Agreement Between Direct Smear Microscopy and GeneXpert MTB/RIF in Diagnosis of Pulmonary Tuberculosis Among Patients Attending Consultory TB Clinic, Kirkuk

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ABSTRACT

Background: Tuberculosis is one of the deadliest diseases causing morbidity and mortality worldwide, as it infects 9 million and kills 3 million annually, yet one third of the world population is infected with TB. This study was planned to compare the accuracy of PCR with direct sputum smear among patients who attended the TB Clinic in Kirkuk.

Patients and Methods: A cross sectional study recruited 958 patients who presented to chest and respiratory diseases clinic for the period from 4th of December till 30th June 2014 for detection of Mycobacterium tuberculosis by direct smear microscopy. Out of these 321 samples were tested by gene Xpert; they were stratified according to site of disease to 249 pulmonary and 72 extra pulmonary cases.

Results: It was found that the percentage of direct smear positive was 3.03% while that for PCR was 11.5%. The percentage of Rifampin resistance was (10.8%) among positive cases.

Conclusions: It is concluded that GeneXpert/MTB/RIF is superior to direct AFB in detecting Mycobacterium tuberculosis, by time and detecting resistance in less than 2 hours.

Key words: Direct smear microscopy, GeneXpert, pulmonary tuberculosis
**Introduction**

Tuberculosis is one of the most deadly diseases worldwide; about 2 billion people in the world carry latent TB; more than nine million will become ill and about three million will die (1).

Poor health system, limited laboratory capacity for case detection, treatment barriers and complications make TB a major challenge for public health programs (2).

Detection of Mycobacterium species is mainly detected by Ziehl-Neelson stain which employs acid-fast staining technique. First the sample is inversed in hot carbolfuchsin that stains red, then the sample is stained with acid-alcohol and counter stained with methylene blue. On examination, the bacteria appear bright red against a blue background (3).

The Xpert MTB/RIF assay is a fully automated molecular diagnostic test for TB disease, developed in partnership with Cepheid Inc, the foundation for innovative new diagnostics (FIND); the university of medicine and dentistry of New Jersey (UMDNJ) and the national institutes of health (NIH). It can detect rifampin resistance from sputum specimens in less than 2 hours and minimizes staff manipulation and biosafety risk (4).

**Objectives**

The study was conducted to:

1. Show the agreement of conventional and new methods in detecting Mycobacterium in sputum and other body fluids.
2. To detect rifampin resistance among smear positive cases.

**Patients and Methods**

A cross sectional study recruited 72 patients who presented to Chest and Respiratory Disease Clinic in Kirkuk for the period between 4.12.2013-4.2.2014. Sputum samples were obtained from each patient for direct smear microscopy and GeneExpert for detection of Mycobacterium tuberculosis, then agreement in results of both investigations was assessed.

Regarding sputum sampling each TB suspect was requested to submit two sputum specimens, one for conventional smear plus Xpert MTB/RIF and the second for the conventional smear alone. When the results of Xpert were positive the Rifampin sensitive patients were registered as Cat 1 or Cat 2 according to drug history. If the specimen proved to be RIF/ resistant, it would be subjected to culture and drug sensitivity test (DST).

**Results**

Table 1 shows that during the period of the study; a total of 958 sputum samples were examined for direct smear microscopy to detect the presence of mycobacterium tuberculosis.

Regarding GeneXpert (PCR), a total of 321 were examined; all smear positive cases, highly suspected smear negative TB cases and other body fluids referred by public and private sectors were included in the study.

Regarding gender difference, the ratio of male to female was 1.9:1. The percentage of positive cases by DSM was 3.03%, while in PCR it was 11.52%.

It was clear that there was significant statistical difference between the two tests (P<0.05).

It is clear that PCR has detected higher positive cases than the conventional method AFB; also it is obvious that males are affected more than females.

It was found that the proportion of positive cases in GeneXpert was greater than that with AFB. There was a statistically significant difference between the two methods (P< 0.007), as indicated in Table 2.

Table 3 illustrates the distribution of positive cases detected by PCR. Out of 321 samples tested for detection of MTB by GeneXpert, 37 cases were positive, distributed as 35 pulmonary TB and 2 extra pulmonary (pus); apart from these 2 extra pulmonary samples , all other samples were negative.

Among the 37 positive cases only 4 (10.81%) cases were proved to be resistant to Rifadin by the new test (GeneXpert). The percentage of Rifadin resistance was 11.42% among pulmonary cases.

It is obvious from table 3 that PCR has advantage to detect Rifampin resistance cases within less than 2 hours.

Regarding other body fluids, all were negative when tested by PCR.
Results

Table 1: Distribution of cases in DSM & PCR according to Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>DSM (958)</th>
<th>PCR (321)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
<td>Negative</td>
</tr>
<tr>
<td>Male</td>
<td>19 (3.67)</td>
<td>499</td>
</tr>
<tr>
<td>Female</td>
<td>10 (2.27)</td>
<td>430</td>
</tr>
<tr>
<td>Total</td>
<td>29 (3.03)</td>
<td>929</td>
</tr>
</tbody>
</table>

Table 2: Agreement between DSM and GeneXpert for study sample

<table>
<thead>
<tr>
<th>GeneXpert</th>
<th>DSM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>37</td>
</tr>
</tbody>
</table>

Table 3: Distribution of types of TB cases according to gender and Rifadin resistance detected by PCR

<table>
<thead>
<tr>
<th>Type of TB</th>
<th>P.C.R. 321</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>249</td>
</tr>
<tr>
<td>Extra pulmonary</td>
<td>72</td>
</tr>
<tr>
<td>Total</td>
<td>321</td>
</tr>
</tbody>
</table>

RFS= Rifadin sensitive; RFR= Rifadin resistant
Discussion

WHO have started the largest roll-out of GeneXpert, as detecting TB rapidly and identifying drug resistance on the spot is an essential step to improve care of the infected people and avoid transmission in the community (5).

The higher percentage of positive cases among males than females detected by PCR, is in agreement with WHO report as males have much higher rates than females.

In a study conducted in Vantanilla, Lima, in 2005 showed that tuberculosis globally kills more women than any other single infectious disease. It is noted that 70% of the poor are women and they face obstacles in seeking medical advice and care (6).

Detection of Mycobacterium TB by PCR was increased in comparison with direct sputum smear; this result is in agreement with a study done in Brazil and published as Clinical trials during 2011.

In a survey conducted among prisoners, in Malaysia, it was documented that a single GeneXpert assay proved to get 53% sensitivity, 100% specificity and concluded that single test yields low screening sensitivity (7).

WHO technical report has concluded that PCR is the most common method of amplification, in spite of many other methods for rapid test for diagnosing MTB which are commercially available as in Roche (Amplicor PCR-FDA-approved), Becton Dickson CBD probe Tec, Standard Displacement amplification (SDA), Gene probe (Amplified Mycobacterium tuberculosis Direct (AMTD),Transcription machinated amplification (TMA) in addition to the assay used in the current study under the commercial name Cepheid (GeneXpert MTB/RIF) nested real time PCR (8).

In addition to excellent sensitivity with smear positive specimens, the device can identify 72.5% of smear negative culture positive samples from single tests. WHO recommended that the GeneXpert MTB/RIF is suitable for use at district and sub -district levels (9&10).

Detection of drug resistance in comparing the conventional (AFB) test and new PCR, showed that males had higher resistance than females although the number of cases is small as tuberculosis is a rare disease and among all notified cases resistance occupies 3% of the TB cases (11).

In MTB, mono resistance to (RIF) is rare and almost 90% of resistant cases to RIF are also resistant to INH so a positive result of RIF is a strong surrogate of MDR-TB, and in resistant isolates it has been shown that from to 95% to 98% RIF resistance is caused by mutations in the proB gene encoding the RNA polymerase B-unit (12&13).

References