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Analysis *Mycobacterium lepromatosis* as the causative agent of diffuse lepromatous leprosy patient in Indonesia

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Abstract. *Adriaty D, Alinda MD, Karim A, Putra BHK, Agusni RI, Iswahyudi, Wahyuningtyas PA, Wahyuni R, Widayati E, Listiawan MY, Prakoeswa CR. 2023. Analysis* Mycobacterium lepromatosis *as the causative agent of diffuse lepromatous leprosy patient in Indonesia. Biodiversitas 24: 4521-4529.* The present investigation deals with identifying, besides clinical signs, both microscopically and molecularly, to clearly differentiate the *Mycobacterium lepromatosis* genome against *Mycobacterium leprae* so that treatment can be carried out properly. To date, there has yet to be a report on this discovery in Indonesia. A 53-year-old Javanese man had ulceration on both legs that had been reoccurring in the past two years. Some of the ulcers were preceded by blisters that rapidly turned into a wound. The ulcers were described as wide and deep, covered with blackish crusts and some exudative areas with irregular edges. He had a history of recurrent epistaxis and had never been treated for leprosy. There was rapid progression and the development of new ulcers on his legs and hands for two weeks before hospitalization. There was body weakness, no fever, and the skin was shiny and ichthyosiform. On the face, diffuse infiltration will appear without nodules, and madarosis also occurs. Examination in this study used the BTA examination method, histopathological examination with HE and Wade-fite staining with biopsy material and the PCR method using skin slit smear material. The results of the BTA examination showed that BI was 3+, and MI showed 7%. Histopathology showed thinning of the epidermis with many foam cells containing BTA, including endnotes and perivascular tissues. Nested PCR examination with LERF2-MLER4 primers for detecting *M. leprae* showed a positive result with amplicon 135bp in size. Advanced PCR examination using LPMF2-MLER4 primers for detecting *M. lepromatosis* also showed a positive result with amplicons 142bp in size. The result of DNA sequencing was consistent with the order of nucleotides of *M. lepromatosis*. Based on the clinical and histopathological results, it was consistent with diffuse lepromatous leprosy and Lucio's phenomenon. The DNA sequencing showed a suitable result with *M. lepromatosis*, which has been reported in Mexico. Thus, it can be concluded that in biomolecular strain, this bacterium is an *M. lepromatosis* that has never been reported in Indonesia.

Keywords: Biomolecular, *Mycobacterium leprae*, new species

INTRODUCTION

Leprosy, or Hansen's Disease, is a chronic dermatologic infection caused by *M. leprae*. The disease is curable yet remains a public health problem even though there is no known ubiquitous reservoir for transmission of *M. leprae* other than human beings (Eichelmann et al. 2013). Leprosy is a serious infectious disease for both the patient (because of disabilities and social consequences) and the community (Scollard 2016). Leprosy still affects millions of people worldwide in the form of new diagnoses and neurologic disability. At the beginning of 2009, the registered prevalence of leprosy globally was 213.036 cases. India, Brazil, and Indonesia are the main contributors to the new caseload. By 2021, Indonesia is still the third-highest country for new cases of leprosy (WHO 2022). Based on the National Leprosy Situation Analysis in 2022, East Java is the highest province for new cases of Leprosy in Indonesia, with 1,839 cases from total 12,288 cases (Scollard 2016; Smith 2011; Chotimah and Mindra Jaya 2022).

The disease manifests a spectrum of clinicopathologic forms, ranging from tuberculoid leprosy (TT), to borderline forms, to lepromatous leprosy (LL), and can be paucibacillary or multibacillary in skin lesions. One form of the disease may markedly dominate others within a geographic region. It has long been thought these variations are due to the individual host immune effects. In polar lepromatous, there are two main clinical forms: nodular lepromatous leprosy and diffuse lepromatous leprosy (DLL) or Lucio's leprosy (Eichelmann et al. 2013; Lee et al. 2011). Lucio's leprosy has a special type of lepra reaction, and the features are similar to those of ENL with histology of vasculitis, thrombosis, and other features of acute inflammation. There is thrombosis of vessels leading to local ischemia and ulceration - commonly known as the Lucio phenomenon (LP). This type of reaction is more common in Mexico; however, other cases have been observed and published in different countries like Costa

Rica, Argentina, Brazil, and India (Kamath et al. 2014).

The different clinical forms of leprosy are mainly related to the various immunological responses to the infection. LP is a rare but distinctive skin eruption in patients with diffuse lepromatous leprosy. LP may not be easily recognized, especially in non-endemic countries, which leads to confusing diagnoses and loss of time for treatment. Lucio's phenomenon usually appears in untreated or inadequately treated non-nodular lepromatous leprosy (DLL) (Jurado et al. 2015). Morbidity and mortality of LP are high. Some case reports reported that 6 of 10 patients died from LP. Reported patient with LP was die due to complications from widespread ulcers, including anemia, sepsis, and shock (Kamath et al. 2014). In patients who recover, the deformity can occur due to ulcers. Thus, it takes special attention to this disease (Jurado et al. 2015; Kamath et al. 2014). *M. leprae* has been known as the leprosy agent since its initial discovery in 1873. In 2008, a new etiologic agent—*M. lepromatosis*—was recognized in two patients of Mexican origin who died of DLL (Han et al. 2012). Although this new bacteria has similarities with *M. leprae*, it still has biomolecular differences (Jurado et al. 2015).

DLL or LP is endemic in Mexico but has been reported in other countries, including Asia (Prakoeswa et al. 2016). In studies conducted on 120 leprosy patients in Mexico, 87 patients showed positive results on PCR examination. Of the 87 patients, 55 patients (63.2%) detected *M. lepromatosis*, 18 patients (20.7%) *M. leprae* and 14 patients (16.1%) were both (Jurado et al. 2015; Kumari et al. 2008). In a retrospective study period from 1997-2006 in Malaysia, only one patient had LP out of 49 leprosy patients (Ang et al. 2003). In Indonesia, there have been several case reports of LP but no report of the discovery of *M. lepromatosis* as a causative agent (Prakoeswa et al. 2016).

It is an interesting case discovery of DLL, *M. leprae* that found together with *M. lepromatosis* as the causative agent of ulceration (dual infection) in a male patient that firstly found in Indonesia. The DNA sequencing showed a suitable result with *M. lepromatosis*, which has been reported mostly in Mexico. The patient is still within observation until now, with an improvement of clinical manifestation and reduction on both bacteriological and morphological index.

MATERIALS AND METHODS

Clinical sign

The hands and legs of a 53-year-old Javanese man have been experiencing recurrent multiple wounds and ulcerations for two years. There was rapid progression and the development of new ulcers on his legs and hands for two weeks before hospitalization. The ulceration is described as a painful scarlet spot that later darkens and ulcerates. At first, the ulceration was just a red patch that appeared on both of his legs. The red patches then gradually progressed to both his legs and then to both of his hands. Blisters preceded some of the ulcers. He also complained of malaise and noticed leg weakness for one week before hospitalization. On admission, there was no fever, the skin was shiny ichthyosiform, and there was eyebrow loss. In the last ten years ago, he had a history suffer flushing on his face, recurrent rhinitis, and epistaxis. Nevertheless, the patient did not worry about it. He had already examined himself to a doctor at public health center about two years before because there were complaints of painless but sometimes painful blackish patches on the back of the right foot, and then became blisters and wounds. However, the patient was denied taking MDT and had never been treated before for leprosy. A year ago, he was treated by a dermatologist and got oral medications, ointments, and a compress with normal saline. The complaint was improving. In the last two months of 2016, the wound reappeared, and he only treated himself with chloramphenicol powder and compressed with normal saline, but the complaint did not improve.

There were no symptoms of numbness patches, and a history of painful nodules was denied. The history of living in leprosy's endemic area was positive. He lives in Surabaya, but he was born and grew up in Sampang-Madura, north coast of Java. The history of the family who suffers similar symptoms was denied. History of close contact with leprosy patients was denied. He has had a long stay in Sumatra (Riau) since the '90s, but since 2014 the patient has returned to Surabaya.

During the physical examination, the patient demonstrated compos mentis status, with normal blood pressure and pulse rate. Additionally, the temperature recorded on the first day was 37.8°C. The cutaneous examination revealed on the facial region that there was diffuse infiltration without nodule (Figure 1). There were extensive multiple deep and irregular ulcers covered with a blackish crust and, in some areas, yellow slough eroding the subcutaneous tissue with ragged margins. These ulcers were distributed symmetrically over both legs and both hands. There were gloves and stockings anesthesia. The skin of the extremities was dry and ichthyosiform. Eyebrow loss and earlobes thickening were positive. The ulnar claw deformity was noted.

Acid-fast bacilli in Ziehl-Neelsen Stain

A thorough evaluation was performed. Blood examination shows that the patient was anemic (hemoglobin 5.8 g/dL) and hypoalbuminemia (serum albumin 1.79 g/dL). Acid-fast bacilli in *Ziehl-Neelsen* Stain: Briefly, the bacteria are first treated with carbol fuchsin (primary stain) and heated on a steam bath (mordant), allowing the stain to penetrate the cell wall. It is followed by applying acid alcohol (decolorizer) and staining with methylene blue (counterstain). The acid-fast bacteria appear red and other structures take on the blue-green color of the counterstain. Modified Ziehl-Neelsen Stain (Wade-Fite Stain): *M. leprae* are much less acid- and alcohol-fast than *M. tuberculosis*. The mycolic acid coat of leprosy bacilli is less strong and is easily decolorized by the standard *Ziehl-Neelsen* technique. A modification is used (Wade-Fite technique) in which peanut oil is used with the deparaffinized solvent (xylene), which minimizes the exposure of the bacterial cell wall to organic solvents and preserves their acid-fastness.

Tissue source

The image shows numerous *M. leprae* singly and in clusters (globi) with Wade-Fite stain in skin biopsy (Figure 3). A slit skin smear revealed the presence of a global form of Acid-Fast Bacilli (AFB) with a bacteriological index (BI) was 3+ from earlobes and skin lesions and a morphological index (MI) of 7%. The histopathologic with HE staining, evidence from the leg's ulcer margin revealed an atrophic epidermis with Green Zone, a group of foam cells with some lymphocyte cells, and vasculitis with endothelium occlusion of thrombus seen on the dermis (Figure 2). There were lymphocyte cell infiltrations on the subcutaneous fat followed by the proliferation of capillary blood vessels. By wade-fite staining, AFB was seen prominently within perivascularly and endothelial cells. The conclusion is that the description is consistent with Morbus Hansen's DLL type with Lucio's phenomenon.

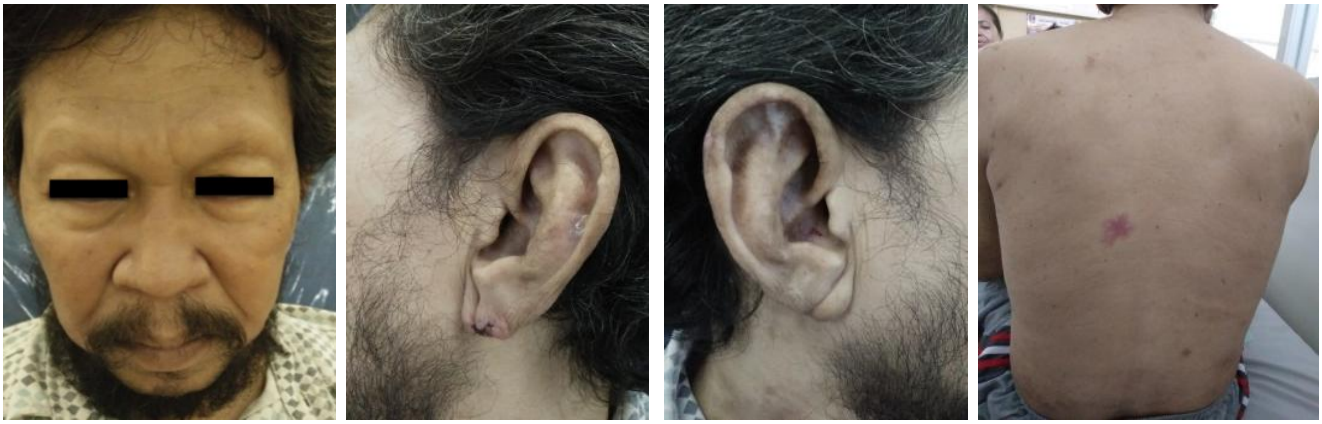


Figure 1. A. Facial diffuse infiltration and madarosis, B-C. Earlobes thickening, D. Purpuric macules on back was negative

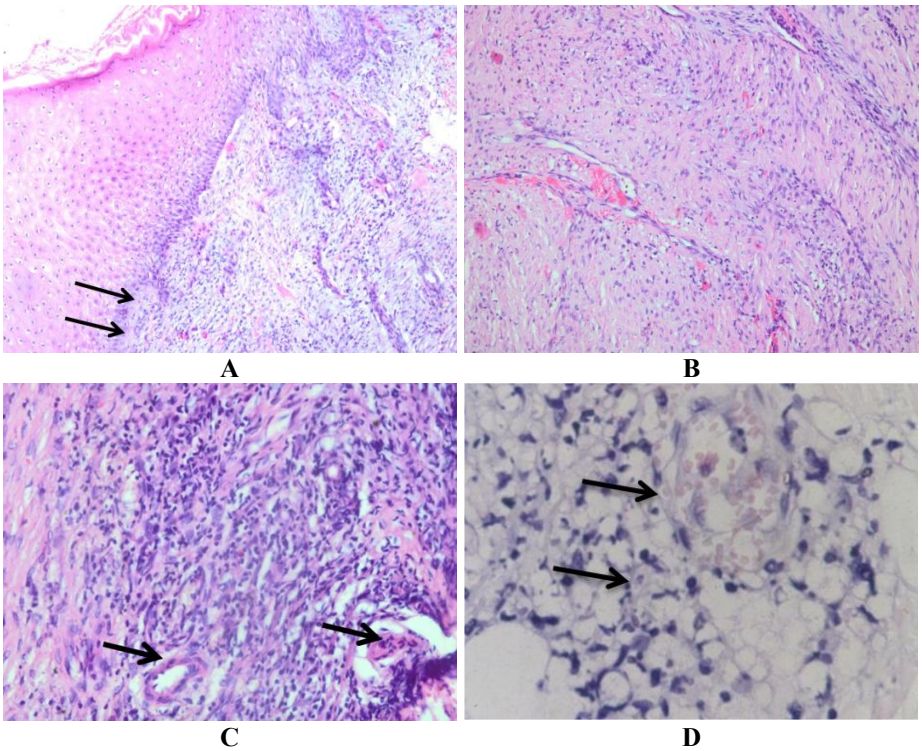
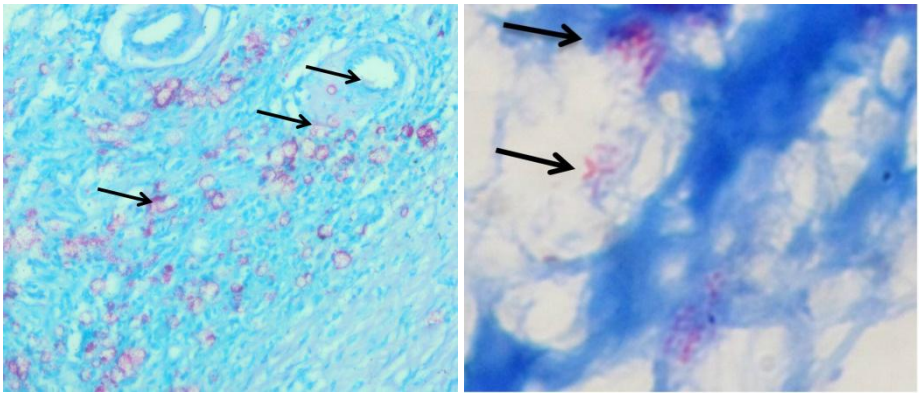
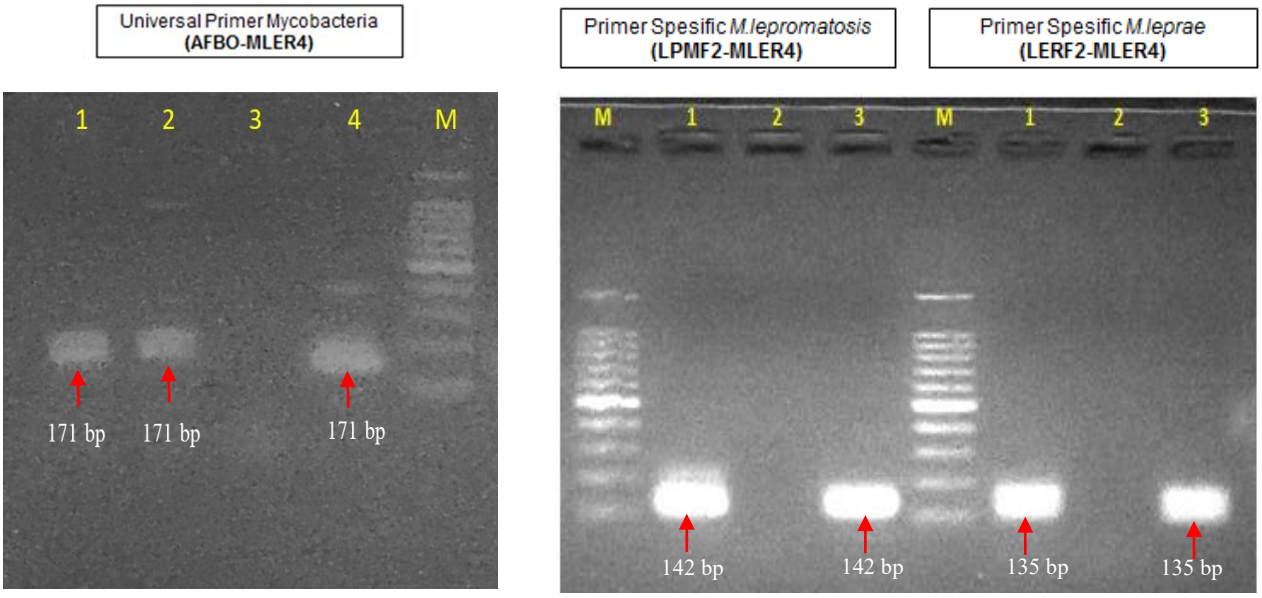


Figure 2.A. HE staining, 100x magnification, atrophic epidermis with Green Zone, B. HE staining, 400x magnification, lymphocyte cells infiltration on the dermis and perivascular and dense histiocytic infiltration of the skin, C. HE staining, Obj 400x magnification, there were lymphocyte cell infiltrations followed by proliferation of capillary blood vessels, D. HE Staining, 1000x magnification, group of foamy macrophages with some vasculitis and endothelium occlusion of thrombus



A **B**

Figure 3. Wade-fite staining, 400x magnification: A. Many acid-fast bacilli were seen prominently perivascular and inside the blood vessels (endothelium). B. Fite-Faraco staining, 1000x magnification: acid-fast bacilli on subcutis



A **B**

Figure 4.A. PCR 1st round (Primers: AFBFO-MLER4): 1st PCR result were 171bp. line 1 (positive control *Mycobacterium lepromatosis*), line 2 (positive control *Mycobacterium leprae*), line 3 (negative control), line 4 (patient), M (100bp DNA ladder). B. PCR 2nd round: 2nd PCR results were 142bp for Specific Primers of *M. lepromatosis* (LPMF2-MLER4): M (100bp DNA ladder), line 1 (patient), line 2 (negative control), line 3 (positive control *M. lepromatosis*). 2nd PCR results were 135 bp for Specific Primers *M. leprae* (LERF2-MLER4): M (100bp DNA ladder), line 1 (patient), line 2 (negative Control), line 3 (positive control *M. leprae*)

PCR and differentiation of species

The polymerase chain reactions (PCR) examination from the skin lesion was already performed and was analyzed by Institute Tropical Disease, Universitas Airlangga. The samples were collected in the same manner as taking skin smears for bacterial index (BI) examination using a disposable stainless-steel blade. Samples were collected from some sites that showed the highest BI in previous examinations (WHO 2012). DNA extraction was done using a tissue kit (DNAeasy Blood & Tissue Kit; QIAGEN, Valencia, CA). The design of PCR primers and assays was described earlier by Han et al. (2014). In brief, two steps of heminested PCR were used to maximize detection sensitivity: 1st PCR used primers AFBFO (5′ GCGTGCTTAACACATGCAAGTC 3′) and MLER4 (5′CCACAAGACATGCGCCTTGAAG 3′) that were common to detect all mycobacteria (~150 species). PCR conditions were used in present investigation described earlier by Han et al. (2014). The results amplicon for genus *Mycobacteria* show positively in a thin lane, it was 171 base pairs (bp) (Figure 4.A), and further PCR was designed to separate species by using MLER4 and LPMF2 (5′′GTCTCTTAATACTTAAACCTATTAA 3′) for detection *M.lepromatosis* (142 bp) and MLER4 and LERF2 (5′CTAAAAAATCTTTTTTAGAGATAC3′) for *M. leprae* (135 bp). The result of the second round of PCR using the LERF2-MLER4 primer was positive for *M. leprae* (Figure 4.B). While the PCR result for *M. lepromatous* using

LPMF2-MLER4 primer (5′ GTCTCTTAATACTTAAA CCTATTAA 3′) was also positive.

The 2nd PCR results continued to confirm the DNA sequences by Sanger Sequencing and show as described (Figures 5 and 6). According to NCBI BLAST data, all the mutations in the DNA sequencing result showed a suitable result compared with *M. lepromatosis* reported in Mexico.

Patient therapy

The patient was treated with multidrug therapy for a multibacillary regimen consisting of rifampicin 600 mg, clofazimine 300 mg once a month, and clofazimine 50 mg/day. The dapsone still has not been given because the patient with anemic, 5.8 g/dL. This therapy combines 2×400mg/day pentoxifylline, wound care, and debridement. In correcting the anemic condition in this patient, the transfusion of packed red blood cells (PRC) was done until the hemoglobin reached 11 grams/dL. Furthermore, albumin transfusion aims to overcome hypo albumin conditions. The patient improved after seven days of treatment and was discharged after three weeks in stable condition. The ulcers healed slowly after the wound care. The following treatment resulted in healing with cicatrization. There were hypochromic scars and a hyperchromic border in the lesion about five weeks after hospitalization.

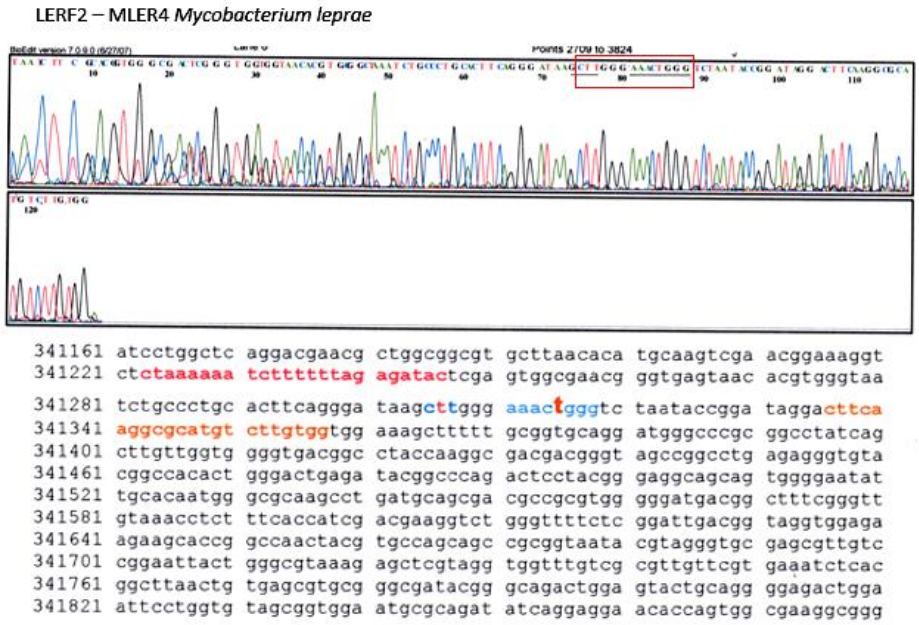


Figure 5. The Sequencing result of *Mycobacterium leprae*: DNA sequencing analysis using the 16S rRNA gene showing a sequence of nucleotides to be consistent to *M. leprae*

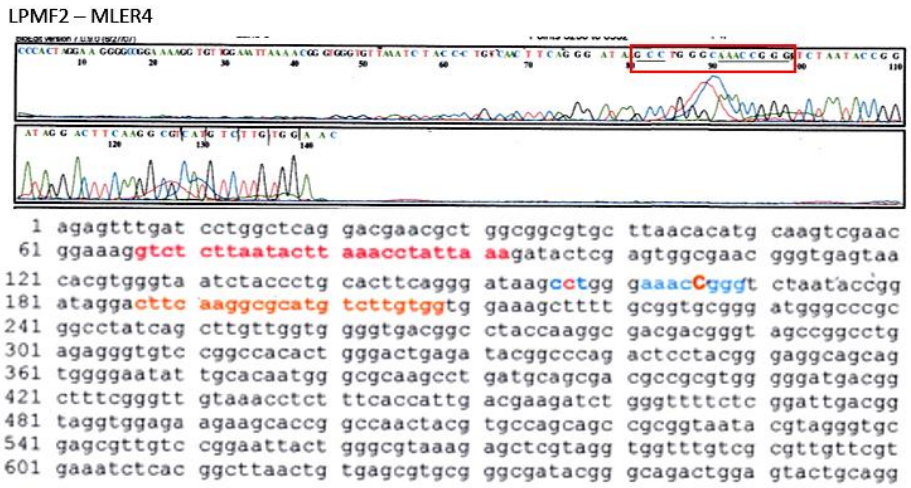


Figure 6. The sequencing analysis result of the 16S rRNA gene *Mycobacterium lepromatosis* showing the unique sequence compared to *Mycobacterium leprae*. The blue area section is the difference nucleotide of *M. lepromatosis* compare to *M. leprae*

RESULTS AND DISCUSSION

Leprosy is a chronic dermatological and neurological disease resulting from the unculturable pathogen *M. leprae* infection. Nonetheless, an average of 250.000 to 300.000 new cases have been reported annually in the last five years worldwide. In Indonesia, East Java is the highest state for new cases of Leprosy (Smith 2011; WHO 2021). Indonesia is still the third largest contributor of leprosy cases in the world after India and Brazil. According to data as of January 24, 2022, there were 7,146 new lepers, with a proportion of children of 11% (Ministry of Health Indonesia 2022).

Established leprosy was classified as TT, BT, BB, BL, and LL in the Ridley-Jopling classification. This classification is based on the immunological spectrum of the disease. LL is a generalized form without cellular immunity to the leprosy bacillus (Han et al. 2012). In 1982, WHO proposed a new valuable classification for newly established multidrug therapy (Prakoeswa et al. 2016). Patients are classified as paucity-bacillary form (PB) and multi-bacillary form (MB). *M. leprae* in LL patients is also localized in internal organs (Ang et al. 2003; Kumari et al. 2008). The bacterium mainly affects the skin, peripheral nerves, mucosa of the upper respiratory tract, and eyes. It can be progressive and cause permanent sequelae without treatment, especially in lepromatous cases. There are enormous quantities of AFB in skin smears done on lesions or cooler body regions (ear lobe elbow, back or finger, knee). The bacterial index is approximately 6+ (the maximum of the scale), and there are large-sized hyperchromic globes (Monteiro et al. 2012).

Lepromatous Leprosy may present as a diffuse variant characterized by diffuse and massive infiltration of the skin, known as diffuse lepromatous leprosy (DLL). DLL is a unique, severe form of leprosy initially recognized by Lucio and Alvarado in 1852 and further described by Latapi and Chevez-Zamora in 1948, both in Mexico. It is thus also called Lucio's Latapi leprosy. This form shows a diffuse cutaneous infiltration, with no nodule or plaque formation, and frequent skin ulceration in the late stage, known as Lucio's phenomenon (Deps and Collin 2021). It is essential to point out that in Lucio's leprosy, there are two clinical forms: (i) pure and primitive diffuse leprosy - "pure" because it has nodules and "primitive" because it begins with diffuse infiltration, without previous lesion in skin or neural; and (ii) secondary pure diffuse leprosy, which comes from an undetermined case without nodules, and before the patient develops the infiltration, hypochromic, dysaesthesia spots, or neuritis (Han et al. 2012). DLL has been endemic in western and central Mexico and Costa Rica but rarely reported elsewhere, including Asia (India, Sri Lanka, Malaysia, and Singapore) (Ang et al. 2003; James et al. 2011; Jurado et al. 2015).

In the beginning, the clinical manifestation of the skin shows wide infiltration that has been compared with myxedema, and the face looks "healthy" (lepra Bonita or pretty leprosy), and the earlobe turns thick (Kavya and Sacchidanand 2013). Later in the progression of the disease, hands become swollen, puffy, and red, and the legs look edematous. In time, all the skin becomes flaccid and atrophic. The general symptoms that are usually seen are fever, arthralgias, myalgias, and the appearance of very painful, red, or purpuric macules of irregular shapes, angulated or "stellar"; these lesions usually begin on the lower legs and continue progressing up to the thighs, hip, trunk, and upper limbs. In a matter of days, these spots exhibit "dryness" and appear as authentic infarcts with a detachment of the necrotic skin and showing ulcers. In the advanced stage, the lesions may become ulcerated, particularly on the lower extremities, or even generalized, leading to fatal secondary bacterial infection and sepsis (Kavya and Sacchidanand 2013; Vargas-Ocampo 2007).

In this case, the patient is a male 53 years old, lives in Surabaya but was born and grew up in an endemic area (Madura). He came to the emergency ward for two years with multiple wounds and ulceration on his legs and hands. He had a history of chronic rhinitis and epistaxis. The skin was dry and ichthyosiform. Eyebrow loss and earlobes thickening were seen on the patient. On palpation, diffuse facial infiltration and necrotizing purpuric tissue on both earlobes. There were no nodules or plaque formations. There were complaints of sensation loss (hand glove and stocking anesthesia), no hypo-pigmented lesion, and no nerve enlargement. Slit-skin smear revealed a BI of 3+ with globi from skin lesions and earlobes and a high MI of 7%. This result supports the features of the DLL type.

Reactional episodes in leprosy are defined as a set of clinical manifestations - general, cutaneous, neural, and visceral - that, in acute and subacute form, interrupt the normally slow and insidious evolution of the disease with polymorphism in its behavior, and that can be seen before, during, or after treatment (Han et al. 2012; Jurado et al. 2015). LP is in the type 2 reaction group, presented in all pure and primitive diffuse cases and in most secondary DLL cases. It normally begins in the feet and goes upward, affecting the legs, tights, arms, trunk, and face. It presents as erythema spots of different shapes, some angular or with amoebic appearance, of variable size, and painful. At 24 or 48 hours later, they are slightly infiltrated; by the third or fourth day, they become dark with a purpuric appearance and subsequently present central necrosis that appears as a small blister (Kamath et al. 2014; Massone et al. 2014). Finally, a red-dark eschar is formed, which falls off a few days later, leaving an atrophic scar of pearl white color. The duration of this pathological process lasts approximately 15 days. On other occasions, in the lower limbs mostly, flaccid blisters form, which, when open, cause ulcerations of variable sizes, more or less deep with well-defined borders as if they were created with a "punch." The previous is followed by general symptoms such as shivers, fever, arthralgia, and bad general condition (Han et al. 2012; Massone et al. 2014).

The histopathologic alterations in LP depend on the clinical evolutionary condition they suffer. Histopathologically there is a necrotizing perivasculitis; the epidermis shows foci of ischemic necrosis, frequently with detachment from the dermis with ulceration and hyperplasia. In the dermis, it is possible to see the features characteristic of DLL, such as wide infiltration by foamy

macrophages, always with abundant acid-fast bacilli inside them (globi) or outside in the stroma in association with lymphocytes, around vessels, nerves, and adnexal structures including erector muscles. It is the kind of reaction in which vasculitis occurs, mostly in venules and the final stage will be unique endothelial proliferation and vascular occlusion (Massone et al. 2014; Peixoto et al. 2013).

The lession involves the legs of the patient has recurred for two years. There was rapid progression and the development of new ulcers on his legs and hands for two weeks before hospitalization. At first, the ulceration was only on both of her legs, but recently, it has spread into both hands. Most striking was the presence of extensive multiple deep and irregular ulcers covered with a blackish crust and, in some areas, yellow slough eroding the subcutaneous tissue with ragged and angular margins. These ulcers were distributed symmetrically. Blisters preceded some of the ulcers. On admission, there was no fever; he complained of malaise and noticed leg weakness for one week before hospitalization. A histopathology examination performed on the edge of his leg's ulcer shows vasculitis with endothelium occlusion of thrombus. The enormous number of acid-fast bacilli inside the blood vessels (endothelium) is also seen in Wade-fite staining. This clinical manifestation and histopathology examination results support Lucio's DLL phenomenon.

The diagnosis of DLL may be delayed, especially in non-endemic areas, resulting in death. Slit skin smear and microscopic observation are relatively easy, but the reliability and sensitivity are limited. Therefore, new techniques using PCR are developed. Among the methods, a nested PCR method targeting the RLEP region of *M. leprae* is the most sensitive and reliable (Curi et al. 2014). *M. leprae* has been known as the leprosy agent since its initial discovery in 1873. In 2008, a new etiologic agent—*M. lepromatosis*—was recognized in two patients of Mexican origin who died of DLL. Further analyses of 20 genes and pseudogenes revealed a 9.1% genetic difference between the two leprosy bacilli to substantiate a species-level divergence that occurred approximately 10 million years ago. The 9.1% sequence difference contrasts starkly with the clonal worldwide *M. leprae* strains that vary by 0.005%, as revealed by extensive genome sequencing and multilocus typing. there is a century-long history of DLL and likely dominance by *M. lepromatosis.*

M. lepromatous has led us to hypothesize that the disease came with the first American settlers from Asia around 13,000 years ago. Finding *M. lepromatosis* in Myanmar and Singapore earlier supports this Asian origin. Finding it in Brazil accords further American spread from North to Central America, such as Costa Rica, where DLL has been endemic (Han et al. 2014; Sehgal 2005).

The 16S rRNA gene, known for all described bacteria (~10,000 species), was selected as the PCR target because of its conserved and variable regions. The designs of PCR primers and assays were described previously (Sharma et al. 2020). The thermocycle was as follows: activation of the enzyme at 95°C for 2 min; 35 cycles of denaturation (95°C for 20 sec), primer annealing (58°C for 20 sec for the first-round PCR or 48°C for 20 secfor the second-round PCR), and extension (72°C for 40 sec and final extension for 5 min). A regular Taq polymerase was used. An agarose gel electrophoresis examined the target amplicons. The heminested PCR, with two 35 doubling cycles, enabled the detection of as low as one to three copies of the target, an exquisite sensitivity shown previously in an *M. leprae* PCR study (Han et al. 2008, 2014).

In this case, PCR examination by two rounds of heminested PCRs was performed to maximize detection sensitivity using the LERF2-MLER4 primer (5′ CTAAAAAATCTTTTTTAGAGATAC 3′) was positive for *M. leprae* and the PCR result for *M. lepromatous* using LPMF2-MLER4 primer (5′ GTCTCTTAATACTTAAACC TATTAA 3′) was positive for *M. lepromatosis* as well (Singh et al. 2015). This means that the patient has a double infection.

The lack of skin nodules is a recognized feature of DLL, based on extensive clinical experience with Mexican patients and the recent studies that also established *M. lepromatosis* as the specific cause of DLL. Han et al. (2012), the dual infections by *M. lepromatosis* and *M. leprae* accounted for 16.1% of all species confirmed cases. The manifestations of these cases were more variable than those of a single infection by either species: some cases exhibited mostly features of *M. lepromatosis* infection, such as DLL, younger age, and the extremities as the biopsy site, whereas others mainly exhibited features of *M. leprae* infection, such as the chest and face/ear as biopsy sites (Han et al. 2014). Several factors may affect the manifestations, including such factors as which species infects the patient first and its duration, whether infection by the second species represents the general vulnerability of the patient to these organisms or a mere chance of exposure due to common living environment, the preferred site of infection of each species, and dominance of one species over the other (Han et al. 2008). The hit-or-miss nature of minute biopsy and the overall lower DNA quality after tissue processing (formalin fixation and paraffin embedment) and long storage also likely undermined PCR detection of the etiologic agents in a handful of cases (Gillis et al. 2011; Han et al. 2014).

Lucio's phenomenon usually appears in untreated or inadequately treated non-nodular lepromatous leprosy (DLL). The treatment for DLL is by MDT, recommended by WHO, as for other multibacillary cases. Until now, thalidomide has been regarded as the best therapeutic option for LP due to the anti-TNF-α effect, so it has been postulated that other anti-TNF-α drugs could be useful, like pentoxifylline, infliximab, and etanercept, mainly in those patients where thalidomide is contraindicated or in countries where it is restricted due to its teratogenicity (Han et al. 2012; Kamath et al. 2014; Kumari et al. 2008). This patient had never had treatment with MDT before. Accordingly, he was treated with a multidrug regimen of MB leprosy and responded appropriately. The drug given is also combined with pentoxifylline 2×400 mg/day, wound care and debridement for ulcers. The ulcers healed slowly with cicatrization about five weeks after hospitalization.

Notably, the appropriate management of comorbidity factors such as anemia and hypoalbuminemia.

In conclusion, Lucio's phenomenon (LP) is rarely reported in East Java despite a high prevalence of multibacillary cases. This could be attributed to a lack of clinical suspicion or inconsistent histopathological features. The diagnosis of LP in this case was based on clinical and histopathological features. The underdiagnosis and obscurity of DLL and LP may be due to a lack of awareness. The significance of *M. lepromatosis* extends beyond Mexico and DLL, as it is also identified as a cause of leprosy in the Americas and Asia. Leprosy, a clinical condition known to humanity for centuries, is caused by *M. leprae, M. lepromatosis*, or both. It is widely recognized that the clinical forms of leprosy vary across continents and countries. For example, in India and Africa, 90% of leprosy cases are tuberculoid, while in Mexico, 80% to 90% are lepromatous (LL and DLL). In Southeast Asia, the two forms are equally distributed. Given the chronic nature of leprosy and the variable time it takes to diagnose, variations in clinical diagnoses and stages of infection are to be expected. However, more cases from different countries need to be examined to confirm the specificity of *M. leprae*-LL and to determine whether *M. lepromatosis* and/or dual infection are the main contributors to these variations. LP should still be considered in the differential diagnosis, even in non-endemic areas of leprosy. The lack of consensus regarding LP therapy has led to controversies in its management. Establishing an early diagnosis and promptly initiating treatment can help reduce morbidity and mortality.

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