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Short Communication

Diagnostic performance of GeneXpert MTB/RIF assay compared to conventional *Mycobacterium tuberculosis* culture for diagnosis of pulmonary and extrapulmonary tuberculosis, Nepal

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Abstract

Tuberculosis is an infectious disease caused by the *Mycobacterium tuberculosis*. It is a global health problem and major cause of death in resource-limited countries like Nepal. Timely diagnosis with sensitive testing methods could assist in early management of the disease. This study was conducted to compare the diagnostic performance of GeneXpert MTB/RIF and conventional acid-fast staining with *M. tuberculosis* culture. The study was carried out in the Department of Microbiology, Shree Birendra Army Hospital, Nepal. Samples (n=500) were tested with a GeneXpert MTB/RIF assay and acid-fast bacilli (AFB) smear microscopy. All samples were sent for *M. tuberculosis* conventional culture by the German-Nepal Tuberculosis Project, Kathmandu, Nepal (GENETUP). Out of a total 500 pulmonary and extrapulmonary samples tested, 97 samples were positive for *M. tuberculosis* by GeneXpert MTB/RIF assay. Out of the positive samples, only 95 samples were found positive by the culture method. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of AFB microscopy was 45.3%, 99.5%, 99.5% and 88.5%, respectively. The sensitivity, specificity, PPV and NPV of GeneXpert MTB/RIF was found to be 100%, 99.5%, 97.5% and 100%, respectively compared to the gold standard culture method. The GeneXpert MTB/RIF test was comparable with culture diagnosis of both pulmonary and extrapulmonary tuberculosis cases.

Keywords: GeneXpert MTB/RIF assay, pulmonary tuberculosis, extrapulmonary tuberculosis, sensitivity, specificity

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Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. According to the World Health Organization (WHO) factsheet data as of October 2020, a total of 1.4 million people died of TB including 208,000 people with human immunodeficiency virus (HIV)

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infection in 2019. Globally, TB is one of the top 10 causes of death and caused by a single infectious agent, *M. tuberculosis* [1]. In Nepal, according to the Annual Report from the National Tuberculosis Center (NTC), during 2020, 65,000 new cases and 17,000 deaths were reported [2]. Timely diagnosis of TB will contribute to achieve the aims of the End Global TB Strategy that aims to drop new TB cases by 80%, mortality due to TB by 90% and a total reduction of poverty experience by TB-affected families by protecting from catastrophic expenses by 2030 [2].

Microscopy, culture, and drug susceptibility testing (DST) are the standard methods for TB diagnosis that are used worldwide. Microscopy, however, has low sensitivity and culture with drug sensitivity testing has a long turn-around time of at least 8 weeks [3]. Rapid and accurate diagnosis of pulmonary and extrapulmonary TB remains a challenge in the context of countries such as Nepal where resources are limited and a lack of specific laboratory expertise exists [4-6]. The implementation of the GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, USA) had been approved by the WHO in 2010 for detecting *M. tuberculosis*. This method is fully automated cartridge-based real-time PCR. The system is designed in such a way that sample processing, DNA extraction and DNA amplification occurs in an integrated manner to diagnose *Mycobacterium* infection and detect rifampicin resistance in one setting [1]. The introduction of GeneXpert MTB/RIF assay for the diagnosis of both pulmonary and extrapulmonary TB has therefore revolutionized the field of TB diagnosis, ultimately improving early management and control of this important disease [7].

This study was conducted to evaluate the performance of GeneXpert MTB/RIF assay for detection of *M. tuberculosis* in clinical samples of suspected pulmonary and extrapulmonary TB cases from Nepal in comparation with the gold standard *M. tuberculosis* culture method.

Methods

Study design and patients

A prospective study to detect *M. tuberculosis* among clinically suspected cases of TB was conducted. All clinically suspected pulmonary and extrapulmonary TB cases attending the Department of Pulmonary Medicine, Shree Birendra Army Hospital, Nepal, were subjects in the study. All the suspected cases of pulmonary TB were advised to give a sputum sample, likewise, for extrapulmonary TB, patients were advised to provide sputum as well as various extrapulmonary samples such as pleural, peritoneal, cerebrospinal fluid, and pus aspirate by invasive methods and gastric aspirate as detailed in the National Tuberculosis Management Guidelines, 2019, Nepal [7]. All samples were then processed for acid-fast bacilli (AFB) and GeneXpert MTB/RIF testing, following the standard manufacturers' protocol [8]. All the samples were then sent for conventional culture and sensitivity testing at the German-Nepal Tuberculosis Project (GENETUP), Kathmandu, Nepal, for confirmation.

Data analysis

The performance of the GeneXpert MTB/RIF assay and AFB microscopy were assessed by calculating the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) to diagnose TB compared to *M. tuberculosis* culture as the gold standard method. The sensitivity, specificity, PPV and NPV of MTB/RIF assay for both pulmonary and extrapulmonary TB case were calculated together.

Results

A total of 500 samples were included in the study of which 473 cases were suspected pulmonary TB cases and 27 were suspected extrapulmonary TB cases (**Table 1**). A comparative analysis of GeneXpert MTB/RIF, AFB smear microscopy and culture are presented in **Table 2.** The sensitivity and the specificity of GeneXpert MTB/RIF, compared to conventional culture, was 100% and 99.5%, respectively. The AFB smear microscopy had a sensitivity of 57.3% and specificity of 99.5%. The PPV and NPV of GeneXpert MTB/RIF were 97.9% and 100%, respectively. The PPV and NPV for AFB microscopy were 99.5% and 88.5%, respectively. None of the samples analyzed demonstrated resistance to rifampicin.

Table 1. Details of distribution of both pulmonary and extrapulmonary samples (n=500)

Type of sample	Number of samples	Number of positive using GeneXpert MTB/RIF	
		Number	Percentage (%)
Pulmonary TB sample			
Sputum	399	87	21.8
Bronchoalveolar lavage	70	6	8.5
Gastric lavage	4	0	0.0
Total for pulmonary samples	473	93	19.6
Extrapulmonary TB sample			
Pleural fluid	12	0	0.0
Pus aspirate	9	3	33.3
Peritoneal fluid	3	0	0.0
Cerebrospinal fluid	2	1	50.0
Joint aspirate	1	0	0.0
Total for extrapulmonary samples	27	4	14.8
Total	500	97	19.4

Table 2. Comparative analysis of GeneXpert MTB/RIF and AFB microscopy and their sensitivity, specificity, PPV and NPV compared to *M. tuberculosis* culture

Diagnosis method		*M. tuberculosis* culture		
		Positive	Negative	Total
GeneXpert MTB/RIF assay	Positive	95	2	97
	Negative	0	403	403
	Total	95	405	
AFB microscopy	Positive	43	2	45
	Negative	52	403	455
	Total	95	405	

Diagnosis method	Sensitivity	Specificity	PPV	NPV
GeneXpert MTB/RIF assay	100%	99.5%	97.9%	100%
AFB microscopy	45.3%	99.5%	99.5%	88.5%

Discussion

TB is an ancient disease that has affected mankind for more than 4000 years [7]. Early diagnosis plays a key role in the management of this disease. WHO data estimates that 58 million lives were saved through early TB diagnosis and treatment between the years of 2000 and 2018 [1]. Readily available testing such as smear microscopy has low sensitivity and the gold standard diagnostic method of culture with antimicrobial sensitivity testing, requires expertise, has a lengthy turn-around time and requires and biosafety laboratory facilities [4].

In the last few decades, there have been several advancements in the development of antibiotic therapies; however, many treatable infectious diseases including TB remain undiagnosed. It is the major problem in resource-limited countries [9]. Following the recommendation by WHO for the use of the GeneXpert MTB/RIF assay in 2010, the field of TB management has been revolutionized. The GenXpert MTB/RIF is a fully automated cartridge based real-time PCR platform. The system is able to simultaneously detect the DNA of *M. tuberculosis* and the *rpoB* gene that is associated with rifampicin resistance [1, 6, 9].

Our study was conducted to compare GeneXpert MTB/RIF assay performance among existing diagnostic methods; smear microscopy and culture. The sensitivity of GeneXpert MTB/RIF, compared with culture, was found out to be 100% whereas its specificity was 99.5%. These findings were similar with the study carried out by Boehme *et at* with a reported sensitivity of 97.6% [4] and to a study of Parsons *et al* reporting a sensitivity of 99.1% [12].

Our data suggests that AFB smear microscopy has a lower sensitivity and specificity as compared to GeneXpert MTB/RIF with a sensitivity of just 57.3% and a specificity of 99.5%. A previous study suggested that the sensitivity of AFB smear microscopy varies between 20% to 80% [13]. Another study found that the sensitivity of smear microscopy was only 46% [14]. In the present study, we could only diagnose approximately 50% of suspected TB cases by smear microscopy as compared to culture results. The sensitivity of smear microscopy is significantly varied as it depends on the quality of sample, method of processing and expertise of the microscopist.

In our study, two TB cases were positive by both GeneXpert MTB/RIF and microscopy but were negative by culture. This type of discrepancy may be due to indiscriminate use of antibiotics resulting in incomplete treatment or dead bacilli. However, the PPV and NPV of GeneXpert were 97.9% and 100%, respectively which was comparable with previous studies [15-16].

There are some limitations of our study. We could not include lymph node extrapulmonary TB cases in this study due to the lack of access to appropriate tissue processing facilities. In this study we could not include clinical, radiological and histopathological findings in the analysis due to incomplete data.

Conclusions

The sensitivity and specificity of the GeneXpert MTB/RIF assay in diagnosing pulmonary and extrapulmonary TB patients are comparable to the gold standard *M. tuberculosis* culture method. Its ability to detect *M. tuberculosis* and rifampicin resistance simultaneously makes it crucial for application in resources-limited countries in Asia such as Nepal as it is rapid, easy to interpret, and user friendly. TB remains a significant burden and Nepal still lacks expertise and well-equipped laboratory facilities where culture could be performed. This could help to achieve global tuberculosis elimination target by 2050.

Declarations

Ethics approval

The protocol of the study was approved by the Institutional Review Committee of Nepalese Army Institute of Health Sciences (NAIHS), Kathmandu, Nepal.

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Conflict of interest

The authors declare that they have no competing interests.

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