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Original Article

Effect of Type 2 Diabetes Mellitus on the Clinical Severity and Treatment Outcome in Patients With Pulmonary Tuberculosis: A Potential Role in the Emergence of Multidrug-resistance

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Background/Purpose: A globally increasing trend of type 2 diabetes mellitus (DM), the rising prevalence of tuberculosis (TB) in many countries, and the emergence of multidrug-resistant TB (MDR-TB) in recent years pose a serious challenge for TB control.

Methods: We investigated pulmonary tuberculosis patients with and without type 2 DM (DMTB and TB, respectively) treated at the Chest Hospital, Taiwan, between November 2004 and October 2005.

Results: One hundred and ninety-two new patients (60 DMTB, 132 TB) were regularly treated for a full course (≥ 6 months) and prospectively followed for more than 1 year. The DMTB patients had more severe infections (far-advanced: 45.0% *vs.* 22.7%, $p < 0.01$), higher mycobacterial loads (sputum smear: $2.9 \pm 1.3^{+}$ *vs.* $1.9 \pm 1.7^{+}$, $p < 0.01$), higher treatment failure rates (17% *vs.* 2%, $p < 0.01$), and longer delayed clearance of mycobacteria than did the TB patients (2.5 ± 3.0 months *vs.* 1.6 ± 1.4 months, $p < 0.01$). After one year, three DMTB patients and one TB patient had MDR-TB (5.0% *vs.* 0.8%, $p = 0.056$). Bacterial genotyping revealed that the proportion of mycobacterial strains was not significantly different in DMTB and TB patients (Beijing strain: 46.7% *vs.* 40.6%, Non-Beijing strain: 53.3% *vs.* 59.4%, $p = 0.632$).

Conclusion: DMTB patients have more severe TB infections, which require longer treatment and are more likely to develop MDR-TB than are patients with TB alone.

Key Words: Beijing strain, diabetes mellitus, multidrug-resistant tuberculosis, pulmonary tuberculosis, spoligotype analysis

The prevalence of tuberculosis (TB) has been rising globally in recent years, with an estimated annual incidence of new TB cases at around nine million and deaths at approximately two million each year.[1–3] The emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) poses a serious challenge for future TB control.[4,5] More than half the TB cases occur in Asian

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and African countries where medical resources tend to be limited and the prevalence of MDR-TB is alarmingly high, which makes the situation even more difficult.[1,6,7]

Diabetes mellitus (DM) has been a well-known risk factor for TB in the past.[8,9] However, the influence of DM on TB was largely ignored during the second half of the past century, after effective treatments for both diseases became available.[10] The situation has changed recently with the global increase in the prevalence of type 2 DM, which has penetrated developing countries.[11,12] The global convergence of the accelerating type 2 DM pandemic, high TB prevalence, and drug-resistant TB during the past couple of decades has become a serious challenge to clinicians worldwide.

Over the past few years, some studies have shown that the treatment failure rate is higher in TB patients complicated with DM. Moreover, there is a significant association between DM and MDR-TB.[13,14] However, there were controversies regarding clinical manifestations and treatment outcomes in diabetic patients among different studies.[15–17] In this study, we prospectively followed-up 192 TB and DMTB patients to determine the influence of type 2 DM on the clinical manifestations and treatment outcomes of pulmonary TB patients.

Patients and Methods

Patients

From November 2004 to October 2005, all pulmonary TB patients treated at the Chest Hospital, a central referral hospital for TB patients in Taiwan, were investigated and classified as having pulmonary TB with (DMTB) or without (TB) type 2 DM. Pulmonary TB was diagnosed using clinical symptoms and chest radiographs, and confirmed using a sputum TB culture with drug-susceptibility testing in each patient. A diagnosis of type 2 DM was confirmed when fasting plasma glucose concentration was > 126 mg/dL at two different time points. The regimen for treating diabetes was determined based on each patient's specific circumstances. Cultures for *Mycobacterium tuberculosis* were performed using a kit (Mycobacteria Growth Indicator Tube (MGIT) 960; Becton Dickinson Diagnostic systems, Sparks, MD, USA) and Lowenstein-Jensen medium. Drug-susceptibility testing was done using proportional methods. Patients confirmed as HIV^+ were excluded. MDR-TB was defined as pulmonary TB that was resistant to two or more TB medications, including at least isoniazid and rifampicin.

The age, sex, diabetic status, hemoglobin A1c (HbA1c) before treatment, status of organ failure [including heart failure (defined as Class III-IV of New York Heart Association Functional Classification)], respiratory failure (defined as dyspnea with $PaO_2 < 50$ mmHg while breathing air, or a $PaCO_2 > 50$ mmHg), and renal failure (defined as established end-stage kidney failure for > 3 months, or urine output < 0.3 mL/kg/hr for more than 24 hr or anuria for more than 12 hr), leukocyte count, severity of pulmonary TB on chest radiograph, sputum-smear acid-fast bacilli (AFB), mycobacterial load, and body mass index (BMI) of each patient were recorded during the diagnostic workup and the beginning of treatment. Modified grading of the severity of pulmonary TB,[18] according to the extent and type of chest radiograph findings, divided the patients into three groups: mild (a single lobe involved); moderately-advanced (unilateral involvement of two or more lobes with cavities, if present, reaching a total diameter no greater than 4 cm); and far-advanced (bilateral disease with massive involvement and multiple cavities). The grading was checked by two chest physicians. A Ziehl-Neelsen-stained sputum smear was performed for microscopic examination, and the mycobacterial load was graded as +, ++, +++, or ++++.[19]

All TB and DMTB patients were treated with the same recommended regimen: isoniazid, rifampicin, pyrazinamide, and ethambutol in the 2-month intensive phase, and then isoniazid, rifampicin, and ethambutol in the 4-month continuation phase, if the regime could be tolerated. Because the national project of directly observed treatment short-course (DOTS) in Taiwan had not

been implemented before 2006, the treatment was based on self-administration. Patients were prospectively followed up for at least 1 year. During the follow-up, the period needed for sputum conversion was documented based on the results of monthly mycobacterial sputum cultures. The treatment outcome was divided into six categories according to modified WHO guidelines[20]: cured (finished treatment with negative bacteriology result at the end of treatment), completed treatment (finished treatment, but without bacteriology result at the end of treatment), failure (remaining culture positive at 5 months or sputum AFB positive at 6 months despite correct intake of medication), defaulted treatment (patients who interrupted their treatment for two consecutive months or more after registration), died (patients who died due to TB or other causes before or during treatment), and transferred out (patients whose treatment results were unknown due to emigration before or during treatment). For treatment-failure patients, the subsequent regime was tailored individually according to clinical conditions and drug-susceptibility tests. A regime containing at least four effective drugs, including second-line anti-tuberculosis agents, was given for 18 to 24 months in accordance with the WHO guideline of MDR-TB.[21] Only patients who followed the treatment protocol were included for analysis. The study was approved by the Human Experiment and Ethics Committee of National Cheng-Kung University Hospital. Written informed consent was obtained from all patients.

Mycobacterial strain genotyping

To compare the differences between mycobacterial strains in the DMTB and TB groups, 62 (30 DMTB, 32 TB) randomly collected isolates of *Mycobacterium tuberculosis* were genotyped. Spoligotyping was done according to the manufacturer's instructions (Isogen Bioscience B.V., Maarsen, Holland). The resulting spoligotypes were documented using a binary code representing either a positive or negative hybridization result and analyzed using Microsoft Excel to group and order the patterns.[22]

Statistical analysis

Data are expressed as percentages for categorical factors and as means ± standard deviations for continuous factors. Comparisons between the DMTB and TB groups used the χ^2 or Student t test, as appropriate. Cumulative incidences of persistent positive sputum mycobacterial cultures over time were analyzed using Kaplan-Meier product limit estimates, and comparisons between groups was performed using the log rank test. Potential predictors for treatment failure at 6 months were evaluated using logistic regression. Statistical significance was set at $p < 0.05$, two-sided. Data were analyzed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) and STATA version 8.0 (StataCorp, College Station, TX, USA).

Results

Clinical manifestations

From November 2004 to October 2005, 438 patients with culture-proven pulmonary TB (including newly diagnosed and previously treated patients) were diagnosed and treated at the Chest Hospital, Tainan, Taiwan. Among them, 129 (29.5%) patients had DM and 309 (70.5%) had TB alone. There were significantly more male patients in both groups. The mean age was similar in both groups. There were 67 (15.3%) multidrug-resistant tuberculosis (MDR-TB) patients in these 438 cases. A higher percentage of patients in the DMTB group, than in the TB group had MDR-TB. Except for gender, none of the other characteristics was significantly different (Table 1).

Because some of these 438 patients had been previously treated in other hospitals before being recruited in this study, we prospectively recruited newly diagnosed patients who were treated for the first time and completed a treatment course (at least 6 months), and were regularly followed-up for a full year in our hospital. Patients who interrupted their treatment for two or more consecutive months, died during treatment, or were transferred out after registration were excluded from further analysis. Thus, we excluded 18 DMTB (12 defaulted

Table 1. Characteristics of 438 patients with culture-positive pulmonary tuberculosis

Characteristics	DMTB ($n=129$)	TB ($n=309$)	p
Sex, men (%)	84	71	0.006
Mean age ± SD (yr)	57.9 ± 12.8	57.2 ± 18.8	0.701
Previously treated cases, *n* (%)	51 (39.5)	118 (38.2)	0.792
MDR-TB, *n* (%)	26 (20.2)	41 (13.3)	0.068
Newly diagnosed cases, *n* (%)	78 (60.5)	191 (61.8)	0.792
MDR-TB, *n* (%)	0	0	

SD = standard deviation; MDR-TB = multidrug-resistant TB; DMTB = pulmonary tuberculosis patient comorbid with type 2 diabetes mellitus; TB = pulmonary tuberculosis patient not comorbid with type 2 diabetes mellitus.

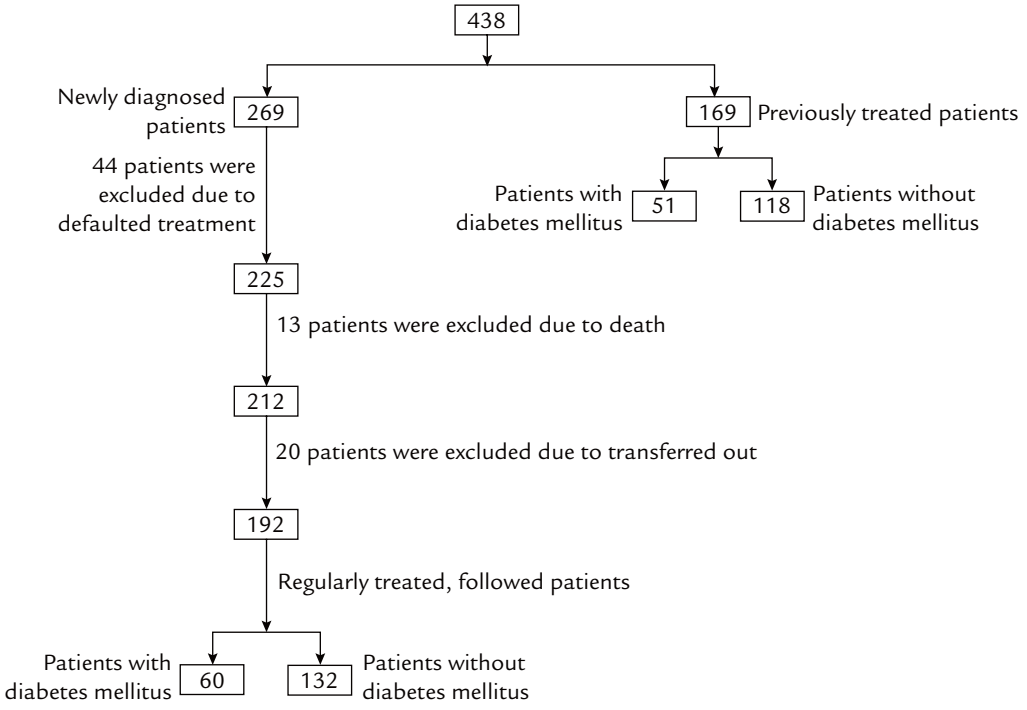

Figure 1. Initially, 438 culture-proven pulmonary tuberculosis (TB) patients were diagnosed during the study period. Among them, 169 patients had received TB treatment before the study. In 269 newly diagnosed TB patients, only 192 patients were enrolled and analyzed after finally excluding defaulted, deceased, or transferred out patients.

treatment, 4 died, and 2 transferred out) and 59 TB (32 defaulted treatment, 9 died, and 18 transferred out) patients. In all, 192 new patients (60 DMTB; 132 TB) met all the criteria and were included for further analysis (Figure 1). Two DMTB patients and nine TB patients associated with pleural effusion during the diagnosis of pulmonary TB (3.3% *vs.* 6.8%, $p=0.336$). No other extrapulmonary TB infection was found.

In the DMTB group, the mean duration of diabetes was 5.0 ± 5.1 years, and the mean initial HbA1c at the time of TB diagnosis was 10.0 ± 2.6% (based on 42 patients with concurrent results). Among the DMTB patients, 52 patients were diagnosed as DM before the TB diagnosis and eight patients were diagnosed at the time when the diagnosis of TB was made. Fifty-six patients (93%) were treated with oral hypoglycemic agents, insulin injection, or both; the remaining four patients received diet control only. The ages of the DMTB and TB patients were comparable. As in the original group, there were significantly more men than women in both subgroups, especially DMTB patients (Table 2). One patient with DM

Table 2. Characteristics of 192 patients with newly diagnosed and regularly treated pulmonary tuberculosis

Characteristics	DMTB ($n=60$)	TB ($n=132$)	p
Sex, men (%)	82	67	0.042
Mean age ± SD (yr)	56.6 ± 12.7	57.5 ± 20.7	0.782
BMI[a] (kg/m^2)	21.8 ± 3.2	20.5 ± 3.3	0.022
Leukocyte count[b] ($\times 10^3$/mL)	9.4 ± 4.1	8.0 ± 2.8	0.008
Chest radiograph severity			< 0.01
Far-advanced	27/60	30/132	
Moderately-advanced	28/60	80/132	
Mild	5/60	22/132	
Cavitary lesion, *n* (%)	45/60 (75)	63/132 (48)	< 0.01
Sputum AFB positive, *n* (%)	53/60 (88)	78/132 (59)	< 0.01
Mycobacterial load (+)[c]	2.9 ± 1.3	1.9 ± 1.7	< 0.01
Primary drug resistance, *n* (%)	10/60 (17)	17/132 (13)	0.484
Isoniazid resistance	10	17	
Rifampicin resistance	0	0	
Ethambutol resistance	0	0	

[a]Only 50 DMTB and 97 TB patients were calculated; [b]only 57 DMTB and 123 TB patients were calculated; [c]the mycobacterial load was graded as +, ++, +++, or ++++, according to the grading of the CDC-USA. DMTB = pulmonary tuberculosis patient comorbid with type 2 diabetes mellitus; TB = pulmonary tuberculosis patient not comorbid with type 2 diabetes mellitus. SD = standard deviation; MDR-TB = multidrug-resistant tuberculosis; BMI = Body mass index; AFB = acid-fast bacilli.

and two without had established end-stage kidney failure for >3 months. The average body mass index (BMI) was higher in the DMTB group (Table 2). The DMTB patients had higher leukocyte counts, more severe chest radiograph manifestations, a higher proportion of cavitary lesions and positive sputum AFB, and heavier mycobacterial loads (Table 2). All these data indicated that DMTB patients had more severe infections at the time of diagnosis. Before treatment, there was no significant difference in the proportion of primary first-line drug resistance between the two groups (Table 2).

Treatment outcomes

After treatment, 50 (83%) DMTB and 119 (90%) TB patients had been cured or completed treatment within one year. However, DMTB patients needed significantly longer treatment to be cured, to complete treatment, and to achieve sputum conversion (Table 3). After the first 6 months of treatment, 10 DMTB and two TB patients who could not be treated successfully were classified as bacteriological treatment failures. They needed regimen adjustments and could not complete the treatment within one year. Among them, eight DMTB and two TB patients had positive mycobacterial culture results during the 5th month of treatment. The remaining two DMTB patients had positive AFB results on their sputum smears in the 6th month of treatment. Additional investigation of those patients with bacteriological treatment failure at 6 months showed that three DMTB patients and one TB patient had developed MDR-TB 1 year after starting treatment. These four patients were the only ones who had still not achieved sputum conversion after 1 year of treatment. The initial mean HbA1c of these eight DMTB patients was 10.3 ± 3.6% and it was 8.6 ± 2.4% after a treatment course. Meanwhile, the initial mean HbA1c of those three DMTB patients who developed MDR-TB was 11.9 ± 2.1% and it was 9.3 ± 3.8% after a treatment course. Another 11 patients in the TB group needed treatment for more than 1 year because of the adverse effects of anti-tuberculosis treatment, rather than the failure

Table 3. One-year outcomes of patients with newly diagnosed and regularly treated pulmonary tuberculosis

	DMTB ($n=60$)	TB ($n=132$)	p
Cured and completed treatment[a], *n* (%)	50/60 (83)	119/132 (90)	0.177
Duration of treatment[b] (mo)	9.9 ± 1.8 ($n=50$)	7.5 ± 1.8 ($n=119$)	<0.01
Treatment failure (at 5–6 mo), *n* (%)	10/60 (17)	2/132 (2)	<0.01
culture/smear positive	8/2	2/0	
Sputum conversion (mo)	2.5 ± 3.0	1.6 ± 1.4	<0.01
MDR-TB, *n* (%)	3/60 (5.0)	1/132 (0.8)	0.056

[a]11 patients in TB group needed treatment after one year due to adverse effect of anti-TB drugs; [b]only those who were cured and completed-treatment within one year were calculated. Cured = finished treatment with negative bacteriology result at the end of treatment; completed treatment = finished treatment, but without bacteriology result at the end of treatment; failure = remaining culture positive at 5 months or sputum acid-fast bacilli (AFB) positive at 6 months despite correct intake of medication; DMTB = pulmonary tuberculosis patient comorbid with type 2 diabetes mellitus; TB = pulmonary tuberculosis patient not comorbid with type 2 diabetes mellitus.

of bacteriological treatment. Although a regimen containing at least four effective drugs (including second-line anti-tuberculosis agents) had been given to MDR-TB patients, one DMTB patient with thick-wall cavitary lesions needed surgical resection to achieve sputum conversion after the 1-year period. A Kaplan-Meier curve of comparisons of the monthly mycobacterial culture results during follow-up showed that mycobacteria from DMTB patients had a higher probability of delayed clearance than those from TB patients (Figure 2, log-rank test, $p<0.01$). All the patients became sputum culture-negative 15 months after the initiation of anti-TB treatment. In univariate analysis for potential predictors, DM and chest radiograph severity were significantly associated with treatment failure at 6 months (Table 4). A multivariate logistic regression analysis on DM and chest radiograph severity revealed that DM is the major contributing factor for treatment failure (Table 4).

Using spoligotype analysis to genotype mycobacterial strains

Sixty-two mycobacterial samples from 30 DMTB and 32 TB patients, including the three samples from the DMTB patients who eventually developed MDR-TB, were analyzed (Table 5). The spoligotype genotyping data revealed that 44% were Beijing type, 13% were East-African-Indian (EAI) type, 13% were Haarlem (H) type, 6% were MANU type, 5% were T type, 3% were U type, and 16% were Orphan (unclassified) type. The results showed similar proportions of Beijing and non-Beijing types in DMTB and TB patients. Of the bacterial strains from the three DMTB patients who developed MDR-TB, two were Beijing type and one was Orphan type.

Discussion

Although type 2 DM has long been recognized as a risk factor of TB, its effects on the clinical picture and treatment response is still poorly understood. In this study, we first performed a one-year cross-sectional investigation of pulmonary TB patients. There was a higher percentage of MDR-TB patients in the DMTB group than in the TB group, which is in accordance with other studies.[14] We then focused our investigation on newly diagnosed patients who had never before been treated for TB. Our prospective study on these TB patients with and without DM provided a unique chance to observe the differences in disease characteristics and treatment response during the first year of treatment.

A recently published Indonesian study[23] reported that DMTB patients presented with more symptoms, but not more severe TB, which differs from results in the present study. It was previously reported[1,24] that TB prevalence is much lower in Taiwan than in Indonesia, and that the age distribution of TB is apparently different in these two countries. Our patients in both groups were significantly older than those in the Indonesian study, in which the TB-only group was much younger

Table 4. Analysis for predictors of treatment failure in patients with pulmonary tuberculosis

Characteristics	Treatment failure ($n=12$)	Non-treatment failure ($n=180$)	p
Sex, men (%)	75	72	0.804
Mean age ± SD (yr)	59.5 ± 15.8	57.1 ± 18.8	0.664
BMI[a] (kg/m^2)	20.0 ± 3.2	21.0 ± 3.3	0.297
Type 2 DM, *n* (%)	10 (83)	50 (28)	< 0.01
Diabetic status			
Duration (yr)	6.0 ± 6.0	4.7 ± 5.0	0.462
HbA1c[b] (%)	9.4 ± 3.8	10.2 ± 2.3	0.455
Treatment			
Diet control	1	3	
OHA and/or insulin	9	47	
Leukocyte count[c] (×10^3/mL)	8.0 ± 2.3	8.5 ± 3.4	0.584
Primary drug resistance, *n* (%)	3 (25)	24 (13)	0.260
Chest radiograph severity			
Far advanced	7	50	0.025
Moderately advanced	4	104	0.098
Mild	1	26	0.555
Cavitary lesion, *n* (%)	9 (75)	99 (55)	0.176
Sputum AFB positive, *n* (%)	10 (83)	121 (67)	0.246
Mycobacterial load (+)[d]	2.8 ± 1.6	2.2 ± 1.7	0.269

Multivariate regression analysis for DM and far-advanced CXR on treatment failure

Variable	Odds ratio	95% CI	p
DM	10.91	2.26–52.76	0.003
Far advanced CXR	2.29	0.65–8.03	0.195

[a]Only 12 treatment-failure and 135 non-treatment-failure patients were calculated; [b]only 8 treatment-failure and 34 non-treatment-failure patients were calculated; [c]only 12 treatment-failure and 168 non-treatment-failure patients were calculated; [d]mycobacterial load, graded as +, ++, +++, or ++++, according to the grading of CDC-USA. SD = standard deviation; DM = diabetes mellitus; HbA1c = hemoglobin A1c; BMI = Body mass index, measured by weight in kilograms divided by the square of height in meters; OHA = oral hypoglycemic agent; AFB = acid-fast bacilli. CXR = chest X-ray. CI = confidence interval.

than the DMTB group. Because age-dependent host-responses to mycobacteria are likely to cause different clinical manifestations via different immunopathogenic mechanisms,[25] the apparent difference in the ages of the TB and DMTB groups in the Indonesian study may make comparison between them difficult. Therefore, our study has the advantage of providing two groups of patients with comparable demographic backgrounds and better reflects the effects of diabetes on pulmonary TB. Although the percentage of DMTB patients in our study (60/192, 31.3%) was higher than that reported in other studies performed in other countries, similar numbers were reported in two recent Taiwanese studies[26,27] from a medical center and a regional hospital in southern Taiwan (74/217, 34.1% and 91/343, 26.5%). Thus, it is unlikely that the high DMTB/TB ratio in the present study reflects a referral bias. Moreover, because our groups of TB-only and DMTB patients showed similar distributions of bacterial strains and primary resistance rates, the clinical severity represented by the higher percentage of more severe chest lesions and mycobacterial loads can be more reasonably attributed to diabetes in the DMTB group. Our multivariate logistic regression analysis also indicated DM as a more important contributing factor of treatment failure.

We showed that there is a higher chance of TB bacilli persistence in the sputum in the DMTB

Table 5. Characteristics and spoligotypes of collected *Mycobacterium tuberculosis* isolates from 62 pulmonary tuberculosis[a]

Characteristics	DMTB ($n=30$)	TB ($n=32$)	p
Sex, men (%)	73	69	0.691
Mean age ± SD (yr)	55.4 ± 13.2	52.1 ± 19.3	0.438
Beijing, *n* (%)	14 (46.7)	13 (40.6)	0.632
Non-Beijing, *n* (%)	16 (53.3)	19 (59.4)	0.632
EAI	6 (20.0)	2 (6.3)	
MANU	4 (13.3)	0	
Haarlem	0	8 (25.0)	
T	0	3 (9.4)	
U	1 (3.3)	1 (3.1)	
Orphan	5 (16.7)	5 (15.6)	

[a]62 (30 DMTB and 32 TB) collected Mycobacterium tuberculosis isolates of pulmonary TB patients were analyzed using spoligotyping: Beijing lineages (44%); EAI (13%); and Haarlem lineages (13%). Ten isolates could not be classified (Orphan; 16%). SD = standard deviation; DMTB = pulmonary tuberculosis patient comorbid with type 2 DM; TB = pulmonary tuberculosis patient not comorbid with type 2 DM; EAI = East-African-Indian.

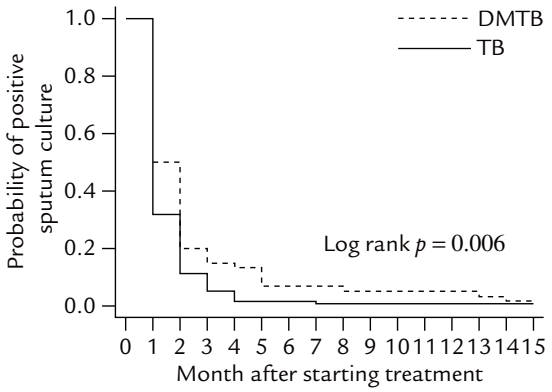

Figure 2. The probability of a positive sputum culture of *Mycobacterium tuberculosis* (TB) during anti-TB treatment [60 patients with pulmonary TB comorbid with type 2 DM (DMTB) and 132 pulmonary tuberculosis patients not comorbid with type 2 DM (TB) patients]. After the treatment had begun, mycobacterial culture results were collected and compared monthly. The percentage of patients positive for sputum TB cultures in the DMTB (--) and TB (—) groups are shown. The DMTB group shows delayed clearance of mycobacteria compared with the TB-only group ($p = 0.006$).

group than in the TB-only group after 5 months of treatment, and that this persistence made it necessary for more patients with diabetes to be treated for more than one year. DMTB patients needed a longer period for sputum conversion. Further statistical analysis confirmed that type 2 DM and the severity of TB were the only related factors of treatment failure in this study. Because we found more severe TB in our patients, we conclude that type 2 DM was the major cause of poor treatment outcomes in DMTB patients. Previous studies showed that a major mechanism for the emergence of drug resistance in TB bacilli is random mutation in the bacterial genome and the pressure of selection by anti-tuberculosis drugs. Pulmonary TB patients with higher mycobacterial loads at the initiation of treatment hence may have higher chance of bacillary mutation and the emergence of MDR-TB.[28] Given that our DMTB patients had higher bacterial loads and needed longer treatment to clear the bacteria, it hence should not be surprising that a higher chance of MDR-TB was observed in the DMTB patient group.

Although DOTS had not been implemented when this prospective follow-up study was performed, the high successful treatment rate in both groups and the conversion of sputum after intensive treatment in all treatment-failure and MDR-TB patients indicate that compliance should not be a significant factor for the different treatment outcomes in this study. Previous genotyping studies[29,30] of *Mycobacterium tuberculosis* showed that the major spoligotypes found in Taiwan include Beijing lineages (44.4–52.5%), Haarlem lineages (13.5%), and EAI plus EAI-like lineages (11%). An association between drug resistance and the Beijing genotype has been well documented.[31,32] As our spoligotype genotyping results in both the TB-only and DMTB groups is in line with previous reported distributions of mycobacterial strains in this country, our results further indicate that the difficulty in treatment and poorer outcome of DMTB patients should be attributed to different host factors in TB-only and DMTB patients rather than to different mycobacterial strains. However, there are several limitations in this study. Although we prospectively followed up the patients, our observations were based on a cross-sectional study design in a single referral center, which precludes

us from understanding the causal relationship between DM and severity of TB from the early stage of the infection. This may hamper the clarification of the role of DM in the early host response to TB, which may lead to major differences in chest X-ray severity and initial bacterial load that we clearly revealed in this study. We are currently working on animal models and *in vitro* leukocyte stimulation tests to directly address these questions. A long-term cohort study on subjects with or without DM for assessing the incidence of TB and MDR-TB is required to obtain definite answers to these questions.

Given the rapidly increasing number of patients with TB, it is apparently critical to investigate the mechanisms that lead to poor treatment outcomes in DMTB patients. A pharmacokinetic study noted that plasma levels of rifampicin were 53% lower in DMTB than TB patients, which might affect treatment outcomes.[33] Moreover, the immune response of the host may also be important in this negative effect of diabetes. Tsukaguchi et al[34] showed a depressed production of IFN-γ in DMTB patients and related this decrease in immune response to poor DM control. Our recent investigations (unpublished data) revealed a depressed IL-12 response to mycobacterial stimulation in leukocytes from DMTB patients and suggested a compromised innate immune reaction in these patients. Therefore, a compromised immune function related to diabetes should be considered an important contributor to the initial severity and poor treatment outcome in DMTB patients. Additional mechanistic studies are hence worthwhile to unravel the influence of type 2 DM on the host defense against TB.

In conclusion, this study showed that DMTB patients had more severe infections, higher mycobacterial loads, higher treatment failure rates, delayed clearance of mycobacteria, and potentially higher probabilities of developing MDR-TB than did TB-only patients. Our data suggest that pulmonary TB patients complicated with type 2 DM may need to be treated with a more intensive anti-TB regimen and be carefully followed-up for MDR-TB.

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