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ORIGINAL ARTICLE

Gene Xpert/RIF Assay: A New Era in Rapid Detection of Pulmonary Tuberculosis

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ABSTRACT

Background*: Tuberculosis (TB) still a major health problem worldwide. Emerging Multidrug-Resistant Tuberculosis-TB (MDR-TB) is an important problem facing the health policy. Actually, less than 10% of MDR-TB cases worldwide are detected. The conventional microscopy ZN (Ziehl-Neelsen) technique lacks sensitivity. The cultural methods which have high sensitive takes as long as 14 days up to 6 weeks to produce results. The Xpert assay is a promising approach for the rapid diagnosis of active TB which provides results within 2 hours.* ***Objectives*** *are to compare the sensitivity and specificity of Gene Xpert/RIF assay versus the traditional methods in detection of Mycobacterium tuberculosis and evaluation of rifampicin resistance in sputum samples.* ***Methodology****: The study was carried out on 30 cases under clinical and radiological doubt of pulmonary tuberculosis from May 2017 to January 2018.three consecutive spontaneously produced early morning sputum samples were collected and subjected to ZN smear microscopy, culture and conventional drug susceptibility test (DST) followed by Gene Xpert MTB/RIF assay. The sensitivity, specificity, PPV, NPV of Gene Xpert & ZN microscopy were calculated using LJ culture of Mycobacterium tuberculosis as standard method* ***Results:*** *A total of 30 sputum samples were tested; among the 30 sputum samples, 8 samples were positive and 18 samples were negative by the three methods used (smear microscopy, culture, gene xpert assay).the overall sensitivity, specificity, PPV & NPV of gene Xpert assay were 100%, 94.7%, 91.7% and100% respectively. The overall sensitivity, specificity, PPV & NPV of smear microscopy were 72.7%, 100%, 100% and 86.3% respectively. Sensitivity, specificity, PPV & NPV of Gene Xpert for detecting RIF resistance compared to phenotypic DST were 100%,100%,100% and100% respectively.* ***Conclusion:*** *GeneXpert MTB/RIF assay is simple, efficient and accurate technique for the rapid diagnosis of MTB. It's simplicity, high sensitivity and specificity for detection of RIF resistance make this technique a very impressive tool for diagnosis of MTB and RIF resistance in MDR cases.*

INTRODUCTION

Tuberculosis is one of the most important health problems in Egypt, TB prevalence rate in Egypt was estimated in the (WHO) global report (2013) to be 29 per 100,000 population [1]. At the same time, multidrug-resistant TB (MDR-TB) cases were estimated worldwide to be 480,000 leading to aproximately 210,000 deaths[2]. MDR-TB is defined as TB disease caused by *Mycobacterium tuberculosis* (MTB) resistant to at least isoniazid and rifampicin (RIF). Actually, less than 10% of MDR-TB casesare detected worldwide. The rapid detection of (MTB) in infected patients is essential for disease management[3]. In most countries the traditional microscopy (Ziehl-Neelsen technique) is the cornerstone in diagnosis of MTB. Microscopy has low sensitivity, it can detect 10-75% of PTB cases. The cultural methods which have high sensitive takes as long as 14 days to 6 weeks to produce results and require specific materials and laboratories[4]. To respond to the necessary need for simple & rapid diagnostic tool at the point of treatment in high-burden countries, a fully automated molecular test for detection of *Mycobacterium tuberculosis* and drug resistance testing was developed through collaboration in a public–private partnership[3].

In December 2010, the WHO endorsed the use of GeneXpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA) for national tuberculosis programs in developing countries. The Xpert assay is an automated nucleic acid amplification test (NAAT) for simultaneous detection of (MTB) and its resistance to rifampin directly from clinical samples. The assay doesn't require sample processing but can be done on chemically inactivated specimen and results are available within 2 hours. Therefore, it is simple, less time consuming and does

not require special technical expertise and biosafety requirements[5,18].

METHODOLOGY

Patients:

This study was carried out on 30 cases under clinical and radiological doubt of pulmonary tuberculosis.The patients were selected from outpatient clinic of EL-Maamoura Chest Hospital over 9 months period of study from May 2017 to January 2018. All cases were subjected to complete history taking & full examination.Written consents were obtained from the patients.The study was approved by the ethical committee of Tanta faculty of Medicine.

Inclusion criteria

Cases under clinical and radiological doubt of pulmonary tuberculosis.

Exclusion criteria

Healthy individuals without any previous history of chest diseases

Laboratory Methods

Three consecutive spontaneously produced early morning sputum samples were collected and tested for ZN smear microscopy, culture & phenotypic drug susceptibility test (DST) followed by Gene Xpert MTB/RIF assay. Xpert MTB/RIF assay was compared with LJ culture method for detecting TB & with phenotypic drug susceptibility testing for detecting RIF's resistance.

Direct smear microscopy was done to investigate the presence of acid fast bacilli in first sample using ZN staining method. Slides showing red coloured acid fast bacilli were taken as positive and negative slides were those without any acid fast bacilli

Gene Xpert test was performed in second sample according to the manufacturer's instructions. Sample reagent was added in a 2:1 ratio to unprocessed sputum in falcon tube and the tube was manually agitated twice during a 15 minute incubation period at room temperature, then 2 ml of the liquefied sputum samples were transferred to the test cartridge by a sterile disposable pipette (provided with the kits).

Cartridges were labeled by the specimen ID and loaded into the Gene xpert instrument and start test within 30 minutes of preparing cartridges [6].

Third sample was decontaminated using the N-acetyl-L-cysteine–sodium hydroxide method (NALC-NAOH) and cultured on Lowenstein –jensen media[7]. All positive cases werechecked for typical AFB by ZN staining and MOTT by subculture on 5% blood agar plates[8].

Phenotypic drug susceptibility testing

Cultures obtained on Lowenstein-jensen media were collected and tested for drug susceptibility to RIF. Drug susceptibility testing was performed using LJ proportional method. The critical drug concentration was 40 microgram/ml

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0 Qualitative data were expressed as frequency & percentage. Sensitivity, specificity, PPV& NPV was calculated.

The sensitivity, specificity, PPV & NPV for the diagnosis of MTB were calculated for AFB smear microscopy & Gene Xpert, using LJ culture of *Mycobacterium tuberculosis* as gold standard.

RESULTS

A total of 30 sputum samples were evaluated. Of these, 8 samples were positive and 18 were negative by the three methods used in our study (smear microscopy, culture, GeneXpert). Four sputum samples were smear negative, one of them was culture negative & Gene Xpert positive while the other three smear negative samples were culture & Gene Xpert positive. One sputum sample was resistant to rifampicin by conventional DST & Gene Xpert while the remaining 11 sputum samples were sensitive to rifampicin by phenotypic conventional DST & Gene Xpert assay.

Table 1: Comparison of results between Gene Xpert and culture as standard method

Gene Xpert assay	Culture		
	Positive	Negative	Total
Positive	11	1	12 (40%)
Negative	0	18	18 (60%)
Total	11 (36.7%)	19 (63.3%)	30

Table (1): shows that among 30 sputum samples, 11 samples were culture and Gene Xpert positive, 18 samples were culture and Gene Xpert negative, one sample was culture negative and Gene Xpert positive leading to 11 samples positive and 19 sample negative for culture compared to12 samples positive and 18 samples negative for Gene Xpert assay.

Table 2: Sensitivity, specificity, PPV, NPV of Gene Xpert with culture as reference

Sensitivity	Specificity	PPV	NPV
100%	94.7%	91.7%	100%

Table (2) shows that the overall sensitivity, specificity, PPV& NPV for Xpert assay when culture method was taken as a reference were 100%, 94.7%, 91.7%,100% respectively.

Table 3: Comparison of results between AFB smear and culture as reference

Smear	Culture		
	Positive	Negative	Total
Positive	8	0	8 (26.7%)
Negative	3	19	22 (73.3%)
Total	11 (36.7%)	19 (63.3%)	30

Table (3) out of 30 sputum samples,8 samples were smear and culture positive and 19 samples were smear and culture negative,3 samples were smear negative and culture positive leading to 8 samples smear positive and 22 samples smear negative compared to11 samples positive and 19 samples negative culture.

Table 4: Sensitivity, specificity, PPV, NPV of AFB smear with culture as reference.

Sensitivity	Specificity	PPV	NPV
72.7	100	100	86.3

Table (4) shows that the overall sensitivity, specificity, PPV & NPV of ZN smear when culture was taken as a reference were 72.7%, 100%, 100%, 86.3% respectively.

Table 5: Comparison of sensitivity, specificity, PPV, NPV of smear and Gene Xpert versus culture as reference.

Culture vs	Sensitivity	Specificity	PPV	NPV
GeneXpert	100	94.7	91.7	100
Smear	72.7	100	100	86.3

Table (5) shows overall sensitivity, specificity, PPV, NPV of smear microscopy and Gene Xpert assay versus culture (gold standard).

Table 6: **Comparison of results between Gene Xpert assay and drug susceptibility test for rifampicin resistance.**

Gene xpert assay	DST		
	Sensitive	Resistant	Total
Sensitive	11	0	11 (91.6%)
Resistant	0	1	1 (8.4%)
Total	11 (91.6%)	1 (8.4%)	12 (100.0)

Table (6) among 30 sputum samples, 11 samples were Gene Xpert and phenotypic DST sensitive to rifampicin, 1sample was Gene Xpert and phenotypic DST resistant to rifampicin (RIF).

Table 7: Sensitivity, specificity, PPV, NPV values of Gene Xpert assay compared to drug susceptibility test of rifampicin (RIF).

Sensitivity	Specificity	PPV	NPV
100%	100%	100%	100%

Table (7) sensitivity, specificity, PPV, NPV of Gene Xpert assay according to phenotypic DST were 100%,100%, 100%,100% respectively.

DISCUSSION

In this study, we have evaluated the diagnostic yield of Gene Xpert to detect MTB in sputum samples and compared it with AFB and culture which was taken as gold standard. In our study, mycobacterial cultures done using solid Lowenstein-jensen media.

The Xpert MTB/RIF is a cartridge-based, automated diagnostic test that can identify MTB and resistance to rifampicin (RIF). The assay does not require sample processing but can be used on chemically inactivated specimen and results are available within 2 hours. Therefore, it is simple, less time consuming and does not require special technical expertise and biosafety requirements [5].

In comparison with culture as gold standard sensitivity, specificity, PPV and NPV of Gene Xpert MTB/RIF assay were recorded as 100%, 94.7%, 91.7%,100% respectively this is in line with previous studies[9,10]which showed sensitivity, specificity, PPV and NPV of gene xpert as100%, 90%, 91.6%, 100% and100%, 99.4%, 98.4%,100% respectively, in other studies Gene Xpert showed lower sensitivity and specificity than our study [11] which were 87.6% and 75% respectively.

One sample was negative culture and Gene Xpert positive (specificity 94.7%), the false positive result of Gene Xpert could be due to that all PCR test amplifies any DNA,live or dead bacilli therefore excretion of residual DNA from dead bacilli explain this discrepant result [9].

Our study demonstrated the overall sensitivity, specificity, PPV, NPV of smear microscopy were 72.7%, 100%, 100% and 86.3% respectively this is in line with other studies [9] which declared that sensitivity, specificity, PPV, NPV of smear microscopy were 72.7%, 100%, 100% and 72,6%, other studies showed lower sensitivity and specificity of smear microscopy [10,12] as they were 26.4%, 98.2% respectively and 53.3%,98.8% respectively.

By comparing the Gene Xpert with smear microscopy, Gene Xpert showed higher sensitivity (100%) then smear microscopy (72.7%) and shared almost same specificity this is in accordance with previous studies [8] which showed the same result.

We compared the ability of smear microscopy with Xpert MTB/RIF assay in MTB detection. Smear microscopy detected 8 MTB cases while Xpert assay detected all 8 positive cases of smear microscopy plus 4 positive cases among 22 suspects with smear negative results. Thus Gene Xpert MTB/RIF out performed smear microscopy and established a diagnosis of presumptive pulmonary tuberculosis for few cases with

smear negative TB, this is compatible with other previous studies [13,14].

Four negative smears were Gene Xpert positive, this discrepant results could be due to the poor quality of sputum samples taken. it is also possible that patients may only excrete bacilli intermittently and so some of samples submitted may have been negative [15], low mycobacterial load could be another explanation for smears negative results as for a sample to be smear positive a bacterial load of 10^4 organism is required [5].

In our study, we demonstrated that sensitivity, specificity, PPV, NPV of Gene Xpert for detecting RIF resistance compared to phenotypic DST were 100%, 100%, 100% and 100% respectively this is similar to results showed in previous studies [16,3].

Other studies showed lower sensitivity and specificity as they were 97.6% & 98.1% respectively and 96.7% , 98.6% respectively[3,17].

CONCLUSIONS

- The present study showed that Gene Xpert and AFB smear microscopy had almost the same specificity but the sensitivity of Gene Xpert is much higher than AFB smear microscopy.
- Despite culture was the reference method but it takes long time to come positive and can't detect Rifampicin resistance simultaneously.
- On the other side Gene Xpert can be a useful diagnostic tool in patients suspected for pulmonary tuberculosis either AFB smear positive or negative due to its rapidity and simultaneous detection of Rifampicin resistance especially beneficial in MDR patients .
- The high sensitivity and specificity of Xpert assay for detection of RIF resistance enhance its use as an initial diagnostic method for RIF resistance, limiting dissemination of MDR TB strains.
- Therefore, GeneXpert MTB/RIF assay is simple, specific and reliable technique for the rapid diagnosis of MTB. The simplicity, high sensitivity & specificity of xpert assay in detection RIF resistance make this technique a very attractive diagnostic method for diagnosis MTB & RIF resistance in MDR cases.

Conflict of Interests

The authors stated that they have no conflict of interests.

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