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GeneXpert assay for rapid detection of *Mycobacterium tuberculosis* complex in respiratory specimens from a high TB endemic area of Pakistan

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ABSTRACT

Tuberculosis is a global health problem, and its early diagnosis is the ultimate strategy for prevention and control. The current study was undertaken to evaluate conventional and molecular diagnostic assays for the detection of mycobacteria in pulmonary tuberculosis (TB) patients from Khyber Pakhtunkhwa region of Pakistan. A total of 259 clinically suspected patients of TB were processed for Zeihl Neelsen (ZN) microscopy, BACTEC MGIT liquid culture and GeneXpert assay. Among 259 samples, 28 (10.81%) were positive for acid fast bacilli (AFB) on ZN microscopy. In liquid culture, the growth of mycobacterium species was obtained in 36 (13.89%) samples while the GeneXpert assay detected *Mycobacterium tuberculosis* (MTB) in 49 (18.91%) samples. Detection rate of MTB was significantly high ($n = 49$, $p < 0.0095$) on GeneXpert as compared to microscopy ($n = 28$); however no significant difference ($p = 0.1230$) was observed on GeneXpert ($n = 49$) and culture ($n = 36$) based detection of MTB. The strength of agreement between GeneXpert and microscopy was also poor (Kappa value < 0.114, 95% CI: −0.72 − 0.301) which support our results. MTB detection rate among female was high as compared to male TB patients while in age wise, the age group 55–64 years has almost high detection rate on microscopy, culture and GeneXpert assay. Findings of the present study highlighted that GeneXpert is more efficient tool for timely diagnosis and proper TB control in high TB endemic area.

1. Introduction

Tuberculosis (TB) is a chronic infectious disease and remains a major global health problem especially in the developing countries. The possible TB transmission from person to person is through spreading of *Mycobacterium tuberculosis* (MTB) into the air by an infected person. Among infectious diseases, TB is the leading cause of deaths worldwide [1,2]. Approximately one-third of the world population is currently infected with TB, and 95% of them are reported in underdeveloped countries. Pakistan ranks sixth among high TB burden countries in the world [3].

Early diagnosis of MTB is potential strategy to control TB. Diagnosis of TB is done by finding of consolidation in the lung apices through X-Ray. Tuberculin skin test (TST) and immune-chromatographic technique (ICT) are also used for the diagnosis of TB. In developing countries like Pakistan, Zeihl Neelsen (ZN) staining of sputum samples followed by acid fast bacilli (AFB)

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detection on microscopy is routinely used which is an inexpensive and rapid test for TB diagnosis [3,4]; however it has limitation of differentiating *M. tuberculosis* from the other mycobacteria. Culturing of mycobacteria is another method through which the detailed diagnosis of TB is possible but it takes longer time and the early diagnosis is required which necessitate the immediate laboratory backup [5]. In addition, a real-time PCR test named as GeneXpert assay is available to detect simultaneously the *M. tuberculosis* and rifampicin resistance which is comparatively quick and accurate method [6,7]. As for as literature mining is concerned there is no data available which compared and evaluated ZN microscopy, liquid culture and GeneXpert assay for TB diagnosis in Khyber Pakhtunkhwa, a resource limited area near to Afghanistan border, and more TB prone area of Pakistan.

The present study compared MTB diagnostic potential of GeneXpert assay with ZN microscopy and culture. Findings of the current study will be helpful for the better management of TB in the area.

2. Materials and methods

2.1. Study area and ethical approval

The study was carried out in Peshawar, the provincial capital city of Khyber Pakhtunkhwa, Pakistan during April 2013 to January 2014. This study was approved by the departmental ethics committee. An informed consent was taken from patient participating in the study.

2.1.1. Sample collection

A total of 259 sputum samples were obtained from patients who visited different hospitals and clinics of Peshawar for TB diagnosis and therapy. The samples were collected in sterile containers and transported in ice box to the laboratory for further processing.

2.2. Zeihl Neelsen (ZN) staining microscopy for AFB detection

Smear was prepared from the sputum sample and routine ZN staining was performed as described earlier [8]. Following staining, identification of AFB was performed under microscope as per guideline of world health organization (WHO).

2.3. Mycobacteria growth indicator tube (MGIT) liquid culturing for mycobacterium isolation

Sputum samples were first subjected to decontamination and then cultured on MGIT liquid culture medium as previously described [9]. Briefly, the samples were decontaminated using NaOH-NALC (*N*-acetyl-*L*-cysteine) sodium citrate solution. The samples were centrifuged at 3000 rpm for 15 min. After centrifugation the supernatant was carefully discarded into the container containing a mycobactericidal disinfectant. Phosphate buffer (pH 6.8) was added to the pellet and used for inoculation on MGIT tubes containing MGIT growth supplement/PANTA. The tubes were incubated in BACTEC MGIT 960 (BD, USA) instrument at 37 °C until the instrument flagged them positive. The tubes with no growth were considered negative after maximum 6 weeks of incubation. The growth with granular appearance and less turbid was further processed for the presence of *Mycobacteria* by preparing a ZN stained smear for microscopy.

2.4. GeneXpert assay for M. tuberculosis detection

GeneXpert is an integrated automated sputum-processing and hemi-nested real-time PCR method (GeneXpert Dx System, Cepheid, USA) for the detection of *M. tuberculosis*. The present study used primers, probes and thermal conditions for the amplification of MTB specific *rpoB* gene and internal control as previously described [10]. Briefly, a sample reagent was mixed with sputum to make 2:1 dilution. After 15 min of incubation with intermittent shaking sample was transferred to the Cepheid cartridge that contain the wash buffer, reagents for lyophilized DNA extraction and PCR amplification. The reaction cartridge was loaded for filtering, DNA extraction and PCR amplification. The three primers (*rpoB* F1, *rpoB* F2, *rpoB* R) were used to amplify *rpoB* gene while five molecular probes (A, B, C, D, E) were used to detect any mutations in the 81 bp RRDR region that are associated with rifampicin resistance. Additionally, three primers and one probe (F, R1, R2, Probe) were used for the detection of *Bacillus globigii* spores gene (internal control for sample processing and PCR) included in GeneXpert MTB/RIF cartridge. In hemi-nested PCR first reaction, initial denaturation at 95 °C for two minutes and then 16 cycles of second denaturation at 95 °C for 5 s, and coupled annealing-extension steps at 72 °C for 40 s was performed. The resulting PCR products were subjected to the second PCR reaction with thermal conditions consisted of initial denaturation at 95 °C for 2 min, and then 45 cycles of denaturation at 95 °C for 5 s, annealing at 64 °C for 20 s and extension at 72 °C for 30 s. The data was analyzed and interpreted by using GeneXpert software.

2.5. Statistical analysis

The statistical differences between different techniques were estimated using the chi-square test and a p value <0.05 was considered as significant. The data was analyzed using *SPSS*, version 13.0. Cohen's kappa correlation coefficients between different techniques were calculated using online tool (http://graphpad.com/quickcalcs/kappa1.cfm).

3. Results

In the current study conventional and molecular diagnostic assays were evaluated for MTB detection. When a total of 259 sputum samples were processed for the AFB microscopy, 28 (10.81%) samples were positive for AFB while 231 (89.18%) samples were AFB negative. On BACTEC MGIT liquid culturing, the detection rate of *Mycobacterium* was 13.89% (n = 36); however 223 (l.10%) samples were culture negative. It is worth mentioning that the detection rate of MTB was higher on culture (13.89%) as compared ZN microscopy (10.80%).

In the present study all the samples were subjected to GeneXpert assay for MTB detection. Although, the GeneXpert detected rifampicin resistance; however the data is not shown in this manuscript. The detection rate was 18.91% (n = 49) while no MTB was detected in 210 (81.08%) samples. Statistical analysis reflected that MTB detection was significantly high (n = 49, $p < 0.0095$) on GeneXpert as compared to microscopy (n = 28); however no significant difference ($p = 0.1230$) was observed in MTB detection rate on GeneXpert (n = 49) and culture (n = 36). Similarly no significant difference ($p = 0.2854$) was observed on culture (n = 36) and microscopic (n = 28) based detection of MTB (Table 1).

To find correlation between any two techniques, a Cohen's kappa coefficients were calculated. On the basis of Kappa value, a “poor” strength of agreement was found between GeneXpert and ZN microscopy (Kappa value = 0.114, 95% CI: −0.72 − 0.301) which confirmed our results. On the other hand the “moderate” strength of agreement was found between GeneXpert and culture (Kappa value = 0.469, 95% CI: 0.281 − 0.658) (Table 2).

When gender wise detection rate of MTB was checked using all the three techniques, MTB was detected more in female, although