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Case Report

Nontuberculous Mycobacterial Lung Disease Caused by *Mycobacterium chelonae*: A Case Report

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Mycobacterium chelonae lung disease is very rare. We report a case of lung disease caused by *M. chelonae* in a previously healthy woman. A 69-year-old woman was referred to our hospital because of hemoptysis. A computed tomography (CT) scan of the chest revealed bronchiolitis associated with bronchiectasis in the lingular division of the left upper lobe. Nontuberculous mycobacteria were isolated three times from sputum specimens. All isolates were identified as *M. chelonae* by various molecular methods that characterized *rpoB* and *hsp65* gene sequences. Although some new lesions including bronchiolitis in the superior segment of the left lower lobe developed on the chest CT scan 35 months after diagnosis, she has been followed up without antibiotic therapy because of her mild symptoms. To the best of our knowledge, this is the first case of *M. chelonae* lung disease in Korea in which the etiologic organisms were confirmed using molecular techniques.

Key Words: Nontuberculous Mycobacteria; Bronchiectasis; *Mycobacterium chelonae*

Introduction

Nontuberculous mycobacteria (NTM) are generally classified as either slow or rapid growers. Rapidly growing mycobacteria (RGM), which are characterized by visible growth on solid media within 7 days, include three clinically relevant species, *Mycobacterium fortuitum, M. abscessus*, and *M. chelonae*[1-3]. All are environmental microorganisms found in soil, bioaerosols, and natural and chlorinated water[1-3]. RGM lung disease is predominantly due to *M. abscessus* (80% of cases) and *M. fortuitum* (15% of cases)[4,5]. *M. chelonae* usually causes skin, bone, and soft tissue infections and is a very rare respiratory pathogen[6,7]. Here, we report a case of *M. chelonae* lung disease associated with bronchiectasis in a previously healthy woman.

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Case Report

In April 2008, a 69-year-old woman was referred to our hospital because of hemoptysis. She had been healthy until one month prior, when intermittent hemoptysis developed. The patient had no smoking history or other medical history. On examination, the patient appeared well. Her weight was 45 kg, height 156.2 cm, temperature 36.2°C, blood pressure 133/75 mm Hg, pulse 73 beats per minute, and oxygen saturation 100% while breathing ambient air. Laboratory results were normal. A human immunodeficiency virus antibody test

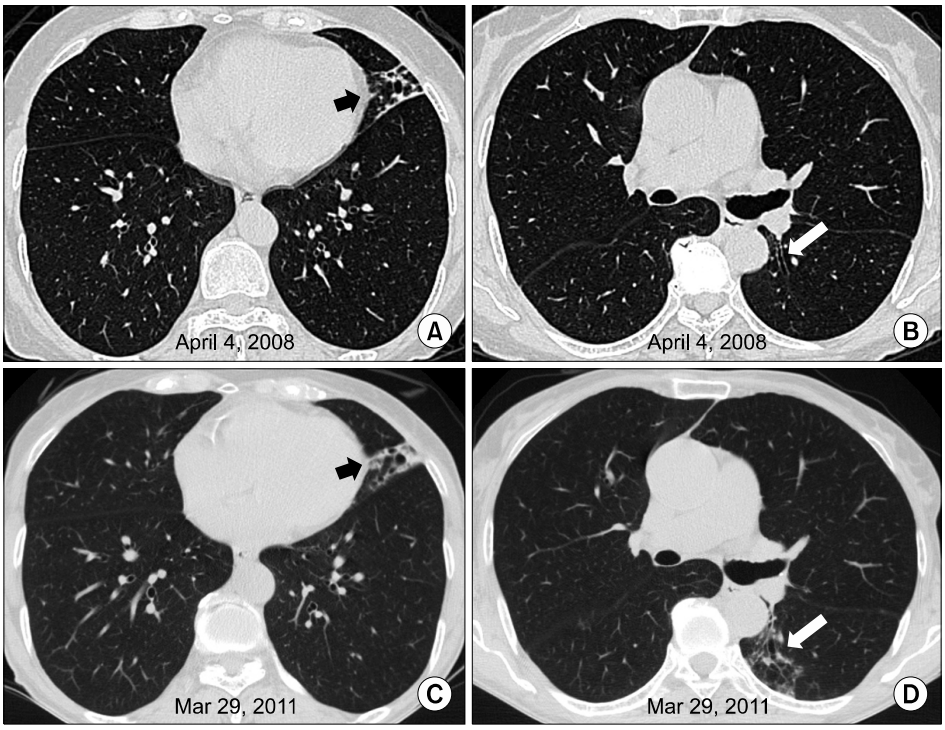

Figure 1. A 69-year-old woman with bronchiectasis and nontuberculous mycobacterial lung disease caused by *Mycobacterium chelonae*. (A) A transverse computed tomography (CT) scan (2.5-mm-section thickness) at the time of presentation reveals bronchiectasis and bronchiolitis in the lingular division of the left upper lobe. (B) Mild bronchiectasis was noted in the superior segment of the left lower lobe at the time of presentation. (C) A CT scan of the same patient at 3 years after diagnosis reveals mild progression of peribronchial infiltration on the lingular division of the left upper lobe. (D) There was newly appearing bronchiolitis in the superior segment of the left lower lobe.

was negative.

A computed tomography (CT) scan of the chest revealed bronchiectasis and bronchiolitis in the lingular division of the left upper lobe (Figure 1A, B). There was no evidence of cystic fibrosis or other common causes of bronchiectasis. NTM were isolated three times from sputum specimens.

NTM species were identified using a polymerase chain reaction-restriction fragment length polymorphism analysis (PRA) based on the partial region of the *rpoB* gene[8]. Colonies were scraped, and genomic DNA of the isolate was extracted using a commercial kit (QIAamp DNA Mini kit; Qiagen, Hilden, Germany). Amplification of the partial *rpoB* gene (360 bp) was performed using primers Rpo5' (5'-TCAAGGAGAAGCGCTACGA-3') and Rpo3' (5'-GGATGTTGATCAGGGTCTGC-3')[8] with subsequent digestion with 5 units of *Msp* I (New England BioLabs, Beverly, MA, USA) for 3 hours at 37°C. The digestion mixtures were analyzed by 3% (w/v) agarose gel electrophoresis. DNA size markers, pBR322-*Msp*I-digested DNA (New England BioLabs) were used to enable estimation of DNA fragment size. The PRA results were determined by comparing their restriction patterns with those available in the National Center for Biotechnology Information GenBank database[9] and referred to as described by Lee et al.[8] for the *rpoB*-PRA. The *rpoB* amplicon restricted by *Msp*I resulted in fragment sizes of 105, 95, 80, 50, and 40 bp in two serial cultures (Figure 2). The *rpoB*-PRA patterns of those cultures exactly matched the known restriction fragment pattern of the reference strain of *M. chelonae* subsp. *chelonae* ATCC35749.

The identification of these NTM isolates was further confirmed by DNA sequence analysis of the partial *rpoB* gene (723 bp) using primers MycoF (5'-GGCAAGGTCACCCCGAAGGG-3') and MycoR (5'-AGCGGCTGCTGG-

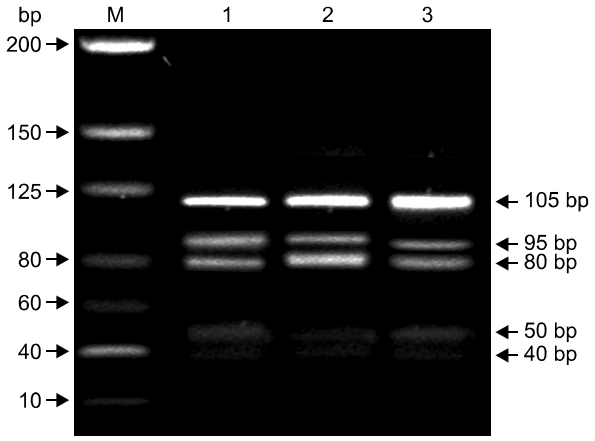

Figure 2. Electrophoresis of the polymerase chain reaction-restriction fragment length polymorphism analysis (PRA) of the *rpoB* gene involving digestion with *Msp*I. *Mycobacteria isolated* from two serial specimens generated the fragments of 105, 95, 80, 50, and 40 bp, which were identical to those of *Mycobacterium chelonae* subsp. *chelonae* ATCC35749. M, size marker; Lanes 1 and 2, patterns of *rpoB*-PRA from two serial samples isolated; Lane 3, the matched pattern of the reference strain *M. chelonae* subsp. *chelonae* ATCC35749.

GTGATCATC-3')[10] and the partial *hsp65* gene (439 bp) using primers Tb11 (5'-ACCAACGATGGTGTGTCCAT-3') and Tb12 (5'-CTTGTCGAACCGCATACCCT-3'). The results revealed sequence similarity (above 99.9%) with *M. chelonae* for the *rpoB* gene (GenBank accession no. EU109300.1) and *hsp65* gene (GenBank accession no. U55832).

Finally, the patient was diagnosed with *M. chelonae* lung disease. Antibiotic therapy was not initiated because of her mild symptoms. Three years after diagnosis of *M. chelonae* lung disease, follow-up chest CT was performed. Although there was mild progression of peribronchial infiltration on the lingular division of the left upper lobe and newly appeared bronchiolitis in the superior segment of the left lower lobe (Figure 1C, D), she did not complain of aggravation of her symptoms.

Discussion

The nomenclature of RGM has changed frequently and has been a source of confusion for clinicians. For instance, within the past approximately 20 years, *M. abscessus*, the most common RGM respiratory pathogen, was labeled as *M. cheloneii* subsp. *abscessus*, *M. chelonae* subsp. *abscessus*, and finally, in 1992, *M. abscessus*[11]. Unfortunately, some mycobacterial laboratories still report *M. abscessus* isolates as *M. chelonae* complex or *M. chelonae/abscessus* group without further species identification[12,13]. The species identification is important because therapy differs significantly depending on the RGM species obtained. Because *M. chelonae* is an extremely rare cause of pulmonary disease, most respiratory isolates that are identified as *M. chelonae/abscessus* complex can be reasonably assumed to be *M. abscessus* isolates[2].

Identifying RGM to the species level is very important[1-3]. Biochemical tests that assess phenotype characteristics have commonly been used by clinical laboratories to characterize RGM. However, these are time-consuming and do not always lead to identification of the organism to the species level[2,3]. High-performance liquid chromatography, which is a useful method for identifying slowly growing NTM, may not enable separation of *M. chelonae* and *M. abscessus*[2,3]. Molecular methods can provide reliable and rapid identification of RGM[1]. In this study, the *M. chelonae* isolates were identified using *rpoB* gene based-PRA and sequence analysis of the *rpoB* and *hsp65* genes.

Skin, bone, and soft tissue disease are the most important clinical manifestations of *M. chelonae* infection. Disseminated *M. chelonae* infection can also occur in immunocompromised patients[1]. *M. chelonae* is an increasingly recognized cause of keratitis, especially after injury with a foreign body or following office ophthalmologic procedures[1]. However, *M. chelonae* is a much less frequent cause of lung disease than *M. abscessus*[1-3].

Reported cases of *M. chelonae* lung disease are usually middle-aged or elderly women with no other co-existing parenchymal lung disease. The symptoms and radiographic presentation of *M. chelonae* lung disease are similar to those of other RGM lung diseases[5]. Bronchiectasis, nodules, and consolidation are the most common

CT features[14].

It is important to distinguish *M. chelonae* from *M. abscessus* because therapy for *M. chelonae* is potentially easier than for *M. abscessus* infection[1]. *M. chelonae* is resistant to anti-tuberculosis agents but is susceptible to a number of traditional anti-bacterial agents such as tobramycin (100%), clarithromycin (100%), linezolid (90%), imipenem (60%), amikacin (50%), clofazimine, doxycycline (25%), and ciprofloxacin (20%)[1]. In addition, inducible resistance to clarithromycin which is conferred by an *erm*(41) gene is found in *M. abscessus* but is absent from *M. chelonae*[15].

In conclusion, *M. chelonae* should be considered a possible etiologic pathogen of the nodular bronchiectatic form of NTM lung disease, despite the rarity of pulmonary *M. chelonae* infection. To the best of our knowledge, this is the first documented case of *M. chelonae* lung disease in an immunocompetent adult in Korea in which the etiologic organism was confirmed using molecular methods that characterized *rpoB* and *hsp65* gene sequences.

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