Evaluating the diagnostic accuracy of Gene Xpert MTB/RIF in Pulmonary Tuberculosis in Kohat Division

Saeed Khan[1], Zia Ullah[2], Lala Ruhk[3], Muhammad Haroon[4], Afsheen Bashir[5], Halima Sadia[6], Maryum Ashfaq[7]

[1]Department of Medicine, KDA Hospital Kohat - Pakistan

[2]Department of Pulmonology, Lady Reading Hospital Peshawar - Pakistan

[3]Hayatabad Medical Complex Peshawar - Pakistan

[4]Quaid E Azam University Islamabad - Pakistan

[5]Basic Health Unit Malana, Dera Ismail Khan – Pakistan

[6]Department of Pharmacology, Bacha Khan Medical College, Mardan-Pakistan

[7]Peshawar Medical College Peshawar-Pakistan

Address for correspondence
Zia Ullah
Department of Pulmonology, Lady Reading Hospital Peshawar - Pakistan
Email: drzia02@gmail.com

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SK ZU conceived idea, ZU MH drafted the study, SK ZU SK collected data, LR AB HS MA did statistical analysis & interpretation of data, SK LR AB critical reviewed manuscript, All approved final version to be published.

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The Authors declares that there is no conflict of interest.

Abstract

Background: Tuberculosis (TB) is considered to be one of the most prominent reasons for death globally caused by a single microorganism [1]. However with the advancement in the field of human health, researchers have mostly focused on the strategies to eradicate the TB but it has not been achieved yet because of the major gaps in terms of resource-constrained settings and is case of huge burden of the disease. This study was conducted with the objective to find out the importance and diagnostic accuracy of Gene Xpert MTB/RIF in Pulmonary Tuberculosis in Kohat Division

Methodology: This is observational study conducted to evaluate suspected MDR-TB patients from Kohat Division. All suspected cases were refered to Xpert Lab and then there sputum were sent to Provincial Referencing Laboratory (PRL lab) Hayatabad Peshawar for Culture and Drug Susceptable TB testing. Data analysis was done in SPSS 21 and MS Excel 2013 version.

Results: A total of 925 patients were included in the study consecutively. In the total patient population 709 (76.6%) were having mean age from 15-44 years. Out of 925 patients 408 (44.1%) were males and 517 (55.1%) were females. The overall sensitivity of Xpert MTB/RIF in detecting culture-positive pulmonary TB was 56.8% (519/925) and culture Negative Pulmonary TB was 43.8% (406/925).

Conclusion: The large-scale demonstration provides a robust data on the potential for increased case finding of TB and DR-TB through routine use of a high sensitivity molecular diagnostic test for TB and DR-TB (Xpert MTB/RIF) in public sector medical services. Our observations may be useful in guiding the decisions on the scale-up of XpertMTB/RIF in high-burden settings.

Key Words: TB; MDR-TB; XpertMTB/RIF; Kohat

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Introduction

Tuberculosis (TB) is considered to be one of the most prominent reasons for death globally caused by a single microorganism.[1] However with the advancement in the field of human health, researchers have mostly focused on the strategies to eradicate the TB but it has not been achieved yet because of the major

gaps in terms of resource-constrained settings and is case of huge burden of the disease. According to the reports of World Health Organization (WHO) approximately 54 million TB deaths were prevented between 2000 and 2017 because of the improvement in the management, preventive and service delivery strategies. Besides the huge declination in the mortality ratio still around 10 million people are affected with TB every year.[1] A significant challenge has been posed by the emergence of highly resistant strains such as Multidrug-resistant tuberculosis (MDR) and extremely drug-resistant (XDR). MDR-TB is described as the resistant against the two most potent drugs including Rifampicin and Isoniazid while the XDR is type of tuberculosis with further bacillary resistance against any fluoroquinolone and against one of the three drugs such as Amikacin, Kanamycin, and Caoreomycin.[2] Therapeutic advancement is still inadequate, but however with the introduction of newer diagnostic strategies such as the Gene Xpert MTB/RIF, DST and use of smear culture the TB incidences have been greatly reduced. Lag time in the initiation of the treatment can be decreased with the early and rapid diagnosis of the TB which will effectively reduce the transmission rates.[3] Chromosomal mutation during mycobacterium replication can lead to genetic resistance against the naturally most potent anti-TB drugs. The inappropriate use of anti-TB drugs has given rise to MDR-TB. MDR-TB is considered one of the most imminent threats in the path of eradicating TB .[4]

Globally, MDR-TB is an emerging health threat. According to the reports of WHO, the incidence rate of new cases of MDR-TB is 2.9%.[5] Pakistan is ranked as the fifth most affected country with TB among the 22 high burdened countries and is at fourth position in the list of 27 most affected countries with MDR-TB.[6] In the list of new TB cases the frequency of MDR-TB is 3.4% and 21% in the list of retreated cases.[7] The main reasons for the emergence of MDR strains of mycobacterium are malnutrition, poverty, poor health facilities, population migration, urbanization, refugee influx, political instability, inadequate housing and sanitation.[4] The treatment of MDR-TB is possible but the cost will be 100 times greater than treating common TB.[8] Treatment failure will cost more and more in the future and 10-15 healthy people will be affected every year with each MDR-TB patient if not treated properly.[5]

In Pakistan, the conventional diagnostic practice is adapted to test all the suspected TB cases with Gene Xpert MTB and sputum smear microscopy. After the initial assessment in case of first-line drug-resistance the TB positive cases are further investigated for first-line DST and second-line DST. Obtaining culture and DST results prolong the diagnosis and treatment initiation process for MDR-TB in Pakistan. The Xpert MTB/RIF assay was endorsed by WHO in December 2010 as a rapid molecular test for the detection of TB and rifampicin - resistant TB.[9]

As compared to the efficiency of the conventional method of smear microscopy the Xpert method has a sensitivity of 89% and specificity of 99% for detecting TB. While in comparison with the method of phenotypic DST the Xpert method can detect the RR-TB with specificity 98% and sensitivity of 95%.[10] In terms of time required for obtaining the results, the DST and culture-based methods take weeks to months while the results with Xpert can be obtained in two hours. MDR-TB is mostly represented with RR-TB, but however in most of the cases the resistance developed against the rifampicin is frequently associated with resistance against isoniazid and this occurs in settings where fixed-dose combination first-line anti-TB drugs are administered.[11]

Xpert MTB/RIF is an advanced automated, Hemi-nested RT-PCR used for the detection of MTB which also uses the molecular beacons to test every positive sample for the assessment of rifampicin sensitivity. Thus the results for MTB and rifampicin resistance can be obtained in less than two hours which is very efficient and short term process as compared to the 8-10 weeks sensitivity testing with conventional drugs. The technique is cartridge-based nucleic acid amplification test (CBNAAT) which does not have any specific requirements for its setup and can be conducted with little technical training. During the processing the tubercle bacilli are inactivated with bactericidal reagents thus eliminating bio-safety risks. The method can be used as rapid point of care diagnostic test.[12]

The study was done at Gene Xpert Lab, located at Lady Reading hospital Peshawar. The objective of the current study was evaluating the performance of culture microscopy, Xpert MTB/RIF assay in diagnosing PTB and detecting resistance to rifampicin, taking culture as the gold standard for the confirmed diagnosis and drug sensitivity.

Methodology

The study included the patient samples from Kohat division including Kohat, Hangu and Karak districts, KP, Pakistan. The samples were selected on the basis of clinical suspicion of PTB (from 2012 to 2019). These subjects were either treatment naïveor were on the anti TB treatment for more than two weeks. The samples collected from suspected TB patients were processed at the Tuberculosis laboratory of Lady Reading Hospital (LRH) with Microscopy culture and Gene Xpert. Samples were also sent to Provincial

Referencing Laboratory (PRL) for DST. A total of 925 patients were included in the study consecutively.

Individual patient data was collected and recorded in the TB 01 file and Electronic Nominal Registration system (ENRS). It included registration number, weight, previous FLD's treatment history, previous SLD's treatment history, demographic data, sex, test results of Xpert, smear microscopy, culture, DST, date of sputum collection, treatment category and date of treatment initiation.

Test Methods

The samples were subjected to Ziehl-Neelsen (ZN) staining, Xpert MTB/RIF (Cepheid, Sunnyvale, US) assay, and culture inoculation. The technicians performing culture inoculation were unaware of Xpert MTB/RIF test results. Culture and Drug Susceptibility Testing (DST) on culture media were taken as the reference standard for MTB detection and rifampicin susceptibility respectively.

Xpert MTB/RIF

The Xpert MTB/RIF test was performed using the G4 version of cartridges according to the manufacturer's instruction (Cepheid, Sunnyvale, CA). Unprocessed samples were used directly for performing the test and no frozen samples were used in the study.

AFB Smear

Two smears were made for each sample. One slide for AFB smear was made directly from the sample while the other slide was made after the sample was decontaminated. After decontamination, the re-suspended pellet in phosphate buffer saline (PBS) was used to make smear on a glass slide and these slides were then stained using the ZN staining method as per the standard protocol and then observed under the microscope.[13]

Statistical Methods

Data analysis was done in SPSS 21 and MS Excel 2013 version.

Results

In the current study, a total of 925 patients were

Table 1: Demographic characteristics of enrolled patients

		No of patients (925)	Percentage
Age (Years)			
	> 14	32	3.4
	15-44	709	76.6
	45-64	163	17.6
	>65	21	2.27
Weight (Kg)			
	<40	291	31.45
	40-60	593	64.1
	>60	41	4.4
Gender			
	Male	408	44.1
	Female	517	55.1
Marital status			
	Married	621	67.1
	Unmarried	301	32.5
	Widow	3	0.4
Characteristics of study population			
Past TB Treatment category			
Newly diagnosed		302	32.6
CAT I		484	52.3
CAT II		139	15.1

Table 2: Overall finding of study

Resistant pattern	Frequency	Percentage
Total patients	925	100
RRD	237	25.62
Fluroquinolone resistant	71	29.3
Extensive Drug - Resistant TB (XDR-TB)	17	1.8

included. In the total patient population 709 (76.6%) were having mean age from 15-44 years. Out of 925 patients 408 (44.1%) were males and 517 (55.1%) were females. After the collection of sputum microscopy culture and Gene Xpert was done from presumptive DR-TB patients. On the basis of past TB treatment category out of total patients 302 (32.6 %) were newly diagnosed while 484 (52.3%) were CAT I patients and 139 (15.1 %) were CAT II patients as shown in Table 1. The overall sensitivity of Xpert MTB/RIF in detecting culture-positive pulmonary TB was 56.8% (519/925) and culture Negative Pulmonary TB was 43.8% (406/925) as shown in Table 2. Among the total patients examined in the current study 237 (25.62 %) Xpert MTB/RIF positive pulmonary TB cases had shown rifampicin resistance.

Discussion

In this large-scale demonstration study across diverse settings in Pakistan, Xpert MTB/RIF arrangement as the initial TB diagnostic test in public health facilities, significantly increased TB case finding. The substitution of smear microscopy by Xpert MTB/RIF on average increased the rate of TB case notification by 16% and of bacteriologically confirmed TB case notification by 39%. Similarly, the proportion of presumptive TB patients with a TB diagnosis increased by 11% for all forms of pulmonary TB, and by 33% for microbiologically confirmed TB cases, taking confounding by age, sex and history of prior TB treatment into account. These findings are similar to those reported from South Africa and Brazil and underscore the potential benefit of using a rapid, high sensitivity TB diagnostic test. [14-15]

The decrease in the proportion of clinically diagnosed TB cases is indicative of the potential benefit in terms of reduced attrition of DR-TB cases, time to diagnosis, and cost of diagnosis, both to the patient as well as to the health system. However, data from South Africa and Brazil also showed an increase in bacteriologically confirmed TB but no increase in persons started on treatment.[16]

There was also a substantial increase in the detection of rifampicin-resistant TB cases by offering a rapid drug resistance test to all presumptive TB patients, using Xpert MTB/RIF, instead of only selectively offering conventional DST to already diagnosed TB patients with a high risk of having drug resistance. Similar findings were documented in the past in studies conducted in South Africa, Uganda and India with Xpert MTB/RIF.[17] In our study, almost 56.8% of rifampicin-resistant TB cases were detected among Xpert MTB/RIF positive TB cases with 32.6 % no prior history of TB treatment. These findings demonstrate the potential impact of extending universal DST to all presumptive TB cases under routine program conditions in improving case finding of TB as well as rifampicin-resistant TB, particularly in areas where drug-resistance in treatment naïve cases is of substantial concern.

Conclusion

The large-scale demonstration provides a robust data on the potential for increased case finding of TB and DR-TB through routine use of a high sensitivity molecular diagnostic test for TB and DR-TB (Xpert MTB/RIF) in public sector medical services. Our observations may be useful in guiding the decisions on the scale-up of XpertMTB/RIF in high-burden settings.

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