Detection of *Mycobacterium tuberculosis* by GeneXpert in Smear Negative Presumptive Case of Pulmonary Tuberculosis Patients

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ABSTRACT

Objectives: This study aimed to determine the efficacy of GeneXpert to identify smear-negative pulmonary tuberculosis patients

Methods: A cross-sectional descriptive study was carried out among 166 outpatients who visited the laboratory Unit of Jalal-Abad Tuberculosis Centre located in Kyrgyzstan. Non- probability; convenient sampling type was used to collect the data for the study. Standard Procedure that was described by the operating manual (Cepheid Inc, Sunny Vale USA) of GeneXpert machine was used for the procedure.

Results: Out of 166 smear negative cases, 45 (27.1%) cases were shown to be positive by GeneXpert. Among the 45 cases, the GeneXpert analysis revealed that 60% were sensitive towards rifampicin and only 24.4% were resistance, remaining were intermediate.

Conclusion: This research shows that Xpert MTB/RIF can identify TB in patients with negative smear tests with a good detection rate, and results would be available the same day, avoiding patient loss and treatment delays.

Keywords: Tuberculosis, Presumptive, Pulmonary, GeneXpert, *Mycobacterium tuberculosis*

INTRODUCTION

Tuberculosis is one of the most widespread infectious illnesses in South Asia. A good management of disease control and transmission necessitates an early and precise diagnosis. It is the primary global cause of morbidity and mortality. Six million people died from TB in 2021. This includes the 187,000 HIV-positive individuals. There were 1.2 million children, 3.4 million women, and 6 million men among them (Bagcchi 2023).

The Xpert MTB/RIF assay is a molecular test that is used for tuberculosis (TB) control by contributing to the rapid diagnosis of tuberculosis also testing for resistant to drug rifampicin. The test detects *Mycobacterium tuberculosis* complex (MTBC) and resistance to rifampin (RIF) in less than 2 hours. In comparison, standard cultures can take 2 to 6 weeks for MTBC to grow and conventional drug resistance tests can add 3 more weeks. The information provided by the Xpert MTB/RIF assay aids in selecting treatment regimens and reaching infection control decisions quickly (CDC 2005). GeneXpert test is highly sensitive and specific test for the diagnosis of TB that detects DNA sequences specific for *M. tuberculosis* and rifampicin resistant. *rpoB* gene (Tamer and Gulekon 2022). According to the best estimates, rifampicin-resistant tuberculosis (RR-TB), the most effective first-line treatment, affected 558 000 persons worldwide in 2017 (range: 483000–639000), and 82% of these individuals had MDR-TB, or multidrug-resistant tuberculosis. Nearly half of all MDR/RR-TB cases worldwide in 2021 were found in three nations: Pakistan (7.9%), China (14%) and the Russian Federation (8.5%), as well as India (26%) (Kostyukova et al. 2023).

Sputum smear microscopy is a quick, easy, and

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affordable method for identifying pulmonary tuberculosis, although prior research has shown that its sensitivity is low and unpredictable. The "gold standard" for MTB detection, mycobacterial culture, which has the highest sensitivity for identifying and confirming active TB, takes 2 to 6 weeks to interpret. In countries like Nepal and Kyrgyzstan, a quick diagnostic method like GeneXpert is highly desired in order to identify the numerous instances of pulmonary tuberculosis, both sputum positive and negative. MTBC DNA is found using the GeneXpert MTB/ RIF test. This study aims to assess GeneXpert's performance in pulmonary TB diagnosis with sputum-negative specimens.

MATERIALS AND METHODS

It was a descriptive cross-sectional study done at Jalal-Abad Tuberculosis Centre located in Kyrgyzstan. It is the largest Centre for the diagnosis and control of tuberculosis. The entire patient who came to the outpatient department and visited the laboratory Unit of Jalal-Abad Tuberculosis Centre located in Kyrgyzstan. The time duration of the study was six months (mid of September to mid of March 2023). Non- probability, convenient sampling type was used to collect the data for the study along with a semi structured close ended questionnaire. A total of 166 samples that were smear negative were taken. Only the symptomatic cases of presumptive pulmonary tuberculosis from 15yrs to 70 years of age group was taken into consideration whereas asymptomatic cases, extra pulmonary tuberculosis and diagnosed case of XDR and MDR were excluded. Data was analyzed by the using Statistical Package for Social Science (SPSS) software.

Xpert MTB/RIF assay

All the procedures for the Xpert assay were conducted as per the manufacturer's instructions (Cepheid 2013). In brief, the sample reagent (mixture of NaOH and isopropanol) in the ratio of 1:2 was added to sputum samples in the Falcon tube. The mixture was vortexed thoroughly until the clear solution was seen and incubated at room temperature for 15 min to minimize biohazard by reducing the viability of *M. tuberculosis*. Then, 2 ml of clear solution was added to the labeled cartridge with the help of a sterile dropper and the cartridge was incubated inside the Xpert machine. Results were obtained in the Xpert system within 2 h. The samples yielding positive results in at least 1 of the 2 cartridges of 2 sputum specimens, i.e. spot and morning, in Xpert was considered as the Xpert-confirmed TB.

Ethical consideration

Approval was taken from the research committee, from the University before conducting the study. Consent was taken from the respondent before conducting the data collection. Permission was taken Jalal-Abad Tuberculosis Centre, Jalal Abad before conducting study (Reg. No.: 340/08)

RESULTS

Distribution of participants by TB in family or close contacts

The figure shows that among 166 respondents 59.1 % (n=98) had history of having their family members or close contact with TB positive whereas remaining 40.9 % (n=68) did not had history of having any family members neither close contact with positive records.

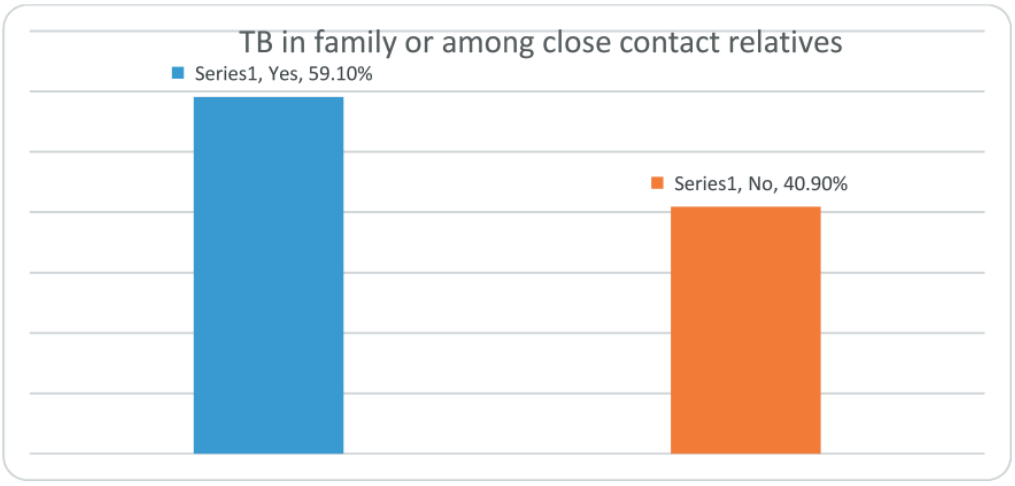

Figure 1: Distribution of suspected TB patients by having TB in family or among close contact.

Distribution of participants by multiple time occurrence of TB

Below figure shows that majority of respondents 62.50 % (n=10) said that they had TB twice before and remaining respondents 37.50% (n=6) said that they had TB thrice before.

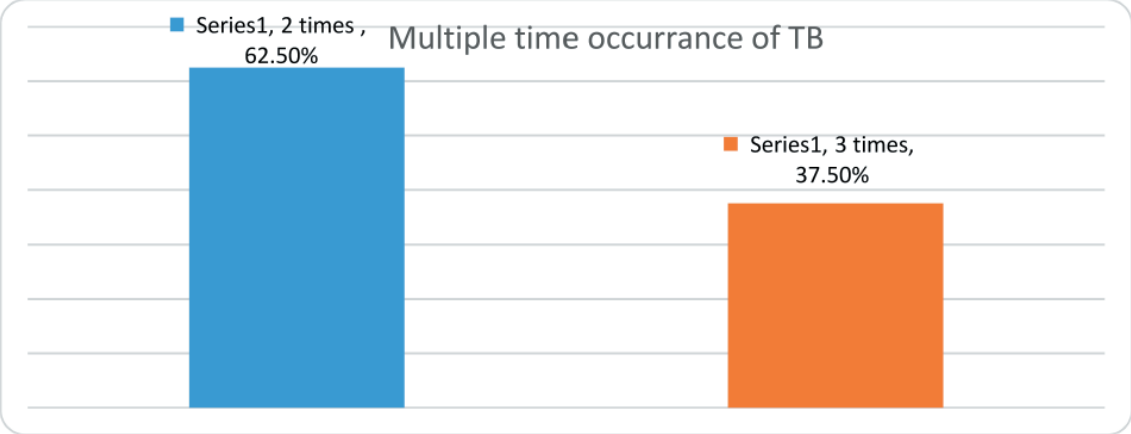

Figure 2: Distribution of suspected TB patients according to multiple time occurrence of TB

Distribution of suspected TB patient by chronic illness

Out of 166, 91 respondents also suffered from other chronic illness. Fifty-eight (63.74%) had Diabetic Mellitus (DM) followed by HIV positive (4%) and remaining 31.86 % (n=29) said they were having multiple chronic problems like kidney diseases, hypertension and renal diseases etc.

Table 1: Distribution of suspected TB patient by chronic illness

Responses	Frequency(n)	Percentage (%)
DM	58	63.74
HIV	4	4.40
Others	29	31.86
Total	**91**	**100.0**

Distribution of suspected TB patient by the result of GeneXpert

The table below show that 72.9% (n=121) of respondents had negative result of GeneXpert whereas remaining 27.1% (n=45) respondent only had positive results.

Table 2: Distribution of suspected TB patient by the result of GeneXpert

Response	Frequency(n)	Percentage (%)
Positive	45	27.1
Negative	121	72.9%
Total	**166**	**100.0**

Distribution of suspected TB patient by the positive result of GeneXpert towards Rifampicin susceptibility

Below table show that out of 45 positive case, 60 % (n=27) of respondents had sensitivity towards Rifampicin while 24.4% (n=11) had resistance and remaining 15.6% (n=7) were intermediate.

Table 3: Distribution of suspected TB patient by the positive result of GeneXpert towards Rifampicin susceptibility

Response	Frequency(n)	Percentage (%)
Sensitivity	27	60%
Resistance	11	24.4%
Intermediate	7	15.6%
Total	**45**	**100.0**

DISCUSSION

The study also revealed that the majority of respondents, 60.24% (n=100) had families with five or more members, followed by 30.12% (n=50) who had three to five, and 9.6% (n=16) who had up to three. Lienhardt et al. (2005) suggested that, the number of households in the compound and the size of each household both raised the risk of TB. More specifically, the risk rose with the number of people living in the home (P-value > 0.01) even though TB was unrelated to the occupancy rate of the home's rooms (Zhe 2022).

In this study, 90.4% (n=150) of the respondents had tuberculosis for the first time, and the remainder 9.6% (n=16) had it more than once, with 62.50% (n=10) having it twice before and the remaining respondents having it three times (n=6). Meta-analysis done by Anwar et al (2013) showed TB recurrence rates (with a 5-year recurrence rate) of approximately 9.5%, which is consistent with our result. In contrast Lin et al. (2021) found incidence of recurrent TB was 15.2%, of the recurrent cases, 55.2% happened within 2-year after completion of anti-TB treatment. Being an older adult (between the ages of 36 and 55), having PTB that was smear positive, having certain co-morbidities, such as HIV and COPD, and being unemployed were all risk factors for TB recurrence (Gadoev et al. 2017).

Moreover, this study shows that 54.8% (n=91) said that they have additional chronic illness other than TB, out of which 63.74% were suffering from diabetes mellitus, 31.86% were having multiple chronic problems like kidney diseases, hypertension etc. and 4.40% were suffering from HIV. Sedky et al. (2018) found diabetes mellitus (26.7%) as common associated disease followed by renal disease which is consistent with our study. Immunosuppressive conditions like HIV, Diabetes and chronic renal disease increase the risk of pulmonary TB by weakening the immune response against bacteria (Kumari and Meena 2014).

GeneXpert was found to be positive in 45 cases (27.1%) of our investigation, whereas it was found to be negative in 121 patients (72.9%). Rai et al. (2022) examined GeneXpert's performance in sputum-negative pulmonary TB detection. Out of 106 of the participants who received a recommendation to take the GeneXpert test for acid-fast bacilli, MTB was detected in 34.9% patients (37/106). According to Vasall et al. (2011), Xpert MTB/RIF resulted in a positive diagnosis for more than 30% of smear-negative individuals. Steingart et al. (2014) demonstrated that GeneXpert has a very high specificity so it can be utilized as a gold standard in situations when culture facilities are not accessible. According to Rimal et al. (2022) the GeneXpert MTB/RIF assay's diagnostic performance was nearly on par with that of culture and can therefore be trusted for MTB identification in sputum samples with negative smear tests. Meyer et al. (2017) concluded that for smear-negative patients, using GeneXpert may provide a quick diagnosis that would otherwise be overlooked. It has the potential to minimize the time and number of visits required to achieve a diagnosis as it replaces smear microscopy in an increasing number of high-burden nations (Pietersen et al. 2014).

In the investigation, the prevalence of Rifampicin resistance/sensitive was as follows, in individuals who tested positive for GeneXpert: 24.4% resistant, 15.6% resistance indeterminate and 60% sensitive. Rai et al. (2022) reported a significant MTB load. Patients with a positive GeneXpert test had a higher prevalence of MDR. 37.83% (14 of 37) of patients were positive and 13.2% (14 of 106) of the overall study population were sputum negative. They concluded that the GeneXpert assay generally shows early detection of MDR-TB especially when used in high-risk individuals as is recommended by WHO. They showed that it is still a useful test to confirm pulmonary tuberculosis even if the smear is negative. The RNA polymerase gene (*rpoB*) encodes the bacterial DNA-dependent RNA polymerase that rifampicin inhibits. Mostly, mutations in a small area of the *rpoB* gene have been linked to resistance to this medicine. Resistance to rifampicin can develop on its own or in combination with resistance to other medications, such as isoniazid. Rifampicin resistance alone may act as a stand-in for MDR-TB in high MDR-TB conditions. Those who have drug-resistant tuberculosis can spread the disease to others. (Beth 2014). RIF is a potent antibiotic that can effectively treat mycobacterial infections as well as other diseases, and the use of the proper combination therapy can typically stop the emergence of resistance while under treatment. Cross-resistance among the rifampicin now, which were used to treat mycobacterial infections seems to be significant. Due to these considerations, PCR-based diagnostics that quickly detect *M. tuberculosis* resistance have been developed (Mabhula and Singh 2019).

CONCLUSIONS

Patients with smear-negative TB benefits from GeneXpert, especially in locations without access to cultures. The assay is quicker than culture, can achieve reasonable sensitivity and specificity, and prevents patient loss and treatment delays. Xpert MTB/RIF treat is helpful tool for quickly identifying RIF-resistant *M. tuberculosis*. The greater prevalence of MTB in the smear-negative sputum sample indicates that authorities should monitor this area for efficient of MTB management. Hence more efforts must be made to carry out educational campaigns using all forms of communication with the goal of raising public awareness of TB.

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CONFLICT OF INTEREST

Authors declared no conflict of interest

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