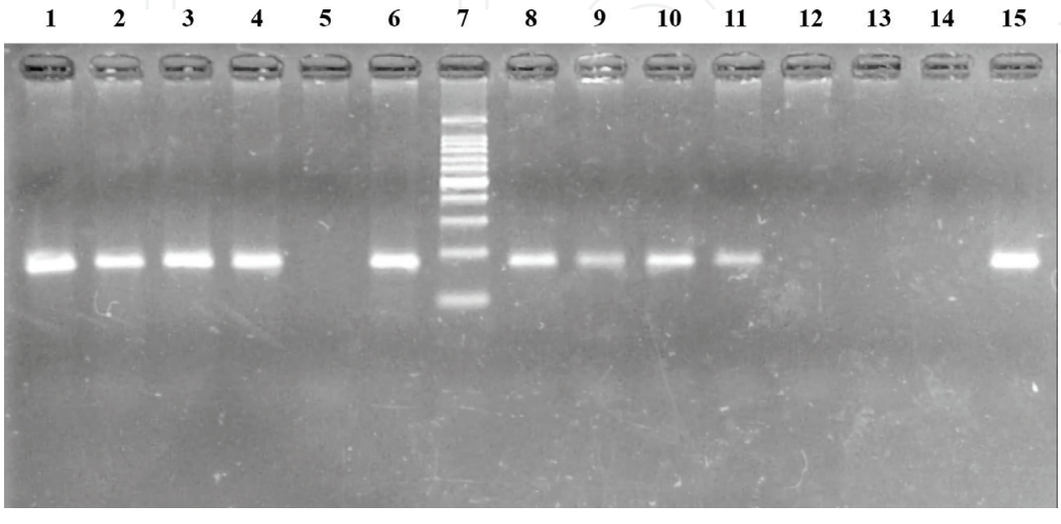

Figure 4. LP1-LP4 PCR product: lanes 1–6 samples; lane 7: 100 bp ladder; lanes 8–13: samples; lane 14: NC; lane 15: PC.

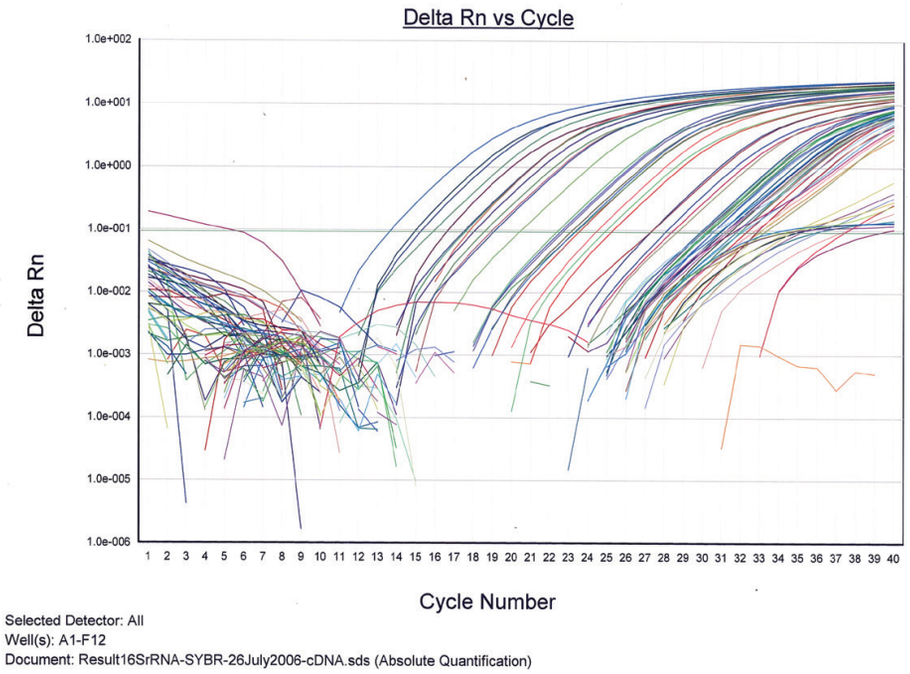

Figure 5. Amplification plot showing increases in fluorescence from each sample.

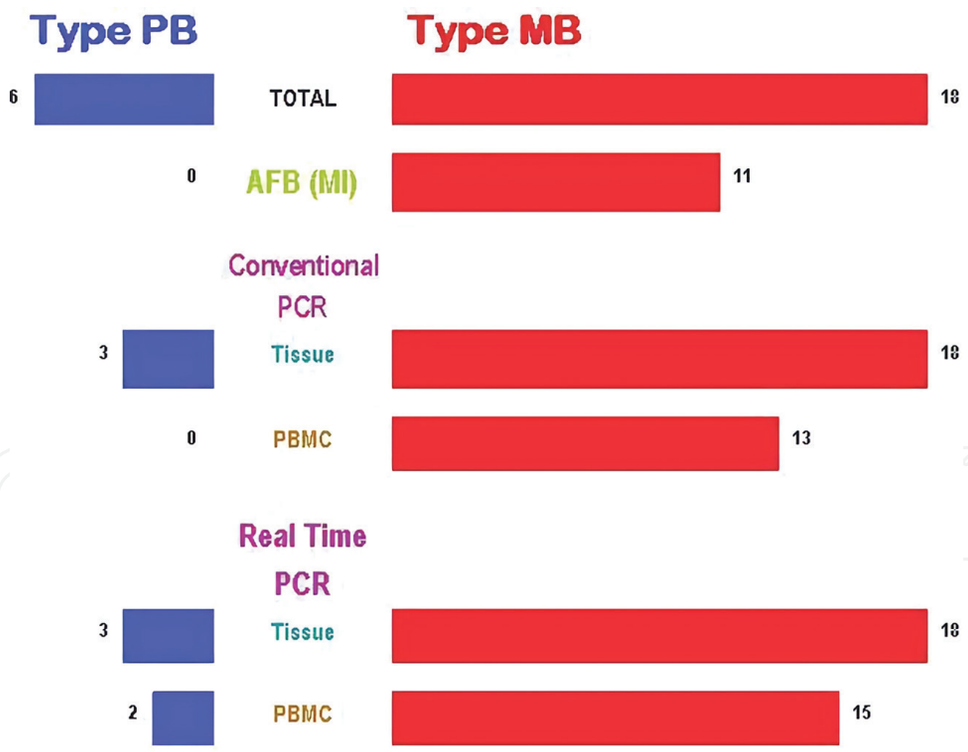

Figure 6. Sample (from skin biopsy and PBMC) showing positive result at leprosy type, AFB examination, 16S rRNA *M. leprae* examination by conventional PCR and teal-time PCR.

between the two groups ($p > 0.05$). Spearman analysis (**Table 2**) revealed that there was a significant correlation between the score of Rab5, Rab7, Lep-LAM, and PGL-1 and the score of 16S rRNA *M. leprae* ($p < 0.05$).

http://dx.doi.org/10.5772/67967

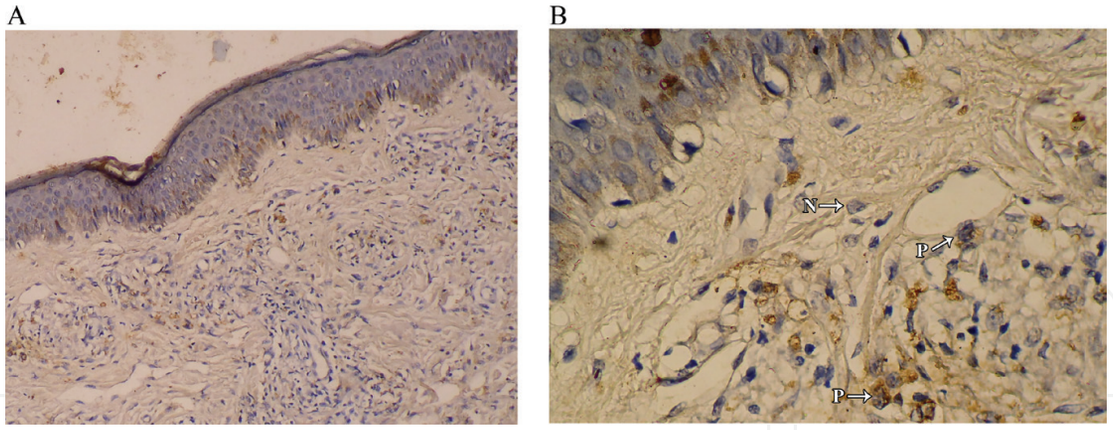

Figure 7. Expression of Rab5 in the skin biopsy section of sample number 14. (A) With the enlargement of 100; and (B) with the enlargement of 450. Note: N = negative reaction (any colors other than brown); P = positive reaction (brown).

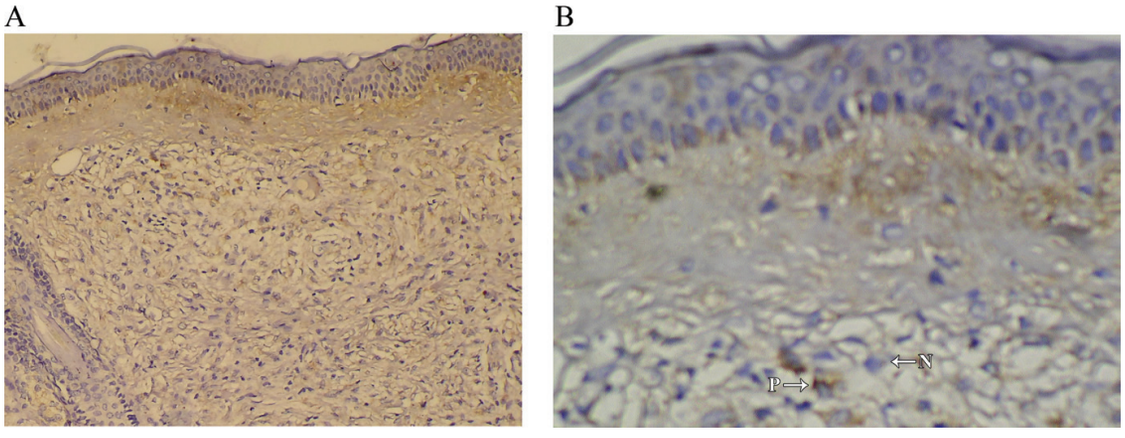

Figure 8. Expression of tryptophan aspartate-containing coat protein in the skin biopsy section of sample number 3. (A) With enlargement of 100 and (B) with enlargement of 450. Note: N = negative reaction (any colors other than brown); P = positive reaction (brown).

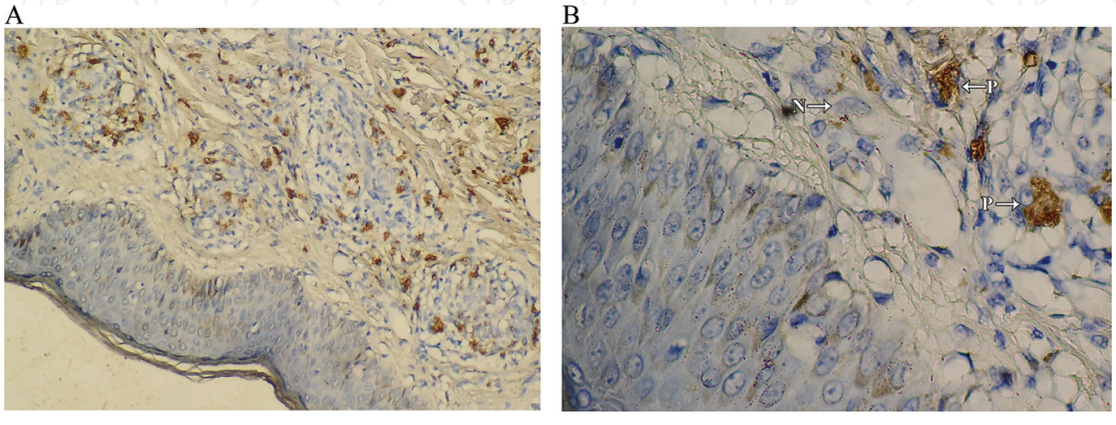

Figure 9. Expression of leprae lipoarabinomannan in the skin biopsy section of sample number 8. (A) With a magnification of 100 and (B) with a magnification of 450. Note: N = negative reaction (any colors other than brown); P = positive reaction (brown).

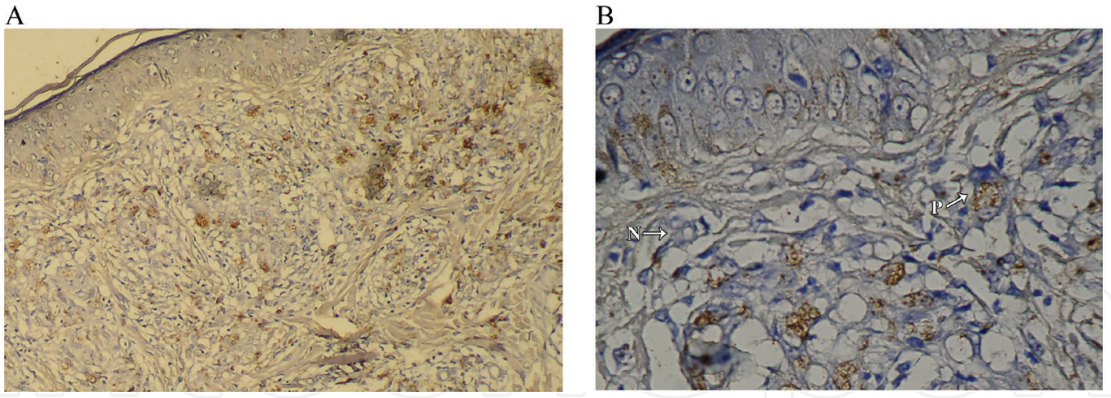

Figure 10. Expression of phenolic glycolipid-1 in the skin biopsy section of sample number 16. (A) With the enlargement of 100 and (B) with the enlargement of 450. Note: N = negative reaction (any colors other than brown); P = positive reaction (brown).

Membrane trafficking in phagolysosome failure is deemed as an important discovery in the study and it is represented by two compounds derived from the host (Rab5 and Rab7) and from the agent (Lep-LAM). PGL-1 role in the inhibition of lysosomes activation pathway in

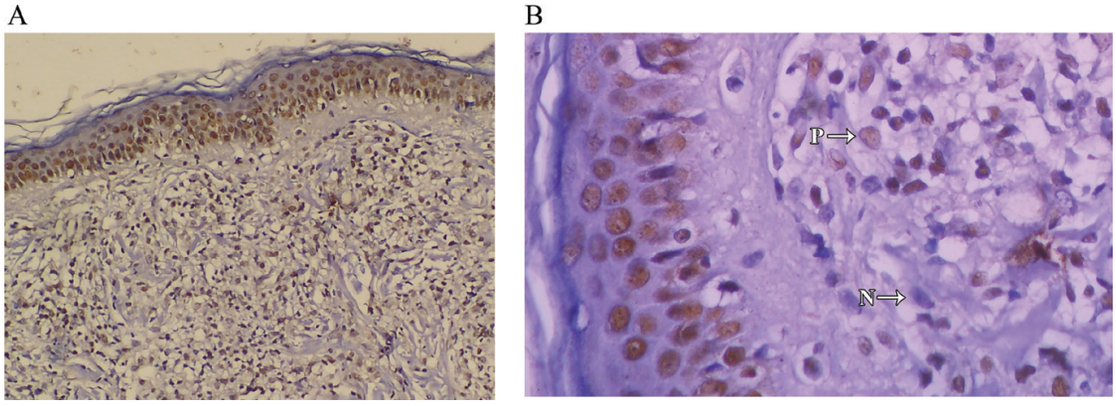

Figure 11. Expression of Rab7 in the skin biopsy section of sample number 6. (A) With enlargement of 100 and (B) with enlargement of 450. Note: N = negative reaction (any colors other than brown); P = positive reaction (brown).

Variables	***p***
Rab5	0.002
Rab7	<0.001
TACO	0.584
Lep-LAM	<0.001
PGL-1	<0.001

Table 1. Mann-Whitney U test results of the differences in the expression of Rab5, Rab7, tryptophan aspartate-containing coat protein (TACO), leprae lipoarabinomannan (Lep-LAM), and phenolic glycolipid-1 (PGL-1) based on positive and negative viability with $p = 0.05$.

http://dx.doi.org/10.5772/67967

Variables	Correlation coefficient	*p*
Rab5	0.483	0.001
Rab7	0.682	<0.001
TACO	0.065	0.662
Lep-LAM	0.608	<0.001
PGL-1	0.491	<0.001

Table 2. Spearman correlation test between combined scores between cell number and staining intensity of the expression of Rab5, Rab7, tryptophan aspartate-containing coat protein (TACO), leprae lipoarabinomannan (Lep-LAM), and phenolic glycolipid-1 (PGL-1) with the score of 16 subunit ribosomal RNA *M. leprae* quantity ($p = 0.05$).

phagolysosome failure was also found from the study. Hence, the expression profiles of Rab5, Rab7, Lep-LAM, and PGL-1 can be used as markers of *M. leprae* viability. From these discoveries, an early diagnostic method for leprosy based on expression pro-files of Rab5, Rab7, Lep-LAM, and PGL-1 is possible. Early diagnosis in leprosy cases is very useful to prevent the occurrence of disability or transmission. In addition to the above discoveries, it is important to study the expression profiles of Rab5, Rab7, Lep-LAM, and PGL-1 in peripheral blood mononuclear cells (PBMCs). Based on the research done by the Leprosy Study Group of the Institute of Tropical Disease, Universitas Airlangga (Prakoeswa, 2011), the results showed no significant differences between expression profiles of 16S rRNA of *M. leprae* in skin biopsy tissue and PBMCs using real-time PCR. Therefore, from the study, the expression profiles of Rab5, Rab7, Lep-LAM, and PGL-1 in PBMCs can be explored to be used as a base. Blood tests without skin biopsy may be sufficient enough and used to create a simplified and noninvasive early diagnostic marker tool for leprosy viability.

Based on the three studies above, early diagnostic method for leprosy can be performed suited to the condition of the facility involved. In a highly qualified laboratorium with skilled analysts, DNA and RNA tests can be performed whereas in a laboratorium with limited facility and analysts, Immunohistochemistry (IHC) test may be used instead based on the phagolysosome failure process.

2.2. Management of leprosy

There has been a decrease in the number of new leprosy patient after MDTL era. However, the number of new cases found is still relatively stable. This means that interventions are still needed to evaluate, and measures to be taken to manage leprosy cases.

2.2.1. Dapsone and rifampicin resistance

Drug resistant cases can be tested by using the biomolecular method as an alternate solution as it is relatively simpler and less time consuming [4]. Based on the detection of mutation in *folp* and *rpoB* gene, we conducted a study about the prevalence of *M. leprae* drug resistance to dapsone and rifampicin in East Java. DNA templates from 153 isolates obtained from MB

leprosy patients from East Java were processed. Polymerase chain reaction (PCR) test was initiated using Lp1–Lp4 primer to show the presence of *M. leprae*. The *folP* and *rpoB* genes were amplified using folP1–folPR and rpoBF-rpoBR primers to obtain the DNA sequence target with 59 isolates for *folP* gene study and 94 isolates for *rpoB* gene study. After purification of PCR product, DNA sequencing was initiated to analyze the mutation on nucleotide sequence.

All isolates showed positive PCR results by Lp1–Lp4. From 59 isolates, 50 isolates showed positive PCR results by folP1–folPR (**Figure 12A**) and the same result goes by rpoBF-rpoBR from 77 out of 94 isolates (**Figure 12B**). In *folP* gene examination, no mutation was found in *rpoB* gene (**Figure 13A**). There are 3 isolates out of 53 that were found to have mutation in amino acid at codon 53; two cases where threonine (ACC) became alanine (GCC) (**Figure 13B** and **C**), and in one case threonine (ACC) became arginin (AGA) (**Figure 13D**). This mutation held responsible for the resistance of *M. leprae* to dapsone. The result suggested that three isolates (6%) from East Java-Indonesia in this experiment are resistant to dapsone and all isolates (100%) are sensitive to rifampicin.

Surprisingly, from three cases that show mutations in the *folP* gene, one of them is a new case with 1 month of multidrug treatment (MDT) duration time. The electropherogram of this sample can be seen in **Figure 13D**. The mutation was detected in amino acid at codon 53 (157–159 nucleotides that are from "ACC" (threonine) to "AGA" (arginine). The isolate is regarded to as a primary diaminodiphenyl sulfone (DDS) resistant. Another mutation in amino acid at codon 53 (157–159 nucleotides) that was detected in two samples is from "ACC" (threonine) to "GCC" (alanine). Based on the clinical data, these two samples are suspected resistant to DDS.

2.2.2. Methylsulfonylmethane treatment in erythema nodosum leprosum

Erythema nodosum leprosum (ENL) is a complex reaction found in the immune system [5]. It causes antibody-antigen complexes to be deposited within various tissues and may cause vasculitis. This condition may occur in MB leprosy patients. While the immunopathology of this disease is not fully understood, the reaction is known as a tumor necrosis factor-α (TNF-α)-mediated process. The severity of the condition, possible complications, limited treatment choices, and recurrent nature of the disease makes it complicated to manage. Research studies to find choices of treatment options for ENL are imperative as the current treatment options

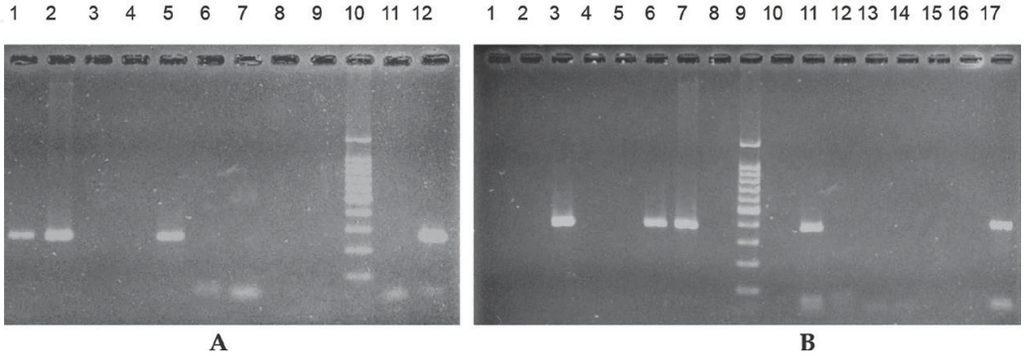

Figure 12. (A). *folP* PCR product, lanes 1–9: samples, lane 10: 100 bp ladder, lanes 11 and 12: NC and PC; (B) *rpoB* PCR product, lanes 1–8 and 10–15: samples, lane 9: 100 bp ladder, lane 16: NC; lane 17: PC.

http://dx.doi.org/10.5772/67967

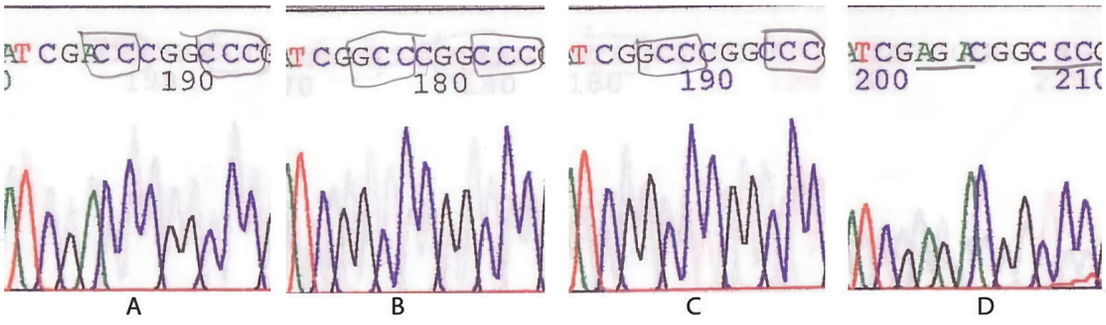

Figure 13. Four *folP* gene sequencing results, (A) No mutation; (B and C). Mutation at codon 53 ACC→GCC; (D) Mutation at codon 53 ACC→AGA.

are limited with high level of morbidity and chronicity. Previous study showed that methylsulfonylmethane (MSM) has strong capability as antiTNF-α properties in vitro. This means that MSM might be useful for treating TNF-α-mediated conditions, such as ENL reaction. Hence, the objective of this study is to establish a correlation whether MSM is effective to treat the clinical signs and symptoms of recurrent ENL reaction in MB leprosy patients.

In this study, patients eligible for the study were all those with MB and admitted for at least a second episode of severe ENL reaction. A total of 10 patients who were eligible for the inclusion and exclusion criteria were enrolled for the study. A standardized history taking using a checklist was recorded from all of the patients chosen for the study. Thorough physical examination was done to look for skin signs, motoric or sensory neuropathy signs, and other possible complications of ENL. After each examination, ENL reaction severity scale was performed and included the basic neurological examination.

Nerve function assessment includes sensory nerve function using the Semmes-Weinstein monofilament test (MFT) and motor nerve function using voluntary muscle tests (VMTs) and all impairments will be recorded. Blood (10 ml) was taken for laboratory assessments on day 1, 7, and 63 for TNF-α examination and routine blood assessment on day 1, 7, 14, 56, and 112. MSM was given to the patients in the study with a dose of 0.1 g/kg bodyweight daily in two divided doses in addition to the World Health Organization's (WHO) multidrug treatment (MDT) and/or additional clofazimine, if a patient has already taken it when the new reaction occurred. If the patient shows clinically significant improvement, the dose would be tapered off by 1 g every 2 weeks, starting from 1 week from the start of MSM treatment. Treatment will be stopped completely in 2 weeks after reaching 1 g/day level.

Graphic of TNF-α in 10 patients is shown in **Figure 14**. Two out of 10 patients showed improvement from ENL reaction. These patients revealed high level of TNF-α, and this value decreased along with lessening of ENL severity scale. First, patient showed increased ENL severity scale within MSM tapering off (full dose of MSM repeatedly and tapered off). The second patient was still in a good condition during follow-up. Eight other patients were dropped on day 3 and 5. In those eight patients, the value of TNF-α showed to be normal and was excluded from the study due to the increase of ENL severity scale. MFT and VMT examinations showed no changes during the study.

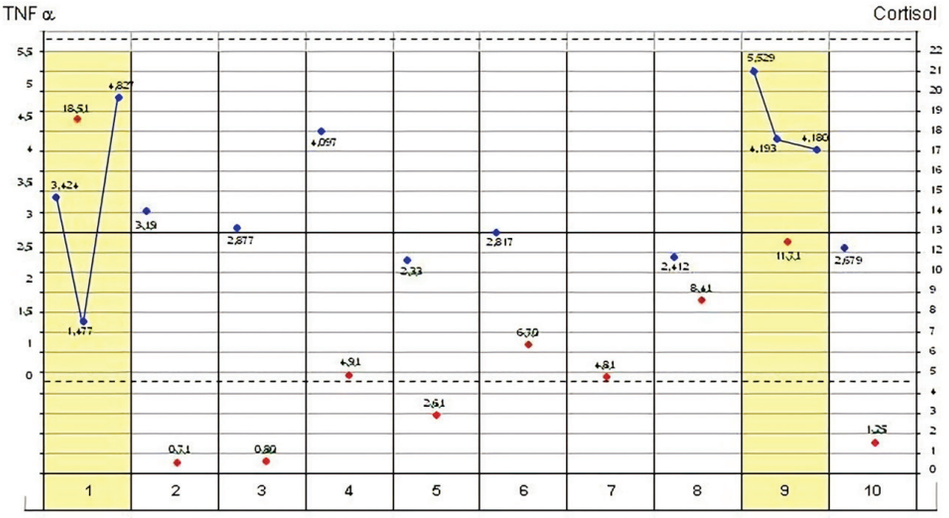

Figure 14. Graphic of TNF-α in 10 patients. Yellow: improving patients; white: dropped out patients; blue: level of TNF-α.

This finding proves that MSM treatment modality is possible as drug of choice for ENL patients with high level of TNF-α in concordance to its mechanism of action as an antiTNF-α.

2.2.3. Chemoprophylaxis in subclinical leprosy

Subclinical leprosy is a person who has high titer of IgM antiPGL-1 without clinical manifestation of leprosy that manifests after several years [6]. Preventive treatment of this leprosy is required especially in children in order to prevent manifest toward leprosy and prevent it from spreading. We evaluate the result of 2 years preventive treatment to subclinical leprosy in elementary school children using special regiment rifampicin and clarithromycin in Raas Island and Nguling, East Java, Indonesia.

Serological surveys for leprosy were conducted and involved a total of 5066 school children, who were screened in 2 leprosy endemic locations in East Java. About 302 elementary school children [109 from Nguling (**Figure 15**) and 193 from Raas Island (**Figure 16**)] were positive for sero (+++) with high IgM antiPGL-1 antibody titer (>3000 U/ml ELISA). Rifampicin 300 mg daily with 250 mg clarithromycin daily for 10 days was given as a preventive treatment, continued with the same drugs administered intermittently every 2 weeks for 3 months. Every year, clinical and serological examination was evaluated.

After 2 years evaluation, none of the children showed any manifestation of leprosy clinically. IgM antiPGL-1 antibody level showed to decrease between these 2 years of evaluation (Raas Island and Nguling: $p = 0.00$). The majority of the children (Raas Island 96.46%; Nguling 94.83%) showed a decrease in IgM antiPGL-1 antibody level, but some of these children (Raas Island 3.54%; Nguling 5.17%) also showed an increased level of IgM antiPGL-1 antibody. All the medications were well tolerated by these children and only a few side effects due to these drugs were reported.

http://dx.doi.org/10.5772/67967

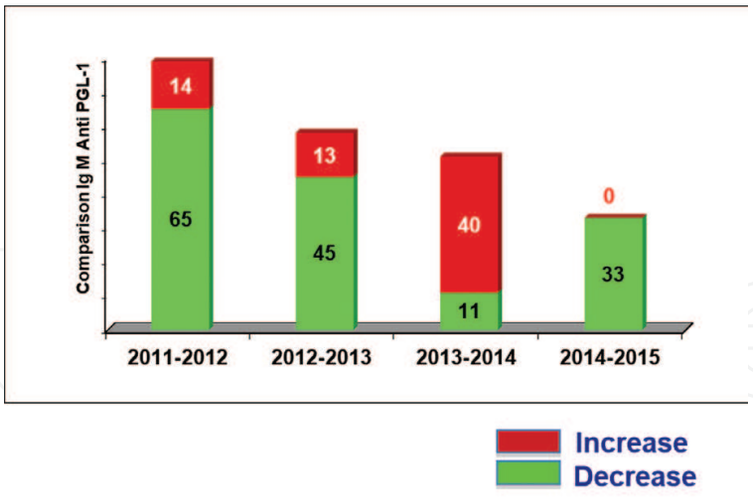

Figure 15. Evaluation of IgM antiPGL-1 in Nguling. Green = decrease; red = increase.

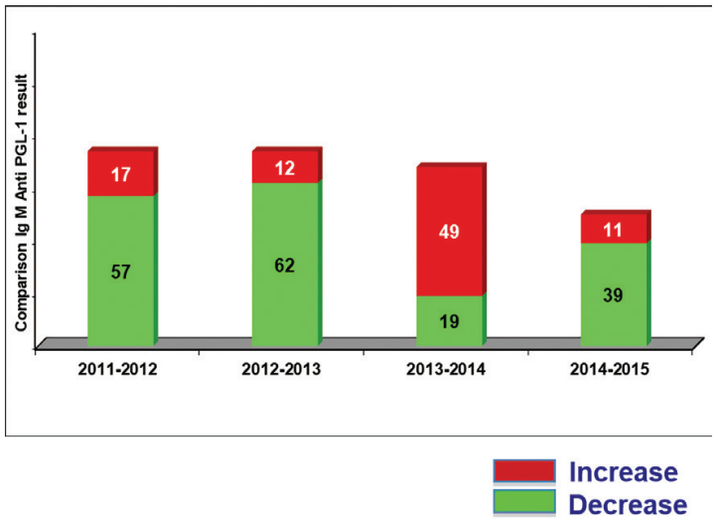

Figure 16. Evaluation of IgM antiPGL-1 in Raas Island. Green = decrease; red = increase.

Chemoprophylaxis for subclinical leprosy in children showed a promising good result after 2 years of evaluation. Further evaluation will be conducted for the next 3 years ahead. Our research about subclinical leprosy in children may support the clinical importance of it in exploring the disease transmission and how we can prevent it.

From these three studies, evaluation of resistance for suspected cases, MSM administration for refractory cases to steroid and administration of chemoprophylaxis for subclinical leprosy, can be implemented on routine leprosy management. Ongoing research is expected to prevent the onset of leprosy. Study on the impact of chemoprophylaxis in household and neighbor contact person with a grant from the Netherlands Leprosy Relief is also being carried out in collaboration with the Ministry of Health and local government.

2.3. Mode of the transmission

During this time, it is believed that the only source of transmission is leprosy MB type, but as we have not achieved elimination, we begin to think about another source of the transmission.

2.3.1. Strain local study in endemic area using PCR sequencing

Multiple locus variable number of tandem repeat (VNTR) analysis has been proposed as a mean of genotyping for tracking leprosy transmission [7]. Many tandem repeats have been reported to be polymorphic with the potential as genetic markers to differentiate strains of *M. leprae*. However, depending on the population, the characteristics of polymorphism may vary. We measured the copy number of repeat in four genetic markers, which are TTC, AC 8a, AC 9, and 6–7 (**Figures 17–23**) in leprosy patients. A number of 23 patients were recruited from outpatient clinic in Department of Dermato-Venereology of Dr Hasan Sadikin Hospital, Bandung. Multiple locus VNTR analysis at four loci was applied using total DNA extracts from skin biopsies.

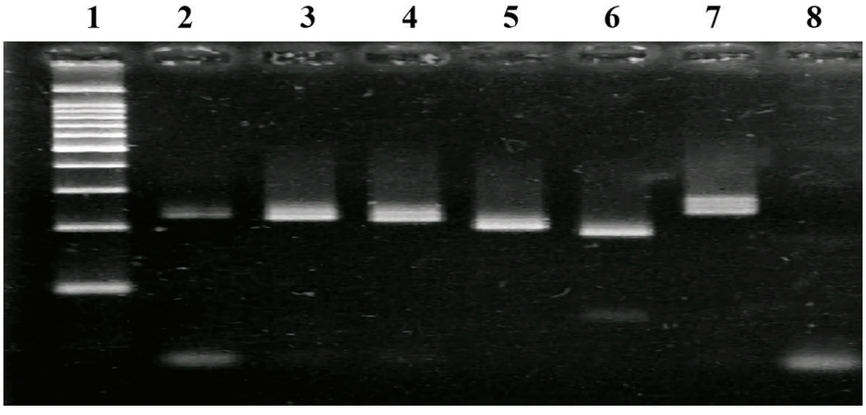

Figure 17. TTC PCR product: lane 1: 100 bp ladder, lane 2: PC, lanes 3–7: samples, lane 8: NC.

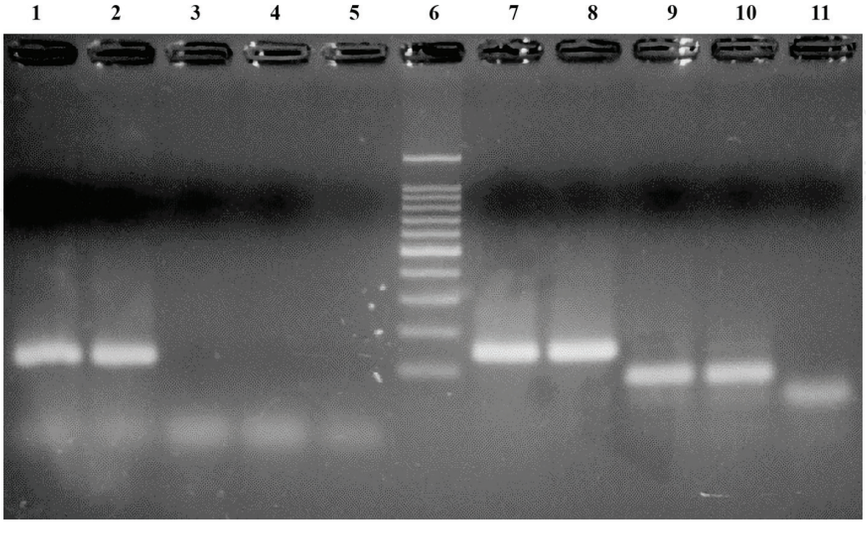

Figure 18. AC 8A and AC 9 PCR product: lane 1: PC AC 8a, lanes 2–4: samples, lane 5: NC AC 8a, lane 6: 100 bp ladder, lane 7: PC AC 9, lanes 8–10: samples, lane 11: NC.

http://dx.doi.org/10.5772/67967

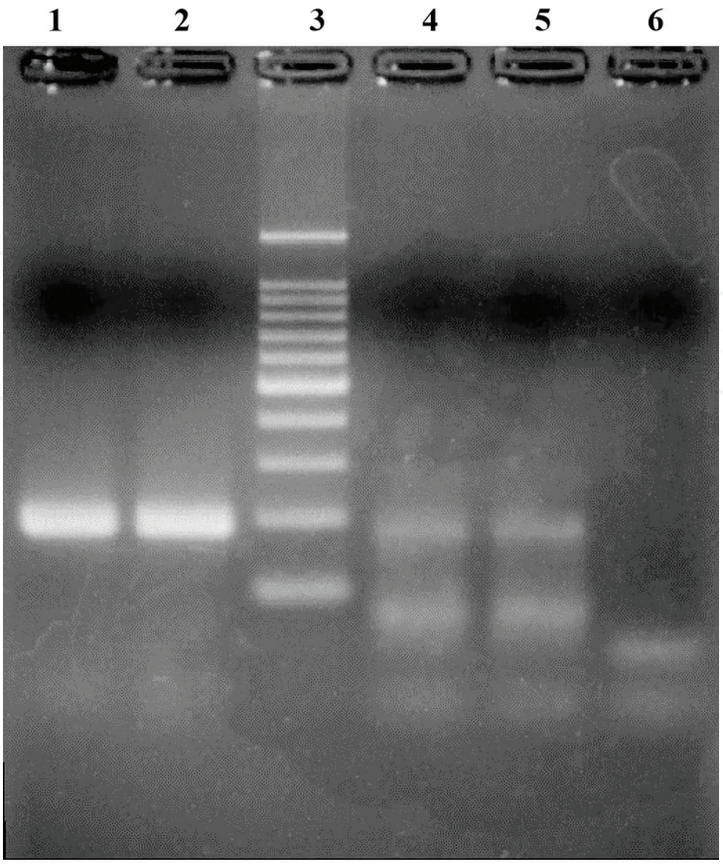

Figure 19. PCR product: lane 1: PC, lanes 2,4,5: samples, lane 3: 100 bp ladder, lane 6: NC.

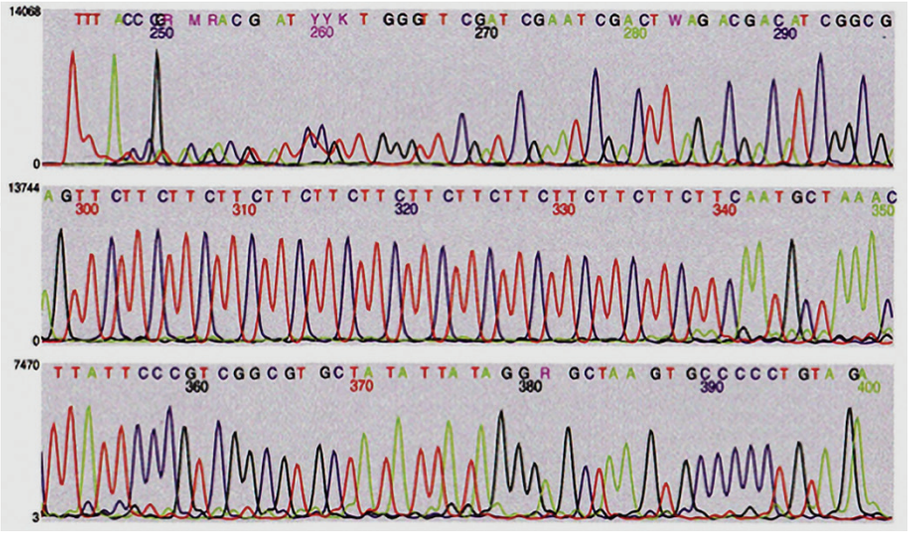

Figure 20. PCR sequencing result: primer TTC.

As shown in **Table 3**, there were five samples showing the same copy number of four genetic marker: TTC = 15; AC 8a = 10; AC 9 = 10, and 6–7 = 6. Two samples showing the same copy

number of four genetic marker: TTC = 16; AC 8a = 10; AC 9 = 11, and 6–7 = 6. The multiple locus VNTR analysis shows two identical *M. leprae* VNTR profiles from Bandung. These attributes support the use of VNTR loci for transmission studies and VNTR analysis can use for multicases family study.

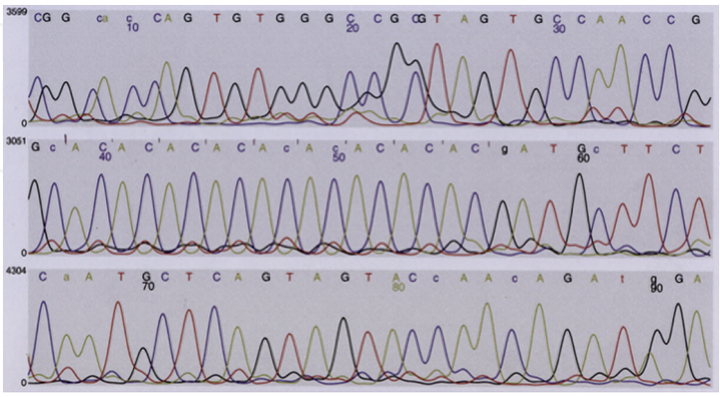

Figure 21. PCR sequencing result: primer AC 8A.

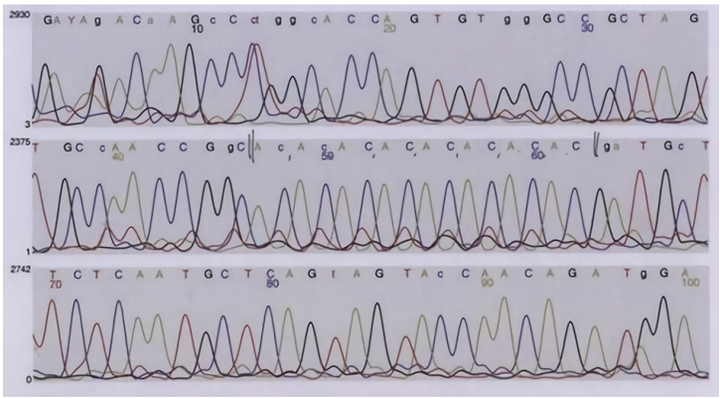

Figure 22. PCR sequencing result: primer AC 9.

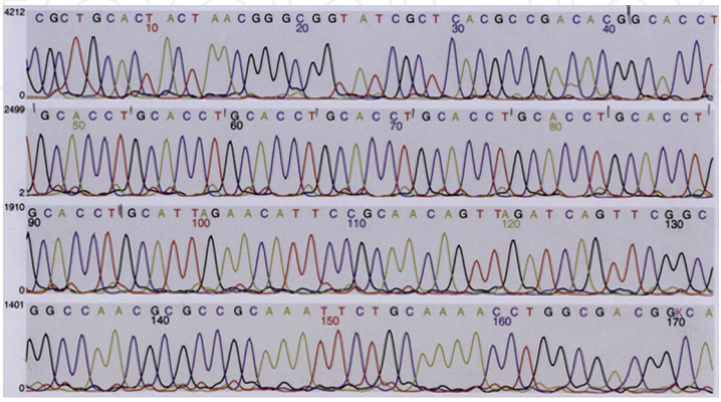

Figure 23. PCR sequencing result: primer 6–7.

http://dx.doi.org/10.5772/67967

Table of VNTR analysis				
No	**TTC**	**AC 8a**	**AC 9**	**6–7**
1	12			
2	13	11		6
3	14			
4	15	10	10	6
5	15	10	10	6
6	15	10	10	6
7	15	10	10	6
8	15	10	10	6
9	15	10	10	6
10	15	10	10	6
11	16	10	11	6
12	16	7	10	
13	16	10	11	6
14	16	10	11	6
15	17	9		6
16	17	8	8	6
17	18			
18	21	8	10	8
19	21	8	9	8
20	22			
21	23			
22	25			8
23	37			

Table 3. VNTR analysis in clinical sample from Bandung isolates.

2.3.2. Environment study and multicase family study using PCR sequencing

East Java is a province in Indonesia that has few endemic areas for leprosy [8]. In order to comprehend this increasing incidence of leprosy, molecular typing will make it feasible to study geographical distributions of *M. leprae*. Genotyping analysis was done by using *variable number tandem repeats* that found in the *rpoT* gene which was followed by the recognition of the TTC triplet in a region of the *M. leprae* genome. The aim of this study is to analyze the number variation of TTC repeats and their distributions in leprosy endemic areas. Poteran

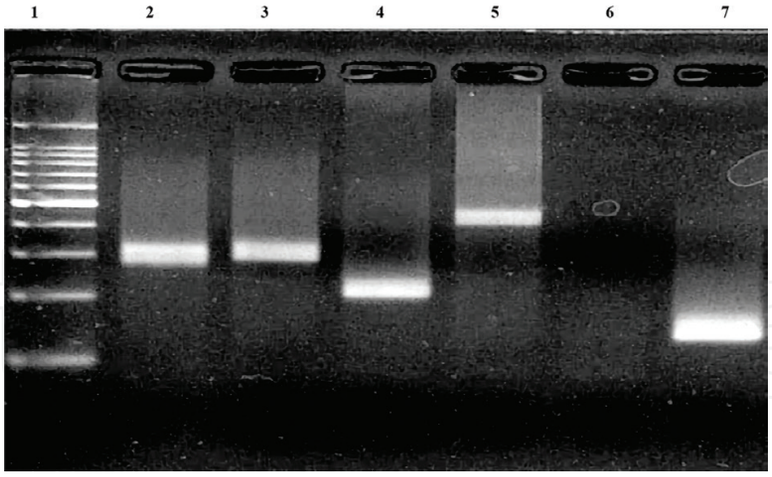

Figure 24. TTC PCR product lane 1: 100 bp ladder; lanes 2–5: sample, lane 6: NC, lane 7: PC.

Island in Madura was chosen for its high prevalence for leprosy and the number has remained stable for the last 5 years. Samples were collected and divide into 3 groups: 91 water sources; 42 nasal swabs of household contact, and 68 skin tissues of leprosy patients. All samples were analyzed by using PCR (**Figure 24**) and the numbers of TTC repeats were confirmed by direct sequencing. From all collected samples, 24 isolates from water resources were positive (26.4%); 26 nasal swabs were also positive (61.9%); and also 24 skin tissues (35.3%).

No. of repeats	Slit-skin specimens	Nasal swabs	Water sources
TTC-9	0	0	4
TTC-10	20	10	8
TTC-11	**32**	**42**	**36**
TTC-12	4	4	0
TTC-13	8	4	12
TTC-14	16	28	32
TTC-15	0	0	4
TTC-16	4	0	0
TTC-17	0	4	0
TTC-24	4	0	0
TTC-28	4	0	0
TTC-40	4	0	0
TTC-44	0	8	0
TTC-49	0	0	4
TTC-60	4	0	0
Total	100% (24 cases)	100%(26 cases)	100% (24 cases)

Table 4. Genotypes TTC frequency (%).

Location	Family member	Relationship	TTC repeat	
			Nasal swab	Slit-skin spec.
House 1	MB patient	Husband	10	10
	Family contact	Wife	11	
	Family contact	Child	- [a]	
	Suspect leprosy	Child	-	11
	Family contact	Mother	10	
	Family contact	Sister in law	11	
	Family contact	Mother in law	10	
	Family contact	Father in law	-	
House 2 (Neighborhood)	MB patient	Child	11	11
	Family contact	Mother	11	
	Family contact	Father	10	
	Family contact	Child	-	
	Family contact	Child	11	

Table 5. Variation of TTC repeats in multicase family.

The copy number of TTC repeats in Talango Island varied from 9 to 60 copies (**Table 4**). The 11-copy TTC genotype was the most frequent in all samples.

The copy number of TTC repeats in Talango Island varied from 10 to 11 copies (**Table 5**). The 11-copy TTC genotype was the most frequent in all samples. There were no differences were found statistically in the pattern distribution of TTC repeats between nasal swab of households contacts and skin tissues of patients (p = 0.594); skin tissues of leprosy patients and water resources (p = 0.441); nasal swab of households contact with water resources (p = 0.906). It can be concluded in endemic areas, transmission of *M. leprae* has strong ties with these three aspects: agent, host, and environment.

2.3.3. Mother-baby transmission in leprosy

Lucio phenomenon is a rare type of reaction in untreated lepromatous leprosy type with diffuse infiltrative form, characterized with ulcerative type of skin lesions [9]. In this study, a case of 29-year-old Indonesian female, 7th month primigravida with lucio leprosy, without prior treatment using WHO-multidrug therapy (MDT). Laboratory examinations reported bacterial index 6+ and morphological index 7% from slit-skin smear; histopathology revealed lucio phenomenon; PCR examination found *M. leprae* DNA on amniotic fluid and skin lesion: positive; umbilical cord membrane and umbilical cord: negative (**Figure 25**). AntiPGL-1 IgM and IgG from patient: 4854 and 1061 U/mL, respectively; from 5-month-old baby: 5 and 1724 U/mL, respectively; from 1-year-old baby: 0 and 3 U/mL, respectively.

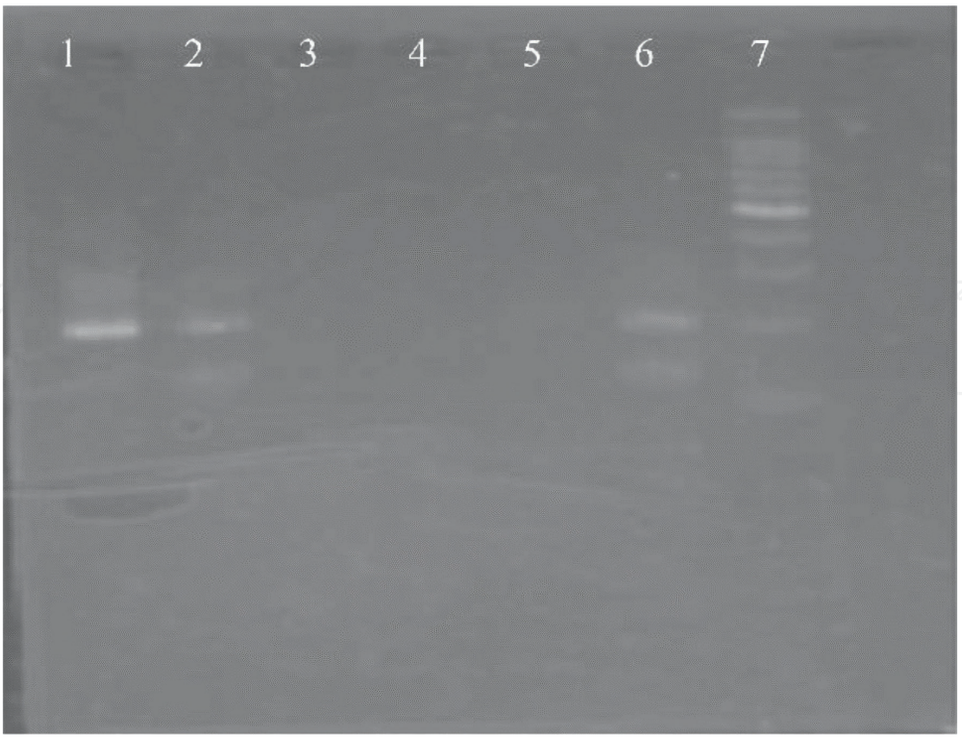

Figure 25. PCR examination: 1: skin lesion; 2: amniotic fluid; 3: umbilical cord membrane; 4: umbilical cord; 5: negative control; 6: positive control *M. leprae* Thai53; 7: DNA ladder.

In **Table 6**, patient's antiPGL-1 IgM and IgG titers collected during caesarian section were way over the cutoff limit, whereas the antiPGL-1 IgM and IgG titers from the umbilical cord blood were below the limit. Hence, placenta is regarded as a protective barrier against fetomaternal transmission of *M. leprae*. The placenta has multiple innate defense properties against pathogens. Only few pathogens can pass through these barriers at low frequencies. DNA of *M. leprae* was found in the amniotic fluid. About 5% of babies born from

Subject	ELISA anti PGL-1 (U/mL)		Cut off (U/mL)	
	IgM	IgG	IgM	IgG
Patient during the caesarian section	4854	1061		
Umbilical cord blood during the caesarian section	0	516		
Patient (after 7 months of therapy)	l912	1505	605	630
Baby (5 month-old)	5	1724		
Baby (1 year-old)	0	3		

Table 6. Results of serological examination using ELISA.

mothers with active leprosy had self-limited indeterminate leprosy before 2 years old and also antiwill have *M. leprae* antibodies of class IgA, IgG, and IgM. The presence of IgA and IgM anti*M. leprae* antibodies in the cord blood of newborn babies from mothers with leprosy might shows an intrauterine immunologic stimulation process that happened due to transplacental transmission of *M. leprae* antibodies. Titers for antiPGL-1 IgM and IgG were reviewed again after the babies reached 5 months old and 1-year-old. On assessment, the titers were found to drop drastically, especially antiPGL-1 IgG titer. Based on these facts, we assume that passive antibody to *M. leprae* from the babies had been acquired from their mothers' blood and transmitted through the umbilical cord blood as shown by the presence of antiPGL-1 IgG antibody.

Studies in genotyping of patients and contact person proved that genotyping is not always appropriate; there is still the possibility of environmental transmission source. From these three studies above, there can be further potential research on transmission of leprosy from nonhuman sources. In the transmission pattern from mother to baby, it shows the importance of the role of placenta as a barrier, therefore, the health of the expecting mother needs to be optimized so as to prevent fetomaternal transmission and to treat the mother as early as possible and closely monitored the baby during incubation phase.

3. Conclusions

Dissemination of an integrated translational research above is done through aspects: education: lectures, case discussions, bed side teaching, and writing research-based textbook for medical students, undergraduates, postgraduates, and dermatology and venereology residency program. Besides, presentations are done at local and international scientific meetings as well as publication of journals. Research: an integrated translational research is a continuous activity with the ultimate goal to eliminate leprosy in Indonesia. Community services: improving services and counseling at social events as well as when we are doing research in endemic pockets area. Besides, counseling is also done through electronic media. Community services can be done in practice of the research results of what they learned through lectures and case discussions in accordance with the level of competence they need to accomplish.

Various educational activities, research, and community service above increase knowledge about leprosy with the ultimate goal of achieving elimination through improvements in the field of preventive, curative, promotive, and rehabilitative. Here, the role of educational institutions is very important in helping to resolve the national problem. In the future, we hope more collaboration research of diagnosis, management, and mode of transmission will be conducted to overcome the problem of leprosy include early detection, management evaluation, and termination of the transmission chain. Integrated translational research is important to be done to resolve leprosy problems both in the community and clinics during the process of medical education.

Author details

Cita Rosita Sigit Prakoeswa

Address all correspondence to: citarositasp@yahoo.com

Faculty of Medicine, Dr. Soetomo Teaching Hospital, Airlangga University, Surabaya, Indonesia

References

[1] Prakoeswa CRS, Agusni I, Izumi S. Detection of DNA *Mycobacterium leprae* in blood of the subclinical leprosy. Folia Medica Indonesiana. 2007;**42**(2):64-67. ISSN 0303-7932

[2] Prakoeswa CRS, Agusni I, Izumi S. Detection of viable *mycobacterium leprae* in skin biopsy and peripheral blood mononuclear cells (molecular biology study of 16s rRNA *M. leprae*). Media Dermato Venereologica Indonesiana. 2011;**38**(3):6-13. ISSN 0216-0773

[3] Prakoeswa CRS, Adriaty D, Wahyuni R, Iswahyudi, Yusuf I, Sutjipto, Agusni I, Izumi S. Expression profile of Rab5, Rab7, TACO, Lep-Lam and PGL-1 on the failure of phagolysosome process in macrophage of leprosy patients as a viability marker of *Mycobacterium lepra.* International Journal of Mycobacteriology. 2016;**5**:155-163

[4] Adriaty D, Wahyuni R, Susari NP, Prakoeswa CRS, Agusni I, Izumi S. Molecular detection of dapsone and rifampicin resistance on *Mycobacterium leprae* from leprosy patients in East Java. Microbiology Indonesia: 2009;**3**(3):115-120. ISSN: 1978-3477

[5] Prakoeswa CRS, Astari L, Citrashanty I, Listiawan MY, Agusni I, Brakel W. Pilot trial: Treatment methyl sulphonyl methane for erythema nodusum leprosum. In: Proceedings of the 18th International Congress of Leprosy; 16-20 September 2013; Brussel. Belgium: International Congress of Leprosy; 2013. pp. 267-278

[6] Prakoeswa CRS, Adriaty D, Wahyuni R, Kusnartedjo, Astari L, Listiawan MY, Agusni I, Izumi S. Three years of evaluation in preventive treatment of subclinical leprosy in elementary school children with subclinical leprosy. In: Proceedings of the World Congress of Dermatology; 8-13 June 2015; Vancouver. Canada: WCD; 2015. p. 60

[7] Prakoeswa CRS, Adriaty D, Wahyuni R, Iswahyudi, Damayanti L Sasmojo M, Agusni I, Izumi S. Genotyping *Mycobacterium leprae* Bandung clinical isolates by multiple locus VNTR analysis. In: Proceedings of the Konas Perdoski XII; 20-22 Juni 2008; Palembang. Indonesia: Konas Perdoski; 2008. pp. 219-220

[8] Adriaty D, Wahyuni R, Iswahyudi, Mudatsir, Prakoeswa CRS, Agusni I, Izumi S. Genotyping analysis by TTC repeat variation of *M. leprae* isolates in leprosy endemic area in East Java (Epidemiology Molecular Study in Poteran Island, Sumenep, East Java Province). In: Proceedings of the 4th Indonesia Biotechnology Conference (International

Forum for Biotechnology); 15-17 February 2008; Bogor. Indonesia: International Forum for Biotechnology; 2008. p. 55

[9] Prakoeswa CRS, Herwanto N, Agusni RI, Rismauli F, Adriaty D, Wahyuni R, Iswahyudi, Listiawan MY, Agusni I, Izumi S. Lucio Phenomenon of leprosy LL type on pregnancy: A rare case. Leprosy Review. 2016;**78**:1-6

IntechOpen

IntechOpen