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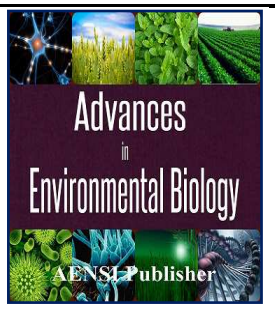

Evaluating the Diagnostic methods used for Tuberculosis (TB) Diagnosis

Nktel Faaz AL-Saad

College of Biotechnology, Al-qasim Green university-Iraq

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ABSTRACT

Tuberculosis(TB) is one of the most common infectious diseases worldwide. Immune anergy caused by several mechanisms increases the severity of this disease. **Aim of this study:** To evaluate the commonly methods used in laboratory for the diagnosis of TB **Methods:** This study was done on 70 patients infected with TB admitted to the Specialized Chest and Respiratory Center in Al-hilla, Iraq. It's also involved 30 obviously healthy control . The patients consists of 43 males and 27 females with age range 8-76 years old, 29 of them were diabetic . Sputum samples were collected from patients and controls for evaluation the diagnostic techniques. **Results:** The results of this study showed that the diagnosis of TB using GeneXpert method was highly sensitive 92.9% sensitivity followed by acid fast bacilli (AFB) smear 50% and culture 31.4% and accuracy rates was 95%, 65% and 52% respectively. **Conclusion:** The current study shows that the GeneXpert is regarded as the best method used for the diagnosis of TB.

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INTRODUCTION

Tuberculosis (TB) remains a major global health problem. Ten million new TB cases and 2 million deaths are estimated to occur each year, more than any time in history [15]. Furthermore, an estimated 2 billion people are thought to be latently infected, providing a large reservoir for active TB that will last for decades [7]. Tuberculosis is an immunological disease and the response to this disease is mediated by the host immune response [11].

The delayed and improper diagnose of active tuberculosis (TB) in developing countries remains a major barrier in the world control of the disease [13]. TB is a curable disease when early and appropriately diagnosed and treated. In 2010, an estimated 8.8 million people became ill with TB, of which 3.1 million with active disease were not diagnosed and notified to national TB control programs; To increase widespread use in resource limited situation, modern tools are required for the diagnosis of TB [14].

The currently available technologies that are use for diagnosis of TB such as smear microscopy and cultur have distinguished limitation. While advanced technologies have results in a large improvements, more clear changes may be noticed with modern or expected diagnostics technologies, particularly nucleic acid amplification technologies (NAAT). In 2010, WHO approved that the tests for TB and rifampicin resistance was reliably done by an automated, bench-top device GeneXpert® MTB/RIF. It is quite easy to use and give the results within hours, and may be used at decentralized levels [5].

MATERIALS AND METHODS

Patients and controls:

This study involved a total of 70 patients (43 males and 27 females) ranged in age from 10 to 75 years , 29 of them were diabetic. They were admitted to the Specialized Chest and Respiratory Center in Al-hilla, Iraq during the period from January to August 2015. A total of 30 apparently healthy individuals (19 males and 11 females) were involved as controls group. The age range of controls was approximately matched to the patients (12-75) years.

Corresponding Author: Nktel Faaz AL-Saad, College of Biotechnology, Al-qasim Green university-Iraq
Tel: 009647831719491; E-mail: n.faaz@yahoo.com

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A special sterile container with a screw cap were used to collected early morning sputum sample (at least one milliliter) for direct acid fast stain test, then the sample processed using 4%NaOH with vortex shaking and refrigerated centrifugation at 3000 Gforce (Gforce=[1.118*10-5]*Radius of the centrifuge rotor*rpm2) for 15 minutes, the supernatant was discard and the alkaline precipitate neutralized by adding fuming of 73%H2SO4 and The resultant neutral suspension became prepared for culture and molecular study [12].

Staining of Sputum Smears with Ziehl-Neelson Stain:

The procedure of staning by Ziehl-Neelsin technique was down according to Varain*et al.*, [12].

Cultivation:

Decontamination and Homogenization of Sputum Specimens (Method-Petroff's Procedure):

The procedure of processing and cultivation of sputum samples was carried out according to Bhawan, [2].

Detection of *Mycobacterium tuberculosis* byXpert®MTB/RIF System:

The method was accomplished according to the orders of the manufacturing company (Cepheid, USA).

Ethical approval:

The essential ethical approval from the Specialized Chest and Respiratory Center in Al-hilla, Iraq was gained. Moreover, all patients collected in this work were knowledged and the agreement was gained from each one before the collection of samples.

Statistical Analysis:

The following equation were used to compute the sensitivity and specificity [10]:

$$Diagnostic\ sensitivity = \frac{True\ psitive}{True\ positive + False\ negative}$$

$$Diagnostic\ specifity = \frac{True\ negative}{True\ negative + False\ positive}$$

$$Diagnostic\ accuracy = \frac{True\ psitive + True\ negative}{True\ positive + False\ Positive + True\ negative + False\ negative}$$

RESULTS AND DISCUSSION

The sensitivity, specificity and accuracy of laboratory diagnostic technique were calculated based on the golden standard which was the clinical diagnosis. The sensitivity of the AFB smear, culture and Gene Xpert of the clinically conformed 70 TB cases in this study were 50%, 31.4% and 92.9%, respectively. The specificity of the AFB, Culture and Gene Xpert methods were 100%. Therefore the accuracy (95%) were obtained from GeneXpert results followed by AFB smear (65%), (Table- 1).

Table 1: specificity, Sensitivity and accuracy of acid fast bacilli (AFB) smear, culture and GeneXpert for clinically diagnosed TB patients.

Test	No. of Patients		Sensitivity	Specificity	Accuracy
	True positive	False negative			
AFB smear	35	35	50%	100%	65%
Culture	22	48	31.4%	100%	52%
GeneXpert	65	5	92.9%	100%	95%

The better sensitivity reported in this study was the real time-PCR based GeneXpert technique, the specificity was 100% table (3-1). These results were in agreement with those results being reported by Raviglion who stated that the real-time PCR assay with an internal control achieved a sensitivity of 96.2% and specificity of 99.2%.

The current results found that (5) patients of (70) clinically definite TB patients were GeneXpert negative, and this result was suggested that those (5) TB infected patients were under TB management and may have arrived to advanced levels of management made their sputum smear, culture and GeneXpert results to be negative.

This study showed that the GeneXpert were positive in 85% (30/35) of smear negative TB cases. The result was higher than that reported by another study of the performance of PCR tests for the diagnosis of tuberculosis which reported that sensitivity was 66-73% in smear-negative samples [6]. Nicol and Zar [8] reported that a sensitivity of 66% for smear-negative TB by using different commercial PCR assays. The rapid identification of *M. tuberculosis* in smear-negative samples as well as in smear-positive samples is important for prevention of tuberculosis transmission, because about 17% of tuberculosis cases involve transmission from persons with negative AFB smear results [4].

The results of AFB smear in this study showed a sensitivity of 50%. The specificity of AFB smear was 100%. Direct microscopic inspection of sputum for AFB was rapid, cheap and quite easy to achieved. Compared to mycobacterial culture, the sensitivity of a single sputum AFB smear is 30% to 40%, but it increased to reach 65% to 80% with multiple specimens or concentrated sputum [3]. The AFB smear mainly has two disadvantages. First, the sensitivity of AFB smear is such that it requires 10^3 to 10^4 organisms per ml of sample to register as positive case. The second disadvantage is that direct microscopy cannot distinguish between *M. tuberculosis* and non-tuberculosis mycobacteria NTM [9].

In the current study, TB culture demonstrated low sensitivity (31.4%) and high specificity (100%). The anti-TB antibiotic administration by many TB patients may be the causative of low sensitivity of TB culture that included in this study. Culture requires an average of 21-30 days to obtain results, however, the sensitivity of culture ranges from 50% to 81% [1].

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