Comparison and evaluation of diagnostic techniques of mycobacterium tuberculosis in Iraq

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Objective Diagnostic tests devoted to the rapid, sensitive, and specific identification of the causative agent are key components of successful wellness plans directed at tuberculosis control. This study focuses on rapid and accurate detection of tuberculosis cases among Babylon population.

Methods The sputum samples were collected from 60 patients suspected to have suffered from tuberculosis infection, in the Specialized Chest and Respiratory Center, Hillah City and Department of Medical Microbiology, College of Medicine, Babylon University, Hilli-Iraq during the period from February to June 2015. Molecular detection of Mycobacterium tuberculosis in patients’ sputum samples using real-time PCR, and gene X-pert for suspected TB-infected patients. The clinical signs were recorded for each patient, including night sweating, fever, loss of weight, and history of cough.

Results Gene X-pert MTB/RIF technique, real-time PCR recording the high sensitivity for AFB positive smear (100%) for both, AFB negative smear (66%, and 58%), respectively. AFB sensitivity was (16.6%), and the specificity was (100%) for all in the present study.

Conclusion The comparison between advance technique (Gene X-pert and real-time PCR) and classical technique (AFB) for the diagnosis of MTB, shows that genetic technique is the best with high sensitivity and specificity.

Keywords tuberculosis, real-time PCR, gene-X-pert

Introduction

Tuberculosis (TB) has been one of the oldest infectious diseases affecting human. It was identified 4000 years ago, from the Middle East and Europe, as the cause of death, suggesting that this disease has been already a widespread health problem back then. In a detailed history, Hippocrates wrote about patients with wasting away associated with coughing and chest pain, often with blood in sputum. These symptoms had been allowed Hippocrates to diagnose TB, which at that fourth dimension has been called “consumption”. The occurrence of descriptions of patients with these signs indicated that the disease was already well entrenched in early times.1,2

TB is a communicable and deadly infectious disease caused by mycobacteria, essentially Mycobacterium tuberculosis.3 TB affects 8.8 million people each year, most of whom dwell in low economic society.4 Additionally, an estimated 2 billion people are believed to be latently infected, providing a great reservoir.3 Tuberculosis typically attacking the lungs (as pulmonary TB) but can affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin (extra pulmonary TB).3 Although in that respect other mycobacteria such as Mycobacterium bovis, Mycobacterium africanum, Mycobacterium canetti, and Mycobacterium microti also can cause tuberculosis, these species are less common. The usual symptoms of pulmonary tuberculosis are a chronic cough with blood-tinged sputum, fever, night sweats and weight loss. Tuberculosis considers an immunological disease and the pathology of disease mediates by the host immune response.5

Diagnostic tests devoted to the rapid, sensitive, and specific identification of the causative agent are key components of successful wellness plans directed at disease control. Moreover, the precise determination of Mycobacterial burden might be beneficial for fast assessment of patient response to standard therapy, particularly in those patients suspected of harboring antibiotic resistant M. tuberculosis strains.6

Chest radiographs may be applied to exclude pulmonary TB disease in a person with a normal immune system who has a positive TST (tuberculin skin test) reaction or IGRA (IFN-γ release assay) and has no symptoms or signs of TB disease.7 Gain techniques for the diagnosis of tuberculosis have attracted considerable interest in diagnosis, particularly with the hope of reducing the time taken to find and identify Mycobacterium tuberculosis in respiratory and non-respiratory specimens.8 Real-time PCR has further advantages, including precision, reproducibility, accuracy, quality control procedures and reduced pollution. In addition, real-time PCR eliminates the need for electrophoresis after the cycling reaction. Furthermore, this technique cuts the analysis time for 3 or 4 days, which is important in lengthy microbiological diagnoses, such as those needed for TB.10 The Gene X-pert system, a real-time PCR that simultaneously detects both Mycobacterium tuberculosis complex (MTBC) and rifampin resistance, was developed.11 In contrast to some real-time PCR instruments, the X-pert MTB/RIF is an on-demand assay described as a simple method that can be performed by personnel with minimal grooming and can provide answers within 2 h.12

The detection of rifampin resistance, as a surrogate for multidrug-resistant TB (MDR-TB), directly from smear-positive respiratory specimens from patients bearing a high risk of MDR-TB has recently been advocated by the World Health Organization.13 Chemotherapy of drug susceptible TB consists of three or four drug regimen, administered for 6 months. The long duration of therapy solutions in poor compliance leading to the emergence of multi-drug resistant forms of M. tuberculosis.14

Reference

1. Descriptions of patients with these signs indicated that the disease has been already a widespread health problem back then. In a detailed history, Hippocrates wrote about patients with wasting away associated with coughing and chest pain, often with blood in sputum. These symptoms had been allowed Hippocrates to diagnose TB, which at that fourth dimension has been called “consumption”. The occurrence of descriptions of patients with these signs indicated that the disease was already well entrenched in early times.

2. TB is a communicable and deadly infectious disease caused by mycobacteria, essentially Mycobacterium tuberculosis. TB affects 8.8 million people each year, most of whom dwell in low economic society. Additionally, an estimated 2 billion people are believed to be latently infected, providing a great reservoir.

3. Tuberculosis typically attacking the lungs (as pulmonary TB) but can affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin (extra pulmonary TB).

4. Although in that respect other mycobacteria such as Mycobacterium bovis, Mycobacterium africanum, Mycobacterium canetti, and Mycobacterium microti also can cause tuberculosis, these species are less common. The usual symptoms of pulmonary tuberculosis are a chronic cough with blood-tinged sputum, fever, night sweats and weight loss.

5. Tuberculosis considers an immunological disease and the pathology of disease mediates by the host immune response.

6. Diagnostic tests devoted to the rapid, sensitive, and specific identification of the causative agent are key components of successful wellness plans directed at disease control. Moreover, the precise determination of Mycobacterial burden might be beneficial for fast assessment of patient response to standard therapy, particularly in those patients suspected of harboring antibiotic resistant M. tuberculosis strains.

7. Chest radiographs may be applied to exclude pulmonary TB disease in a person with a normal immune system who has a positive TST (tuberculin skin test) reaction or IGRA (IFN-γ release assay) and has no symptoms or signs of TB disease.

8. Gain techniques for the diagnosis of tuberculosis have attracted considerable interest in diagnosis, particularly with the hope of reducing the time taken to find and identify Mycobacterium tuberculosis in respiratory and non-respiratory specimens.

9. Real-time PCR has further advantages, including precision, reproducibility, accuracy, quality control procedures and reduced pollution. In addition, real-time PCR eliminates the need for electrophoresis after the cycling reaction.

10. Furthermore, this technique cuts the analysis time for 3 or 4 days, which is important in lengthy microbiological diagnoses, such as those needed for TB.

11. The Gene X-pert system, a real-time PCR that simultaneously detects both Mycobacterium tuberculosis complex (MTBC) and rifampin resistance, was developed.

12. In contrast to some real-time PCR instruments, the X-pert MTB/RIF is an on-demand assay described as a simple method that can be performed by personnel with minimal grooming and can provide answers within 2 h.

13. The detection of rifampin resistance, as a surrogate for multidrug-resistant TB (MDR-TB), directly from smear-positive respiratory specimens from patients bearing a high risk of MDR-TB has recently been advocated by the World Health Organization.

14. Chemotherapy of drug susceptible TB consists of three or four drug regimen, administered for 6 months. The long duration of therapy solutions in poor compliance leading to the emergence of multi-drug resistant forms of M. tuberculosis.
Materials and Methods

Study Population
A total of 60 patients suspected with tuberculosis infection, in the Specialized Chest and Respiratory Center, Hilla City during the period from February to June 2015. The clinical signs were recorded for each patient, including the night sweating, fever, loss of weight, and history of dry cough. Checklist sheets were drawn out for each patient including age, gender, radiological finding, and incidence area. Sputum samples were collected from TB according to the stated procedure.

AFB Smear
The slides were flooded with carbol fuchsin dye. The slides are heated slowly until it is steaming with direct flame for at least 5 minutes. The smear was decolorized with a decolorizing agent and left until the color of the solution is gone. Then it was washed with tap water. Methylene blue was applied to slides by which the dye covered the entire slides surface and left for maximum 60 seconds than washing with water. Microscopically examination: The positive acid fast bacilli smear appears as pink or red rods in a blue background. The number of AFB was calculated by country number of cell in smear according to standard method.

Real Time PCR Assay

Extraction of DNA
The required number of 1.5 ml disposable polypropylene micro centrifuge tubes was developed, including single tube for Negative Control of Extraction (Negative Control, c-). Ten microliter of the MTB IC (Internal Control) and 300 µl of Lysis Solution was added to each pipe. One hundred microliter of samples was added to the appropriate tubes using pipette tips with aerosol barriers. The control was prepared as follows: Add 100 µl of Negative Control C- to the labeled Cneg. Vortex the tubes and incubated for 5 min at 65°C. Centrifuged for 10 s. Four hundred microliter of Prec solution was added and mixed by vortex. Centrifuged all tubes at 13,000 rpm for 5 min and using a micropipette with a plugged aerosol barrier tip, carefully removed and discarded supernatant from each tube without disturbing the pellet. Changed tips between the tube. Five hundred microliter of Wash Solution was added into each tube. Vortex vigorously rinsed to ensure pellet washing. Centrifuged at room temperature for an additional 5 minutes. The test is finished, the light turns off. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge. Reading the Results: The Gene X-pert Instrument system generates the results from measured fluorescent signals and embedded calculation algorithms. The results can be seen in the View Results window.

Results
Among 60 samples, only 10 samples which give a positive AFB, 50 samples give a negative AFB result, 39 samples positive,
21 samples negative results for Real-Time PCR, 43 samples positive, 17 negative results for Gene X-pert respectively as shows in Table-1.

**Discussion**

Gene X-pert MTB/RIF technique, Real-Time PCR recording the high sensitivity for AFB positive (100%) for both, for AFB negative (66%, and 58%) respectively, AFB sensitivity was (16.6%), and the specificity was (100%) as shown in Table 1. The sensitivity of real-time PCR and X-pert MTB/RIF are given similar results with smears positive, but it is low in real-time technique for smears negative. These are matching with Armand et al. who suggested that both methods were highly specific and exhibited excellent sensitivity (100%) with smear-positive specimens, but does not match in the sensitivity of the X-pert MTB/RIF test with the smear-negative specimens were more reduced than that of the real-time PCR assay (48 versus 69%, P < 0.005).

American Thoracic Society Workshop, who estimated that the Gene X-pert assay was introduced lately. Its advantage lies in higher sensitivity and specificity for the diagnosis of TB, together with the detection of drug resistance, and in smear-positive specimens. The sensitivity and specificity of PCR are in the range 90–100%, with a positive prognostic value of > 95%, whereas in smear-negative specimens, the sensitivity of PCR was reduced to < 50%.

Our study found that the sensitivity of Gene X-pert for AFB smear negative was (66%). These results agreed with the work reported by Zeka et al. who suggested that the X-pert MTB/RIF assay have reported test sensitivities of 57 to 76.9% in cases of smear-negative.

In our study, real-time PCR has a second value of sensitivity (69%) for smear negative after the Gene X-pert. These results were in accordance with those results reported by Raviglion, who proposed that the real-time PCR assay with internal control achieved a sensitivity of 96.2 % and specificity of 99.2%. Templeton et al. explains that internal control reaction has been included to determine the robustness of the PCR resulting by monitoring the nucleic acid extraction as well as the presence of inhibitors.

Raipal et al. stated that direct microscopy may give negative results if the number of AFB less than 500 bacilli/ml, consistently positive specimens would have to contain 105 bacilli per ml varying with the extent of the wound or the presence citations. The scanty or numerous AFB smear test, which differs according to the number of bacilli in microscopy filed.

In our study, the sensitivity for the diagnosis of *M. tuberculosis* of Gene X-pert and real-time PCR higher than AFB because the Gene X-pert and real-time PCR depends on diagnosis on DNA amplification and have ability to detect less than 10 of acid fast bacilli per ml in a sputum sample while AFB smear depending on the ability of *M. tuberculosis* to take stain and, may give a negative result if there few number of acid fast bacilli less than 5000 bacilli per ml in the field.

**Conclusions**

The comparison between advance technique (Gene X-pert and Real time PCR) and classical technique (AFB) for the diagnosis of MTB, show that genetic technique is the best with high sensitivity, and specificity.

**Consent**

All authors declare that written informed consent was obtained from the patients for publication of this research article.

**Competing Interests**

Authors have declared that no competing interests exist.

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**Table 1. Results of diagnostic techniques for TB**

| Test     | No. of patients | True Positive | False Negative | Total | Sensitivity | Specificity |
|----------|-----------------|---------------|----------------|-------|-------------|-------------|
| AFB      | 10 (16.7%)      | 50 (83.3%)    | 60             | 100%  | 16.6%       | 100%        |
| Real-time PCR | 39 (65%)        | 21 (35%)      | 60             |       | 100%        | 58%         |
| Gene X-Pert | 43 (71.7%)     | 17 (28.3%)    | 60             |       | 100%        | 66%         |

The chi-square statistic is 43.2658. The p-value is < 0.0001. The result is significant at p < 0.005.
Comparison and evaluation of diagnostic techniques of Mycobacterium tuberculosis in Iraq

Abdul-Sattar A. K. AL-Seedi et al.

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