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Case Report

Mycobacterium leprae BACILLEMIA IN BOTH TWINS, BUT ONLY MANIFEST AS LEPROSY IN ONE SIBLING

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ABSTRACT

Leprosy in twins is rarely reported. A 19 years-old male student, from Lamongan district, was diagnosed as Multibacillary (MB) leprosy in the Skin and STD Clinic of Dr. Soetomo General Hospital Surabaya. Multiple anesthetic skin lesions were found, but the bacteriologic examination was negative for Acid Fast Bacilli (AFB). Histopathology examination support the diagnosis of BL type of leprosy. His twin brother that has been lived together since born until present seems healthy without any complaints of skin lesions and have no signs of leprosy. When a serologic examination for leprosy was performed, a high anti PGL-1 antibody level was found in patient (IgM anti PGL-1 2937 and IgG anti PGL-1 3080 unit/ml) while his healthy twin brother showed only low level (IgM 745 and IgG 0 unit/ml). Interestingly when a PCR study was performed to detect M.leprae in the blood, both of them showed positive results. Using the TTC method, a genomic study of for M.leprae, it is revealed that both samples were identic (27x TTC repeats). According to patient's history, he had a traffic accident and got a wound in the knee seven years ago, while the skin lesions seems started from this area around three years ago before it spread to other parts of the body. The patient was treated with Multi-drug therapy (MDT) while his sibling got a prophylactic treatment for leprosy. After 6 months of treatment, the leprosy skin lesions were diminished and the serologic anti PGL-1 has been decreased. His healthy brother also showed a decrease in anti PGL-1 level and no skin signs of leprosy.

Key words: *leprosy, twin, bacillemia, PCR, prophylactic treatment*

ABSTRAK.

Penyakit kusta pada pasien bersudara kembar merupakan peristiwa yang jarang terjadi. Dilaporkan seorang pemuda berumur 19 berstatus mahasiswa yang datang berobat ke RSUD Dr Soetomo Surabaya dengan keluhan bercak di kulit kaki, badan dan muka. Pasien berasal dari daerah Lamongan dan bersaudara laki-laki kembar, tetapi dalam keadaan sehat. Diagnosa penyakit kusta ditegakkan berdasarkan lesi kulit yang anestesi, meskipun tidak ditemukan Basil Tahan Asam (BTA) dari lesi kulit. Pemeriksaan histopatologis menunjang diagnosa yang sesuai dengan kusta tipe BL. Saudara kembarnya yang telah tinggal bersama sejak kecil tidak menunjukkan adanya lesi kulit ataupun BTA. Pada pemeriksaan serologi anti Phenolic Glycolipid-1 (PGL-1) pada pasien didapatkan kadar yang tinggi (IgM 2937 u/ml dan IgG 3080 u/ml) sedangkan saudara kembarnya menunjukkan IgM anti PGL-1 745 u/ml, sedangkan IgGnya 0. Yang menarik adalah saat dilakukan pemeriksaan PCR untuk mendeteksi adanya M.leprae dalam darah, ternyata keduanya sama-sama menunjukkan hasil PCR yang positif. Selanjutnya dengan metode TTC dilakukan studi genomic dari M.leprae yang ditemukan. Hasil sekuensing pengulangan TTC menunjukkan bahwa ke 2 sampel tersebut identik (27x pengulangan TTC). Pasien diobati dengan obat Multi-drug Therapy (MDT) sedangkan untuk saudara kembarnya diberikan obat pencegahan kusta. Evaluasi setelah 6 bulan menunjukkan perbaikan klinis pada pasien dan penurunan titer antibodI anti PGL-1, sedangkan saudara kembarnya tetap tidak menunjukkan adanya gejala kusta serta semakin rendahnya titer antibodi.

Kata kunci: *kusta, saudara kembar, basilemia, PCR, terapi pencegahan*

BACKGROUND

Leprosy is a chronic disease caused by Mycobacterium leprae that primarily effects the peripheral nerves and secondary affects the skin and other organs.[1] Transmission leprosy dependent on immunological status and susceptibility, household contact, the environment and social conditions such as economic status, lacking of ventilation at home or poor hygiene.[2] Genetic factors are also to be a important factor in transmission of leprosy disease. Studies suggest that, among monozygotic (identical) twins if one had leprosy, the other almost always had leprosy, while this was not the case with dizygotic twins.[3] It is also influenced by human leukocyte antigen (HLA) that affect susceptibility.[4]

The main transmission route of M.leprae is droplet infection, but transmission such as skin contact, through the placenta during pregnancy, breast-feeding and trauma should not be ruled out even though there is no conclusive evidence.[5]

WHO recommends the Multi-drug Therapy (WHO-MDT) regiment for leprosy and the program have began since 1980 in Indonesia.[6] Although most of leprosy cases have been treated, there are still new leprosy cases were detected every year, indicating that transmission of leprosy still occurred in the community.[7] One of the reasons for explaining the continuing of new detected leprosy cases is the non-human resource of M.leprae. Since the human source (leprosy patients) are already treated by MDT and become non-infectious anymore, the role of non-human resources should be kept in mind.. These non-human resources including water, soil or other contaminated agents.[7] Several studies reports existence of viable M.leprae outside the human body. Detection of viable Mycobacterium leprae (RNA M.leprae) found in soil samples in Ghatampur India.[8] DNA M.leprae also found in water sources (wells) in along coast of East Java[9] M.lepra in soil and wells water were reported in leprosy endemic areas of East Java, including Lamongan Regency.[10]

CASES

Twins (Y and D) , 21 years-old students, unmaried, from Lamongan, visited the Skin and VD Clinic of Dr Soetomo General Hospital Surabaya. One sibling, Y, complained anasthetic red patch, which firstly appeared in front of right knee since 3 years ago. Y and D was born on 1994 in Payaman, Solokuro, part of Lamongan distric. Both of them were normaly born in one placenta (monozygotic). They spent time together in one house and sharing one bedroom since childhood. When they were 13 years old, they had junior school in Sendang agung village, Paciran, part of Lamongan district. In 2012 they became students in Malang and still lived in one the dorm rooms.

In 2007, Y was 14 years old, he got accident falling to the ground in Lamongan. He got trauma behind the right knee. At that time the wound just treated with antiseptic and healed. Six years later, in 2013, Y complained red patch which first appeared in front of the right knee. He went to a doctor and got some medications but the skin lesions still persist. Then the patient and his sibling visited the Out patient Clinic of Dr. Soetomo Hospital Surabaya.

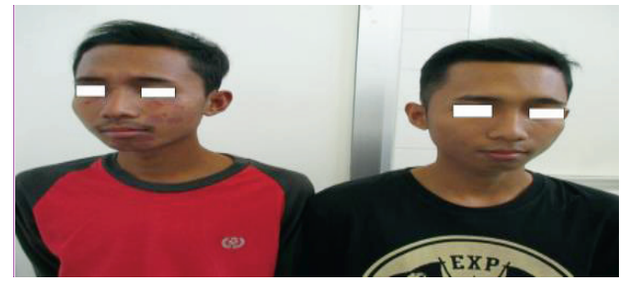


Figure 1. Twins A. Y (leprosy patient) B. D (healthy twin).

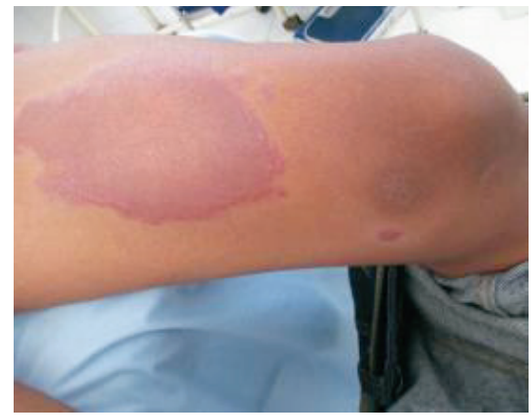


Figure 2. A. First anesthetic lesion on the knee

Multiple anaesthetic skin lesions were found over the right extremity and face. Negative result of skin slit smear for Acid Fast Bacilli (AFB) were noted from bacteriological examination using Ziehl Neelsen staining. Skin biopsy from the skin lesion at the right extremity revealed a BL type leprosy. Serological examination (ELISA anti PGL-1 antibody) serology to both of twins, ELISA results of IgM anti PGL-1 in Y patient was 2937 unit/ml and IgG anti PGL-1 was 3080 unit/ml. In the other health twin, serology results showed levels of IgM anti PGL-1 was 745 unit/ml and IgG anti PGL-1 was 0.

Skin biopsy from the skin lesion at the right extremity revealed a BL type leprosy. (Figure 3 & 4)

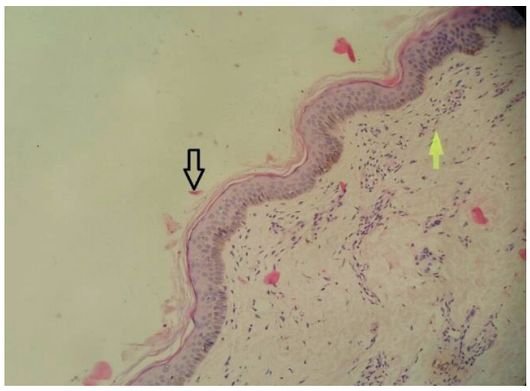


Figure 3. Epidermal atrophy, flattened rete ridges and grenz zone were observed (H/E 400x)

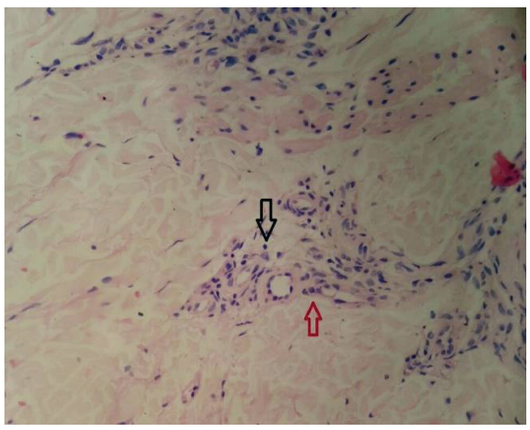
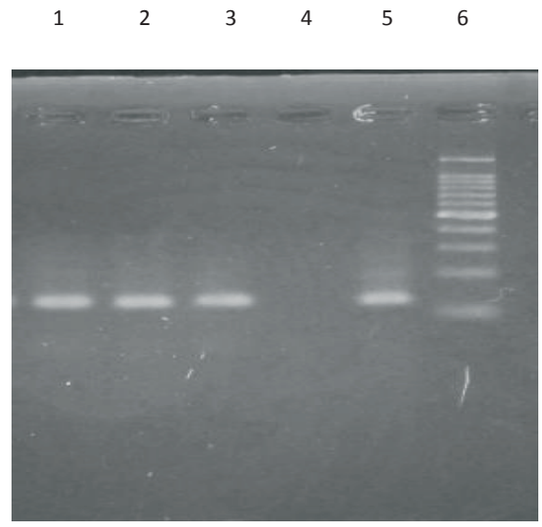


Figure 4. Dilated capillary blood with lymphocyte infiltration. . (H/E 400x)

Polymerase Chain Reaction (PCR) study was performed to the bloods of the twin, using the LpF and LpR nested primers to the bloods of the twin (Figure 5)



Note :
1. Pbmc from leprosy patient (Y)
2. Pbmc from healthy sibling (D)
3. Skin lesion of patient (Y)
4. Neg Control
5. Pos Control – M.leprae Thai53
6. 100bp DNA ladder

Figure 5. PCR results from blood and skin lesion (LpF –LpR nested primers) .

Further study was conducted to compare the genomic pattern between the two M.leprae DNA from the amplicon

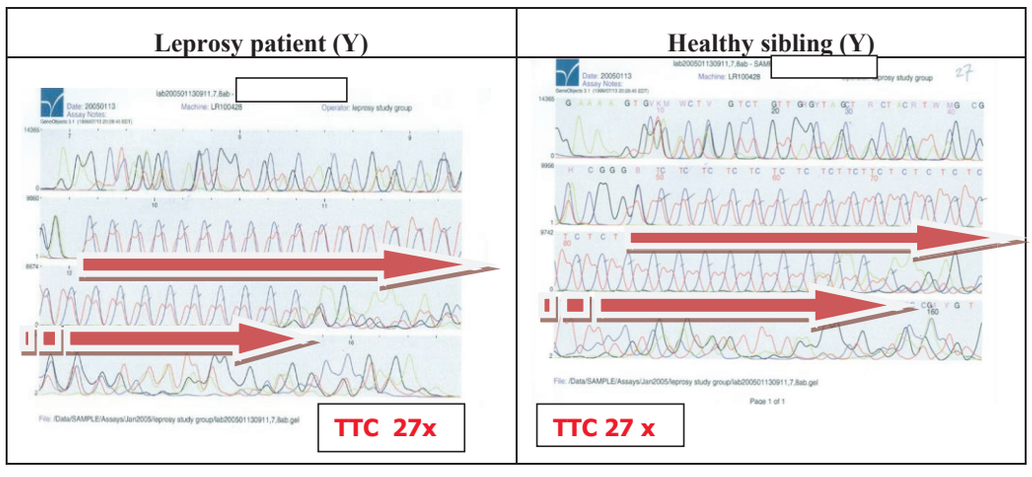


Figure 6. Direct sequencing of TTC area from both samples and number of TTC repeats

products of PCRresults . (Figure 6). Using the TTC method, the number of TTC repeats from both of samples were similar, 27x repeats, which indicates the two samples were identic or similar strain.

The examination results of these twins can be summarized as follows :

Data	Mr. Y (leprosy patient)	Mr. D (healthy siblings)
Skin lesions	Multiple anesthetic macules	No skin lesions
Bacterial examination (AFB)	Negative	Negative
Histopathology from skin lesion	BL type of leprosy	Not done
Serology (anti PGL-1)	IgM 2937 IgG 3080 u/ml	IgM 745 IgG 0 u/ml
PCR from skin lesion	Positive	Not done
PCR from blood (pbmc)	Positive	positive
Direct sequencing TTC area	27 x repeats	27 x repeats

The leprosy patient (Mr. Y) was treated with Rifampicine, Dapsone and Lamprene (WHO-MDT regiment) for 12 months while his healthy sibling was treated with a prophylactic dose of Rifampicine and Ofloxacine for two weeks. After six months later, the skin lesions disappear and the titer of anti PGL-1 antibodies were decreased. His healthy sibling does not develop any sign of leprosy and the anti PGL-1 titer became normal.

DISCUSSION

Leprosy in twin is relatively rare and seldom reported in the literature. Chakravartti & Vogel (1973) conducted an epidemiologic study leprosy in twins. Among 62 pairs of monozygotic twins and 40 pairs of dizygotic, they found that the monozygotic twins have a greater risk to get leprosy if the sibling affected the disease.3 Several studies reported several genes and substance may have a role in the susceptibility to leprosy, including HLA, TAP2, VDR, PTPN22 in adaptive immunity and NRAMP1, TLR2,MICA etc. in innate immunity.[4]

In our case, they are monozygotic twin which is theoretically will have a similar pattern. They live together since birth until adolescent in leprosy endemic area of Lamongan. This area has been known as leprosy endemic area in East Java since a long time ago.11 If the source of infection is the same, usually via droplet infection, they will got the same exposures and same long time duration. Then the incubation period will be the same and both of them will manifest leprosy on the same time. But in fact, leprosy manifest only in one sibling and the different life experience between them is the traffic accident seven years previously. The site of the first skin lesion of leprosy was very close with the scar of the wound during the accident three years ago. It might be possible that M.leprae entered the body via the wound and then spread to other organ. Non-human resource of M.leprae have been reported from some leprosy endemic areas and also some of them found the viable M.leprae from the soil and water.12. In our case, Mr. D who got traffic accident probably infected by the bacilli from environment, which become manifest leprosy after 4 years. The diagnosis of leprosy in this case is confirmed by the typical anesthetic skin lesions and histopathological examination. Although the other cardinal signs of leprosy (peripheral nerves enlargement and the present of Acid Fast Bacilli / AFB) was negative, the PCR results showed that the specific DNA of M.leprae was present in the skin lesion and peripheral blood. The serological test result of Mr.Y supported the diagnosis of manifest leprosy (high titer of IgM and IgG anti PGL-1) while the antibody titer of Mr.D showed a low sero-positive result (IgM anti PGl-1 745 u/ml with cut off 605 u/ml) that indicated a subclinical leprosy. One can assume that the process of leprosy in Mr.D is still in the initial stage, which will progress to manifest leprosy within certain years ahead.13 The use of the TTC technique, one procedure of Variable Number Tandem Repeat (VNTR) method for genetic study of M.leprae. This technique was chosen because it is relatively easy, simple and relatively low cost.14 The results showed 27x TTC repeats in both samples indicated similar pattern of the strain, which means they were originated from one similar source.

After got the disease, Mr. D became a source of infection for his twin brother. Positive PCR test from the blood indicates that the healthy brother was in subclinical stage of leprosy. This stage will develop toward the manifest leprosy after certain years, if no prophylactic treatment was given.15 Up to present time. there is still no guidance yet about chemoprophylactic treatment in Leprosy, therefore the use of Rifampicine and Ofloxacine for the subclinical leprosy in this case was based on the author's experience.[16]

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