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Evaluating the Diagnostic Accuracy of GeneXpert MTB/RIF Assay for the Diagnosis of Tuberculosis

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AU and MWK developed the idea and concept of the paper. AU and ML collected the data. MWK and HU performed the statistical analysis. MWK, AKT and INS complied the paper and wrote the literature. All authors edited and approved the final manuscript.

Key words
Accuracy, Diagnosis, GeneXpert, Sensitivity, Specificity, Tuberculosis

ABSTRACT

Tuberculosis (TB) is one of the major global public health concerns particularly affecting population of low-income countries. Early detection of disease coupled with other parameters help in treatment and reducing disease transmission. The current study was conducted to assess the sensitivity and specificity of the GeneXpert MTB/RIF (Cepheid Sunnyvale, CA, United States) in comparison to conventional techniques used for the diagnosis of TB. Our study is one of the first ones from Pakistan investigating and assessing the performance of GeneXpert. We recruited eight hundred clinically TB suspects initially and included seven hundred and sixteen clinically TB suspects in the final analysis. The results of GeneXpert were compared with Mycobacteria Growth Indicator Tube (MGIT) and Ziehl-Neelsen (ZN) staining. In comparison to MGIT and ZN staining the sensitivity of GeneXpert with 95 % confidence interval (CI) was (99.7 %, CI 0.98-0.99) and (95.1 %, CI 0.92-0.97) respectively. The positive and negative predictive values with 95 % CI were (97.1 %, CI 0.94-0.98) and (99.7 %, CI 0.98-0.99) when results of GeneXpert were compare with MGIT results. The results of this study confirm the performance of GeneXpert. With high sensitivity and rapid detection, GeneXpert is ready to be considered as preferred diagnostic tool for TB.

INTRODUCTION

Tuberculosis (TB) is a chronic contagious disease instigated by Mycobacterium tuberculosis (MTB). TB spreads through aerosols of TB germs into the air and typically affects the lungs and other organs of the human body (WHO, 2016).

Tuberculosis is a major worldwide public health issue and is graded in top ten causes of mortalities worldwide. In 2016, World Health Organization (WHO) reported 10.4 million new cases of TB and 1.7 million mortalities related to TB. More than 95% of mortalities reported were from low-income and middle-income countries. Moreover, 56% of these cases were reported in five Asian countries (China, India, Indonesia, Pakistan and Philippines). Pakistan is included in list of seven most endemic countries where each year an estimated 510,000 new TB cases are identified (WHO, 2016, 2018).

Conventional methods of diagnosis including sputum microscopy and mycobacterial tuberculosis culturing

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are mostly in use particularly in developing countries. Also, multidrug resistant tuberculosis (MDR-TB), where bacterial strains become resistant to first-line anti TB drugs (Isoniazid, Pyrazinamide, Rifampicin), has emerged as threats to TB treatment (Chang *et al*., 2012; Jacobson *et al*., 2010; Vaghela and Anand, 2020). In 2016, globally 600,000 new cases of rifampicin were notified in which 0.46 million cases had MDR. Moreover, approximately 15,000 emerging new drug resistant TB cases occur each year (WHO, 2016, 2018). For all new TB cases, WHO recommends standardized short course chemotherapy based on a regimen of four first-line drugs taken for 6-8 months (Dye and Williams, 2000).

GeneXpert MTB/RIF (Cepheid Sunnyvale, United States) is a novel revolutionary development in tuberculosis diagnostics with endorsement from WHO (Lawn *et al*., 2013; Tortoli *et al*., 2012; WHO, 2020). The accuracy of GeneXpert has been reported as high in many previous studies (Christopher *et al*., 2019; Sharma *et al*., 2015). GeneXpert may be used for diagnosis of MTB and particularly rifampicin (RIF) resistant TB cases. Early detection of MTB and MDR is important in diagnosis to improve the successful treatment rate and decrease TB transmission (Boehme *et al*., 2010, 2011).

Diagnosis with GeneXpert is also becoming common in developing world including Pakistan. The main objective of this study was to compare the performance of GeneXpert with common conventional methods of Mycobacteria Growth Indicator Tube (MGIT) and Ziehl-Neelsen (ZN) staining for smear microscopy.

MATERIALS AND METHODS

Study group

The study was carried out by collecting blood, urine, fluid and sputum samples from clinically TB suspects having symptoms (i.e., coughing with/out expectoration more than 3 weeks, fever, night sweats, chills, loss of appetite, fatigue and weight loss) visiting Provincial Reference Laboratory (PRL) at Fatima Jinnah Chest Hospital Quetta, Pakistan. The study was approved by the Institutional Review Board of Balochistan University of Information Technology Engineering and Management Sciences (BUITEMS; No: 10/2017). All the study participants provided valid informed consent.

Sample collection and processing

Blood, urine, fluid and sputum specimen were collected in sterile leak proof container, while tissue specimen was placed in sterile saline to protect from dehydration. All the specimens were initially screened for presence of mycobacteria. The respiratory specimen was based on sputum, broncho alveolar lavage fluid and bronchial aspirates while specimens from tissue included blood, sterile body fluids and urine. Non respiratory specimens were also collected for the testing of Mycobacterium tuberculosis complex (MTBC) and other mycobacteria. Most common specimen for pulmonary infection was sputum. To enhance sensitivity through smear, sputum samples were taken early morning. Three sputum samples were collected from each patient at different time intervals (before breakfast, after breakfast and at laboratory by lab staff). For the analysis of social and demographical factors subjective information was also collected with the help of questionnaire. All specimens were processed through Acid-Fast Bacillus (AFB) smear microscopy, GeneXpert assay and inoculate on the day of collection (IUATLD, 2000).

Sample decontamination

Sputum samples were decontaminated through sodium hydroxide-N-acetyl-Lcystein (NaOH-NALC) method. For this purpose, sputum samples (10 ml) were taken into a follicle tube, an equal volume of NaOH (final concentration of 1 %) and sodium citrate solution was added. After vortexes for 15 seconds samples were kept at 25 °C for 15 min for decontamination. The tube was filled 2 cm from top with phosphate buffer. Vortexes and Centrifuge at 3000 xg for 15 min. Supernatant was discarded and 2.5 ml phosphate buffer saline (pH 6.8) was added (IUATLD, 2000).

Microscopy

According to World Health Organization (WHO) guidelines, smears were screened through Auramine-O-fluorescence microscopy while positive smears were re-examined with ZN staining for AF (IUATLD, 2000).

Mycobacterium inoculation on Lowenstein-Jensen medium (LJ Media)

Decontaminated samples 10 ml were transferred to follicle tube. Inoculated into two slants of LJ media for liquid medium and smear microscopic examination. Using a pipette, 3-4 drops (0.4 ml) were inoculated in each LJ slant. All LJ slants were incubated at 37 °C and examined daily. After formation of mycobacterium colonies, ZN staining was carried out to identify AFB, while samples were considered culture negative if there were no colonies formed after 8 weeks of incubation time (Chihota *et al*., 2010).

Mycobacterium inoculation on liquid medium (MGIT)

Decontaminated samples 3-4 drops (0.5 ml) through pipette inoculated into MGIT. The MGIT tubes were kept

in BACTEC 960 for scanning purpose. The tubes were incubated at 37°C and examined daily up to eight weeks. When mycobacterium growth appears in MGIT tube, automated mycobacterial detection system of BACTEC 960 indicated the MGIT tube with the help of green signal (Chihota *et al*., 2010).

GeneXpert assays

The GeneXpert MTB/RIF test was performed using GeneXpert Model GX-XVI GXXVI-16-LXX as per the manufacturer's instruction. The decontaminated samples were kept for 15 min at rest. After 15 min pulmonary samples were centrifuged for 5 min at 1000 relative centrifugal force (RCF), while extra pulmonary sample was centrifuged for 15 min at 3000 RCF. Through pipet 2.5 ml sample was taken to cartridge and placed in modules of GeneXpert. The cartridge was scanned by GeneXpert, and required 1 h and 52 min for detection.

Statistical analysis

All the statistical analysis were performed using R 3.5.0 (R Core Team, 2018). Sensitivity, Specificity, negative and positive predictive values (NPV/PPV) were also calculated. MGIT culture was used as reference method.

RESULTS AND DISCUSSION

The current study was conducted for the evaluation and performance of diagnostic accuracy of GeneXpert MTB/RIF in detection of TB. The results of GeneXpert were also compared with other diagnostic methodologies including MGIT culture method and ZN staining method.

A total of 800 clinically TB suspects were initially included in the study 84 (10.5%) samples of clinically TB suspects were discarded due to technical shortcomings (insufficient in quantity, errors in diagnostic modalities, culture contamination). 716 clinically TB suspects samples were included in the final analysis of the study. All the samples were subjected to ZN staining, GeneXpert assay and culture inoculation.

Out of the total 716 clinically TB suspects, 48.6% (N= 348) individuals were confirmed as positive for TB. The prevalence of TB positive individuals was higher in the middle age group individuals.

The mean age ± SD of the clinically TB suspects was 43.6 ± 20.9, of which 48.7% (N= 349) were males and 51.3% (N= 367) were females. Characteristics of study participants are presented in Table I which also shows the comparison of TB positive and TB negative individuals.

The characteristics of the clinically TB suspects between the TB positive and TB negative groups were similar except from smoking status. Smoking status in TB positive group individuals was significantly higher (27.3%) than TB negative group individuals (20.9%). Age and gender of the individuals between the groups did not differ significantly.

Table I. Characteristics of the TB suspects (N = 716), divided into groups according to diagnosis as TB positive or TB negative by GeneXpert method.

	TB positive individuals	TB negative individuals	*p*
Age at diagnosis (years)	43.8 (18.7)	43.4 (22.9)	0.775†
Gender: N (%)			
Male	167 (48.0)	182 (49.5)	
Female	181 (52.0)	186 (50.5)	0.695‡
Smoking status: N (%)			
Smoking	95 (27.3)	77 (20.9)	
No smoking	253 (72.7)	291 (79.1)	0.046‡

†, p-value based on Student's t-test; ‡, p-value based on Chi square test.

Table II. TB detection analysis of all the three diagnostic methods (GeneXpert, MGIT† and ZN‡) using MGIT† as reference method.

	MGIT† culture positive	MGIT† culture negative
GeneXpert Positive: N (%)	338 (99.7)	10 (2.7)
GeneXpert Negative: N (%)	1 (0.3)	367 (97.3)
ZN‡ Positive: N (%)	290 (85.5)	15 (4)
ZN‡ Negative: N (%)	49 (14.5)	362 (96)

†, mycobacteria growth indicator tube culture; ‡, Ziehl-Neelsen staining for smear microscopy.

Out of total 716 samples, 51.4% (N= 368) were GeneXpert negative and 48.6% (N= 348) were GeneXpert positive. 47.3% (N= 339) were screened positive and 52.7% (N= 377) as negative by MGIT culture method. Whereas, 57.4% (N= 411) were detected as negative by ZN staining and 42.6% (N = 305) as positive (Table II).

Results of both GeneXpert and ZN staining were compared with MGIT culture. MGIT culture results were used as reference. In comparative analysis, the sensitivity and specificity of GeneXpert with 95% confidence interval (CI) was comparatively higher (99.7%, CI 0.98 - 0.99) and (97.3%, CI 0.95 - 0.98) respectively than that of ZN staining (85.5%, CI 0.81 - 0.89) and (96.0%, CI 0.93 - 0.97), respectively (Table III).

GeneXpert results were accurate and reliable with the sensitivity of 99.7% and specificity of 97.3%. The positive

Table III. Diagnostic performance of GeneXpert and ZN†, using MGIT‡ culture as a reference method.

	GeneXpert * MGIT‡ culture	ZN† * MGIT‡ culture
Prevalence	47.3 (0.44-0.51)	47.3 (0.43-0.51)
Sensitivity % (95% CI)§	99.7 (0.98-0.99)	85.5 (0.81-0.89)
Specificity % value (95% CI)§	97.3 (0.95-0.98)	96.0 (0.93-0.97)
Positive predictive value % (95% CI)§	97.1 (0.94-0.98)	95.1 (0.91-0.97)
Negative predictive value % (95% CI)§	99.7 (0.98-0.99)	88.1 (0.84-0.90)
False positive	0.03 (0.01-0.05)	0.05(0.03-0.08)
False negative	0.00 (0.00-0.02)	0.12(0.09-0.16)

†, Ziehl-Neelsen staining for smear microscopy; ‡, mycobacteria growth indicator tube culture; §, confidence interval.

predictive values and negative predictive values were comparatively higher, 97.1% and 99.7% respectively in GeneXpert result analysis. The chances of tested as being false positive and false negative were also as low as 2.7% and 0.3%, respectively. The results of current study are in line with previous studies conducted for the performance of GeneXpert diagnostic method (Christopher *et al*., 2019; Sharma *et al*., 2015). According to Sharma *et al*. (2015), the test showed the overall sensitivity and specificity of 95.7% and 99.3%, respectively. Similarly, Christopher *et al*. (2019) reported (99.5%) valid results for GeneXpert.

Although the difference was not statistically significant, the disease was comparatively more prevalent in females than males. The reason for high prevalence in female gender might be inaccessibility to health care facilities (Yousafzai, 2011). Women often face hindrances in gaining approach to diagnostic services, health examinations and in completing sufficient treatment (Razaq, 2015). Furthermore, the burden of household assignments and childcare leave them with meager time to access health care and particularly tuberculosis care for themselves (Feng *et al*., 2012).

Smoking is an independent risk factor not only for active TB but also for latent TB cases. Smoking significantlyincreases the risk of acquiring and development of TB. According to study results, smoking status of individual was statistically significantly different between TB positive and TB negative groups (Table I). Higher rates were observed in TB positive individuals. We did not have information about passive smoking that could have explained the results further. Non-smoking individuals are easily exposed to passive smoking especially in the current study population as smoking indoor and in public places is not very rare event (Goel *et al*., 2019; Thomas *et al*., 2019; Ullah *et al*., 2020).

The strength of the present study is the large sample size and comparison with conventional TB diagnosis methodologies. Along with performance of GeneXpert techniques, we also performed comparative analysis between different available diagnostic techniques including ZN staining and MGIT culture. We also collected different socioeconomic parameters of study individuals that help in exploring the trends.

The limitations of our study included no information of human immunodeficiency viruses (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV). Although HIV is not very common in the study population, but the prevalence of HBV and HCV is higher in the study population (Ullah *et al*., 2020).

CONCLUSION

In conclusion, Although MGIT culture method is considered currently as the gold standard method for the detection of TB, diagnostic accuracy of GeneXpert coupled with rapid detection and easy to use technology is gaining popularity in the field of TB diagnosis. Also, the detection of rifampicin resistance and MDR associated TB cases are of great advantages of GeneXpert.

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IBR approval

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Statement of conflict of interest

The authors have declared no conflict of interest.

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