Evaluation of GenoType MTBDR*plus* assay for rapid detection of isoniazid- and rifampicin-resistance in Mycobacterium tuberculosis isolates from diabetes mellitus patients

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Background: To evaluate the performance of GenoType MTBDR*plus* assay for rapid detection of isoniazid (INH)- and rifampicin (RIF)-resistance in Mycobacterium tuberculosis (M.TB) isolates from diabetes mellitus (DM) patients, a retrospective study was conducted.

Methods: Between May, 2012 and Sep, 2016, 227 tuberculosis (TB) patients with DM were enrolled in the study. Culture and phenotypic drug susceptibility test were performed in all cases. The GenoType MTBDR*plus* assay were done on sputum or culture specimens and carried out according to manufacturer's instructions. The sensitivity, specificity, false positive ratio, false negative ratio and diagnostic odds ratio for detecting INH- and RIF-resistance by GenoType MTBDR*plus* assay were calculated using the phenotypic drug susceptibility test as the gold standard.

Results: The total sensitivity, specificity, false positive ratio, false negative ratio and diagnostic odds ratio of GenoType MTBDR*plus* assay: (I) for RIF-resistance detection, were 96.7% (83.3%, 99.4%), 97.5% (94.2%, 98.9%), 38.1 (16.0, 90.7), 0.034 (0.005, 0.235) and 1,113.6 (125.6, 9,873.5), respectively; (II) for INH-resistance detection, were 82.1% (64.4%, 92.1%), 91.9% (87.3%, 95.0%), 10.2 (6.2, 16.8), 0.194 (0.088, 0.43) and 52.3 (17.5, 156.2), respectively; (III) for multiple drug resistance (MDR) detection, were 92.9% (68.5%, 98.7%), 96.2% (92.8%, 98.0%), 24.7 (12.3, 49.5), 0.074 (0.011, 0.491) and 333.1 (38.7, 2,868.7), respectively.

Conclusions: GenoType MTBDR*plus* assay is highly sensitive and specific for rapid diagnosis of MDR-TB in DM patients. The application of this assay could be considered for some settings. Further studies were needed to assess the role and value of GenoType MTBDR*plus* assay in treatment for MDR-TB with DM patients.

Keywords: GenoType MTBDR*plus* assay; sensitivity; specificity; diabetes mellitus (DM)

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Introduction

Tuberculosis (TB) is one of the most serious infectious diseases, with an annual incidence of 9 million new cases, killing more than 1.5 million people annually (1).The multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid (INH) and rifampicin (RIF), is an increasing threat to TB control as the treatment is difficult, expensive, and a major health care cost burden

for developing countries (2). According to World Health Organization (WHO), in the year 2014, an estimated 3.3% of new cases and 20% of previously treated TB cases have MDR-TB (3).

Diabetes mellitus (DM) is a non-communicable chronic disease whose incidence is increasing globally (4). Several studies have elucidated an association between DM and TB. The prevalence of DM among TB patients around the world varies according to different regions that range from 12% to 44% and tended to increase in the past decade (5). DM increases the risk of developing active TB by a factor of 2–3 as compared with the general population (6). Except accelerating TB disease, DM is also an increasingly recognized comorbidity that can complicate its treatment and increases the risk of a poor TB outcome (6-9). Among MDR-TB patients, DM is a relatively common comorbidity in patients undergoing treatment for MDR-TB, it was found to be independently associated with an increased risk of both treatment failure and death (10). Moreover, DM increases the risk of developing serious adverse events in the therapy against drug-resistant TB, such as nephrotoxicity and hypothyroidism (9).

Current studies support that transmission of drug-resistant TB strains drives the drug-resistant TB epidemic, including MDR and extensively drug-resistant TB (11,12). Early detection of MDR-TB is essential for starting an effective treatment regimen and reducing its transmission in the population. However, for drug susceptibility testing (DST), conventional methods relying on solid or liquid culture are time-consuming and labor-intensive. GenoType MTBDR*plus* assay (Hain Lifescience GmbH, Nehren, Germany) which is designed for the rapid detection of Mycobacterium tuberculosis (M.TB) complex and INH- and RIF-resistance was endorsed by WHO (13). The molecular line probe assay detects mutations associated with the *rpoB* gene for RIF-resistance, *katG* genes and *inhA* regulatory region gene for INH-resistance (14). In a meta-analysis, GenoType MTBDR*plus* showed excellent pooled sensitivity and specificity for detection of resistance to INH (91%, 99%), RIF (96%, 98%), and MDR-TB (91%, 99%) (15). The MTBDR*plus* assay demonstrated excellent performance and offers great promise in improving MDR-TB care and prevention.

The aim of this study was to evaluate the performance of GenoType MTBDR*plus* assay for rapid detection of INH- and RIF-resistance in M.TB isolates from DM patients.

Methods

Subjects

This study was approved by the Human Research Ethics Committees of Shandong Provincial Chest Hospital. The Ethics Approval ID number is: SPCHEC 2016-11-01. Because of the retrospective nature, written consent was waived.

Between May, 2012 and Sep, 2016, 227 TB patients with DM were enrolled in the study. Culture and phenotypic DST were performed on all cases. The absolute concentration method (INH: 1 μg/mL, RIF: 50 μg/mL) on Löwenstein-Jensen medium was used to screen M.TB isolates (16). Their clinicopathological characteristics were reviewed and analyzed.

All TB patients were confirmed by mycobacterial culture (Löwenstein-Jensen medium). DM was diagnosed according to the WHO criteria, i.e., a fasting blood glucose ≥7 mmol/L or HbA1c ≥6.5%. Also, DM was diagnosed if patients had a history of known DM or were receiving anti-diabetic agents.

GenoType MTBDRplus assay

The GenoType MTBDR*plus* assay was done on sputum or culture specimens and carried out according to manufacturer's instructions. Briefly, three steps are required: (I) DNA extraction from specimens; (II) amplification of target region by PCR; (III) hybridization of PCR product to the specific oligonucleotide probes, immobilized on the strip. Drug resistance was read as the absence of wild-type band and/or presence of mutation band.

Statistical analysis

Statistical analysis was carried out using SPSS 17.0 software. Data were expressed as mean ± standard deviation (SD), all calculations were estimated at a 95% confidence interval (95% CI). The result of the phenotypic DST assay was used as the gold standard to calculate the sensitivity, specificity, false positive ratio, false negative ratio and diagnostic odds ratio for detecting INH- and RIF-resistance by GenoType MTBDR*plus* assay.

Results

A total of 227 specimens (40 culture and 187 sputum samples) collected from DM patients were enrolled in the study. Their mean age was 52.2 (13.2, SD) years. Of these

Table 1 The clinical characteristics of the participants

Specimens	Number	Sex, male (%)	Age (years)	HIV negative status (%)	Resistance			Underlying diseases	
					INH	RIF	MDR	PTB	PTB + EPTB
Culture	40	35 (87.5)	52.0±14.1	0 (34/34)	4	7	2	35	5
Sputum	187	162 (86.6)	52.2±13.0	0 (169/169)	24	23	12	152	35
Total	227	197 (86.8)	52.2±13.2	0 (203/203)	28	30	14	187	40

INH, isoniazid; RIF, rifampicin; MDR, multidrug resistance; PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis.

Table 2 The performance of GenoType MTBDRplus assay for detection of INH- and RIF- resistance in M.TB isolates from DM patients

Specimens	Resistance	Sensitivity (95% CI)	Specificity (95% CI)	FPR	FNR	DOR
Culture	INH	0.50 (0.15–0.85)	0.89 (0.74–0.96)	4.4 (1.1–16.8)	0.565 (0.210–1.515)	7.6 (0.8–71.3)
	RIF	0.86 (0.49–0.97)	0.67 (0.85–1.00)	28.3 (4.0–199.5)	0.147 (0.024–0.905)	192.0 (10.5–3,509.5)
	MDR	1.00 (0.34–1.00)	1.00 (0.91–1.00)	–	0	–
Sputum	INH	0.88 (0.69–0.96)	0.93 (0.88–0.96)	11.9 (6.8–20.9)	0.135 (0.047–0.389)	88.1 (23.0–338.1)
	RIF	1.00 (0.86–1.00)	0.98 (0.94–0.99)	41.0 (15.6–107.9)	0	–
	MDR	0.92 (0.65–0.99)	0.95 (0.91–0.97)	17.8 (9.2–34.4)	0.088 (0.013–0.574)	202.9 (23.5–1,749.1)
Total	INH	0.82 (0.64–0.92)	0.92 (0.87–0.95)	10.2 (6.2–16.8)	0.194 (0.088–0.43)	52.3 (17.5–156.2)
	RIF	0.97 (0.83–0.99)	0.98 (0.94–0.99)	38.1 (16.0–90.7)	0.034 (0.005–0.235)	1,113.6 (125.6–9,873.5)
	MDR	0.93 (0.69–0.99)	0.96 (0.93–0.98)	24.7(12.3–49.5)	0.074 (0.011–0.491)	333.1 (38.7–2,868.7)

M.TB, mycobacterium tuberculosis; DM, diabetes mellitus; INH, isoniazid; RIF, rifampicin; 95 CI, confidence interval; FPR, false positive ratio; FNR, false negative ratio; DOR, diagnostic odds ratio; MDR, multidrug resistance.

patients, 86.8% [197] were male, all (203/203) tested for HIV status were HIV-negative, 58 patients were retreatment cases, 187 patients have isolated pulmonary TB, 40 pulmonary + extra-pulmonary TB (25 pleural, 5 lymph node, 4 meningitis, 7 others). Amongst the 227 onset isolates, 182 (80.2%) were sensitive, 30 (13.2%) mono-resistant (14 INH, 16 RIF), 14 (6.2%) MDR. The clinical characteristics of the participants were presented in *Table 1*.

The total sensitivity, specificity, false positive ratio, false negative ratio and diagnostic odds ratio of GenoType MTBDR*plus* assay: (I) for RIF-resistance detection, were 96.7% (83.3%, 99.4%), 97.5% (94.2%, 98.9%), 38.1 (16.0, 90.7), 0.034 (0.005, 0.235) and 1113.6 (125.6, 9873.5), respectively; (II) for INH-resistance detection, were 82.1% (64.4%, 92.1%), 91.9% (87.3%, 95.0%), 10.2 (6.2, 16.8), 0.194 (0.088, 0.43) and 52.3 (17.5, 156.2), respectively; (III) for MDR detection, were 92.9% (68.5%, 98.7%), 96.2% (92.8%, 98.0%), 24.7(12.3, 49.5), 0.074 (0.011, 0.491) and 333.1 (38.7, 2,868.7), respectively. The diagnostic performance of GenoType MTBDR*plus* compared to DST in solid medium, for RIF- and INH-resistance detection in M.TB isolates from DM patients, is summarized in *Table 2*.

Discussion

Although from 1990 to 2010, the prevalence of TB decreased significantly, China ranked the second largest country in terms of the number of TB patients (17). A cohort study showed that the prevalence of latent TB infection ranged from 13% to 20% (18). Since TB with a high prevalence in many developing countries, it enables the coexistence with chronic diseases, which increased in the last decades (19,20).

In China, the prevalences of total diabetes and pre-diabetes were 9.7% and 15.5%, respectively (21). Therefore, it was concluded that there are much TB patients with TB-DM comorbidity. DM and TB affect vulnerable groups, such as older adults and people with other morbidities

(hypertension, respiratory diseases, mental disorders, cancer) (22). Diabetes contributes to worsen outcomes and increased severity, when active TB disease develops (23). Thus, there is an urgent need to implement strategies for TB prevention and control among the millions of DM patients exposed to M.TB.

In our study, the GenoType MTBDR*plus* assay demonstrated excellent performance in detecting MDR-TB in DM patients, with a sensitivity of 92.9% (68.5%, 98.7%) and a specificity of 96.2% (92.8%, 98.0%). In various other studies, it was shown that the GenoType MTBDR*plus* assay achieved a high sensitivity and specificity for MDR-TB (15). The results in this study differ slightly, with a sensitivity and specificity for RIF of 96.7% (83.3%, 99.4%) and 97.5% (94.2%, 98.9%) and for INH of 82.1% (64.4%, 92.1%) and 91.9% (87.3%, 95.0%), respectively. The sensitivity and specificity for the detection of INH-resistance are slightly lower than that published in the meta-analysis [sensitivity: 91% (88%, 94%), specificity: 99% (98%, 99%)] (15).

In general, there still remain several problems difficult to be solved, for example, few mutant probes, hetero-resistance and requirement of high bacillary load. The assay is designed to detect the more frequent mutations related to INH- and RIF-resistance, not to detect the whole mutations. Therefore, this would decrease the sensitivity in detection of drug resistance, especially of INH-resistance. Until now, alterations in multiple genes (24), like *kat*G, *inh*A, *oxy*R-*aph*C, *kas*A and *ndh* (25-27), have been reported to be associated with INH-resistance. However, the MTBDR*plus* assay for the detection of INH-resistance is designed to detect only one mutation in *katG* and three in *inhA*. Moreover, the limited numbers of probes in GenoType MTBDR*plus* restricted its detection of all mutation loci, which might also have decreased its sensitivity. Hetero-resistance is the phenomenon of simultaneous occurrence of drug resistant and drug sensitive TB isolates in the same sample (28,29). The phenomenon has been reported in clinical practice in China (30). It may also contribute to the discordant results between the molecular assay and phenotypic DST. Bacillary load is also an important factor influencing the performance of the test. A study conducted by Seifert M. *et al.* showed that smear gradation appeared to influence test sensitivity and specificity, indicating a significant association between bacillary load and test performance (31).

Our study also has several limitations. First, because of its retrospective nature, it may produce selection bias. However, consecutive subjects were enrolled, which should reduce the likelihood of a selection bias. Although the MTBDR*plus* assay demonstrated excellent performance, its impact on TB control strategies for DM patients haven't been addressed yet. In the next studies, we would conduct studies evaluating the role of GenoType MTBDR*plus* assay in reducing adverse events in MDR-TB treatment for MDR-TB with DM patients.

Overall, the molecular assay is rapid, reliable and easy to interpret, although the test requires more technical expertise. The results of diagnosis thus have been found to be significantly shorter than the conventional DST method. It is concluded from the present study that GenoType MTBDR*plus* assay is highly sensitive and specific for rapid diagnosis of MDR-TB in DM patients. The application of this assay could be considered for some settings.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2017.03.01). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional/regional/national ethics committee/ethics board of Human Research Ethics Committees of Shandong Provincial Chest Hospital (No. SPCHEC 2016-11-01). Because of the retrospective nature, written consent was waived.

References

1. WHO. Global Tuberculosis Report 2014. World Health Organization, 2014.
2. Nations JA, Lazarus AA, Walsh TE. Drug-resistant tuberculosis. Dis Mon 2006;52:435-40.
3. WHO. Global tuberculosis report 2015. World Health Organization, 2015.
4. Goldhaber-Fiebert JD, Jeon CY, Cohen T, et al. Diabetes mellitus and tuberculosis in countries with high tuberculosis burdens: individual risks and social determinants. Int J Epidemiol 2011;40:417-28.
5. Ko PY, Lin SD, Tu ST, et al. High diabetes mellitus prevalence with increasing trend among newly-diagnosed tuberculosis patients in an Asian population: A nationwide population-based study. Prim Care Diabetes 2016;10:148-55.
6. Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. PLoS Med 2008;5:e152.
7. Alkabab YM, Al-Abdely HM, Heysell SK. Diabetes-related tuberculosis in the Middle East: an urgent need for regional research. Int J Infect Dis 2015;40:64-70.
8. Muñoz-Torrico M, Caminero-Luna J, Migliori GB, et al. Diabetes is Associated with Severe Adverse Events in Multidrug-Resistant Tuberculosis. Arch Bronconeumol 2017. [Epub ahead of print].
9. Viswanathan V, Vigneswari A, Selvan K, et al. Effect of diabetes on treatment outcome of smear-positive pulmonary tuberculosis--a report from South India. J Diabetes Complications 2014;28:162-5.
10. Kang YA, Kim SY, Jo KW, et al. Impact of diabetes on treatment outcomes and long-term survival in multidrug-resistant tuberculosis. Respiration 2013;86:472-8.
11. Yang C, Luo T, Shen X, et al. Transmission of multidrug-resistant Mycobacterium tuberculosis in Shanghai, China: a retrospective observational study using whole-genome sequencing and epidemiological investigation. Lancet Infect Dis 2017;17:275-84.
12. Shah NS, Auld SC, Brust JC, et al. Transmission of Extensively Drug-Resistant Tuberculosis in South Africa. N Engl J Med 2017;376:243-53.
13. Barnard M, Gey van Pittius NC, van Helden PD, et al. The diagnostic performance of the GenoType MTBDRplus version 2 line probe assay is equivalent to that of the Xpert MTB/RIF assay. J Clin Microbiol 2012;50:3712-6.
14. Hillemann D, Rüsch-Gerdes S, Richter E. Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of Mycobacterium tuberculosis strains and clinical specimens. J Clin Microbiol 2007;45:2635-40.
15. Bai Y, Wang Y, Shao C, et al. GenoType MTBDRplus Assay for Rapid Detection of Multidrug Resistance in Mycobacterium tuberculosis: A Meta-Analysis. PLoS One 2016;11:e0150321.
16. Deng Y, Wang Y, Wang J, et al. Laboratory-based surveillance of extensively drug-resistant tuberculosis, China. Emerg Infect Dis 2011;17:495-7.
17. Wang L, Zhang H, Ruan Y, et al. Tuberculosis prevalence in China, 1990-2010; a longitudinal analysis of national survey data. Lancet 2014;383:2057-64.
18. Gao L, Lu W, Bai L, et al. Latent tuberculosis infection in rural China: baseline results of a population-based, multicentre, prospective cohort study. Lancet Infect Dis 2015;15:310-9.
19. Marais BJ, Lönnroth K, Lawn SD, et al. Tuberculosis comorbidity with communicable and non-communicable diseases: integrating health services and control efforts. Lancet Infect Dis 2013;13:436-48.
20. Stevenson CR, Forouhi NG, Roglic G, et al. Diabetes and tuberculosis: the impact of the diabetes epidemic on tuberculosis incidence. BMC Public Health 2007;7:234.
21. Yang W, Lu J, Weng J, et al. Prevalence of diabetes among men and women in China. N Engl J Med 2010;362:1090-101.
22. Pereira SM, Araújo GS, Santos CA, et al. Association between diabetes and tuberculosis: case-control study. Rev Saude Publica 2016;50:82.
23. Alisjahbana B, Sahiratmadja E, Nelwan EJ, et al. The effect of type 2 diabetes mellitus on the presentation and treatment response of pulmonary tuberculosis. Clin Infect Dis 2007;45:428-35.
24. Seifert M, Catanzaro D, Catanzaro A, et al. Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review. PLoS One 2015;10:e0119628.
25. Mathuria JP, Nath G, Samaria JK, et al. Molecular characterization of INH-resistant Mycobacterium tuberculosis isolates by PCR-RFLP and multiplex-PCR in

North India. Infect Genet Evol 2009;9:1352-5.
26. Hristea A, Otelea D, Paraschiv S, et al. Detection of Mycobacterium tuberculosis resistance mutations to rifampin and isoniazid by real-time PCR. Indian J Med Microbiol 2010;28:211-6.
27. Kim SY, Park YJ, Kim WI, et al. Molecular analysis of isoniazid resistance in Mycobacterium tuberculosis isolates recovered from South Korea. Diagn Microbiol Infect Dis 2003;47:497-502.
28. van Rie A, Victor TC, Richardson M, et al. Reinfection and mixed infection cause changing Mycobacterium tuberculosis drug-resistance patterns. Am J Respir Crit Care Med 2005;172:636-42.
29. Morgan M, Kalantri S, Flores L, et al. A commercial line probe assay for the rapid detection of rifampicin resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis. BMC Infect Dis 2005;5:62.
30. Mei Z, Sun Z, Bai D, et al. Discrepancies in Drug Susceptibility Test for Tuberculosis Patients Resulted from the Mixed Infection and the Testing System. Biomed Res Int 2015;2015:651980.
31. Seifert M, Ajbani K, Georghiou SB, et al. A performance evaluation of MTBDRplus version 2 for the diagnosis of multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 2016;20:631-7.

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