Int J Clin Exp Med 2019;12(6):7739-7745
www.ijcem.com /ISSN:1940-5901/IJCEM0079411

Original Article

Study on drug resistance and *Mycobacterium tuberculosis* L-form monitoring in diabetic patients with pulmonary tuberculosis

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Received April 25, 2018; Accepted June 8, 2018; Epub June 15, 2019; Published June 30, 2019

Abstract: Objective: The objective of this study was to investigate drug resistance in diabetic patients with pulmonary tuberculosis (TB) and to monitor cultivation of *Mycobacterium tuberculosis* L-form. Methods: Retrospective analysis was conducted in 62 sputum-positive TB patients complicated with diabetes and 89 non-diabetic TB patients. Bronchoalveolar lavage fluids of patients were inoculated on two Lowenstein-Jensen mediums for bacterial susceptibility testing, including rifampicin (RFP), isoniazid (INH), streptomycin (SM), para-amino salicylate (PAS), ofloxacin (OFX), propionyl isobutylamine (PTO), and ethambutol (EMB). Smear testing was carried out for all patients. Results: In the study group, a total of 46 cases with sputum smears were positive for acid-fast bacilli, accounting for 74.19% of the total number of patients. This was higher ($P<0.05$) than the control group (26.97%). Total drug resistance rates in the study group were significantly higher than the control group ($P=0.02$). Single drug resistance in the study group was significantly less than the control group ($P<0.05$). Of the 62 study subjects, 46.77% were positive for *Mycobacterium tuberculosis* L-form, 25.81% (16 cases) were negative for all three tests, and 17.74% (11 cases) were positive for all three tests. Conclusion: Drug resistance in diabetic patients with pulmonary TB was significantly greater than patients with simple pulmonary TB. MTB-L should be more strictly tested in determining bacteriological evidence for curing TB.

Keywords: Diabetes, tuberculosis, drug resistance, Mycobacterium tuberculosis L-form

Introduction

Diabetes is the most common chronic disease in the world and often occurs in middle-aged and elderly populations [1]. Since the beginning of 19th century, the incidence of diabetes has remained high. With the development of society and improvement in living standards in recent years, the incidence of diabetes has increased year by year [2, 3]. According to Zinman et al. [4], the global population with diabetes exceeded 400 million in 2015. China ranks the highest in the world in terms of the number of patients. Treatment of diabetes has encountered great difficulty and there has been a constant search for means of effectively preventing and treating diabetes. However, no significant breakthroughs have been made [5].

Diabetes leads to various complications, including cardio-cerebrovascular disease, nervous system disease, and nephropathy. If the disease is not treated in time, it can directly lead to malignant tumor disease [6]. Diabetic patients are prone to a variety of infections. The most common is tuberculosis [7]. According to Ronacher et al. [8], about 26.75% of diabetic patients have tuberculosis and risk of tuberculosis infection increases with age. Existence of diabetes accelerates progression of pulmonary tuberculosis, making it more likely to deteriorate into lung cancer disease. Similarly, tuberculosis worsens drug resistance in patients with diabetes and increases difficulty in treatment [9]. Treatment of coinfection with both diseases has become a hot research topic in clinical practice. With continuous advancement in research, many studies at home and abroad [10-12] have proven that the causative pathogen of pulmonary tuberculosis in diabetic patients was *Mycobacterium tuberculosis* L-form (MTB-L).

Table 1. MTB-L classification criteria

Grading	Judgment standard
- (Negative)	0 bacteria/50 views
± (False positive)	1~3 bacteria/50 views
+ (Positive)	10~99 bacteria/50 views
++ (Strong positive)	≥100 bacteria/50 views

This present study was conducted to provide effective reference and guidance for prevention and treatment of patients with both diabetes mellitus and pulmonary tuberculosis through analyzing drug resistance and monitoring MTB-L of these patients.

Methods

Study design

This retrospective analysis was conducted among patients admitted to the Tuberculosis Prevention and Treatment Center of Binzhou Central Hospital. The study group consisted of 62 cases with both diabetes and sputum positive tuberculosis. Patients were aged 35-60 years old with a mean age of 46.87±11.53 years. Of the total 62 cases, 41 were male and 21 were female. In addition, 89 patients with non-diabetic pulmonary tuberculosis, admitted at the same time, were selected as controls, including 56 males and 33 females, aged 35-60 years old with a mean age of 47.63±9.81 years.

Inclusion and exclusion criteria

Diabetes was diagnosed in strict accordance with the Standards of Medical Care in Diabetes-2014 [13]. In addition, pulmonary tuberculosis was diagnosed in strict accordance with the Standards of Medical Care in TB-2012 [14]. Sputum cultivation for MTB-L was performed by the Chief Physician in the Laboratory of Microbiology. Complete medical histories were obtained from all patients. Patients complied with the arrangement of our medical professionals. Exclusion criteria: concomitant cardio-cerebral vascular disease, combined respiratory tract infections, combined digestive tract infections, combined neoplasms, combined autoimmune related diseases, and malignant histiocytosis. Other exclusion criteria included administration of other immunological drugs before treatment, experience with chemotherapy, pregnancy, physical disability, family history of genetic disease, hospital transfer midway, and being bedridden.

All included patients completed the acid-fast bacilli sputum smear, sputum cultivation, and bacterial susceptibility testing and signed informed consent.

Laboratory tests

All procedures were performed in strict adherence to 2014 Guidelines on Bacterial Culture for the Diagnosis of Tuberculosis [15]. Bronchoalveolar lavage fluid was treated with 4% sodium hydroxide (NaOH), inoculated onto two Lowenstein-Jensen mediums, and incubated in an incubator (37°C) for 24 hours for bacterial susceptibility testing, including rifampicin (RFP), isoniazid (INH), streptomycin (SM), para-aminosalicylate (PAS), ofloxacin (OFX), propionyl isobutylamine (PTO), and ethambutol (EMB). Sputum samples were stained for detection of acid-fast bacilli and viewed by fluorescent microscopy. Smear testing under a microscope was carried out and the Lowenstein-Jensen medium and trypticase soy agar (TSA-L) and Roche medium were used for cultivation.

Determination criteria

For positive/negative grading criteria for microscopy of MTB-L, please refer to the study performed by Skrahin et al. [16] (**Table 1**). Positive TSA-L medium culture was adopted as the final determination criteria. Resistance to one anti-tuberculosis drug was regarded as single drug resistance. Resistance to one or more anti-tuberculosis drugs, other than INH or RFP, was designated as multi-drug resistance. Resistance to both INH and RFP was regarded as multidrug resistant tuberculosis (MDR-TB). Simultaneous resistance to INH, RFP, and fluoroquinolones was regarded as extensively drug resistant tuberculosis (XDR-TB).

Statistical analysis

SPSS 22.0 statistical software was used to analyze and process data. Categorical data, such as drug resistance and sputum smear results, are expressed in the form of rates. Chi-square tests were used for comparison between groups. Continuous data, such as patient age, are presented as mean ± standard

Table 2. Comparison of clinical data between the two groups of patients [n (%)]

	Research group (n=62)	Control group (n=89)	X^2 or t	P
Age (year)	46.87±11.53	47.63±9.81	0.44	0.66
Body weight (KG)	83.24±12.83	79.68±14.57	1.55	0.12
Disease course (weed)	5.29±2.87	5.86±3.04	1.16	0.25
Gender			0.16	0.69
Male	41 (66.13)	56 (62.92)		
Female	21 (33.87)	33 (37.08)		
Living Environment			0.09	0.77
City	54 (87.10)	76 (85.39)		
Countryside	8 (12.90)	13 (14.61)		
Smoking			0.35	0.55
Yes	55 (88.71)	76 (85.39)		
No	7 (11.29)	13 (14.61)		

Table 3. Antibacterial sputum smear results from sputum [n (%)]

	Research group (n=62)	Control group (n=89)	X^2	P
Acid-fast bacillus smear positive	46 (74.19)	24 (26.97)	32.17	<0.01
Acid-fast bacillus smear negative	16 (25.81)	65 (73.03)		

deviation and t-tests were used to compare means between groups. P<0.05 indicated a statistically significant difference.

Results

General data comparison

No significant differences in age, weight, course of disease, gender, living environment, and smoking status between the two groups of patients were observed (P>0.05), proving that the two groups were comparable (**Table 2**).

Results of sputum smear for acid-fast bacilli

In the study group, a total of 46 cases (74.19%) with a sputum smear were found to be acid-fast bacilli positive. Sixteen cases in the study group (35.8%) with a sputum smear were shown to be acid-fast bacilli negative. Twenty-four cases in the control group (26.97%) had a sputum smear that was acid-fast bacilli positive while 65 cases (73.03%) had a sputum smear that was acid-fast bacilli negative. Patients presenting with positive acid-fast bacilli sputum were significantly higher than the control group (*P*<0.01) (**Table 3**).

Bacterial susceptibility testing

In the study group, 41 cases developed drug resistance, accounting for 66.13% of the total. Of these, 9.68% (6 cases) were single drug resistant, 12.90% (8 cases) were multidrug resistant, 17.74% (11 cases) were MDR-TB, and 25.81% (16 cases) were XDR-TB. In the control group, 42 cases displayed drug resistance, accounting for 47.19% of total controls. Of these, 22.47% (20 cases) were single drug resistant, 4.49% (4 cases) were multidrug resistant, 17.98% (16 cases) were MDR-TB, and 2.25% (2 cases) were XDR-TB. The total proportion of drug resistance was significantly higher in the study group than the control group (P=0.02). In terms of types of drug resistance, the study group had the highest occurrence of XDR-TB, followed by MDR-TB, while the control group had the highest occurrence of single drug resistance, followed by MDR-TB. No significant differences were found in MDR-TB between the study and control groups (P>0.05), but the proportion of XDR-TB and multidrug resistance in the study group was significantly higher than the control group (all P<0.05). Single drug resistance was significantly lower in the study group than the control group (P<0.05) (**Table 4** and **Figure 1**).

Results of MTB-L infection

Further, MTB-L infection was tested. MTB-L can be diagnosed in an accurate and reliable way as the 20% serum added to the culture medium provided necessary L-type bacteria growth substances. Within the 62 patients, 46.77% were positive for MTB-L, including 17.74% (11 cases) positive for all three tests, while 25.81% (16 cases) were negative for three tests. There were 14.52% patients in this experiment with false positives for MTB-L (sputum smear and roche medium results were positive, while TSA-L was negative) (**Table 5**).

Table 4. Drug sensitivity test results in the two groups [n (%)]

	Research group (n=62)	Control group (n=89)	X^2	P
Single drug resistance	6 (9.68)	20 (22.47)	5.21	0.02
Multiple drug resistance	8 (12.90)	4 (4.49)	4.32	0.03
MDR-TB	11 (17.74)	16 (17.98)	0.20	0.67
XDR-TB	16 (25.81)	2 (2.25)	8.23	0.01
Total (%)	66.13	47.19	5.30	0.02

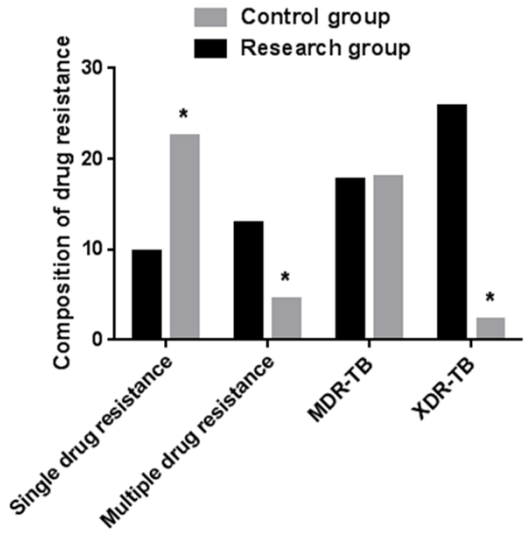

Figure 1. Drug sensitivity test results. XDR-TB was highest in the study group, followed by MDR-TB, while single-drug resistance was dominant in the control group, followed by MDR-TB. The study group's XDR-TB and multi-drug resistance was significantly more than the control group, while single drug resistance was significantly less than the control group, *P<0.05.

Discussion

The most common complication of diabetes is damage to the immune system, leading to reduced immune function. This reduced immune function can greatly increase the risk of MTB infection and occurrence of tuberculosis [17]. With a rising incidence of tuberculosis in recent years, MTB-L's impact on tuberculosis and the relationship between the two have been increasingly valued by clinicians. With further research, there are already many relevant documents [18, 19], at home and abroad, proving that not only does MTB-L impact occurrence of tuberculosis, but it is of potential significance to lung cancer, malignant lymphoma, prostate cancer, and other diseases. With an increase in drug resistance categories in recent years, the positive rate of MTB-L has remained high. Results of VanderVen et al. [20] demonstrated that patients diagnosed with MTB-L-positive pulmonary tuberculosis are much more difficult to treat than patients with MTB-L negative TB, suggesting that MTB-L may affect treatment of tuberculosis. At present, treatment of chronic disease remains extremely difficult. It is much worse in diabetic patients with pulmonary tuberculosis. The most commonly used method for treatment of tuberculosis in clinical practice is chemotherapy [21]. However, during the course of chemotherapy, patients will generate tremendous drug resistance to isoniazid plus rifampin, directly affecting the prognosis of patients. Therefore, studying drug resistance in diabetic patients with pulmonary tuberculosis has always been an important topic. This study aimed to analyze drug resistance characteristics of patients with both diabetes mellitus and pulmonary tuberculosis, aiming to provide reference and guidance for prevention and treatment through drug susceptibility testing and monitoring of cultivation results of MTB-L in patients with diabetes mellitus complicated with pulmonary tuberculosis.

Results of this study revealed that patients with diabetes mellitus complicated with pulmonary tuberculosis were more resistant to XDR-TB than those with simple pulmonary tuberculosis, while single-drug resistance was less than that of patients with pure tuberculosis. MDR-TB showed no significant differences, consistent with the findings of Parida et al. [22]. However, Pérez-Navarro et al. [23] demonstrated that there was a difference in drug resistance between diabetes mellitus patients with pulmonary tuberculosis and those with simple tuberculosis, but there was no significant differences between single drug resistance, XDR-TB, and MDR-TB. The reason for this may be that Pérez-Navarro et al. failed to exclude patients that had undergone prior immunological drug administration, as chemotherapy and tuberculosis chemotherapy will greatly increase drug resistance in patients and can consequently cause discrepancies in experimental results. This experiment was carried out based on strict selection of subjects, following inclusion and exclusion criteria to ensure maximum stringency and accuracy of the experiment. In the

Table 5. L-type culture

Training situation			n (%)
Smear (-)	Roche medium (-)	TSA-L (-)	16 (25.81)
Smear (-)	Roche medium (-)	TSA-L (+)	11 (17.74)
Smear (-)	Roche medium (+)	TSA-L (-)	2 (3.23)
Smear (-)	Roche medium (+)	TSA-L (+)	7 (11.29)
Smear (+)	Roche medium (+)	TSA-L (-)	9 (14.52)
Smear (+)	Roche medium (+)	TSA-L (+)	11 (17.74)
Smear (+)	Roche medium (-)	TSA-L (-)	6 (9.68)
L-type bacteria positive (%)			46.77

present study, patients in the research group with negative MTB-L sputum smears accounted for 25.81%. MTB-L is a variant of *Mycobacterium tuberculosis* caused by immunodeficiency or improper treatment with mycelium that results in morphologic changes and serious deficiencies in cell walls [24]. Therefore, failure to detect MTB-L with acid-fast staining may be due to its morphological differences with the original bacteria, which may easily be confused with another bacterium. Culturing of L-type bacteria requires a hypertonic, low-agar, and serum-containing environment [25]. The agar content in traditional culture medium was 1.5%~2.0% and L-type bacteria failed to penetrate and grow on its surface, resulting in failure of cultivation. TSA-L medium reduced the density of agar to 0.8%~1.0%, which ensured the growth of L-type bacteria. Additionally, the 20% serum added to the culture medium provided necessary L-type bacteria growth substances so that MTB-L could be diagnosed in the most accurate and reliable way. However, there were still 14.52% patients in this experiment with false positives for MTB-L (sputum smear and Roche medium results were positive, while TSA-L was negative). This may have been due to failure to exclude infections caused by mistakes or by errors in experimental operations.

This present study compared drug susceptibility testing in patients with both diabetes mellitus complicated with pulmonary tuberculosis and patients with simple tuberculosis. This study cultured MTB-L in diabetic patients with pulmonary tuberculosis. However, there were still deficiencies in the experiment due to limitations with experimental conditions. For example, the study population was quite small and was examined for a relatively short amount of time. Subjects of this study should have been investigated for a longer period of time to achieve the best experimental results.

In summary, drug resistance in diabetic patients with pulmonary tuberculosis is significantly higher than that of patients with just tuberculosis. Occurrence of MTB-L should be more strictly examined in determining criteria for treatment of bacteriological tuberculosis.

Disclosure of conflict of interest

None.

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