

Research Article



Comparison of Gene Xpert MTB/RIF Assays with Conventional Standard Proportion Method for Determination of Drug Susceptibility In Multidrug Resistant TB Suspects

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Abstract | Background: Tuberculosis (TB) is a contagious disease and multidrug resistant tuberculosis is an emerging global issue. Rapid detection of such type of tuberculosis is necessary for timely control of the disease. GeneXpert test has already been implemented by World Health Organization to diagnose the infection on urgent basis.

Objectives: This study was designed to apply GeneXpert MTB/RIF assays for the detection of rifampicin resistant tuberculosis and validation of assays by comparing with conventional standard drug proportion method. Additionally, to explore whether the assay can be utilized in treatment of Multidrug Resistant TB

Settings: This study was undertaken in Pakistan Health Research Council TB Research Centre in collaboration with Department of Pulmonology, King Edward Medical University, Mayo Hospital, Lahore.

Methods: Sputum samples from 125 patients were collected from confirmed pulmonary TB patients who were not responding to standard regimen of first line anti-tubercular treatment. Smears were stained by Ziehl-Neelsen method. All specimens were processed for culture and drug sensitivity by drug proportion method using Lowenstein Jensen medium as well as GeneXpert MTB/RIF assay.

Results: A total of 125 subjects were registered in present study including 64 (51.2%) males and 61 (48.8%) females of age 15 years and above with mean age of 36.9 ± 14.99 . Sensitivity and specificity of the assay was observed as 92.1% and 93.5%, respectively. Association of rifampicin resistant by MTB/RIF assay and isoniazid resistance was found to be 88.1% and an agreement rate of rifampicin resistance by GeneXpert MTB/RIF assay with isoniazid resistance was 81.25%. A total of 56 (44.8%) cases were found to be multidrug resistant patients and an agreement rate of 92.9% (52/56) was demonstrated in multidrug resistant patients which was found to be rifampicin resistant by GeneXpert in present study.

Conclusion: GeneXpert MTB/RIF assay shown high sensitivity (96.7%) and specificity (98.6%). This most modern and latest technique, particularly in smear negative patients, helps rapid detection of TB and rifampicin resistance, which facilitates prompt diagnosis of multidrug resistant TB. These results propose that effective treatment can be initiated at an early stage, which will greatly help in reducing multidrug resistant TB.

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Introduction

Tuberculosis (TB) is a communicable disease that is caused by *Mycobacterium tuberculosis* (MTB). Disease ranks the fore most causes of death due to any microorganism. Initially, TB attacks the lungs, however it can infect other organs including lymphatic system, central nervous system, blood circulation, alimentary system and hence can affect almost any part of the body⁽¹⁾. Nearly 1/3 of total world's population has been infested with MTB and at danger to develop TB⁽²⁾. The estimated global incidence rate of TB remained 128 patients among one hundred thousand people and 1400 thousand died of TB in 2010⁽³⁾.

Incidence of TB in Pakistan is as high as 80/100,000 population with smear positive pulmonary TB, 177/100,000 over all pulmonary TB⁽⁴⁾ and 231/100,000 of all kinds of TB including pulmonary and extra-pulmonary TB every year till 2010, moreover death rate due to TB remained 34/100,000 population in Pakistan is higher than the neighbor country India that comprises 20/100,000⁽⁵⁾. Economic burden of TB has considerable impact in Pakistan as country spends 5.1% of the total health budget and contains high rank of 6th among high burden of TB countries around the Globe⁽⁴⁾.

Multi drug resistant TB may be defined as any strain belongs to MTB complex that is resistant to rifampicin and isoniazid simultaneously, either resistant to other first line drugs or not⁽⁶⁾. In such cases, it became necessary for the TB patient to undergo drug susceptibility testing. Standard drug proportion method on Lowenstein Jensen media has been extensively used and thought to be the gold standard drug susceptibility testing of MTB⁽⁷⁾. But this technique necessitates about six weeks for isolation of MTB stains and further 4-5 weeks for sensitivity testing hence causes significant delay in detection from clinical specimens. During this time clinicians have only option to treat patients with standard regimen as susceptible TB. During this delay in desired treatment may affect badly in final outcome as well as transmission of primary drug resistant types⁽⁸⁾.

Numerals of modern diagnostic methods brought new approaches not only in diagnosing but drug susceptibility testing of MTB. Therefore, practical complexities of these tests and their reliability on excited Lab substructure limit implementation espe-

cially among high burden setting with low economic structure⁽⁹⁾. GeneXpert MTB test has been recently introduced which detects the existence of MTB complex and rifampicin resistance simultaneously⁽¹⁰⁾. This is an automated system and relies on hemi nested/real time PCR assay assimilated in one cartridge. Direct clinical specimen or decontaminated centrifuged specimens are recommended for processing that takes only 2 hours to provide final results⁽¹⁰⁾. This assay has been recommended by the World Health Organization (WHO) and its utility has been expected three times increase for diagnosing drug resistant TB patients⁽¹¹⁾.

Mutations in *rpoB* gene 81 bp region confer the resistance to rifampicin and the region is called rifampicin resistance determining region (RRDR). However, the nature and incidences of alterations in the *rpoB* gene of rifampicin resistant MTB vary considerably according to the geographical location. GeneXpert MTB/RIF assay is new in Pakistan and uses RRDR for diagnosis of rifampicin resistant isolates. No doubt GeneXpert MTB/RIF assay is a great achievement; however its validation and agreement rate should be monitored and calculated at local level, which should ultimately help the clinician in rapid treatment and provides a clue towards the multidrug resistant TB. However it is not necessary that all the cases, which are resistant to rifampicin, should also be resistant to isoniazid as a study from this area showed resistance of about 40% in rifampicin, which were not resistant to isoniazid with or without resistance to other first line drugs⁽⁶⁾.

Multidrug resistant TB is a fatal disease and threatens the whole world. Moreover extra drug resistance (XDR) is also emerging which is quite alarming and can lead to untreatable TB. Its prompt diagnosis and treatment is extremely important. GeneXpert assay is very handy technique and its application in laboratories may revolutionize the tendency of TB management in Pakistan and other countries. Therefore on the basis of above mentioned references it is necessary to validate the utility of GeneXpert MTB/RIF assay kit on local level as manufacturer validate it by taking the samples from South Africa, Azerbaijan, Peru and India⁽¹²⁾.

Current research was carried to assess the application of GeneXpert MTB/RIF assay in the diagnosis of rifampicin resistant TB and its utilization in treatment of multidrug resistant TB and validation of the meth-

od by comparing it with conventional standard drug proportion method.

Methodology

A cross-sectional study was undertaken in Pakistan Health Research Council TB Research Centre in collaboration with Department of Pulmonology King Edward Medical University, Mayo Hospital Lahore during May 2013 to August 2014. Sample size of 125 patients was estimated by taking 5% level of significance, prevalence of rifampicin resistance 65% using 8% precision level and expected sensitivity and specificity of 86% and 90% simultaneously. Non-probability convenient sampling technique was used in present study.

Multidrug resistant TB patient is defined as person who remains sputum positive after 3 months of standard regimen i.e. first line anti-tubercular drug (ATT). These confirmed TB patients and their contacts after screening for AFB were included in this study.

Patients already taking 2nd line of ATT were not included in this study, as they have already been declared confirmed multidrug resistant TB patients. Structured questionnaire was used to gather the information. Sputum samples from 125 confirmed pulmonary TB patients who were not responding to standard regimen of first line ATT, Contacts of known multidrug resistant TB patients or health workers which are at high risk of acquiring multidrug resistant TB were collected from out patients and inpatients of Department of Chest Medicine, Mayo Hospital Lahore.

Both direct smears and concentrated smears were performed. Sputum specimens were added an equal amount of 4% NaOH to digest and decontaminate. Samples were then treated with phosphate buffer PH 6.8 that neutralizes the basic effect of NaOH. Samples were then centrifuged at 3000rpm for 15 minutes. After concentration supernatant were discarded and sediment were re-suspended in small amount (1-2 ml) of phosphate buffer. Half of the re-suspended sediment was used for inoculation on Lowenstein Jensen media slants and concentrated smear preparation and other half shall be processed for GeneXpert MTB/RIF assay as prescribed by in the protocol⁽¹³⁾. Further steps for staining, isolation and drug sensitivity testing shall be as follows.

Smears were covered by liquid 1% carbolfuchsin. For

decolonization and secondary staining, 25% sulphuric acid and 0.3% methylene were used simultaneously. A 100X oil immersion lenses were used to observe the smears. Reporting was done according to WHO/IUTALD recommendations⁽¹⁴⁾.

Culture was reported positive on appearance of even single colony. Culture reporting criteria shall be used as already explained in the other study⁽⁷⁾. For internal quality control each known negative and positive smears were stained in every batch. For external quality assurance smears were also shared with provincial TB control program. Known American Type culture control (ATCC) was used as positive control and sterile distil water was used as negative control for internal quality assurance of Lowenstein Jensen media. Random slants of Lowenstein Jensen media inoculated with sterile distilled water are also be incubated from each batch as negative controls. Culture positive isolates are run on *para-nitro benzoic acid* containing media to differentiate *Mycobacterium tuberculosis* complex from non-tubercular *Mycobacteria*.

After primary isolation, pure growths thus obtained were sub cultured on drug containing Lowenstein Jensen medium. Recommended concentrations of the drugs were used in the medium as described in previous studies⁽⁷⁾. For internal quality assurance sensitive and resistant specimens from previous batches are run parallel to the current batches while for external quality assurance National Reference Laboratory Islamabad send 30 strains which they import from Belgium each year and then results are shared and they send their report back to the research centre.

Results

A total of 125 subjects were registered in present study including 64 (51.2%) males and 61 (48.8%) females of age 15 years and above with mean age of 36.9±14.99. Most of 73 (58.4%) subjects were of age 35 or below and 64 (51.2%) were illiterate. A total of 111 (87.8%) subjects belong to poor or lower middle class. Demographic characteristics of study subjects are shown in Table 1.

Family history of tuberculosis was present in 81 (64.8%) subjects while 65 (52.0%) had history of contact with multidrug resistant TB patients. Almost all of 125 (100%) patients had fever, fatigue 123 (98.4%) and cough in 120 (96.0%) subjects as shown in Table 2.

Table 1: Demographic characteristics and smear results of study subjects (N=125).

Demographic factors	Characteristics	Mean \pm SD	N	%
Gender	Male		64	51.2
	Female		61	48.8
Marital status	Married		79	63.2
	Unmarried		42	33.6
	Others*		4	3.2
Age in years	Mean	36.9 \pm 14.99		
	Range	16-78		
Age classes	<25		42	33.6
	26-35		31	24.8
	36-45		18	14.4
	46-55		19	15.2
	56-65		11	8.8
	66+		4	3.2
Family size	Mean	8.2 \pm 2.36		
	Range	4-14		
Education	Illiterate		64	51.2
	Primary		2	1.6
	Middle		14	11.2
	Matriculation		31	24.8
	Intermediate		5	4.0
	Graduate and above		9	7.2
Socio economic status	Poor class		46	35.8
	Lower middle class		65	52.0
	Upper middle class		11	8.8
	Higher class		3	2.4
History of smoking	Smokers		53	42.4
	Non smokers		72	57.6
Smear result	Scanty		6	4.8
	1 ⁺		59	47.2
	2 ⁺		38	30.4
	3 ⁺		22	17.6
History of anti TB treatment	Cat-I		69	55.2
	Cat-II		42	33.6
	Cat-I & Cat-II		14	11.2
Previous anti TB treatment outcome	Cured		39	31.2
	Defaulted		10	8.0
	Failure		72	57.6
	Others		4	3.2

*Divorced=1, Separated=1, Widows=2

Table 2: Clinical history, sign and symptoms of study subjects (N=125)

Factors	Present		Absent	
	N	%	N	%
Family history of tuberculosis	81	64.8	44	35.2
History of MDR contact	65	52.0	60	48.0
Fever	125	100.0	0	-
Fatigue	123	98.4	2	1.6
Cough	120	96.0	5	4
Weight loss	114	91.2	11	8.8
Chest pain	112	89.6	13	10.4
Expectoration	105	84.0	20	16.0
Anorexia	97	77.6	28	22.4
Breathlessness	96	76.8	29	23.2
Hemoptysis	32	25.6	93	74.4

A total 63 (50.4%) subjects shown resistance on culture by drug proportion method, while 58 were found to be resistant by GeneXpert MTB/RIF assay showing an agreement rate of 92.07%. Two out of 5 subjects, who showed intermediate results by GeneXpert MTB/RIF assay, were also found to be resistant on culture by drug proportion method. Comparison of rifampicin resistance by both methods is shown in Table 3.

Table 3: Comparison of GeneXpert MTB/RIF assay with drug sensitivity testing of rifampicin on culture.

Rifampicin on GeneXpert	Rifampicin on culture		Total	
	Resistant	Sensitive		
	N	%	N	%
Resistant	58	98.3	1	1.7
Sensitive	3	4.9	58	95.1
Intermediate	2	40.0	3	60.0
Total	63	50.4	62	49.6

Sensitivity = 92.1%, Specificity = 93.5%, PPV = 98.3%, NPV = 95.1%, Accuracy = 96.7%

A total of 64 (51.2%) study subjects shown resistance by drug proportion method of which 52 subjects were also found to be rifampicin resistance on GeneXpert. This picture shows an agreement of 81.25%. This elaborates that there are 81.25% chances of rifampicin resistance on GeneXpert if the specimen is resistant to isoniazid by drug proportion method as shown in Table 4. There are 7 (11.9%) cases, which were resistant to rifampicin by GeneXpert method, but sensitive to isoniazid by drug proportion method moreover one of these 7 cases was also sensitive to

rifampicin by drug proportion method.

A total of 56 (44.8%) out of 125 cases were found to be multidrug resistant patients in present study. A high agreement rate of 92.9% (52/56) was demonstrated in multidrug resistant patients found to be rifampicin resistant by GeneXpert in present study.

Table 4: Comparison of GeneXpert MTB/RIF assay with drug sensitivity testing of isoniazid on culture.

Rifampicin on GeneXpert	Isoniazid on culture				Total	
	Resistant		Sensitive			
	N	%	N	%	N	%
Resistant	52	88.1	7	11.9	59	100.0
Sensitive	10	16.4	51	83.6	61	100.0
Intermediate	2	40.0	3	60.0	5	100.0
Total	64	51.2	61	48.8	125	100.0

Sensitivity = 83.9%, Specificity = 87.9%, PPV = 88.1%, NPV = 83.6%, Accuracy = 85.8%

Discussion

The main hindrance in controlling multidrug resistant TB is its delayed diagnosis which is further necessary to start the speedy treatment and protect the public from direct exposure from this deadly microorganism. It takes only two hours for GeneXpert MTB/RIF assay to show the results of rifampicin susceptibility in smear positive TB patients. There is strong endorsement from WHO to use this rapid technique for initial detection of multidrug resistant TB suspects, Human immunodeficient virus (HIV) infection with TB and conditionally recommended where multidrug resistant TB and/ or HIV are not important and plenty of smear negative specimens⁽¹¹⁾.

Sensitivity and specificity of GeneXpert MTB/RIF assay was found to be 92.1% and 93.5%, respectively in the present study are comparable with sensitivity (96.7%) and specificity (98.6%) claimed by the manufacturer⁽¹³⁾ however; study is not comparable with a study which presented much lower sensitivity of 81.3% from London⁽¹⁵⁾. Few other studies from various parts of the world, which used RRDR's of rpoB gene, had also shown lower level of sensitivities ranging 78.0%-86.0%^(16,17). Positive predictive value (PPV) 98.3 while, negative predictive value (NPV) 95.1 of this technique is also in agreement with efficiency report of the instrument⁽¹³⁾.

Association of rifampicin resistant by MTB/RIF assay and isoniazid resistance was found to be 88.1% and an agreement rate of rifampicin resistance by GeneXpert MTB/RIF assay with isoniazid resistance was 81.25% in present study. However an overall resistance to rifampicin and isoniazid by drug proportion method was found to be 51.2% and 50.4% respectively was almost same. A study based on thirteen years data from same settings has already revealed almost an equal resistance to rifampicin (26.45%) and isoniazid (24.12%) however shown a lower agreement rate of 59.5% (419/704) rifampicin resistant cases, which must be isoniazid resistant⁽⁷⁾.

GeneXpert MTB/RIF assay has been designed to reduce the delay in prompt diagnosis and precludes the drug susceptibility by providing the rapid rifampicin resistance status as is shown in present study. This fact has already revealed and in another study from these settings where 692 contacts of 112 index patients were screened and 17 contacts were found to be smear positive of which 4 (23%) were rifampicin resistant by GeneXpert MTB/RIF assay⁽¹⁸⁾. Hence this technique provided quick detection and makes the choice for clinicians to take it up and participate in controlling the multidrug resistant TB measures. As the definition of multidrug resistant TB is "*Mycobacterium tuberculosis* organism resistant to both most powerful drugs i.e. rifampicin and isoniazid, either resistant or sensitive to other first line anti TB medicines".

In present study a total of 59 cases were found to be rifampicin resistant by GeneXpert MTB/RIF assay of which 88.1% were declared as multidrug resistant and 11.9% were resistant to rifampicin only and were not declared as multidrug resistant by drug proportion method. Moreover out of 5 specimens who were shown as intermediate results by GeneXpert MTB/RIF assay 2 were found to be multidrug resistant TB by drug proportion method. Therefore exemption to get final results by culture method could not be lounged yet moreover if organism is mono resistant to isoniazid only then other first line anti TB drugs are effective as is shown in present study where 3.2% cases are only resistant to rifampicin. Other studies have also shown mono resistance to rifampicin and other first line anti TB drugs^(7,19).

Although GeneXpert MTB/RIF assay is a great achievement and supporting on much higher levels in diagnosing drug resistant TB than convention-

al techniques even then this does not mean to stay calm against this deadly organism. Each and every effort in diagnosing multidrug resistant TB should be appreciated. Genotype MTBDR *plus* is another molecular technique which diagnoses both isoniazid and rifampicin resistance simultaneously and has been tested to a minimal level in developing countries. Munir *et. al.* in their study have validate this technique by comparing the results with conventional drug proportion method in which various pulmonary and extra pulmonary specimens were used. The study demonstrated 100% sensitivity, 66.7% specificity, 86.9% PPV and 100% NPV⁽²⁰⁾.

Demographic characteristics revealed in present study like gender, mean age, education, socio-economic status, signs symptoms and history of smoking etc. were much likely to other studies^(18,20).

Conclusion

GeneXpert MTB/RIF assay shown high sensitivity (96.7%) and specificity (98.6%). This most modern and latest technique particularly in smear negative patients helps rapid detection of MTB and rifampicin resistance, which facilitates prompt diagnosis of multidrug resistant TB. This means that effective treatment can be initiated at an early stage, which will greatly help in reducing multidrug resistant TB.

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Author's Contribution

Muhammad Kashif Munir: Presented the idea of research and wrote the article.

Sana Rehman: Reviewed the literature and collected the data.

Rizwan Iqbal: Supervised the project and drafted the manuscript.

Muhammad Saqib Saeed: Revised the final draft.

Muhammad Asim: Analysed the data.

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