Jpn. J. Infect. Dis., 75, 427-430, 2022

Short Communication

Mycobacterium leprae and *Mycobacterium lepromatosis* Infection: a Report of Six Multibacillary Cases of Leprosy in the Dominican Republic

Juan Daniel Periche Fernández[1], Víctor Enrique Pou-Soarez[1], Roberto Arenas[2], Eder Rodrigo Juárez-Duran[2], Sofía Lucila Luna-Rojas[3], Juan Xicohtencatl-Cortes[4], Luary Carolina Martínez-Chavarría[5], Fernando Martínez-Hernández[3], and Rigoberto Hernández-Castro[3]*

[1]Dominican Dermatological Institute and Skin Surgery Dr. Huberto Bogaert, Santo Domingo, Dominican Republic; [2]Mycology Service and [3]Department of Ecology of Pathogens, General Hospital Dr. Manuel Gea González, Mexico City; [4]Intestinal Bacteriology Laboratory, Children's Hospital of Mexico Dr. Federico Gomez, Mexico City; and [5]Department of Pathology, Faculty of Veterinary Medicine, National Autonomous University of Mexico, Mexico City, Mexico

ABSTRACT: The causative agents of leprosy are *Mycobacterium leprae* and *M. lepromatosis*. *Mycobacterium lepromatosis* was found in 2008 to cause diffuse lepromatous leprosy in Mexican patients. This study aimed to identify *M. leprae* and *M. lepromatosis* in paraffin-embedded skin samples from Caribbean patients with leprosy. A total of six skin samples were obtained from the Dominican Republic. All cases presented the multibacillary form; five were nodular lepromatous leprosy, and one was borderline lepromatous leprosy. All patients received multidrug therapy. Molecular identification was achieved using the *M. leprae*-specific repetitive element for *M. leprae* and the *hemN* gene for *M. lepromatosis*. *Mycobacterium leprae* was identified in two lepromatous leprosy cases, and one borderline lepromatous leprosy case; *M. lepromatosis* was found in one nodular lepromatous leprosy case. Both *Mycobacterium* species were present in two nodular lepromatous leprosy cases. This is the first report of *M. lepromatosis* in the Dominican Republic.

Leprosy or Hansen's disease is a chronic infectious granulomatous disease caused by *Mycobacterium leprae* or *M. lepromatosis* (1). They mainly affect the skin, peripheral nerves, mucosa of the upper respiratory tract, and eyes. In general, the disease does not cause disabilities if it is diagnosed and treated early, but otherwise, it evolves and generates severe complications and leprosy reactions that incapacitate the patient and, in the most serious cases, culminate in death (2).

The disease is classified into five clinical forms according to immunity: tuberculoid leprosy (TT), borderline tuberculoid (BT), borderline-borderline (BB), borderline lepromatous (BL), and lepromatous leprosy (LL) (3). This study aimed to identify *M. leprae* and *M. lepromatosis* in paraffin-embedded skin samples from Caribbean clinical cases of leprosy.

We studied six patients with leprosy from the Dominican Republic, four males and two females, with an age range of 26–48 years. All cases were multibacillary; clinically, five of them corresponded to LL, and one corresponded to BL. Acid-fast bacilli (AFB) were detected in all cases with a bacteriological index (BI), examined using slit skin smear, from 1+ to 4++++, and morphological index of 0–20%, 50–75%, or 75–100%. BI test and biopsy were performed before treatment; in all cases, granulomas with distinctive foamy (vacuolated) macrophages were found (Virchow's cells granuloma). All patients received multidrug therapy (MDT) with a single monthly dose regimen containing 600 mg of rifampicin, 300 mg of clofazimine, and daily doses of dapsone 100 mg plus clofazimine 50 mg. Three patients finished treatment after 24 months, and three patients were still under treatment at 6 months (Table 1).

Case 1: A 40-year-old male patient had a 4-year history of hypoesthesia of the lower limbs. He presented erythematous plaques on the face and nodular lesions on the buttocks and lower limbs (Fig. 1A). He had alopecia of the eyebrows, eyelashes, and body hair, as well as dry skin and muscle wasting of the hands. He also presented painful thickening of the cubital, sciatic, and posterior tibial nerves, with the decreased muscular

Received October 28, 2021. Accepted February 28, 2022.
J-STAGE Advance Publication March 31, 2022.
DOI: 10.7883/yoken.JJID.2021.709
*Corresponding author: Mailing address: General Hospital Dr. Manuel Gea Gonzalez, Tlalpan 14080, Mexico City, Mexico. Tel: (+52 55) 40003000, E-mail: rigo37@gmail.com; rhc@unam.mx

Table 1. Clinical, histopathological and molecular findings of Dominican leprosy patients

Case	Age years/ gender	Clinical diagnosis	Evolution	BI	MI	Histopathology	PCR identification
1	40/Male	Nodular lepromatous leprosy (DLL)	4 years	4+	0–20%	VCG + AFB	*M. lepromatosis*
2	35/Male	Nodular lepromatous leprosy (DLL)	8 months	4+	50–75%	VCG + AFB	*M. lepromatosis* + *M. leprae*
3	26/Female	Nodular lepromatous leprosy (DLL)	1 year	4+	50–75%	VCG + AFB	*M. lepromatosis* + *M. leprae*
4	48/Male	Borderline lepromatous leprosy (BL)	1 year	3+	0–20%	VCG + AFB	*M. leprae*
5	27/Male	Nodular lepromatous leprosy (DLL)	1 year	3+	75–100%	VCG + AFB	*M. leprae*
6	39/Female	Nodular lepromatous leprosy (DLL)	1 year	1+	50–75%	VCG + AFB	*M. leprae*

BI, bacteriological index; MI, morphological index; VCG, virchow's cells granuloma; AFB, acid-fast bacilli.

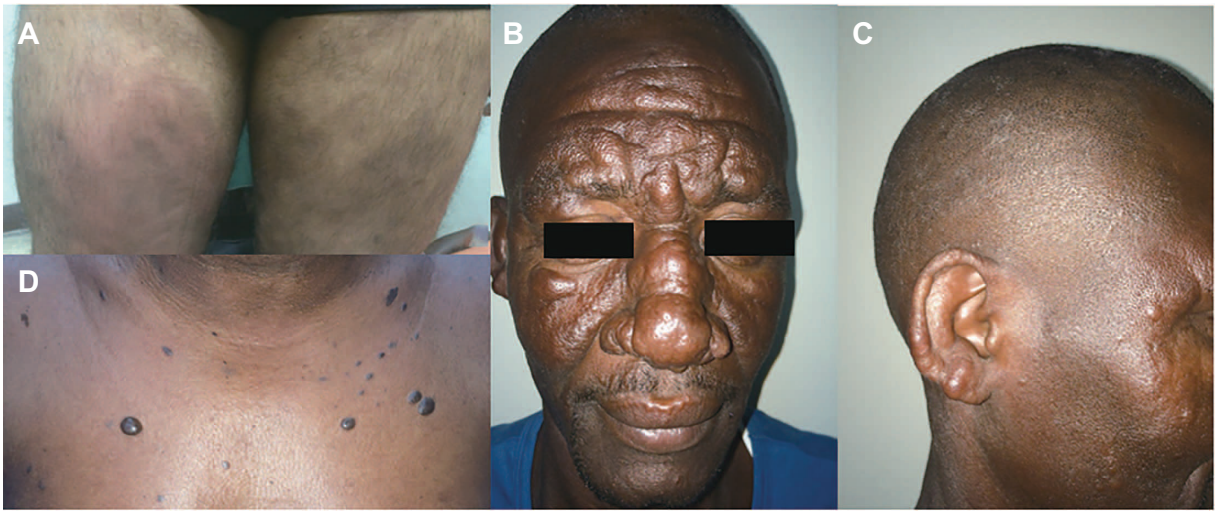

Fig. 1. (Color online) Cases of leprosy in the Dominican Republic. (A) Nodular lesions and erythema nodosum leprosum; (B) Nodular lesions on the face ("leonine facies"); (C) Infiltrated nodules on the forehead and ears, dry skin, and alopecia; (D) Pigmented nodular lesions of different sizes.

strength of the right hand. The MDT was administered for 24 months, resulting in clinical improvement, a negative AFB test, and no disabilities.

Case 2: A 35-year-old male patient presented an 8-month history of infiltrated plaques and nodular lesions on the face (Fig. 1B–C), trunk, and limbs, affecting mainly the nasogenian grooves and auricular pavilion. He presented generalized dry skin and body alopecia, edema, and hypoesthesia on the hands with interosseous atrophy, as well as thickening of the auricular nerve branch of the cervical plexus and nonpainful thickening of the cubital nerves. The sensitivity and muscular strength of his feet were conserved. The MDT was administered for 24 months, resulting in a negative AFB test and no disabilities.

Case 3: A 26-year-old pregnant female patient with a 1-year history of leprosy was detected in a "contact exam" for a family member with leprosy. She presented nodular lesions on the face (affecting mainly the nose), trunk, and limbs, as well as generalized dry skin and body alopecia. She also presented nonpainful thickening of the cubital and sciatic nerves, with the conserved muscular strength of the hands and feet. After delivery, she received MDT. She is still under treatment, with evidence of clinical improvement.

Case 4: A 48-year-old male patient with a 1-year history of leprosy was detected in a "contact exam" of a family member with leprosy. He presented infiltrated plaques on the trunk, limbs, and lower limb hypoesthesia. He had no thickening of the cubital or sciatic nerves and had conserved muscular strength of the hands and feet. The MDT was administered for 24 months, resulting in clinical improvement, a negative AFB test, and no disabilities.

Case 5: A 27-year-old Haitian man with a 1-year history of leprosy was detected in a "contact exam" for a brother with LL. He presented infiltrated nodules on the forehead, ears, and buttocks, as well as hand and foot hypoesthesia but conserved muscular strength (Fig. 1D). There was no thickening of cubital or sciatic nerves. The MDT was administered for 6 months. He currently has skin pigmentation and grade 2 disability of the hands and feet.

Case 6: A 39-year-old female patient with a 1-year history of leprosy was seen for dermatological consultation. She presented infiltrated nodules on the face, ears, hands, buttocks, and limbs. She had no thickening of cubital or sciatic nerves but conserved muscular strength of the hands and feet. Currently, she continues under MDT treatment.

From all paraffin-embedded skin samples, paraffin removal was performed with a xylene-alcohol protocol. Then, total deoxyribonucleic acid (DNA) was extracted using a DNeasy blood and tissue kit (Qiagen, Ventura,

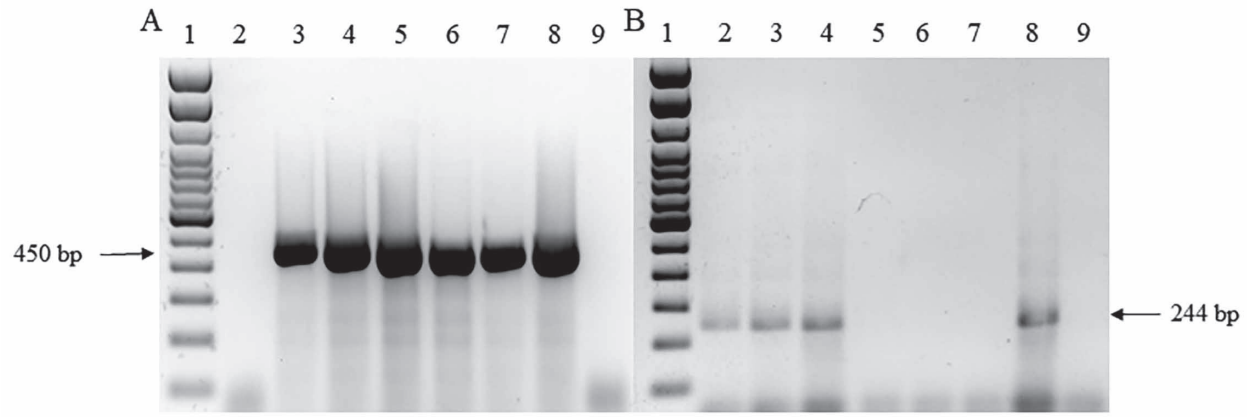

Fig. 2. PCR amplification of the 450 bp fragment of *M. leprae* specific repetitive element (RLEP) (A) and the 244 bp fragment of *hemN* gene for *M. lepromatosis* (B). (A) Lane 1: 100 bp DNA ladder; lane 2: case 1 negative for *M. leprae*; lanes 3–7: cases 2–6 positives for *M. leprae*; lane 8: positive control; lane 9: negative control. (B) Lane 1: 100 bp DNA ladder; lanes 2–4: cases 1–3 positives for *M. lepromatosis*; lanes 5–7: cases 4–6 negatives for *M. lepromatosis*; lane 8: positive control; lane 9: negative control.

CA, USA). Molecular identification was achieved using polymerase chain reaction (PCR) amplification of the *M. leprae*-specific repetitive element (RLEP) for *M. leprae* and *hemN* gene for *M. lepromatosis*. A set of primers RLEP-7 (5′-TGAGGCTTCGTGTGCTTTGC-3′) and RLEP-8 (5′-ATCTGCGCTAGAAGGTTGCC-3′) were used for *M. leprae*, which amplified a 450 bp fragment (Fig. 2A), and primers LPM244-F (5′-GTTCCTCCACCGACAAACAC-3′) and LPM244-R (5′-TTCGTGAGGTACCGGTGAAA-3′) which amplify a 244 bp fragment, were used for *M. lepromatosis* (Fig. 2B) (4).

Mycobacterium. leprae was identified as the only etiologic agent in two nodular or diffuse LL (DLL) cases and one BL case, while *M. lepromatosis* was identified as the only etiologic agent in one DLL case. Mixed infection with both *M. lepromatosis* and *M. leprae* was identified in two DLL cases (Table 1).

Leprosy has been reported worldwide throughout history, from biblical to contemporary times, and the Caribbean is no exception. In 2019, the Caribbean region was the second place where more new cases of leprosy were reported worldwide (1,501 new cases reported) (5). Some time ago, *M. lepromatosis* was restricted to Mexico (1,6–8), but there are recent reports of this etiological agent in Canada (9), Singapore (10), Brazil (11), Myanmar (11), USA (imported case) (12), and Italy (Cuban patient) (13).

Mixed infection by *M. leprae* and *M. lepromatosis* has been previously reported; most of them have been observed in Mexican patients with LL (6,8,14). However, it has been reported in patients from Brazil (11), Indonesia (15), and Paraguay (16), all with the same presentation of LL.

Even though the LL form of leprosy and these species are no longer considered endemic to the Caribbean, the reported cases from this area still show similarities. Our patients were within an age range of 26–48 years; this coincides with those cases reviewed in Puerto Rico (17), Mexico (7,8), and Colombia (18), where most of the leprosy cases occurred in the adult population. In addition, most reported affected patients have been male, as seen in our patients. All the cases showed a clinical and bacteriological effective response to the standard MDT according to the World Health Organization (WHO) recommendations.

When comparing the clinical diagnoses, we observed that five of our six patients showed nodular LL, and only one manifested borderline LL, but the causal agents were different between them (Table 1). This pattern has been observed previously in the study carried out by Torres-Guerrero et al. (8), but here, we showed that both species could cause DLL and BTL, regardless of whether they are alone or in a dual infection.

In general, in the Dominican Republic and the Caribbean region, cases of leprosy are only diagnosed using slit skin smear, they are reported as paucibacillary or multibacillary, and they have been associated with *M. leprae*. Our results provide the first evidence of the presence of *M. lepromatosis* in the Dominican Republic.

Conflict of interest None to declare.

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