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Original Research Article

Molecular Detection of Extra-pulmonary Tuberculosis by the Automated GeneXpert MTB/RIF Assay: 3-years' experience in a teaching hospital, Saudi Arabia

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Abstract

Background: rapid and accurate diagnosis of extrapulmonary tuberculosis (EPTB) continues to be a challenge. Although culture remains the most sensitive method for confirmation of TB, the prolonged time required for culture, limit its contribution to clinical decision making. Our study aims to assess the performance of Xpert MTB/RIF test against smear and culture-confirmed cases of extrapulmonary TB. ***Methods:*** A total, 272 non-respiratory specimens (tissues, 88 (32.4%), pleural fluids, 60 (22.1%), CSF, 48 (17.6), aspirate 36 (13.2), ascetic fluid, 20 (7.4), urine, 8 (2.9), body fluid and pus 5 (1.8) each, and blood 2 (0.73) submitted to the laboratory for Mycobacteria over two-year period were comparatively investigated with the molecular-based Gene Xpert MTB/RIF assay system and conventional smear and solid culture methods. ***Result:*** The reliability indices of the Gene Xpert MTB/RIF assay are higher compared to smear. The overall sensitivity (82.1%) of the Gene Xpert MTB/RIF assay is significantly higher than smear (46.9 %). The highest Mtb positivity agreement between the Xpert MTB/RIF assay and Mtb culture was found in pus and CSF specimens (100 % [95% CI, 91.1 % to 100 %]) each, while the lowest Mtb positivity agreement was found in the ascetic fluids specimens (50% [95% CI, 44% to 58%]). The Area under the curve (AUC) of the Receiver Operator Characteristic (ROC) curve to assess the accuracy of smear was 0.737 (with 95% CI of 0.600-0.874), whereas the AUC of the ROC curve for Xpert MTB/RIF was 0.946 (with 95% CI of 0.000-1.00). ***Conclusion:*** The Xpert assay showed superior performance over the conventional smear for the rapid detection of M. tuberculosis, in (EPTB).

Keywords: GeneXpert MTB/RIF assay, smear, *Mycobacterium tuberculosis*, extrapulmonary tuberculosis; sensitivity, specificity, culture.

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INTRODUCTION

Tuberculosis (TB) remains an important cause of death worldwide. In 2015, the World Health Organization (WHO) reported 10.4 million TB new infection and 1.8 million died from TB [1]. Large number (0.8 million) of new cases of extrapulmonary tuberculosis (EPTB) were reported globally [2]. The 2004 estimation rate of TB incidence reported in Saudi Arabia was 9,471 (40/100,000). This rate and the mortality caused by tuberculosis rose by 6.2% between 1990 and 2004 [3]. Diagnosis of EPTB is a major challenge and frequently presented with atypical presentation mimicking other inflammatory diseases or malignancies [4, 5]. Sensitive laboratory testing is essential to make an early diagnosis and reduce morbidity and mortality caused by TB particularly in HIV patients where the mortality of untreated TB is high [6, 7]. Conventional methods used for the diagnosis of EPTB such as microscopy, culture, histopathology and serological assays are limited by the long period required for detection of positive TB cases. Culture is frequently negative and only 28% of suspected cases of TB are detected and reported as smear positive [13]. In recent years, nucleic acid amplification diagnostic technologies have been evaluated and employed largely for early diagnosis and reporting of TB including EPTB [8-13]. The Xpert MTB/RIF (Cepheid Inc.) is an automated, rapid test based on nested real-time PCR assay for MTB and RIF resistance detection. The utility of the Xpert MTB/RIF assay is well established for the diagnosis of pulmonary TB (sensitivity 89%, specificity 99%) [11, 12]. The high accuracy of the assay has led the WHO to formulate a policy suggesting the implementation of Xpert MTB/RIF assay as a replacement of the conventional diagnostic methods [13]. However, the diagnostic accuracy for EPTB, especially smear-negative cases, is not yet well established and needs

further studies [10]. In many studies, the Xpert assay has been compared with other molecular assays for M. tuberculosis detection using culture as the reference method [15-17]. However, there are few data on direct comparisons with the standard culture MTB test for EPTB [18]. The aim of this prospective study was to evaluate the performance of the Xpert assay, compared to that of the standard test smear and culture for the detection of extra pulmonary M. tuberculosis.

MATERIALS AND METHODS

Patients

Two hundred and seventy-two extrapulmonary specimens obtained from 253 patients were included in the study. Patients were considered to have extrapulmonary tuberculosis based on bacteriological, clinical, pathological, or radiological evidence of tuberculosis.

Specimens

All non-respiratory specimens received in our Reference Tuberculosis Laboratory obtained from patients attended our Institute, a Tertiary University Hospital, Riyadh, Saudi Arabia, were included. The study was conducted during the period from May 2015 to May 2018.

Culture medium inoculation, incubation, and test duration

All specimens were processed by the standard N-acetyl-L-cysteine and sodium hydroxide (NALC/NaOH) method with a final NaOH concentration of 1% (according to the Deutsches Institutfur Normung guidelines [19]. After the centrifugation step, the sediment was suspended in 1.0 to 1.5 ml of sterile phosphate buffer (pH 6.8).This suspension was used for inoculation of culture media. Different culture media were used. Specimens from sterile sites (cerebrospinal fluid (CSF), pleural, peritoneal, and synovial) were centrifuged. Tissue samples such as lymph node and bone were ground with sterile grinders. Smears were made and stained by auramine O and examined for the presence of AFB using a fluorescence microscope. Solid culture was performed on Lowenstein–Jensen (LJ) slants and liquid culture on the Mycobacteria Growth Indicator Tube (MGIT) 960 automated system (Becton Dickinson Biosciences, Sparks, MD, USA) as per manufacturer's instructions. Positive growth on MGIT tubes and/or LJ slants was examined microscopically for acid-fast bacilli (AFB). Further confirmation of M. tuberculosis was performed with an immunochromatographic test (SD, Bioline). The tubes were incubated in the MGIT 960 instrument at 37°C.

MGIT 960

MGIT tubes were inoculated with 0.5 ml of the processed specimen. The tubes were incubated in the MGIT 960 instrument at 37°C. For tissue samples, a further MGIT tube was inoculated with 0.5 ml specimen and incubated at 31°C. For tubes identified as positive, a smear of a sample from the tube was prepared for examination for acid-fast bacilli (AFB), and further differentiation of mycobacteria was performed with molecular methods.

Solid Media

For each specimen, one Lo¨wenstein-Jensen (LJ) slant and one broth of Bactec MGIT 960 tube (MGIT 960; Becton Dickinson Diagnostic Systems) are inoculated. Bacterial colonies were investigated by AFB smear and were further investigated by molecular methods. For the purpose of data analysis, each of the different media was regarded as a single culture medium system.

AFB Smears

After processing of the specimens, smears were prepared from all samples other than urine and were examined for Mycobacteria (NRC) for the presence of AFB. All smears were stained by the Kinyoun method and examined with a light microscope.

Antituberculosis Susceptibility Testing

Drug susceptibility testing (DST) for RMP was performed with the Bactec MGIT 960 method (MGIT 960; Becton Dickinson Diagnostic Systems) with the standard critical concentration of 1 microgram/ml RMP.

Xpert Procedure

The Xpert assay was performed as recently described [20]. Sample reagent was added in a 3:1 ratio to microgram 0.5 ml of decontaminated specimen. The closed tube was manually agitated twice during a 15-min incubation period at room temperature before 2 ml of the inactivated sample reagent-sample mixture was transferred to the Xpert test cartridge. Cartridges were inserted into the GeneXpert device, and the automatically generated results were read after 90 min.

Statistical Analysis

A statistical package program (SPSS version 17.0) was used. A Receiver operator characteristic (ROC) curve was plotted to assess the accuracy of both smear and the Xpert MTB/RIF.

Ethical Consideration

The Institutional Ethics Committee approved the study.

RESULTS

Patients

The age of the patients range from one year to 90 years (median age of 43 years, with 22.2 standard deviations); 58% were males.

Type of Specimens

A total of 272 EPTB specimens were received in the laboratory within the study period and categorized as listed in Table-1. Tissue accounted for (88 %) of the samples, followed by pleural fluid (22.1 %), Cerebrospinal fluid (CSF) (17.6 %), aspirate including fine-needle aspirates [FNA] (predominantly lymph nodes) (13.2%), ascetic fluid (7.4 %) and urine (2.9%). The remaining samples include pus and body fluids (dialysis, synovial, and miscellaneous fluids) comprising 1.8 % of the specimens.

Sensitivity and specificity of the Xpert MTB/RIF assay

The reliability indices of Xpert MTB/RIF are higher than smear. Compared with culture results as the gold standard, the overall sensitivity of the Xpert MTB/RIF test was 82.1% (23 / 28) and the specificity was 98.3% (236 / 240). On the other hand, the sensitivity and specificity of fluorescent staining smear microscopy were 46.9 % (15/ 32) and 99.6 % (239 / 240) respectively. The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for smear and MTB/RIF assay are detailed in (Table-2**)**.

The overall sensitivity of the Xpert MTB/ RIF assay according to the culture result was 100% (15/15) for smear-positive extrapulmonary specimens and 47.1 % (8/17) for smear-negative extrapulmonary specimens.

The performance of Xpert MTB/RIF from different EPTB specimens

Compared to MGIT culture; Xpert MTB/RIF assay had the highest sensitivity on pus (100 % [95% CI, 91.1 % to 100 %]), CSF (100 % [95% CI, 91.1 % to 100 %]), followed by tissue (84.6% [95% CI, 76% to 94%]). Pleural fluids and aspirates accounted both for (75% [95% CI, 64% to 85%]), with least sensitivity was obtained from ascetic fluids (50% [95% CI, 44% to 58%]).The specificity values for Xpert MTB/RIF assay compared to culture were high for all types of specimens (Table-3).

The reliability indices of smear compared with Xpert MTB/RIF

A Receiver operator characteristic (ROC) curve was plotted to assess the accuracy of both smear and Xpert MTB/RIF. The area under the curve (AUC) for smear was 0.737 (with 95% CI of 0.600-0.874), while the AUC for the Xpert MTB/RIF was 0.946 (with 95% CI of 0.000-1.00) (Figure-1**)**.

Table-1: Extrapulmonary Specimens Stratified By Disease Site

Type of specimens	N	P	T (n= %)
Tissue	74	14	88 (32.4)
Pleural Fluid	55	5	60 (22.1)
CSF	47	1	48 (17.6)
Aspirate	31	5	36 (13.2)
Ascetic Fluid	17	3	20(7.4)
Urine	8	0	8 (2.9)
Body Fluid	5	0	5 (1.8)
Pus	1	4	5 (1.8)
Blood	2	0	2 (0.73)
Total	240	32	272 (100)

Table-2: sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for smear and MTB/RIF assay with the culture method as reference

Test	Sensitivity	Specificity	PPV	NPV
Smear	(15/ 15 + 17) 46.9 %	(239 / 239 + 1) 99.6 %	(15 / 16) 93.8%	(239 / 256) 93.4 %
Gene-expert	(23 / 23 + 5) 82.1%	(236 / 236 + 4) 98.3%	(23 / 27) 85.2 %	(236 / 241) 97.9 %

Table-3: Sensitivity and specificity of MTB/RIF assay from different EPTB specimens

Type of specimen	Sensitivity		Overall specificity	Overall sensitivity
	Smear positive	Smear negative	N (%)	N (%)
CSF (48)	1/1 (100.0%)	-	47/47 (100.0%)	1/1 (100.0%)
Ascetic fluid (20)	-	1/1 (100.0%)	17/17 (100.0%)	1/2 (50%)
Pleural fluid (60)	1/3 (33.3%)	2/3 (66.7%)	55/55 (100.0%)	3/4 (75%)
Tissue (88)	8/11 (72.7%)	3/11 (27.3%)	73/73 (100.0%)	11/13 (84.6%)
Pus (5)	2/4 (50%)	2/4 (50%)	1/1 (100.0%)	4/4 (100.0%)
Aspirate (36)-1	3/3 (100.0%)	-	30/30 (100.0%)	3/4 (75 %)

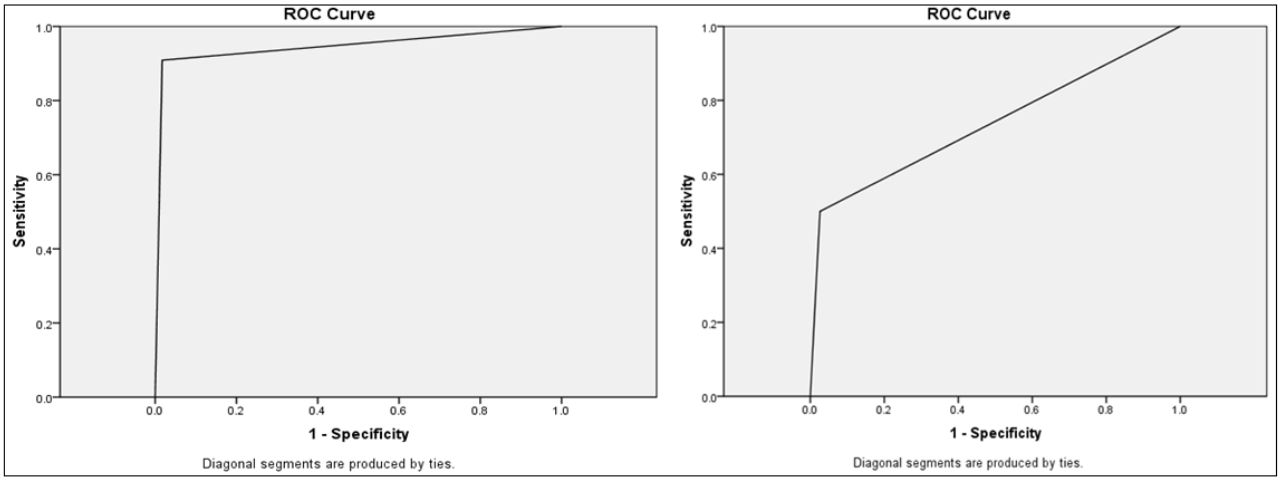

Fig-1: The Receiver Operator Characteristic (ROC) curve for smear and Xpert MTB/RIF assay revealed The Area Under the curve (AUC) for smear is 0.737 (with 95% CI of 0.600-0.874), while the AUC for Xpert MTB/RIF is 0.946 (with 95% CI of 0.000-1.00)

DISCUSSION

Tuberculosis (TB) remains a major global important health issue with 9 million new cases and 1.4 million deaths in 2013 [21]. The rapid diagnosis of patients with extrapulmonary disease and detection of rifampin (RIF) resistance are essential for early disease management. The GeneXpert MTB/RIF assay is a new molecular diagnostic test for the rapid diagnosis of both pulmonary and extrapulmonary tuberculosis and detection of RIF resistance in clinical specimens. Previous studies have evaluated the performance of the Xpert assay in comparison with those of smear and culture results for either PTB or EPTB specimens [15, 17, 18]. A study by Vadwai *et al.*, investigated five hundred forty-seven extrapulmonary specimens for both culture (solid and liquid) and Xpert testing. Xpert sensitivity and specificity results were assessed in comparison to smear, culture, clinical, radiological, and histological findings. For culture, the sensitivity was low, 53 % (150/283 specimens). The sensitivity of the Xpert assay was 81% (228/283 specimens) (64% [89/138] for smear-negative cases and 96% [139/145] for smear-positive cases), with a specificity of 99.6 [22]. In another study evaluating GeneXpert MTB/RIF Assay for rapid diagnosis of Pulmonary and Extrapulmonary Specimens, Two hundred fifty-three pulmonary and 176 extrapulmonary specimens obtained from 429 patients were included in the study. Sensitivity with extrapulmonary specimens: 100% for smear-positive specimens (4/4) and 47.7% for smear-negative specimens (21/44) [23]. In a similar study, the sensitivities of the Xpert MTB/RIF test for smear-positive and smear-negative extrapulmonary specimens have been reported to be 100% and 37%, respectively [24]. In those studies, the Xpert MTB/RIF assay showed excellent sensitivity with performance higher than that of the smear assays for EPTB in smear-positive specimens with sensitivities reported between 96 and 100%. On the other hand, the sensitivity of smear-negative specimens has been variable ranged from 64 to 46.7 %. In the present study, the sensitivity of the MTB/RIF Xpert assay compared to culture was 82.1%, which is compatible with those reported in other studies [22, 24]. In addition, in our study the Area Under the Curve (AUC) of the Receiver Operator Characteristic (ROC) curve to assess the accuracy of smear was 0.737 (with 95% CI of 0.600-0.874), whereas the AUC of the ROC curve for PCR was 0.946 (with 95% CI of 0.000-1.00). Interestingly, this factor explains that the Xpert assay has better accuracy than smear specimens.

In conclusion, the performance of the GeneXpert MTB/RIF assay was superior to that of the smear and culture and it is useful for the rapid detection of *Mycobacterium tuberculosis* in EPTB samples. Better surveys are needed in high burden countries requiring high quality routine surveillance. Ultimately effective control of the disease requires an understanding of the dynamics of disease transmission, implementation of accurate and rapid diagnostics and typing methodologies, and efficacious treatment. Saudi Arabia has interesting and special population dynamics. There are up to six million imported populations mainly from endemic regions, in South and South East Asia and over two million pilgrims visiting the holy cities located in the western region of the Kingdom each year, with the majority of pilgrims coming from endemic areas.

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