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Brief report

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Comparison of culture, microscopic smear and molecular methods in diagnosis of tuberculosis

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ABSTRACT

Objectives. Tuberculosis (TB) is a public health problem worldwide, with the highest mortality . The development of nucleic acid-based tests for detection of *Mycobacterium tuberculosis* complex (MTBC) has significantly increased sensitivity compared to conventional smear microscopy and provides results within a matter of hours compared to weeks for solid culture, which is the current gold standart. The aim of this study was to compare the culture, microscopic smear and molecular method in the diagnosis of TB .

Material and methods. Seven hundred ninety specimens belonging to clinically suspected cases of TB were studied retrospectively. The specimens were grouped as respiratory and non-respiratory and the groups were compared for mycobacterial detection assays. The culture and the molecular diagnostic GeneXpert MTB/RIF (GX) assay method were compared.

Results. When culture was used as the reference standart, 32 (4.05%) specimens were positive for MTBC. Of the 32 culture positive clinical specimens 24 (3.03%) were respiratory and 8 (1.01%) were non-respiratory specimens. All 24 of the 24 respiratory specimens were positive by the GX test, Seven of the eight non-respiratory specimens positive for culture were positive by GX assay. Five of the seven hundred fifty-eight samples of culture negative were positive with GX assay. Sensitivity and specificity of GX were found to be 96.8 % and 99.3 %, respectively.

Concluisons. Molecular methods to acquire time in diagnosis as well as the increase in linearity gives a different perspective to the diagnosis of tuberculosis. The GX assay has a diagnostic utility for rapid diagnosis of TB.

Key Words: Tuberculosis, GeneXpert, Culture, Acid-fast Bacilli

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Comparación del cultivo, frotis y un método molecular en el diagnóstico de la tuberculosis

RESUMEN

Objetivos. La tuberculosis (TB) es un problema de salud pública a nivel mundial, con la mortalidad más alta. El desarrollo de pruebas basadas en ácido nucleico para la detección de complejo de *Mycobacterium tuberculosis* (MTBC) ha aumentado significativamente la sensibilidad en comparación con la microscopía de frotis convencional y proporciona resultados en cuestión de horas en comparación con semanas para cultivo sólido, que es la prueba de referencia. El objetivo de este estudio fue comparar el cultivo, el frotis microscópico y un método molecular en el diagnóstico de la tuberculosis.

Material y métodos. Se estudiaron retrospectivamente 790 especímenes pertenecientes a casos clínicamente sospechosos de TB. Las muestras se agruparon como respiratorias y no respiratorias y los grupos se compararon para los ensayos de detección de micobacterias. Se comparó el cultivo y el método de análisis molecular GeneXpert MTB / RIF (GX).

Resultados. Cuando se utilizó el cultivo como prueba de referencia, 32 (4,05%) muestras dieron positivo para MTBC. De las 32 muestras clínicas con cultivo positivo, 24 (3,03%) fueron respiratorias y 8 (1,01%) fueron muestras no respiratorias. Todas las 24 muestras respiratorias fueron positivas mediante la prueba GX. Siete de las ocho muestras no respiratorias positivas para cultivo fueron positivas mediante la prueba GX. Cinco de las setecientas cincuenta y ocho muestras del cultivo negativo fueron positivas con la prueba GX. La sensibilidad y la especificidad de GX fueron del 96,8% y 99,3%, respectivamente.

Conclusiones. Los métodos moleculares para ganar tiempo en el diagnóstico así como el aumento en la linealidad dan una perspectiva diferente al diagnóstico de tuberculosis. La prueba GX tiene una utilidad diagnóstica para el diagnóstico rápido de la tuberculosis.

Palabras Claves: Tuberculosis, GeneXpert, cultivo, bacilos ácido-alcohol resistentes

INTRODUCTION

Tuberculosis (TB) is a chronic disease caused by a type of *Mycobacterium tuberculosis* (MTB). TB is spread from person to person through the air. TB is the most common cause of death from infectious disease. In 2016, 6.3 million new cases of TB were reported (up from 6.1 million in 2015), equivalent to 61% of the estimated incidence of 10.4 million; the latest treatment outcome data show a global treatment success rate of 83%, similar to recent years . In 2016, a total of 12.417 TB cases were reported in Turkey, with an Incidence rate of 14: 100 000 patients with suspected TB [1].

Clinicians evaluate patients with suspected TB by medical history, physical examination , chest radiograph and checking up on patiens symptoms . TB is diagnosed by detecting of Mtb bacteria in a clinical specimen. Culture are remains the gold standard for laboratory confirmation of TB disease, and growing bacteria are required to perform drug-susceptibility testing. GeneXpert MTB/RIF (GX) (Cepheid, Sunnyvale, California, USA) assay is a new molecular test for TB which diagnoses Mtb by detecting the presence of Mtb bacteria, as well as testing for resistance to the drug rifampin [2,3].

In this study, we retrospectively evaluated the performance of solid and liquid culture media, acid-fast bacilli (AFB) testing and GeneXpert methods for respiratory and non-respiratory specimens for the diagnosis of TB.

MATERIAL AND METHODS

Clinical specimens. A retrospective study was conducted from January 2016 to June 2017 at the Ataturk Research and Traning Hospital, Department of Medical Microbiology , Izmir, Turkey. Respiratory and non-respiratory clinical specimens collected from patiens with suspected Mtb or nontuberculous mycobacterial (NTM) infection. A total of 790 specimens were assessed by solid (Löwenstein-Jensen), liquid (Bactec MGIT960) culture media and GX assay. Of the 790 specimens 483 were respiratory (sputum, broncho alveolar lavage, tracheal aspirate), and 307 were non-respiratory (urine, pleural fluid, ascites, tissue biopsy, abscess, bile fluid, cerebro spinal fluid) specimens.

Laboratory methods. Clinical specimens were decontaminated using the N-acetyl-L-cysteine sodium hydroxide method (NALC-NaOH). After the centrifugation step, the sediment was resuspended in 1 to 1,5 of sterile phosphate buffer (pH 6.8). This suspension was used for inoculation of culture media. A smear of the processed sediment was prepared and examined for the presence of AFB.

Liquid culture media based on fluorometric detection of growth. Mycobacteria Growth Indicator Tube (MGIT) tubes were inoculated with 0.5 ml of the processed specimen. The tubes were incubated in the MGIT 960 instrument at 37°C.

Solid culture media, Lowenstein-Jensen (LJ) (Salubris , Turkey) was inoculated with 0.25 ml suspension processed for each specimen and incubated at 37°C. For tubes identified as positive, a smear of a sample from the tube was prepared for examination for AFB. All smears were stained by the Kinyoun method and examined with a light microscope.

MTB strains isolated from culture were identified using the MGIT TBc ID method (MPT 64: Becton Dickinson, Sparks, Maryland, USA). After identification of MTB complex strains, drug susceptibility test (DST) was performed using MGIT SIRE (Becton Dickinson-Sparks, Maryland, USA) according to the manufacturer's recommendations. Tests were performed using the final concentration (83 µg/ml) of streptomycin (STR), (8,3 µg/ml) isoniazid (INH), (83 µg/ml) rifampin (RIF), (415 µg/ml) etambutol (EMB).

The GX for MTB/RIF assay procedure, the GX assay was performed following the manufacturer's recommendations. Decontaminated samples were mixed with a sample reagent containing sodium hydroxide and isopropanol alcohol (GX reagent). Two milliliters of each sample was transferred to a test cartridge and inserted into the GX platform. Results were available 1 hour and 55 minutes later.

RESULTS

A total of 790 specimens with suspected TB infection which were assayed by liquid and solid culture, smear microscopy, GX method and conventional drug susceptibility testing. The results of culture, smear microscopy, and GX for all specimens are presented in table 1. Of the 790 specimens, 32 (4.05%) were culture positive for MTB. Of the 32 culture positive specimens, 24 (3.03%) were respiratory and 8 (1.01%) were non-respiratory.

Two specimens were culture-positive for non-tuberculosis mycobacteria (NTB). These two bacteria outside of MTBC were not detected by molecular methods. Because only MTBC types could be detected with the GX assay. Because of this, these two bacteria were considered out of the evaluation. According to culture results, the overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of GX and smear microscopy are shown in table 2.

Thirty two Mtb isolates were tested for RMP resistance by the conventional drug susceptibility testing. Twenty nine (90.6 %) were found to be susceptible to RMP, while three (9.4 %) were resistant to RMP. All of the three samples identified as resistant by conventional methods were also found to be resistant by the GX method.

DISCUSSION

Classic laboratory techniques such as direct microscopy for the diagnosis of tuberculosis are far from being sensitive. Furthermore, cultures are time-consuming, they require biosafety precautions and need educated laboratory personnel [4].

Table 1 The results of culture, AFB and GX tests for all specimens

Method	All specimens (n=790)	Respiratory specimens (n=483)	Non-respiratory specimens (n=307)
Culture (+)	32	24	8
AFB (+)	17	13	4
GX (+)	31	24	7
Culture (-)	758	459	299
AFB (+)	0	0	0
GX (+)	5	4	1

AFB: acid-fast bacilli

Table 2 According to culture results, the overall sensitivity, specificity, PPV and NPV of GX

Method	Sensitivity % (95% CI)	Specifitiy % (95% CI)	PPV %	NPV %
AFB	53 (34 - 70)	100 (99 - 100)	100	98
GX	96 (83-99)	99 (98-99)	86	99
AFB (Respiratory)	54 (32 - 74)	100 (99 - 100)	100	97
AFB (Non-Respiratory)	50 (15 - 84)	100 (98 - 100)	100	98
GX (Respiratory)	100 (87-100)	99 (97-99)	85	100
GX (Non-Respiratory)	87 (51-99)	99 (98-99)	87	99

AFB: acid-fast bacilli, PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval

Molecular techniques have substantially changed in the field of tuberculosis diagnosis and they have been proven to yield rapid results as well as being highly sensitive.

Culture continues to be the gold standard for the diagnosis of TB, but isolation can take up to 6 weeks due to slow growth rate of the organism [5]. Smear microscopy to detect acid-fast bacilli in clinical specimens is a rapid and inexpensive test, although our study showed that microscopic detection sensitivity was 54 % in respiratory samples and 50 % in non-respiratory samples.

However, despite having been proved to be a sensitive and rapid method when compared to the other methods evaluated in this study, GX proved to be more sensitive in both respiratory (100 vs. 54 %) and non-respiratory (87 vs. 50 %) specimens than smear testing.

Ionniadis *et al.* Analyzed 80 respiratory and 41 non-respiratory samples, and reported the sensitivity, specifity, PPV, NPV of the GX system for respiratory and non-respiratory samples as 90%, 94%, 93%, 91%, 100%, 91%, 50%, 100% respectively. The GX system was found to be an advantageous technique for the identification of MTB , especially in smear-negative samples [6].

Bunsow *et al.* performed a study including 290 respiratory and 305 nonrespiratory. They reported the sensitivity and specifity, PPV, NPV values of GX as for respiratory specimens 97%, 98%, 95%, 99%, respectively and for non-respiratory specimens as 33%, 99%, 80%, 97% respectively. The values for respiratory samples were higher than the values of our study. The GX system was reported to be a rapid and it gave accurate results in identifiyng MTB particularly in smear positive respiratory specimens [7].

Zeka *et al.* performed a study including 253 respiratory and 176 non-respiratory specimens. They found the sensitivity and specificity, PPV, NPV values of GX for respiratory and non-respiratory specimens as 86%, 99%, 96%, 98% and 67%, 96%, 93%, 80% respectively. They reported that the GX assay was a rapid and useful technique in the identification of MTB [8].

Bilgin *et al.* performed a study including 243 respiratory, 684 non-respiratory specimens. The sensitivity, specificity, PPV, NPV values of GX for respiratory and non-respiratory samples were 100%, 98%, 87%, 100% and 71%, 98%, 71%, 98%, respectively. The GX method was reported to be a practical technique because it has a high sensitivity and gives rapid results for identification of MTB [9].

Wadwai *et al.* performed a study consisting of 547 non-respiratory specimens and they found the sensitivity and specificity of GX as 77% and 75%, respectively [10]. In another study, Tortellini *et al.* evaluated 1476 non-respiratory specimens and reported the sensitivity and specificity of the GX as 81% and 99%, respecticely [11]. Both studies concluded that the NALC-NaOH decontamination could affect the quality of the specimens reducing the sensitivity of the GX for MTB detection.

The main purpose of this study was to assess the effectiveness of the GX assay in testing AFB-negative specimens collected from patients with clinical signs highly suggestive of active TB.

The results of the culture, smear microscopy and GX assay in our study correlate with those reported by other studies when the effectiveness of the GX assay in detecting the presence of MTB bacilli in AFB negative specimens is considered.

In our study, since culture was accepted as a standard, a total of five false positives were detected. A total of five samples were identified from four respiratory specimens from one non-respiratory specimen. Contamination in molecular methods is a consideration. In addition, live bacteria may not be taken as a specimen in treated patients. Since live and dead bacilli can not be discriminated by PCR methods, it is known that false positivity can be seen in patients with a history of MTB [9].

In our study, seventeen AFB positive samples was detected

in culture-positive 32 samples. The sensitivity and specificity of AFB were found to be 53% and 100%, respectively. Similar results were found in the studies. In a study in Thailand, sensitivity and specificity of the sputum AFB smear and GeneXpertMTB/ RIF assay test were 48% and 84%, and 94% and 92%, respectively [12]. Although AFB is effective in eliminating tuberculosis-negative patients, it is less effective in detection than Genexpert.

Thirty-two patients were diagnosed as TB in our hospital in this study period. We think that tuberculosis cases will increase due to immigration from Middle East (especially, Syria) and frequent use of immunosuppressant therapy.

In conclusion, early diagnosis has great importance for the treatment of tuberculosis and the GX system is an easy and helpful tool for rapid and reliable results with high specifity and sensitivity.

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None to declare

CONFLICT OF INTEREST

The author declare that they have no conflicts of interes

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