Detection of drug-resistant *Mycobacterium tuberculosis* from TB suspected diabetic patients by GeneXpert MTB/RIF method

Asmaul Husna[1,2], Tushar Kanti Dhar[2] and Saurab Kishore Munshi[2‡]

[1]Department of Microbiology, BIRDEM General Hospital, 122, Kazi Nazrul Islam Avenue, Shahbagh, Dkaka-1000, Bangladesh; [2]Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh

Multidrug-resistant tuberculosis (MDR-TB) has become one of the major public health menace in the developing countries which have destructive consequences in immunocompromised patients. Rapid detection of such cases is indispensable to initiate the early and proper treatment of the infected individuals. Present study was conducted to determine the frequency of drug resistant *Mycobacterium tuberculosis* among the TB suspected diabetic patients employing GeneXpert MTB/RIF method. A total of 1311 sputum specimens were tested among which 154 (11.7%), 109 (8.3%) and 92 (7%) were found to carry *Mycobacterium tuberculosis* by GeneXpert, auramine O staining and Zeihl-Neelsen (Z-N) staining methods, consecutively. The relative positivity of the GeneXpert method was 40.3% and 29.2% higher than that of Z-N and auramine O staining methods. The sensitivity, specificity and accuracy of GeneXpert method were found to be higher when compared to these microscopic techniques. An estimation of 12.9% (n=132) of the positive cases was found among the diabetic patients (n=1027), whereas 7.7% (n=22) of the non-diabetic patients (n=284) showed positivity by GeneXpert method. Four (2.6%) among the MTB positive cases exhibited rifampicin resistance of which 3 were diabetic patients.

Key words: Multidrug-resistant tuberculosis (MDR-TB); GeneXpert MTB/RIF method; Diabetes mellitus

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Tuberculosis has remained to be a major health problem worldwide (1-4). Nearly one-third of the global population have encountered *Mycobacterium tuberculosis* infections (2-4). After the human immunodeficiency virus (HIV), it stands to be the second leading cause of death from an infectious disease (3-6). Till 2012, an estimation of 8.6 million people had TB infection and 1.3 million died as a consequences of the disease (7). More than 90% of global TB cases and TB related deaths recover in the developing world with limited facilities (7, 8). TB is highly prevalent in Asian countries. Bangladesh holds the sixth position among the 22 TB burdened countries enlisted by WHO with an estimation of 880 new TB cases and 176 TB deaths per day (2, 9-12). The control and elimination of TB has still remained to be a major challenge for the country due to the lack of initiating early and proper management of the infected individuals (1, 2). The constant rise in the multidrug resistant TB cases in Bangladesh (2.2% of the new TB cases and 14.7% of previously treated TB cases) often heighten difficulties in TB control (1, 2).

The emergence of type 2 diabetes in TB prevalent countries could hinder the efforts to control and eliminate TB (13). Diabetic patients have a 2-3 times higher risk of experiencing MDR-TB than the non-diabetic ones (13, 14). Globally, about 15% of TB cases are pretended to be associated with diabetes (13). Patients having both diabetes and TB, are often misdiagnosed or experiencing delay in diagnosis which consequently results in delay in treatment and thereby the chances of disease transmission and difficulty in recovery for patients may extend (1, 10, 13). Proper and early diagnosis of such cases can aid in the advancement of treatment and management of both diseases (14, 15).

The currently available methods for the routine diagnosis of TB includes chest X-rays, microscopic methods, culture based methods and the Mantoux test (16). Although the microscopic techniques based on auramine O staining and Ziehl–Neelsen staining are known to be the simplest and rapid as well as economical means for TB diagnosis, these require 5,000 to 10,000 bacilli per ml to be detected (11, 17-21). On the other hand, culture based methods which are considered to be the gold standard for TB diagnosis have higher sensitivity and allow drug resistant testing (DST) but these methods take 4 to 8 weeks and further more for DST for the recovery of slow growing *M. tuberculosis* (1, 11, 20-22). Such delay or fail in diagnosis by the routinely applied methods often result in the mismanagement and

‡Corresponding Author. Mailing address: Saurab Kishore Munshi, Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka, Bangladesh; Email: kishore016@yahoo.com.

maltreatment of TB patients, especially the MDR ones (1, 20, 21, 23, 24). The GeneXpert MTB/RIF assay permits the rapid diagnosis of TB disease and drug resistance cases (1, 25-28). The test simultaneously allow the detection of *M. tuberculosis* complex (MTBC) and rifampicin (RIF) resistance in less than 2 hours with higher sensitivity (1, 16, 25, 28, 29).

The diagnosis of TB by such molecular method is quite new in Bangladesh, and only a few diagnostic laboratories have started using GeneXpert for TB diagnosis in recent years (1). A few studies still have been conducted in Bangladesh to evaluate the application of this technique for the diagnosis of suspected TB patients (1). Therefore, the present study was designed to detect drug resistant *M. tuberculosis* rather rapidly and accurately from the TB suspected diabetic patients by the GeneXpert MTB/RIF method and also to evaluate the efficacy of the method as a regular diagnostic tool.

MATERIALS AND METHODS

Settings. The study was carried out at the Department of Microbiology of Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) Hospital where patients having diabetes mellitus and other diseases came from different areas of Bangladesh.

Study Population. A total of 1311 new TB suspected patients were enrolled for this study from November 2013 to April 2014. Patients of all ages and both sexes were tested among which 1027 had been suffering from diabetes mellitus (DM) and had a suspicion of TB. Another 284 samples were collected from non-diabetic individuals for the comparative analysis.

Sample collection, smear preparation, staining and microscopic observation. A volume of 3-5 ml of deep sputum specimens was collected in clean, dry, wide necked, and leak-proof containers. Yellow purulent portion of the sputum was taken using a sterile bamboo stick and was spread evenly to prepare a good smear of 3cm by 2cm in size. Then the slides were allowed to be air dried for 15 minutes and afterwards were placed on the hot plate at 85 °C for 3 minutes (1, 2, 11, 19). After air drying and heat fixation the slides were stained by Z-N and Auramine O methods.

For Ziehl–Neelsen (Z–N) staining, smears were covered with carbol fuchsin stain, and were heated until the first vapor appeared. After 10 min, smears were washed up, covered with 25% sulfuric acid for 3 min, and 0.1% methylene blue was flooded over them for 1 min. Finally, the washed and dried smears were examined under the bright field (BF) microscope (Olympus, CX 21) at 1000· magnification (10, 19). For auramine O staining, smears were covered with 0.1% Auramine solution for 15 min, decolorized with 0.5% acid–alcohol for 3min, and then 0.3% methylene blue was flooded over them for 1 min (1, 10, 11, 19, 30). After drying, smears were examined under the LED fluorescence microscope (Primostar, Carl Zeiss LED, Germany) at 400· magnification (455 nm).

GeneXpert MTB/RIF. First, Sample reagent was mixed with sputum sample in a vial. It was shaken vigorously 10-20 times. Then the specimen was incubated at room temperature for 10 minutes. Again the specimen was shaken vigorously 10-20 times and afterwards incubated at room temperature for an additional 5 minutes. The test was started within 4 hours of adding the sample to the cartridge. The cartridge lid was opened up and then the sample container was uncovered. Using the sterile transfer pipette, the liquefied sample was aspirated. 2ml of sample was transferred into the open port of the Xpert MTB/RIF cartridge (version 3). The sample was slowly dispensed to minimize the risk of aerosol formation. After closing the cartridge lid firmly, the cartridge was loaded into the GeneXpert Dx instrument (Cepheid, USA) and started the test within 5 hours of preparing the cartridge. The results were recorded within 2 hours (1, 25, 31, 32).

RESULTS AND DISCUSSION

Onset of TB in diabetic patients extends the patient complications and often feature a higher risk of death during TB treatment and of TB relapse after treatment (13, 14). To minimize the health hazards of such patients, proper care and treatment regimens have to be implemented more rigorously against both the diseases (14). Therefore, routine screening for TB in diabetic patients is crucial, particularly in high TB prevalent settings (14). Effective TB diagnosis including the drug resistant ones in a relatively short period of time should also be accounted for the management of patients having both diseases. However, the currently available TB diagnosis means in Bangladesh are still either less efficient or time consuming as described previously (1, 2, 10, 11, 19, 30). Whereas, GeneXpert MTB/RIF assay offers rather rapid and precise detection of drug resistant TB cases (1, 16, 25, 28). Hence, the present study was undertaken to evaluate the efficacy of GeneXpert MTB/RIF method in the screening of drug resistant TB from diabetic patients as well as from non-diabetic individuals.

Relatively higher detection frequency of *Mycobacterium tuberculosis* by GeneXpert method compared to microscopic techniques. Previous data suggested that GeneXpert method can effectively recover *M. Tuberculosis* from smear negative cases (25, 27, 28, 33). Present study evidenced similar kind of findings. Out of 1311 samples, 154 (11.7%) were found to harbor *Mycobacterium tuberculosis* by GeneXpert methods, while Z-N staining (Bright field microscopy) and Auramine O staining (LED fluorescence microscopy) recovered 92 (7%) and 109 (8.3%) positive cases, respectively (Table 1). The relative positivity through the Z-N staining and Auramine O staining methods were 59.7% and 70.8%, respectively when compared to the GeneXpert method.

TABLE 1. Comparative detection frequency of *Mycobacterium tuberculosis* by different methods (n = 1311)

Type of patients	No. of positive cases (%)		
	Z-N staining	Auramine O staining	GeneXpert MTB/RIF
Diabetic (n = 1027)	83 (8.9)	97 (9.4)	132 (12.9)
Non-diabetic (n = 284)	9 (3.2)	12 (4.2)	22 (7.7)
Total (n = 1311)	92 (7.0)	109 (8.3)	154 (11.7)

More precisely, 62 and 47 cases which were found to be negative in Z-N staining and auramine O staining, respectively were recovered as positive in GeneXpert method. The GeneXpert method was 100% and 95.3% sensitive and accurate in detecting *M. tuberculosis* when compared to Z-N staining method (Table 2). In comparison with auramine O staining, the sensitivity and accuracy of GeneXpert method were found to be 99.1% and 96.3%, respectively (Table 2). Such findings supported our knowledge based on previous data on the

TABLE 2. The efficacy of GeneXpert MTB/RIF method in detecting *M. tuberculosis* when compared to Z-N staining and auramine O staining methods (n = 1311)

	Z-N staining	Auramine O staining
True positive (n)	92	107
True negative (n)	1157	1156
Discordant (n)	62	48
Sensitivity (%)	100	99.1%
Specificity (%)	94.9	96.1%
Accuracy (%)	95.3	96.3%

TABLE 3. Rifampicin (RIF) resistance/sensitivity of *M. tuberculosis* isolates from diabetic and non-diabetic patients by GeneXpert method (n = 1311)

Type of Patients	RIF resistant (%)	RIF sensitive (%)	Not detected by GeneXpert (%)	Indeterminate (%)
Diabetic (n=1027)	3 (0.3)	129 (12.6)	891 (86.8)	4 (0.4)
Non-diabetic (n=284)	1 (0.4)	21 (7.4)	261 (91.9)	01 (0.4)
Total (n=1311)	4 (0.3)	150 (11.4)	1152 (87.9)	5 (0.4)

RIF = Rifampicin

efficacy and accuracy of GeneXpert method compared to the microscopic techniques (25, 27, 28, 33). Relatively higher frequency of positive cases were found among the diabetic patients compared to the non-diabetic individuals by all the tested methods (Table 1). The detection frequencies of 12.9%, 8.9% and 9.4% for diabetic patients by GeneXpert, Z-N staining and Auramine O staining, consecutively were dropped to 7.7%, 3.2% and 4.2%, consecutively for non-diabetic individuals (Table 1). Based on the efficacy of the GeneXpert method along with their rapidity, the present study claim its competence to be the method of choice for *M. tuberculosis* detection from smear positive and negative sputum samples of diabetic patients.

Frequency of drug resistant cases. The GeneXpert assay employed in this study is designed as such that can detect the mutations associated with rifampicin resistance in *rpoB* gene region by heminested PCR allowing the combined recovery of *M. tuberculosis* and rifampicin resistance (34, 35). Moreover, the automated bacterial lysis, DNA extraction, real-time PCR amplification, and amplicon detection in a single system aid in attaining result in a relatively short period of time with higher sensitivity compared to culture based methods which allow the treatment to be started instantly (16, 33, 35). The findings of recent studies have also supported these facts as those researches documented nearly 100% sensitivity and higher specificity of the GeneXpert assay in detecting drug resistant TB with vastly reduced turnover time (1, 16, 25, 28, 34, 35). In the present study, the rifampicin resistance was found to be minimal (4, 0.3%) in the 1311 tested patients by GeneXpert method among which 3 (0.3%) and 1 (0.4%) were encountered in the diabetic patients (n=1027) and non-diabetic individuals, respectively (Table 3). More specifically, the frequency was 2.6% among the MTB positive cases (154). In this viewpoint, 2.3% and 4.5% of the *M. tuberculosis* isolates from diabetic patients (n=132) and non-diabetic (n=22) individuals showed rifampicin resistance.

CONCLUSIONS

GeneXpert method was found to be effective method in the detection of *M. tuberculosis* as relative positivity

through this method was found much higher than that by Z-N staining and Auramine O staining. Although the frequency of rifampicin resistance was found to be minimal, the accuracy and rapidity of GeneXpert method in detecting drug resistance was evaluated. Overall, this study demonstrates that the GeneXpert MTB/RIF assay were able to detect the presence of *M. tuberculosis* more efficiently than the microscopic methods with least turnover time, and successfully identified the rifampin resistance conferring mutations in *rpoB* gene regardless of the patients' category. Considering the urge for early and precise detection of TB infected cases, especially from immunocompromised patients having diabetes mellitus, and the higher efficacy of GeneXpert MTB/RIF method in doing so, the present study suggested its frequent endorsement as the efficient tools for routine screening of TB cases as well as drug resistant ones in Bangladesh.

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