Tuberculosis Treatment Failure Due to Multiple-Strain *Mycobacterium tuberculosis* Infection – A Case Report

You-Cheng Chang, Ruwen Jou*, Shun-Tien Chien, Ying-Hsun Wu, Ruay-Ming Huang

Treatment failure in a tuberculosis patient has been defined as continued or recurrent positive cultures after 4 months of treatment. The common reasons for this are the poor compliance of the patient or an inappropriate regimen of anti-tuberculosis drugs. Multiple-strain *Mycobacterium tuberculosis* infection with different drug susceptibility results is another reason that has seldom been considered in the past. The development of genotyping methods, such as restriction fragment length polymorphism, has considerably improved the ability to distinguish *M. tuberculosis*. ***(Thorac Med 2010; 25: 155-160)***

Key words: tuberculosis, *Mycobacterium tuberculosis*, multiple-strain *Mycobacterium tuberculosis* infection, restriction fragment length polymorphism

Introduction

Tuberculosis (TB) can develop through progression of a recently acquired infection (primary disease), reactivation of a latent infection, or exogenous re-infection. In the past, it was estimated that approximately 90% of adult cases of TB were the result of endogenous reactivation of latent infection. More recently, with the aid of molecular genotyping techniques, it was found that approximately 60% to 70%, and 30% to 40% of TB cases, respectively, were reactivation and recent infection [1]. Multiple-strain *Mycobacterium tuberculosis* infection can also be identified by using molecular biological methods. We herein describe a case of pulmonary TB treatment failure due to a multiple-strain infection.

Case Report

A 33-year-old female diabetes mellitus patient under insulin control for 5 years visited the Chest Hospital, Department of Health, Executive Yuan on 16 February 2005 with the chief complaint of cough and generalized malaise for 2 months. Chest radiography (CXR) demonstrated right upper lung consolidation with cavity and satellite lesions, and left upper lung infiltration (Figure 1). Sputum acid-fast stain was positive, and anti-TB drugs, including ethambutol and rifater (containing isoniazid, rifampin, and pyrazinamide) were prescribed at that time. Mycobacterial culture revealed a

Chest Hospital, Department of Health, Executive Yuan; * Reference Laboratory of Mycobacteriology, Research and Diagnostics Center, Center for Disease Control, Department of Health
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positive *M. tuberculosis* complex that showed sensitivity to first-line anti-TB drugs in conventional 7H10 agar proportion drug susceptibility testing (DST). The patient's symptoms improved after anti-TB treatment. Sputum conversion was noted after 15 June 2005. The anti-TB regimen was shifted to ethambutol and rifinah (containing isoniazid and rifampin) after 1 June 2005. Follow-up CXR showed improvement on 9 December 2005 (Figure 2). Since the sputum mycobacterial culture was still positive after a 2-month treatment, we extended the treatment duration to at least 9 months.

Unfortunately, she suffered from hemoptysis in February 2006. CXR demonstrated increased right upper lung infiltration (Figure 3), and the sputum acid-fast stain was positive on 23 February 2006. Under the impression of treatment failure, she was admitted to our hospital. First and second-line anti-TB drugs, including ethambutol, rifater, kanamycin, levofloxacin, para-amino-salicylic acid (PAS), prothionamide, and cycloserine, were prescribed. The mycobacterial cultures remained *M. tuberculosis* complex-positive throughout February 2006. Cultures from sputum collected on 23 and 24 February 2006 were both resistant to isoniazide, rifampin, and streptomycin. However, the culture of the sputum sample on 26 February 2006 was *M. tuberculosis* complex and was resistant to isoniazide, rifampin, ethambutol,

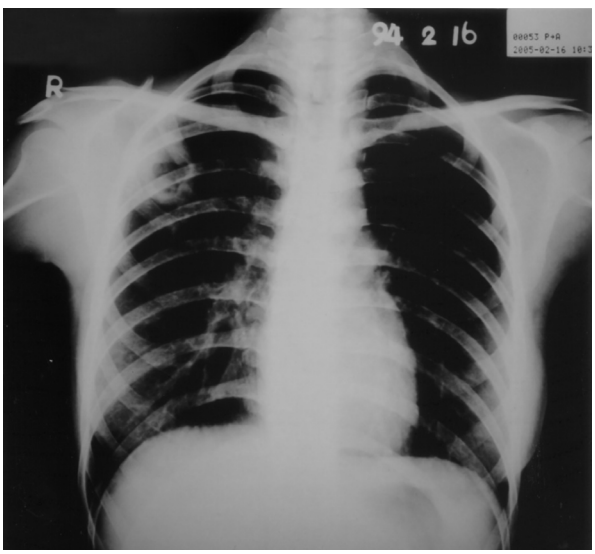

Fig. 1. Posteroanterior chest radiograph shows right upper lung consolidation with cavity and satellite lesions, and left upper lung infiltration.

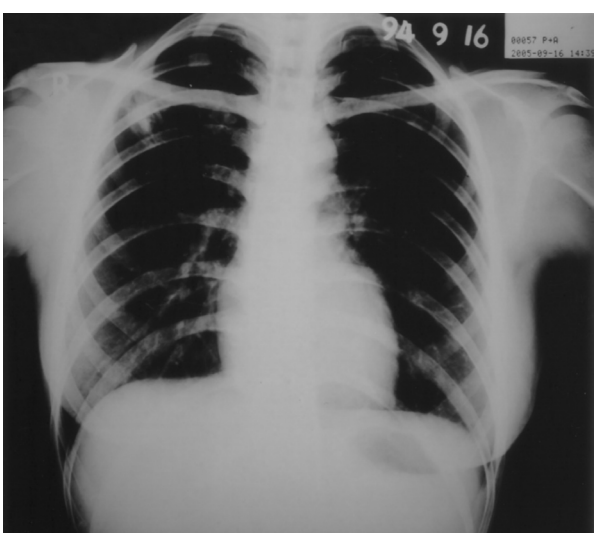

Fig. 2. Posteroanterior chest radiograph shows right upper lung fibrocalcified lesion and left upper lung fibrosis change.

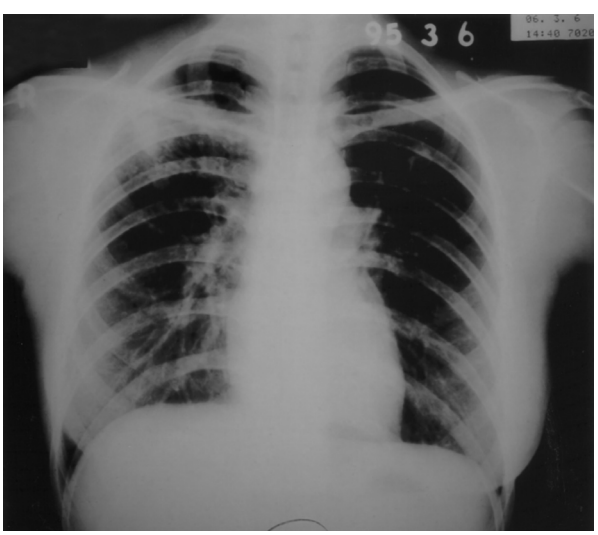

Fig. 3. Posteroanterior chest radiograph shows increased right upper lung infiltration.

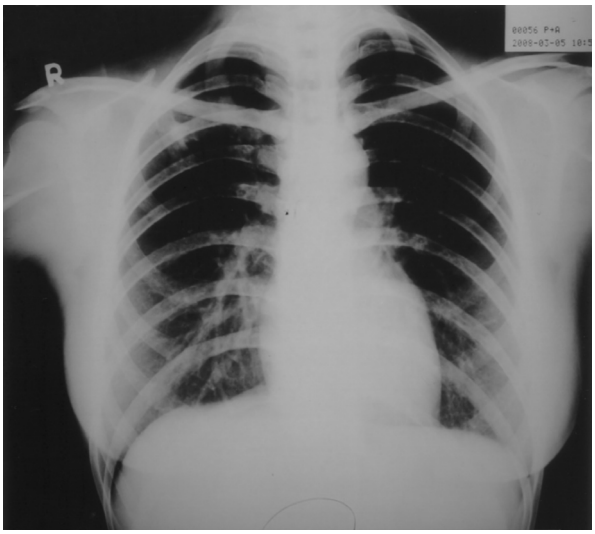

Fig. 4. Posteroanterior chest radiograph shows right upper lung fibrocalcified lesion and left upper lung fibrosis change.

and streptomycin. The sputum mycobacterial culture converted to negative after May 2006. Pulmonary TB treatment was completed in March 2008, and the follow-up CXR showed improvement (Figure 4). The treatment course, including regimens and sputum smear with culture results, is listed in Table 1.

The 4 isolates were sent for genotyping using the restriction fragment length polymorphism (RFLP) method. The RFLP pattern of the first isolate cultured on 16 February 2005 was different from that of the other 3 cultures in 2006. The RFLP results were compatible with DST profiles and the clinical course (Figure 5). Multiple-strain *M. tuberculosis* infection was confirmed in the patient.

Discussion

For years, there has been much discussion and many opinions about the relative importance of exogenous re-infection and endogenous reactivation in the development of clinical TB disease following the initial infection with *M. tuberculosis* [2]. Patients with TB have often been assumed to be infected with single *M. tuberculosis*, and infection with 1 strain was thought to confer immunity to additional *M. tuberculosis* infection [3]. Therefore, recurrence of disease after treatment is often considered to be caused by the same strain that caused the initial infection. Mankiewicz and Liivak

Table 1. Clinical course of anti-TB treatment included anti-TB drugs, and sputum smears with culture results. INH = isoniazid, RIF = rifampin, EMB = ethambutol, PZA = pyrazinamide, KM = kanamycin, LVX = levofloxacin, TBN = prothionamide, CS = cycloserine, PAS = para-aminosalicylic acid.

date	year	2005											2006											2007											2008		
	month	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3
sputum	smear	+	+	+	+	−		−	−		−		−	+	+	−	−	−		−		−	−		−	−			−		−	−		−	−		−	−	
	culture	+	+	+	+	−		−	−		−		−	+	+	−	−	−		−		−	−		−	−			−		−	−		−	−		−	−	
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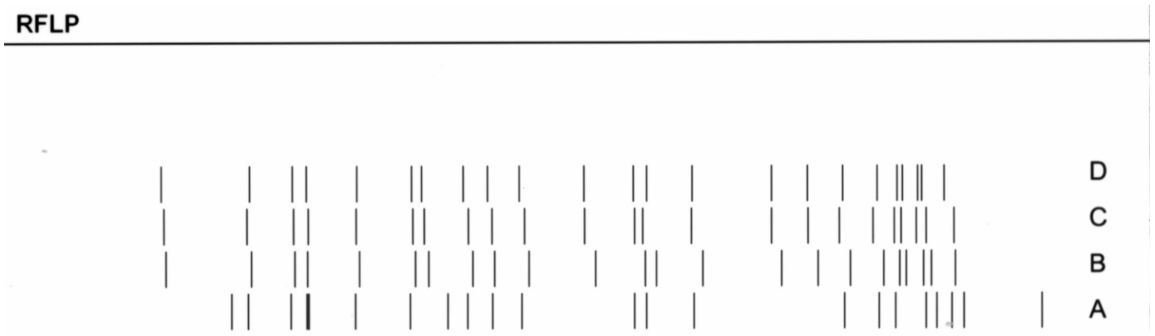

Fig. 5. Specimen A was cultured on 16 February 2005. Specimen B was cultured on 23 February 2006. Specimen C was cultured on 24 February 2006. Specimen D was cultured on 26 February 2006. The RFLP pattern of the first specimen which was cultured on 16 February 2005 was different from the other 3 sets of specimens which were cultured from 23 February 2006 to 26 February 2006.

used phage typing to analyze the heterogeneity among individual colonies obtained from cultures of specimens isolated from 233 Eskimo patients, leading to the conclusion that 14.1% of patients tested were simultaneously infected with more than 1 strain of *M. tuberculosis* [4]. The development of molecular biological methods, including RFLP, has considerably improved the capacity to distinguish *M. tuberculosis* [5-6]. Multiple-strain *M. tuberculosis* infection with distinct DST results seriously confuses the prognosis of treatment [7-8]. Multiple infections in patients with TB have been described in the form of mixed or simultaneous infections with 2 or more different strains, or in the form of exogenous re-infection, in which an initial infection with a strain is followed by a second infection with a new strain. Mixed infections are defined as simultaneous infection by 2 or more *M. tuberculosis* strains, as evidenced by very distinct molecular biological methods. When we provide regular treatment for a TB patient but fail, multiple-strain infection should be considered [9]. Furthermore, it is possible that undetected drug-resistant strains may emerge under the pressure of initial antibiotics. Our findings suggested that antibiotics pressure with a standard first-line regimen might have led to a reduction in the growth of the drug-susceptible strain, and the selection of a previously undetected genetically distinct drug-resistant strain [10]. To understand the causes of TB treatment failure, we recommend performing conservative bacteriological examinations, including mycobacterial culture, DST, and genotyping to analyze the possibility of multiple-strain infection.

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結核病治療失敗導因於多重菌株感染：病例報告

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結核病治療失敗的定義是經治療四個月後，痰培養持續或再次陽性，常見的原因為病人醫囑順從性不好或處方的不適當。此外，多重結核菌株感染合併不同的藥物抗藥性，也可能是治療失敗的一種可能，過往這種情況較少被考慮到，但是伴隨著分子生物學的發展，使用基因分型方法（如限制酵素片段長度多形性），可以鑑別及釐清不同的結核菌感染導致的治療失敗。*(胸腔醫學* ***2010; 25: 155-160)***

關鍵詞：結核病，結核菌，多重結核菌株感染，限制酵素片段長度多形性

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