Three Different Genotyping of *Mycobacterium leprae* in a Family: A Case Report

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ABSTRACT

Background: Indonesia is the third country with the highest prevalence of leprosy worldwide after India and Brazil. The risk of transmission is higher in household contacts, siblings, and neighborhoods. **Purpose:** Familial leprosy due to household contacts has been considered as the main transmission in leprosy. The aim of the examination is to detect the presence of *Mycobacterium leprae* and analyze the variation number of TTC repeats. **Case:** A family, consisted of mother, 35 year-old, was diagnosed with lepromatous leprosy (LL) type and erythema nodosum leprosum (ENL) necroticans. Her husband, 36 year-old, was diagnosed with tuberculoid leprosy. Daughter, 4 year-old, was diagnosed as indeterminate leprosy due to white small patches on her left cheek, arm, and leg, but there was no complain about anesthesia. **Case management:** Enzyme-linked immunosorbent assay (ELISA), histopathological examination, and polymerase chain reaction (PCR) for detection of *M. leprae* were performed. All of PCR results were positive. After sequencing of the TTC area, it revealed that the number of TTC repeats were different. **Conclusion:** Transmission from mother to others was suspected in family with leprosy living in the same house. PCR examination revealed 16 times TTC repeats on mother, 18 times on father, and 13 times on daughter. It was proven that infection of *M. leprae* originated from different genomes, which means different source of infection.

Key words: familial leprosy, household contact, genotyping, PCR TTC, case report.

ABSTRAK

Latar belakang: Indonesia merupakan peringkat ketiga di dunia dengan angka prevalensi kusta tertinggi setelah India dan Brazil. Faktor risiko penularan meningkat pada kontak serumah, saudara, dan tetangga. **Tujuan:** Kusta pada keluarga dengan kontak serumah diperkirakan menjadi sumber penularan utama. Tujuan dari pemeriksaan *polymerase chain reaction* (PCR) adalah untuk mendeteksi adanya kuman *Mycobacterium leprae* dan menganalisis variasi dari pengulangan TTC. **Kasus:** Keluarga yang terdiri dari ibu berusia 35 tahun didiagnosis dengan kusta tipe *lepromatous leprosy* (LL) dan eritema nodosum leprosum (ENL) nekrotikan, suami usia 36 tahun dengan kusta tipe tuberkuloid (T), dan anak usia 4 tahun dengan kusta tipe *indeterminate* (I) berupa bercak putih pada pipi sebelah kiri, lengan, dan paha, namun tidak didapatkan keluhan mati rasa pada bercak tersebut. **Penatalaksanaan:** Dilakukan pemeriksaan serologis *enzyme-linked immunosorbent assay* (ELISA), histopatologis, dan PCR untuk mendeteksi adanya kuman *M. leprae*. Hasil pemeriksaan PCR menunjukkan hasil yang positif pada ketiganya. *Sequencing* pada area TTC menunjukkan perbedaan angka pada pengulangan TTC. **Simpulan:** Keluarga dengan penyakit kusta yang tinggal serumah, pada kasus ini diduga transmisi berasal dari ibu. Pemeriksaan PCR pada ibu menunjukkan 16 kali pengulangan TTC, 18 kali pada ayah, dan 13 kali pada anak, sehingga menunjukkan perbedaan *strain* dari *M. Leprae*. Dapat disimpulkan bahwa penularan berasal dari sumber yang berbeda-beda.

Kata kunci: *familial leprosy*, kontak serumah, *genotyping*, PCR TTC, laporan kasus.

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INTRODUCTION

Leprosy affects approximately 700.000 new patients world wide annually. The prevalence in 2010 were 228.474 new cases as reported by 130 countries. In Indonesia there were 17.012 new cases.[1] These numbers show that leprosy is still a major health problem in the world, especially in Indonesia, and the transmission is still high. It is thought that in endemic areas many

individwils are infected *withkrycobacterium /eprae,*but only a few of them actually develop the disease."

Epidemiological evidence indicates that familial occurrence accounts for approximately half of cases in leprosy.' This is not suxprising, given the fact that leprosy is an infectious disease caused by *M /eprae,* which is spread from person to person mainly through nasal discharge or respiratory tract, therefore contact with a leprosy patient is essential for the transmission of *M /eprae."* The most important contact-related factors are the closeness, intensity of the contact, and inherited susceptibility, which make household contact is the first place of high risk transmission. Other factors which influence the development of infection and disease, such **as agenutritional status, contact with other** mycobacteria, the type of leprosy, and the bacterial index, and also patient-related factors which is involved **intransmission.**[2]

Definitive identification of *M leprae* is sometimes problematic, since the organism is not cultivable. Rapid molecular-type assays have been developed for detection of *M leprae* directly from patient specimens using available genetic data. These assays have been primarily on the amplification of *M* leprae-specific sequences using polymerase chain reaction (PCR) and identification of theM *leprae* DNA fragment.' Basic PCR consists of three steps: thermal denaturation of the target DNA, primer annealing of synthetic oligonucleotide primers, and extension of the annealed primersby a DNA polymerase.'

Shio *et al,* discovered a genomic divergence of *M leprae* by the variation of TIC repeats. Variety in the copy numbers ofTTC repeats can be used to classif'y *M leprae* into a considerable number of subtypes.' According to the report from Shin, the gene location of the TIC repeats were not found in *krycobacterium* ***tuberculosis, Mycobacterium avium, Mycobacterium*** *marinum,* or human tissues, which indicated their specificity *toM leprae.'*

The aim of the examination is to detect the presence of *M leprae* and analyze the variation number ofTIC repeats. It is reasonable to assume that the iodex case in the same house is the source of infection. Further investigation with PCR and TIC are needed, because of the fact that household patient is not the only source of infection ofleprosy bacilli.

CASE REPORT

A 35 year-old woman from Surabaya came to Dr. Soetomo Outpatient Clinic on September 2010 with chief complain of pain erythematous nodnles on almost all over her body since 2.5 years ago. The erythematous nodule often occurred and became nlcerated, while she also experienced fever. The nodules were painful and warm on palpation. Before the nodnle occurred, she complained of erythematous patches on her face and extremities since 2009. There were slightly loss of sensation on the patches.

There were no history thickening of her earlobes, eyebrows loss, and eye deformity. She never complaint **about nasal congestion, epistaxis, or visual** disturbances. There were hipoesthesia on her palms and soles. No complain about defecation and mixturation. No complaint about cough, sore-throat, dental caries, and discharge from vagina. She went to general practitioner 2.5 years before and received some **medication, but there were no improvement.**

Since 2 years before the nodules had broken and became ulcers, then she came to Dr. Soetomo Outpatient Clinic of Dermatology Department and was diagnosed as morbus Hansen (MH) lepromatous leprosy (LL) type with erythema nodosum leprosum (ENL) necroticans. She was hospitalized in our ward 4 times since January 2011 because of the reaction and necroticans ulcer. She received first multi drug therapy of leprosy (MDTL) from September 2010 and prolonged until presently, 22"' MDTL.

Physical examination revealed an alert, weak female, with the blood pressure 100/70 mmHg, pulse rate 100 times per minute, respiration rate 20 times per minute, and body temperature 38.5°C. From heed and **neck examination, there was anemic condition, no** icterus, cyanosis, nor dyspnea. Thorax, heart, and lungs were within normal limit. From abdomen, liver and spleen were not palpable. From her upper and lower extremities there were no edema and warm on palpation. **There were xerosis cutis on her lower extremities and** hypestesia on both palms and soles. No thickening or pain on palpation on *nervus auricularis magnus dextra* **and *sinistra*both *nen;us medianus, nervus peroneus*** *latera/is,* and *nervus tibialis posterior.*

Dermatological examination on almost all over her body, there were erythematous nodules, with diameters of 1-2 em, warm and tender on palpation, some nodules **became ulcers, there were erosionseasy to bleed, no**

pus, and there were scars.

From the laboratory examination results there were anemia with Hb 10,3 g/dl, increased leucocytes to 25.000/ul, thrombocyte 561.000, liver function test (LFT) 20 U/L, SGPT 30 U/L, blood urea nitrogen (BUN) 11 mg/dL, SK 1,2 mg/dL, and slight hypoalbuminemia 3.0 g/dL. Urine examination was within normal limit. Slit skin smear revealed that acid fast bacilli (AFB) was positive with 3+ bacteriological index (BI) and 0% morphological index (MI). Enzyme-linked immunosorbent assays (ELISA) to detect antibodies to phenolic glycolipid 1 (PGL-1) of *M. leprae* showed immunoglobulin M (IgM) 837 u/ml and immunoglobulin G (IgG) 188 u/ml.

A year later after first cycle of multidrug treatment of lepromatous leprosy (MDTL) treatment, the serologic test and the results were IgM=749 u/ml and IgG=297 u/ml. Biopsy from the lesion showed elongation of the rete ridges in epidermis. In dermis there was Grenz zone area with wide group of foamy macrophages, infiltration of lymphocytes and lots of bacillis. For the treatment, MDTL was continued, and added methyl prednisolone 28 mg in divided dose and tapered off, cystenol 500 mg, amoxycillin 3 times 500 mg, wet dressing with normal saline for macerated

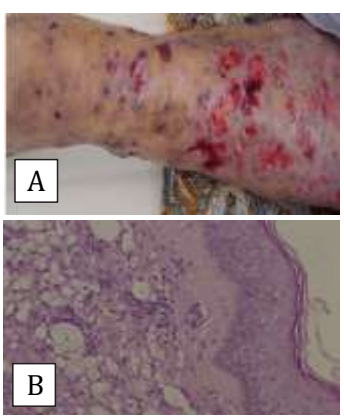

Figure 1. (A) Necroticans ulcer on almost all over the body, easy to bleed and painful. (B) Histopathology result (100x) wide group of foamy macrophages with infiltration of lymphocyte, lots of bacilli (AFB), grenz zone.

lesions, sulfas ferrosus, vitamin complex B, and high calorie high protein diet.

Father, 36 year-old, visited Dermatology and Venereology Outpatient Clinic to accompany his wife for routine control and revealed that he had three white anesthetic patches on his right and left cheeks. The lesions appeared 6 months ago. Firstly they were small white patches then in 3 months, they became larger and accompanied with lost of sensation, and eventually became more red in color. History of eyebrow loss, thickening of the earlobes, and eye deformity were denied. No complain of nasal congestion and epistaxis. History of taking topical or oral medication was denied.

Physical examination of general status was alert with blood pressure, pulse rate, respiration rate, and body temperature within normal limit. From head and neck, there were no anemic, icterus, cyanotic, nor dyspnea. From thorax and abdomen there were no abnormality, from upper and lower extremities there were no edema and warm on palpation.

There were no thickening or pain in palpation of superficial nerves such as *nervus auricularis magnus dextra* and *sinistra*, both *nervus medianus, nervus peroneous lateralis* and *nervus tibialis posterior*. There were no gloves and stocking anesthesia on both palmar and soles.

Dermatological examination on his right and left cheeks, there were two erythematous plaques on the right cheek and one plaque on the left cheek with 3-4 cm diameter, distributed symmetrically, anesthetic, and sharply marginated. From blood and urine examination there were no abnormality. The earlobes and skin smears from lesion for AFB were negative, with 0 BI and MI. ELISA showed IgM titer of 495 u/ml and IgG titer was 139 u/ml. This patient was treated with multidrug therapy tuberculoid (MDTT) for 6 months.

Histopathology examination from the lesions showed full of epitheloid cells that appeared as granuloma and datia langhans cell in dermis, with focus of lymphocyte infiltration, and there were few bacillis.

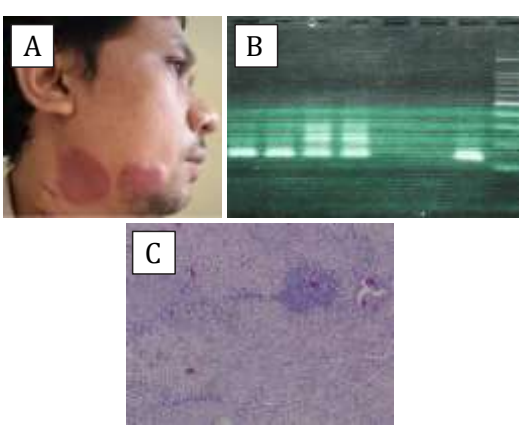

Figure 2. (A) plaque erythematous, anesthetic, sharply marginated, diameter 3-5 cm. (B) PCR result 150 bp. (C) Histopathology result (100x) epitheloid cells appeared as granuloma, datia langhans cells, infiltration of lymphocyte.

PCR examination from lesion swab was positive, marked with band in gel electrophoreses.

Child, 4 year-old, with small white patches on her left cheek, left arm, and left upper leg since 3 months, but no complain of anesthesia. The first patches appeared on her left cheeks, then during the time they also appeared on her upper arm and upper leg. Physical examination of general status was composmentis with blood pressure, pulse rate, respiration rate, and body temperature within normal limit. From head and neck, there were no anemia, icterus, cyanotic, nor dyspnea. From thorax and abdomen there were no abnormality, from upper and lower extremities there were no edema, and palpation was warm. There were no thickening or pain in palpation on superficial nerves such as *nervus auricularis magnus dextra* and *sinistra*, both *nervus medianus, nervus peroneous lateralis* and *nervus tibialis posterior*. Bacillus Calmette-Guerin (BCG) scar was positive. From laboratory result, blood, and urine examination were within normal limit. The AFB was negative. IgM antibody PGL-1 titer was 59 u/ml, IgG titer was 10 u/ml.

We continued the examination with biopsy from the lesion, and the result did not clearly explain the diagnosis of leprosy, but still there was possibility and suspicion of leprosy. From the conclusion of histopathology result there were atrophy and shortening of rete ridge in epidermis, while in dermis there were wider capillary blood vessels, infiltration of lymphocytes, perivascular histiocytes, but no tubercle nor granuloma and bacilli were found. PCR examination was also performed due to suspicion of indeterminate

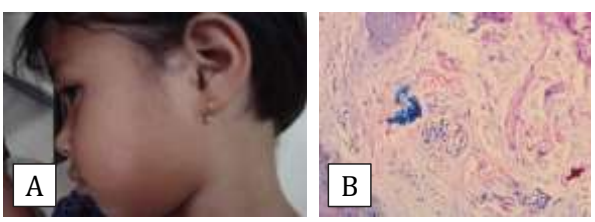

Figure 3. (A) Hypopigmented patches, sharply marginated, no loss of sensation. (B) Infiltration of lymphocyte and perivascular histiocyte. No tubercle/granuloma/AFB.

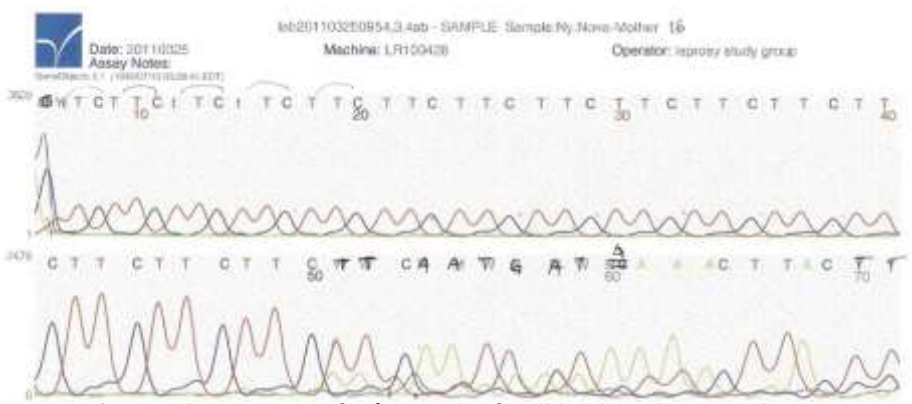

Figure 4. Sequencing result of Mrs. N/mother (36 y.o), TTC repeats 16 times.

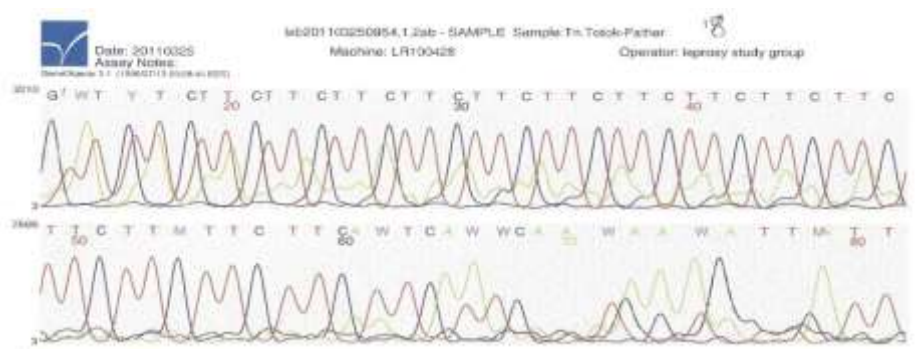

Figure 5. Sequencing result of Mr. T/father (38 y.o), TTC repeats 18 times.

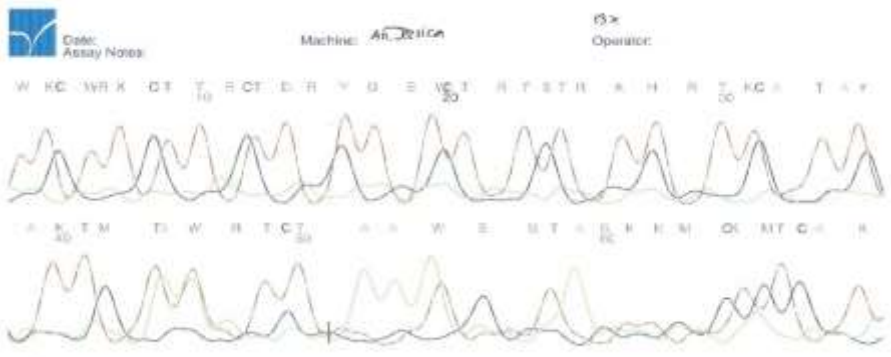

Figure 6. Sequencing result of child J/daughter (4 y.o), TTC repeats 13 times.

leprosy, and the result was positive with TTC repeats 13 times.

From the socio-economic condition, they lived in a small house at Surabaya. There are 6 members of the family who lived in the same house, including mother, father, 2 daughters, mother-in law, and nephew. There were only 2 bedrooms in the house, and the child often slept with the parents. There was no bathroom or water closet, only draw-well to take a bath and to wash the clothes, so they had to go to the public restroom to take a bath or defecate. The housing condition were poor of hygiene, circulation, and lighting inside the house, with room temperature of 33°C.

DISCUSSION

Hansen's disease is well known for its family accumulation, in which genetic factors of characteristics to define immunological competence might play certain roles.[10] Polymorphisms of the human leucocyte antigen (HLA), which constitute the major histocompatibility complex (MHC) in humans, are the most important genetic factors in the immunogenetic. More recent studies concluded that both HLA (DR2) and non-HLA genes contribute to a genetic susceptibility in type of leprosy.[11]

Lepromatous leprosy as the index case was related with increased risk of leprosy, as well as inherited susceptibility.[5] In this case, this family is in high risk of transmission, the main family were considered to be examined, including the husband and child. The mother was suspected as the index case with leprosy LL type and ENL necroticans, the father with leprosy BT type, and the daughter with indeterminate leprosy. Based on reference, the highest risk of leprosy was associated with households of MB patients and the risk of leprosy for households of PB patients was similar to the risk of leprosy for direct neighboring houses of MB patients.

ENL can occur in untreated LL type or during MDT. In this case, the mother developed ENL during MDT after several months of treatment. In severe ENL, the nodules may changes to blisters and become ulcerated (ENL necroticans). Chronic recurrent ENL may cause fibrosis of the skin and leaving scars.[8] From the clinical manifestation of the father, he was suspected to suffer leprosy BT type, because of the BI/MI and serologic test were negative, then histopathology and PCR examination were performed to establish the diagnosis, and the result were positive. For the treatment, MDTT was given for 6 months and followed up afterwards. From the child there were also small hypopigmented lesions. The problem was all the examination results were negative. Biopsy showed there was possibility of indeterminate leprosy, therefore PCR examination were performed, and the result was positive, thus the patient was assessed as indeterminate leprosy. These differences in the phenotype of the disease between MB, PB, and indeterminate leprosy are believed to be caused by individual variation in the immune response induced by various components of *M. leprae*, so genetic factors substantially affect the pathogenesis and were responsible for the differences in leprosy susceptibility and type.[3]

The theory of indeterminate leprosy shows single or few flat hypopigmented macule with minimally altered sensation of the skin, no peripheral nerve enlargement, and negative slit skin smear, which is consistent with the patient. Since the histopathological features of indeterminate leprosy are often unspesific, the PCR test to detect *M. leprae* will be helpful.[12] The majority of indeterminate cases resolve spontaneously. If the lesions do not resolve, the disease will progress into one of the leprosy types depending on the cellular mediated immunity (CMI). If the lesion persist until more than 6 months of monitoring, and histopathology

can not be performed, then leprosy trealment should be considered.

PCR have shown I00% specificity and sensitivity ranging from 34 to 80% in patients with PB and more than 90% in patients with MB.' Comparative genome analysis identified *M. leprae* specific peptides or proteins, and some of them specifically react with T-cell derived from leprosy patients. Those peptides are potentially eligible for developing rapid diagnostic test for the early detection of *M. leprae* infection."

PCR for *M. leprae* was explored based on the sequencing data. From the *M. leprae* genome sequence database, TTC DNA repeats were identified, indicating the number of repeats at each locus variable among *M. leprae* strain. The major strength of PCR is the ability of the reaction to produce incredibly large amounts of DNA of defined length and sequence from small quantities of DNA through enzymatic amplification using reasonably inexpensive equipment and reagents."

In this case, there were 3 slit-skin smear specimens collected from the skin lesion of the patients. All the positive results in PCR continued to be analyzed by TIC repeats genotyping with sequencing of the TTC area. The result of the PCR revealed 16 times TTC repeats on mother, 18 times on father, and 13 times on daughter. The positive control uses *M. leprae* strain *Thai-53* from Thailand, which is mostly found in South East Asian region with TTC-14 copy.' DNA sequencing result showed that the number of TTC repeats of each individual were different, which means that the genotype of *M. leprae* was also different. It referred to the different sources of infection. Such case was also reported by Matsuoka *eta/* in 2004, in which there was a family with different TTC genotype strains. This result disagreed with the conventional conception that a heavy household contact with MB patient is the source of infection and suggest existence of other infection **sources. In some cases, the genomic does not match** between the patient who is presumed to be the source of infection and the co-habiting family patient, these facts show the possibility of direct infection by sbeding from MB patients or inapparent infection and indirect infection by *M. leprae* that survive in environment. Additionally, the bacteria survived for 7 days in environment after discharge from nasal and dried.[10]

Environment also has influence to be a competent **reservoir of the leprosy transmission.[9] Socio-economic** factors may be important in determining the risk of developing leprosy. Study by Ponnighaus *et a/* showed there is a strong inverse relation between good housing condition and decreased risk of leprosy. Socio-economic factors such as sanitation, housing condition, economic status, literacy, and nutrition also influence the risk of transmission especially in airborne disease like leprosy.' This family lived in a small house with 6 family members. They were of low economic status, poor sanitation, poor hygiene, lack of circulation, and high humidity with room temperatore of 33°C. These conditions are related with the viability of *M. leprae* outside the body. Based on literatore, *M. /eprae* can survive for *9* days in a dried condition, at 24-33°C, *5* months in the dark at 28-44% humidity, and 7 days with exposure to sunlight for 3 hours per day." It can be concluded that the existence of *M /eprae* is closely related to 3 aspects, which are agent, host, and **environment.**

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