Mycobacterium leprae deoxyribonucleic acid positivity on skin lesion of untreated leprosy patients and its route to the skin surface

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Abstract

The route of transmission of leprosy is still unclear. Multiple opinions exist regarding the environmental dissemination of *Mycobacterium leprae (M. leprae)*. This research aims to identify the positive proportion of *M. leprae* deoxyribonucleic acid (DNA) on skin lesion surface of untreated multibacillary leprosy patients by polymerase chain reaction (PCR), and its association with the examination result of acid-fast bacilli (AFB) in epidermis, pilosebaceous unit, and sweat glands. The *M. leprae* DNA sample was taken from smears of the skin lesion surface by using PCR and the skin biopsy test was done to detect AFB in epidermis, pilosebaceous unit, and sweat glands. From 28 subjects, there was 82.1% positive proportion of *M. leprae* DNA on the skin lesion surface of untreated multibacillary leprosy patients, where all were found in the lepromatous leprosy and borderline leprosy type. The result of AFB was positive in epidermis (71.4%), pilosebaceous unit (46.4%) and sweat glands (53.6%). We found M. *leprae* by using PCR technique on skin lesion surface of untreated multibacillary leprosy patients, and its route which most likely was through the epidermis.

Introduction

Leprosy or *Morbus Hansen* (MH) is a chronic granulomatous infectious disease caused by *Mycobacterium leprae* (*M. leprae*).[1] Leprosy remains a health problem in Indonesia, despite the fact that leprosy elimination in Indonesia was accomplished in 2000 with the average of elimination 0.86 per 10.000 persons per year.[2]

At present, the route of *M. leprae* transmission is still unclear. The most believable theory is dissemination through respiratory tract.[1,3-5] Other theories believe that the dissemination is through skin lesions of a leprosy patient to other individual's skin, yet this theory has still been debated until now.[1] Some leprosy experts say that skin can be one source of leprosy dissemination that cannot be ignored.[6-8] Ridley, 1976 found in the multibacillary (MB) leprosy patient's fingers there were so many intact bacilli that could be the source of infection. A year later, Leiker, 1977 collected evidences of epidemiological, clinical, and bacteriological supporting the infection theory through skin. Furthermore, Kotteeswaran *et al.*, 1980 from their study in India concluded that in lepromatous and borderline leprosy patients, *M. leprae* could be spread through secretion of sweat and sebum to skin surface. Satapathy *et al.*, 2005 in India acquired acid-fast bacilli (AFB) groups on epidermis of skin lesions of one lepromatous leprosy (LL) patient and suspected that skin lesions can be one source of leprosy transmission.[9] In Indonesia, Ghozali *et al.*, 1982 found the AFB on stratum corneum in one case of LL. Bacilli on the stratum corneum was believed as the source of infection through the skin.

Following the disagreements, further research is needed to clarify the possibility of leprosy transmission through skin. Now that Polymerase Chain Reaction (PCR) technique has found to detect *M. leprae* deoxyribonucleic acid (DNA). The specificity of PCR technique for *M. leprae* reaches 100% while the sensitivity is more than 80%.[10] Before PCR for *M. leprae* found, histopathological examination with acid fast staining had become the best way to detect the existence of *M. leprae* in the tissues.

This research's purpose was to know whether skin lesions of untreated leprosy patients could be a source of dissemination, by justifying the existence of *M. leprae* on the skin lesion surface of leprosy patients through better techniques than in the previous studies. Moreover, it is to know its association with some skin structures like epidermis, pilosebaceous unit and sweat glands or sweat ducts as the origin or the exit of *M. leprae* to the skin surface.

Materials and Methods

This research used descriptive study with cross-sectional design, conducted in Dermatology and Venereology Clinic, Cipto Mangunkusumo Hospital, Jakarta, from May to September 2008; and was approved by the ethical committee of medical research ethics of the faculty of medicine, Universitas Indonesia. Subjects were leprosy patients who came or were referred to our department. The inclusion criteria of the research subjects were aged 15 to 59 years old, had been diagnosed with leprosy anddivided into categories of WHO classification (multibacillary/MB, paucibacillary/PB) and Ridley-Jopling classification (lepromatous leprosy/LL, borderline lepromatous/BL, mid borderline/BB, borderline tuberculoid/BT), having skin lesion leprosy on their back, not having got any leprosy treatments, and agreed to be the research subject by signing consent form. Meanwhile, the exclusion criteria were patients who had a history of bleeding disorders or consuming drugs that can cause disorders such as acetosal, warfarin, or heparin.

The specimen of skin lesion smears and skin biopsy were taken from the same lesion. Skin lesions were the surface of the

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Key words: *Mycobacterium leprae*, deoxyribonucleic acid, skin surface, skin structures.

Contributions: EN collecting, analyzing, and references search; PM manuscript writing; BW, SM manuscript reviewing.

Conflict of interest: The authors declare that no potential conflict of interest.

Acknowledgement: We would like to thank Professor Dr. dr. Indropo Agusni, SpKK(K), Professor Shinzo Izumi, MD, PhD (Tropical Diseases Centre, Surabaya, Indonesia), and dr. Emmy Soedarmi Sjamsoe Daili, SpKK(K) (FKUI, Jakarta, Indonesia) for their participation, support and insightful discussions in this study.

Conference presentation: Part of this paper was presented at the 23rd Regional Conference of Dermatology, 2018 August 8-11, Surabaya, Indonesia.

Received for publication: 1 February 2019.
Accepted for publication: 7 February 2019.

Licensee PAGEPress, Italy
Dermatology Reports 2019; 11(s1):8024
doi:10.4081/dr.2019.8024

skin lesions of leprosy, located on the back, intact, no erosions, excoriation, or ulcers. The procedure of taking the skin lesion surface smears is as follows: the specimen was taken from the most active leprosy lesion minimum 2 hours after bathing or cleaning the lesion area. Swabbing on the defined skin lesion surface by using wet cotton buds soaked in NaCl 0.9% sterile liquid – the cotton bud is cut and dipped into the plastic tube containing 2 mL of alcohol 70% and sealed tightly – after that, the specimens were stored at temperatures between 24^{0}C and 30^{0}C until DNA extraction and PCR tests are done. The DNA extractions and PCR were done in the Tropical Diseases Center laboratory at Universitas Airlangga, Surabaya.

Skin biopsy was done based on our standard procedures. The biopsy sample was taken with ellipse incision; the longest diameter is 1 cm, and subcutis depth.

DNA extraction from the skin lesion surface smears was done by using QIAprep Spin Miniprep KIT®. The PCR process used Takara® PCR Therma Cycler TP 600 machine and its equipment (Takara Bio Inc., Japan). PCR product was retrieved with the machine of electrophoresis Mupid J®. The result of amplification DNA was visualized by ethidium bromide coloring and ultraviolet light, and then photographed with digital camera Kodak Edas 2910 connected to the computer. Researchers and expert staffs of laboratory did the readings by looking at the positive and negative controls. The result of PCR is positive if it is the same with the positive control at the reading.

Skin tissues that had been fixed by formalin 10% for 24 hours, were processed into a paraffin block and then made into three slides, and colored by Fite-Faraco staining. AFB was looked for in epidermis, pilosebaceous units, and sweat glands or sweat ducts, valued qualitatively (if AFB found, it was considered positive; and if not found, negative), and then it was recorded. The result of AFB is positive when there are intact rods shaped like red stain in epidermis, pilosebaceous unit, and sweat glands or sweat ducts.

The data was analyzed by using software program *Statistical Package for Social Sciences* /SPSS 11.5. To compare the two paired-qualitative variables, McNemar test was used with $p<0.05$ considered significant difference.

Results

From the period of May to September 2008, there were 28 subjects that fulfilled the research criteria. In this research, the number of males (67.9%) were more than females (32.1%). The average age was 35.18 years old and the median was 33.5 years old, with the youngest 17 and the oldest was 59. Most subjects (53.6%) were between 25 and 44 years old. The leprosy type of subject research was mostly BL (71.4%), followed by LL (10.7%), BB (10.7%), and BT (7.1%).

The positive proportion of *M. leprae* DNA on skin lesion surface of untreated MB leprosy patients by PCR technique was 82.1%. It was found in all LL and BL type patients. Positive proportion of AFB in epidermis was 71.4%, sweat glands or sweat ducts 53.6%, and pilosebaceous unit 46.4%. The comparison of AFB and M. leprae DNA PCR results on the surface of untreated skin lesions MB leprosy are represented in Table 1.

Discussion

In this research, *M. leprae* DNA was detected in 82.1% of sample from the skin lesion surface of untreated leprosy. That result is similar with the previous pilot study done by the author: the positivity of *M. leprae* DNA on the skin lesion surface accounted 80% (4 out of 5 patients). Other similar research done by Job *et al*., 2008 in India detected the existence of *M. leprae* DNA accounted 80% (8 out of 10 people) that was taken from washing the back skin and behind the arms of the untreated MB leprosy patients, regardless of the presence or absence of skin lesions.[11] Another research by Goulart *et al*., 2007 found the positivity of 73.6% from 110 samples of skin biopsy from untreated leprosy patients.[12] As far as the author knows, there is no published research yet justifying the existence of *M. leprae* DNA on skin lesion surface of leprosy patients by using samples taken from non-invasive method before 2008.

In this study, *M. leprae* DNA was only detected in the LL and BL patients. It has been found that skin tissues have been infiltrated by *M. leprae* in higher amounts in LL and BL type than in BB and BT type; therefore, the possibility of *M. leprae* exit to the skin surface in LL and BL is higher than in BB and BT type. Goulart *et al.*, 2007 used two primers to amplify the fragment 372 and 130 pairs of base until the final result of PCR reached 40 cycles. They found that the fragment 130 pairs of base were successfully detected in 73.6 samples (81/110) with classification 40% TT, 55.5% BT, and 100% BB, BL and LL. Meanwhile, the fragment 372 pairs of base were detected in 52.7% cases and classified as 13.3% TT, 33.3% BB, 64.7% BB, 83.3% BL, and 95.2% LL. However, the biopsies have shown to be more effective than skin smears. The smaller quantity of cells in skin smears may possibly be the cause of poor detection of *M. leprae* DNA using this type of sampling.[12]

Some skin structures are suspected as the way out of *M. leprae* to the skin surface, which are epidermis, pilosebaceous units, and sweat glands or sweat ducts. The valuation of this AFB positivity is done with the same lesions as the source taken from for the sampling of test *M. leprae* DNA on skin lesion surface. As far as the author knows, there is no published research yet to know the relationship between the existence of AFB in epidermis, pilosebaceous unit and sweat glands or sweat ducts and the findings of *M. leprae* DNA on skin lesion surface; thus, there is no comparable data.

There is a compatible result between the PCR of *M. leprae* DNA on skin lesion surface and the AFB in epidermis. This finding strengthens the assumption that epidermis

Table 1. Comparison of acid-fast bacilli in biopsy result and skin surface *Mycobacterium leprae* deoxyribonucleic acid.

Variable	Skin surface *M. leprae* DNA		X^2 (McNemar)	p
	Positive n=23	Negative n=5		
Epidermis				
AFB Positive	19	1	1,8	0.375*
AFB Negative	4	4		
Pilosebaceous unit				
AFB Positive	13	0	10	0.002*
AFB Negative	10	5		
Glands or sweat glands duct				
AFB Positive	15	0	8	0.008*
AFB Negative	8	5		

M. leprae= *Mycobacterium leprae*, AFB=acid-fast-bacilli, n= number of subjects, PCR= polymerase chain reaction, DNA= deoxyribose nucleic acid, *McNemar's Test.

could act as the source and one of the ways out of *M. leprae* to the skin surface. Seo *et al*. tried to explain how *M. leprae* exits from the epidermis to the skin surface, which is through keratinization process. *M. leprae* will also going up to the keratin layer and detached from the skin even though it has not been known yet whether the bacillus is alive or dead.[12] On the other hand, there is no compatible result between the PCR result of *M. leprae* DNA on skin lesion surface and the AFB in pilosebaceous unit and sweat glands or sweat ducts. This does not support the theory of pilosebaceous unit and sweat glands or sweat ducts as the origin or exit of *M. leprae* to skin lesion surface. Nonetheless, this result is still needed to be studied further for some reasons. Firstly, the positivity of AFB test result in pilosebaceous unit and sweat glands or sweat ducts are quite high, respectively 46.4% and 53.6%. Secondly, all the specimens with the positive result of AFB test on the pilosebaceous unit glands and sweat glands or sweat ducts also give positive result in the test of PCR *M. leprae* DNA on skin lesion surface. Thirdly, theoretically *M. leprae* could go out from pilosebaceous unit to the skin surface through the process of hair growth and secretion of sebaceous glands. In addition, anatomically and physiologically there is a possibility that the secretion of sweat glands may be identified as the origin or exit of *M. leprae* to the skin surface. Desikan and Iyer, 1972 acquired AFB in 38% sample from hair follicle of BL and LL patients, and also stated that the structure as one of the ways out of *M. leprae* to skin surface. Kotteeswaran *et al*., 1980 acquired the positivity of AFB in 45% hair follicle, 20% sebaceous glands, 100% *m. arrector pili,* 40% sweat glands, and 10% sweat ducts in LL patients. Meanwhile, in BL respectively accounted 35%, 15%, 40%, 25%, and 10% of patients.[8] Although the positivity proportion of *M. leprae* DNA on skin lesion surface is quite high, that DNA couldn't be differentiated, whether it comes from the living or the dead *M. leprae*. This is realized as the limitation of the research. Other limitation is that the PCR of *M. leprae* DNA was done only qualitatively. Therefore, it cannot be summarized that *M. leprae* found on the surface of skin lesions of leprosy patients whether alive and with a considerable amount of role as a source of infection. Besides that, the comparison between the test result of AFB in epidermis, pilosebaceous unit, and sweat glands or sweat ducts and the PCR result of *M. leprae* DNA on skin lesion surface does not necessarily describe the actual state.

Conclusions

Using PCR technique, the positivity proportion of *M. leprae* DNA on skin lesion surface of untreated leprosy patients was found quite high in LL and BL type, but not detected on BB and BT type. There is a compatible result between the AFB in epidermis and the PCR result of *M. leprae* DNA on the skin lesion surface, strengthen epidermis as the way out of *M. leprae* to the skin surface. Further research is needed using *Reverse Transcription* PCR to prove the viability of *M. leprae* and PCR *Real Time* to estimate the number of *M. leprae* on the surface of skin lesions of patients with untreated leprosy and their role as leprosy transmission sources. Development of sensitivity of PCR technique is needed to detect *M. leprae* DNA, especially on BB and BT leprosy.

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