Original Article

Pulmonary disease caused by *Mycobacterium simiae* in Iran's national referral center for tuberculosis

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Abstract
Introduction: Several species of non-tuberculosis mycobacteria (NTM) can affect humans and can cause either symptomatic or asymptomatic infection. This study aimed to determine the clinical and radiological manifestation, as well as the treatment, of *M. simiae* in patients in Masih Daneshvari Hospital, a TB referral hospital in Iran.
Methodology: This retrospective study involved all patients presenting to our referral center from 2002 to 2009, with confirmation of *M. simiae* pulmonary infection. For all patients, sputum smear and culture for identification was performed, as was drug susceptibility testing. Additionally, PCR identification methods for NTM, and high-resolution CT scan were conducted. All patients were treated according to American Thoracic Society recommendations.
Results: In total, 26 cases of *M. simiae* were identified in our center. The mean age of the patients was 58.23 ± 16.9years. Only one patient was HIV positive, and all but one were Iranian. The most frequent symptom was coughing (92.3%), and 100% of the patients had nodular lesions. In addition, bronchiectasis and cavitation were present in 84.6% and 88.5% respectively. All the patients were resistant to every first-line drug. Two patients failed the treatment, and twenty-four were cured, after which no recurrence of the disease was observed.
Conclusion: *M. simiae* may present with clinical and radiological manifestations consistent with tuberculosis, and be resistant to anti-TB agents. A more efficient treatment for NTMs such as *M. simiae* is needed, to shorten the period of treatment and proved fewer adverse effects than current therapies.

Introduction

Various types of non-tuberculosis mycobacteria (NTM) have been recognized as a cause of symptomatic or asymptomatic pulmonary infection in humans[1] Increasing rates worldwide of NTM infections in recent decades can be attributed to two major factors: the growing epidemic of HIV infection and the significant improvements in laboratory diagnostic methods [1,2,3].

Mycobacterium simiae, one of the non-tuberculous mycobacteria (NTM) that can affect both humans and animals was described as a new species in 1965 when it was isolated from a Macacus rhesus monkey [4]. This bacterium is phylogenetically classified in the same complex as other mycobacteria such as *Mycobacterium triplex*, *Mycobacterium genavense*, *Mycobacterium heidelbergense*, and *Mycobacterium lentiflavum* [5]. With regard to its chemical and bacteriologic characteristics, it is a slow-growing, rust-colored photochromogenic mycobacterium and is niacin positive. *M. simiae* infections have been found in various regions such as the southern United States, Israel, and Cuba [6-10]. The infections are most commonly found in immunosuppressed hosts such as HIV-positive individuals, the elderly, children, and those with a history of tuberculosis (TB) [6,11-13]. Furthermore, those with other diagnosed diseases such as diabetes mellitus, cardiovascular disease, and malignancy are more prone to acquiring *M. simiae* infection [11].

M. simiae can cause infections in diverse organs, especially in the respiratory system, causing variable

clinical manifestations. A recent study indicated that individuals of certain ethnic origins, particularly those from the Middle East region, seemed to be more prone to contracting the disease [11].Only a few cases of *M. simiae* infections have been documented, and there is only one established case report of *M. simiae* in Iran [14].

In this particular study, we aimed to determine the clinical and radiological manifestations, as well as the treatment outcome, of patients with *M. simiae* who presented to our referral center in Iran.

Methodology

Setting

This study was conducted in Masih Daneshvari Hospital, Tehran, Iran, which is the national referral center for tuberculosis and possesses the national reference mycobacteriologic laboratory. Most patients who saw no improvement in their symptoms (such as cough, sputum, fever, weight loss, dyspnea and anorexia) after TB treatment, or the conversion of sputum smear or culture at the end of TB treatment did not happen, were referred to our center from peripheral health centers around the country, with several new cases presenting directly to our institution.

Patient recruitment

All patients presenting to our center from 2002 to 2009 with confirmed infection with *M. simiae* were included in the study. Sputum smear and culture were performed for all patients. Three cultures were considered for every patient for controlling and confirmation of *M. simiae*.

Identification using Bergey's Manual of Systemic Bacteriology

Sputum inocula produced up to 120 colonies on Lowenstein-Jensen (LJ) slants after incubation for 4 to 8 weeks**.** Samples which tested negative using polymerase chain-reaction for IS6110 sequences and spoligotyping, both indicating presence of *M. tuberculosis*, were studied further. Bergey's Manual of Systemic Bacteriology [15,16] was used to determine that the specimen in question was atypical *Mycobacterium*. Conventional biochemical tests for photo induction, niacin production, nitrate reduction, catalase (heat stable and semiquantitative), Tween (Roche, Mannheim, Germany) hydrolysis (for a period of 10 days) and urease (Murphy-Hawkins disk

method, Merck, Germany) tests were performed [17]. Controls comprising standard negative atypical mycobacterial specimens, obtained from both the Department of Mycobacteriology at the National Institute of Public Health and the Environment of the Netherlands were used. The specimens were also grown on LJ medium at the temperatures of 25°C, 37°C, 40°C, and 45°C.

Species identification by PCR

Bacterial DNA was prepared according to van Soolingen *et al.* [18]. A segment of the 65-kDa heat shock protein gene (*hsp65*) was amplified by specific primers and digested using two restriction enzymes (*Bst*E II and *Hae*III) as described by Telenti *et al.* [19]. Band sizes were estimated and compared to mycobacterial reference strains provided by the American Type Culture Collection or the Collection of the Italian Reference Laboratory for Mycobacteria [20]. Clinical isolates from the Mycobacteriology Laboratory of Verona Hospital were also used [20].

Further testing

Drug susceptibility testing (DST) was performed on all *M. simiae* cultures. Culture identification by PCR was done for patients whose infections were resistant to all first-line drugs. Tuberculin skin test (TST) was done with pure protein derivation (PPD), and indurations more than 10 mm were considered positive. Furthermore, all patients underwent high resolution computed scan (HRCT).

Results

Twenty-six cases of *M. simiae* infection were identified from among 1,850 patients who were admitted to our centre with suspected tuberculosis between 2002 and 2009. Diagnosis of NTM was based on clinical and microbiological criteria as stated in American Thoracic Society (ATS) guidelines [20].Thirteen patients were female (50%), and all but one were Iranian (96%). The mean age of the patients was 58.23 ± 16.9years. Only one patient was HIV positive (4%).Twenty-one patients (81%) had a history of pulmonary illness and treatment, whereas five patients (19%) were newly presenting cases. Twelve patients (46%) had concomitant disease. The tuberculin skin test was positive in 20 patients (76.9%).

Table 1. Characteristics and demographic factors of the patients

		Number of patients	Percent value
Age		58.23 ± 16.9yr	
Sex	Male	13	50
	Female	13	50
Smoking	Yes	9	34.6
	No	17	65.4
Opium addiction	Yes	5	19.2
	No	21	80.8
History of TB treatment	Yes	21	80.8
	No	5	19.2
Previous TB Regimen			
	New	5	19.2
	Cat I	9	34.6
	Cat II	8	30.8
	Irregular	4	15.4
Symptoms			
	Cough	24	92.3
	Sputum	19	73.1
	Fever	22	84.6
	Perspiration	17	65.4
	Dyspnea	17	65.4
	Chest pain	7	26.9
	Weight loss	20	76.9
	Loss of appetite	11	42.3
TST	Positive	20	76.9
	Negative	6	23.1
Co-disease			
	No Disease	14	53.8
	Diabetes	2	7.7
	HIV	1	3.8
	CRF	2	7.7
	Heart disease	1	3.8
	Pulmonary disease	4	15.4
	Others	2	7.7

Cat I (standard regimen) includes: Isoniazid (H), Rifampin (R), Pyrazinamide (Z), and Ethambutol (E) for 2 months/HR for 4 months [2HREZ/4HR]
Cat II: 2 HRZES (Streptomycin)/ 1 HREZ/ 5 HRE
Irregular regimen: The patients used Cat I or II irregularly
HIV: Human Immunosuppressive Virus, CRF: Chronic Renal Failure, Pulmonary disease includes: Asthma(1), Bronchiectasis (1), Corpulmonel(2)

Demographic factors and characteristics factors are summarized in Table 1. Additionally, the clinical manifestations were determined. The most frequent symptom was coughing (92.3%). All patients had at least one side nodular lesion in HRCT (Table 2).

Drug susceptibility testing was also performed on strains isolated from sputum using all first-line anti-TB agents. Interestingly, strains from all patients (100%) were resistant to all first-line drugs (isoniazid, rifampin, ethambutol, pyrazinamide, and streptomycin).

All patients with confirmed *M. simiae* infection were treated according to the ATS recommendations on NTM treatment, with a regimen consisting of clarithromycin, ofloxacin, and co-trimoxazole. This regimen was continued for twelve months after culture conversion. After administering the treatment, 24 patients were cured and two patients failed the treatment. There was no recurrence of the disease in any of the patients during two years of follow-up.

Discussion

After laboratory examination, it was determined that 26 patients were infected with *M. simiae*. These individuals were equally females and males (50%), between the ages of 17 and 84, and had a mean age of 58.23 (± 16.9 years). As discussed by Shitrit *et al.*, infection with *M. simiae* is predominantly seen in individuals aged 19 to 87 with a mean age of 44 years [11].

Table 2. High resolution computed scan (HRCT) of *M. simiae* infected patients

HRCT Results		Number of patients	Percent value
Nodules	unilateral	4	15.4
	bilateral	22	84.6
Cavity	none	3	11.5
	unilateral	13	50
	bilateral	10	38.5
Bronchiectasis	none	4	15.4
	focal	9	34.6
	multi-focal	13	50

The ATS, on the other hand, reported a mean age of 57 years [21], and their figures closely match the findings in our patients.

In our study, patients infected with *M. simiae* had either been previously diagnosed as being infected with multi-drug resistant tuberculosis (MDR-TB), had received other types of TB treatments, or were new TB cases. Most of our patients were referred to our center after having received prior treatment against TB. Also, all the cases were resistant to all first-line anti-TB agents. As previously established, this reveals that NTMs such as *M. simiae* should be sought meticulously in patients who fail standard anti-TB regimens, and those who are suspected as being infected with MDR-TB [22].

It has also been reported that patients with clinically significant pulmonary diseases are middle-aged or older (55 to 80 years), and either have a history of TB or pre-existing lung abnormalities [3] are prone to *M. simiae* infection. A study by Maoz *et al.* showed that underlying conditions or diseases such as smoking, diabetes mellitus (DM), ischemic heart disease (IHD), solid and hematologic malignancies, and chronic obstructive pulmonary disease (COPD) were all associated with *M. simiae* infection [23]. With regard to the medical histories of the patients in this study, it is noteworthy that all but one were HIV-negative. Although the HIV epidemic is suggested as one of the causes for increased NTM infection, Maoz *et al.* showed that all their 102 patients with *M. simiae* were HIV negative and HIV infection rates were thus significantly lower among *M. simiae* patients compared to *M. tuberculosis* patients [23]. These results may show that, while other NTMs may be suspected in HIV-positive patients, a clinical suspicion for *M. simiae* infection should also be considered more commonly in non-HIV cases.

Our radiological findings showed that nodular lesion (100%), cavitation (88.5%), and bronchiectasis (84.6%) are the most common radiographic patterns in our patients. Likewise, in other studies, upper lobe involvement, nodular lesions, and cavitations have been described as the most common radiographic findings [24, 9]. According to Shitrit *et al.*, 38% of *M. simiae* patients in their setting had normal chest radiographs. Their study also states a higher occurrence of infection in the upper and middle lobes [11].

Symptoms of the infection that are commonly reported in the literature include sweating, low-grade fever and weight loss, with coughing, hemoptysis, and sputum production [11] less frequently reported. These statements are consistent with symptoms described by ATS for respiratory NTM infections [21]. In our study, fever and weight loss were common symptoms along with sputum expectoration, dyspnea, and loss of appetite. In addition, almost all our patients suffered from coughing. This is in contrast to the findings by Maoz *et al.*; in their study, only 17% of the patients had a cough and this symptom was significantly higher in tuberculosis patients compared to *M. simiae* cases [23].

Our study bears some limitations as well. This study was conducted at Masih Daneshvari Hospital which is a referral center for tuberculosis, meaning that only a subpopulation of *M. simiae* patients presented to our center, mostly those suffering from more serious conditions. There may have been more *M. simiae* cases that were not referred to our center. Furthermore, it is possible for NTMs to infect patients following a previous infection with *M. tuberculosis*. Therefore, it is difficult to determine whether the patient was primarily infected with *M. simiae* or if the present manifestations were the result of *M. simiae* super-infection on the previous tuberculosis.

Therapy of *M. simiae* pulmonary infection remains an issue. Similar to many other NTMs, most of the isolates are resistant to all first-line anti-mycobacterial drugs and their response to chemotherapy is variable and is mainly considered as poor [3]. Furthermore, to the best of our knowledge, there have not been any studies on the genotype of *M. simiae* isolates [11]. The patients in our study received a treatment of clarithromycin, ofloxacin, and co-trimoxazole. It is suggested that a three-drug regimen of clarithromycin, moxifloxacin and co-trimoxazole should be administered, as this is known to be successful [3]. Moxifloxacin is not available in Iran. We therefore had to replace moxifloxacin with ofloxacin. We treated our patients according to the American Thoracic Society (ATS) guidelines [21]. Nonetheless, after 24 months follow-up, they all recovered with no recurrence of tuberculosis or other lung disease.

Conclusion

M. simiae may present with clinical and radiological manifestations consistent with tuberculosis. In most cases, *M. simiae* is resistant to first-line anti-TB medications and therefore anti-TB medication will lead to treatment failure. *M. simiae*, in contrast to many other NTMs, is frequently seen in HIV-negative patients. Therefore, in cases where a patient fails standard anti-TB treatment, even if HIV-negative, NTMs such as *M. simiae* should be suspected and necessary evaluations should be undertaken.

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Conflict of interests: No conflict of interests is declared.