THE EARLY DETECTION OF MYCOBACTERIUM TUBERCULOSIS AND RIFAMPICIN RESISTANCE IN SMEAR NEGATIVE TUBERCULOSIS

Original Article

YAYMA NEGATİF TÜBERKÜLOZDA MİKOBAKTERİ TÜBERKÜLOZ VE RİFAMPİSİN DİRENCİNİN ERKEN SAPTANMASI

Meltem Agca

Sureyyapasa Chest Diseases and Thoracic Surgery Teaching and Research Hospital, Istanbul, Turkey

Suna Turk

Sureyyapasa Chest Diseases and Thoracic Surgery Teaching and Research Hospital, Istanbul, Turkey

Dildar Duman

Sureyyapasa Chest Diseases and Thoracic Surgery Teaching and Research Hospital, Istanbul, Turkey

Kadriye Terzioglu

Uludağ University, Faculty of Medicine, Bursa, Turkey

Merve Tepetam

Sureyyapasa Chest Diseases and Thoracic Surgery Teaching and Research Hospital, Istanbul, Turkey

Aysun Misirlioglu

Sureyyapasa Chest Diseases and Thoracic Surgery Teaching and Research Hospital, Istanbul, Turkey

Mualla Partal

Süreyyapaşa Göğüs Hastalıkları ve Göğüs Cerrahisi Eğitim ve Araştırma Hastanesi, Istanbul, Turkey

Turan Karagöz

Süreyyapaşa Göğüs Hastalıkları ve Göğüs Cerrahisi Eğitim ve Araştırma Hastanesi, Istanbul, Turkey

Corresponding Author

Merve Tepetam
Süreyyapaşa Göğüs Hastalıkları ve Göğüs cerrahisi Eğitim ve Araştırma Hastanesi
İmmunoloji ve Allerji Kliniği, Maltepe Başıbüyük Mah. B blok. Kat:5 34854. Istanbul, Turkey
Tel: 02164214200-1177
Email: fatmamervealan@hotmail.com

ABSTRACT

Introduction: Early diagnosis and accurate treatment of tuberculosis is the most effective way to control the disease and prevent transmission. Xpert Mycobacterium tuberculosis (MTB)/ rifampicin (RIF) system is a nucleic acid amplification technology-based real time polymerase chain reaction (PCR) test, which can detect mycobacterium tuberculosis and rifampicin resistance in clinical specimens within two hours. In this study we aimed to evaluate diagnostic performance of Xpert MTB/RIF assay.

Materials and Methods: 129 clinical samples including sputum, bronchial lavage and pleural fluid. Of these samples, 40 were smear positive and culture positive, 41 were smear-negative and culture-positive. In the study the results of X-pert MTB/RIF assay was compared to those obtained by culture (Löwenstein Jensen medium).

Results: All smear positive and culture positive cases has been determined by Xpert MTB/RIF test. Xpert MTB /RIF test reached 95.12% sensitivity, 97.82% specifity in the diagnosis of smear-negative pulmonary tuberculosis. The sensitivity of the test in detecting rifampicin resistance was found as 81.8% with a rate of 96.92% spesificity.

Conclusion: Reliable results for detection of mycobacterium tuberculosis and rıfampicin resitance can be obtained with Xpert MTB/RIF test within two hours of receipt.

Key words: Tuberculosis, rapid diagnosis, Xpert MTB/RIF test, rifampicin resistance.

ÖZET

Amaç: Erken tanı ve doğru tedavi tüberküloz hastalığının kontrolü ve bulaştırıcılığını önlemek için en etkili yoldur. Xpert Mycobacterium tuberculosis MTB / Rifampisin (RIF) sistemi Xpert (MTB) iki saat içinde klinik örneklerde mikobakteri tüberküloz ve rifampisin direncini saptayabilen nükleik asit amplifikasyon teknolojisi tabanlı gerçek zamanlı polimeraz zincir reaksiyonu (PCR) testidir. Bu çalışmada Xpert MTB / RIF testinin tanısal performansının değerlendirilmesi amaçlandı.

Materyal ve Method: Balgam, bronş lavajı ve plevra sıvısı içeren 129 klinik örnek alındı. Bu örneklerin, 40 ı yayma ve kültür pozitif, 41 i yayma negatif ve kültür pozitif idi. Çalışmada X-Pert MTB / RIF test sonuçları ile kültür (Löwenstein Jensen ortamı) sonuçları karşılaştırıldı.

Bulgular: Tüm yayma ve kültür pozitif örnekler Xpert MTB / RIF testi ile tespit edilmiştir. Yayma negatif kültür pozitif akciper tüberkülozu olgularında Xpert MTB / RIF testi %95,1 duyarlılığa, %97,8 özgüllüğe ulaştı. Rifampisin direncinin saptanmasında testin duyarlılığı %96,9 özgüllük oranı ile %81.8 olarak bulundu.

Sonuç: Xpert MTB / RIF testi ile mikobakteri tüberküloz ve rifampisin direncinin saptanmasında iki saat içinde güvenilir sonuçlar elde edilebilir.

Anahtar kelimeler: Tüberküloz, hızlı tanı, X-pert MTB/RIF test, rifampisin direnci

INTRODUCTION

Incidence of drug resistant tuberculosis infection increase globally and this situation has created a critical need for new methods that can rapidly identify Mycobacterium tuberculosis and drug resistance (1). Rate of tuberculosis cases is 22.5/100.000, rate of multidrug resistant cases in new cases is 2.5 %, 22.8% in previosly treated cases and 5% in all cases in Turkey in 2010 (2). Traditional methods used in the diagnosis of M. tuberculosis are direct microscopic examination and culture (2). Direct microscopic examination can be performed guickly and cheaply but it has low sensitivity and low positive predictive value (2). Lowenstein-Jensen Culture was accepted gold standard for diagnosis of M. tuberculosis but it has long waiting time as 4-6 weeks (2). Rapid detection of M. tuberculosis and rifampicin (RIF) resistance is important for determination of multidrug resistant tuberculosis and effective treatment and prevention of spread of disease.

GeneXpert MTB/RIF test is a nucleic acid amplification technology-based heminested real-time polymerase-chain-reaction (PCR) assay for rapid detection of tuberculosis and RIF resistance in smear-positive and smear-negative respiratory and non-respiratory clinical specimens. Mutations in the 81-bp rifampin resistance determining region (RRDR) of the rpoB gene exist in 95-98% of all rifampin-resistant strains and this test assay to amplify an MTB-specific seguence of the rpo B gene (3,4) The aim of this study is to determine the sensitivity, specifity, positive predictive value (PPV), negative predictive value of Xpert MTB/RIF test for detection of tuberculosis and RIF resistance.

MATERIALS AND METHODS

Samples of 129 patients (103 sputum, 23 bronchiolar lavage, 3 pleural fluid) were included in this study at the Sureyyapasa Chest Diseases and

Thoracic Surgery Teaching and Research Hospital, Istanbul, Turkey. This is the largest chest disease hospital of the country. Of these samples, 40 were smear positive and culture-positive ones, 41 were smear-negative and culture-positive ones. Patients who were suspected of having pulmonary TB based on clinical evaluation and who have: 1) not received anti-tuberculosis therapy, 2) had < 7 days of therapy or 3) have not received anti-tuberculosis therapy in the last 60 days evaluated by Xpert MTB/RIF test. 46 samples were smear negative and culture negative ones which were obtained from non-TB patients whose clinical and radiological findings were not compatible with tuberculosis diagnosis.

Direct microscopic examination of all samples was determined by staining Ziehl -Neelsen. All of the samples were inoculated in Löwenstein-Jensen medium. Negative cultures were identified and drug susceptibility testing of Mycobacterium tuberculosis complex have been studied in 76 samples. These 76 culture positive specimens also have been investigated by Xpert MTB/RIF test for rifampicin resistance.

XpertMTB/RIF method

The Cepheid GeneXpert System integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR. The system consists of an instrument, personal computer, barcode scanner, preloaded software for running tests on collected samples and viewing the results. The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. The primers in the Xpert MTB/RIF assay amplify a portion of the rpoB gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance.

In our study, sample reagent was added in a 2:1 ratio to untreated sputum sample, replace the lid and shake vigorously 10 - 20 times. Than were incubated for 15 minutes at room temperature. At one point between 5 and 10 minutes of the incubation again shake the specimen vigorously 10-20 times. Samples should be liquefied with no visible clumps of sputum. 2-3 ml aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark. We opened the cartridge lid. Transfer sample into the open port of the Xpert MTB/RIF cartridge than closed the cartridge lid. Make sure the lid snaps firmly into place. All other test were done automatically by GeneXpert instrument. Results were obtained with the GX2.1 software, after 1 hour 55 minutes of laboratory arrival.

In XpertMTB/RIF test, detection of Mycobacterium tuberculosis complex in case has been viewed as 'MTB Detected on the the computer screen; if not detected it has been viewed as 'MTB not detected'. For rifampicin resistance the presence of rifampicin resistance has been viewed as 'Rif resistance detected'; absence 'Not Detected Rif resistance' (5).

Statistical Analysis

Chi-square test was used for the evaluation of diagnostic ratio of XpertMTB/RIF 's in the detected culture-positive samples and relationship with direct microscopy, $p<0.05$ was considered significant. In this study; we compared the results of Xpert MTB/RIF system with the Löwenstein Jensen culture results which is the gold standart in the diagnosis of pulmonary tuberculosis and compare the system's sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV).

RESULTS

Of the 129 patients, 83 were men and 46 were women. The mean age of the

patients was 45.3 SD = 19.9 and the median value was 42.

83 patients were diagnosed as tuberculosis according to culture results, among them 72 were new cases of and 11 were recurrent cases. In the study, direct microscopic examination results of the cases had been 89 smear-negative and 40 smear-positive.

In 40 smear-positive culture-positive cases, M. tuberculosis complex positivity has been determined as 100% by the XpertMTB/RIF test. Of the smear-negative 89 cases, growth of Mycobacterium tuberculosis complex were detected in the culture result of 41 cases, any growth of Mycobacterium tuberculosis complex was not detected in 46 cases. XpertMTB/RIF were detected Mycobacterium tuberculosis complex in the 39 of the 41 culture positive cases, the presence of the Mycobacterium tuberculosis complex has not been detected in 4 of the culture positive cases. Among the 46 culture negative cases M. tuberculosis complex were not detected in 45 cases by XpertMTB/RIF, only one sample was found positive for M. tuberculosis complex. The results of smear, culture and XpertMTB/ RIF method was shown in Table 1. Sensitivity, specifity, positive predictive value and negative predictive value of XpertMTB / RIF method in smear negative cases was shown in Table 2.

Smear	**XpertMTB/RIF**	**Culture**		**Total**
		Positive	**Negative**	
Smear negative	Positive	39	1	**40**
	Negative	4	45	**49**
	Total	**41**	**46**	**89**
Smear	**XpertMTB/RIF**	**Culture**		**Total**
		Positive	**Negative**	
Smear positive	Positive	**40**	**0**	**40**
	Negative	0	0	**0**
	Total	**40**	**0**	**40**

Table 1. The results of smear, culture and XpertMTB / RIF test.

Calculated value	**Result**	**%95 CI**
Sensitivity	%95,12	%(83,4 – 99,4)
Specifity	%97,82	%(88,4 – 99,9)
Positive predictive value	%97,5	%(86,8 – 99,9)
Negative predictive value	%91,86	%(80,4 – 97,7)

Table 2. Sensitivity, specifity, positive predictive value and negative predictive value of XpertMTB/RIF method in smear negative cases

Among smear-negative samples, XpertMTB/RIF test results were evaluated according to the type of sample. XpertMTB/RIF test results in 64 sputum and 22 bronchial lavage samples were shown in Table 3-4.

Sample	**XpertMTB/RIF**	**Culture**		**Total**
		Positive	**Negative**	
Sputum	Positive	34	0	**34**
	Negative	4	26	**30**
	Total	**38**	**26**	**64**
Sample	**XpertMTB/RIF**	**Culture**		**Total**
		Pozitive	**Negative**	
Bronchial lavage	Positive	5	1	**6**
	Negative	0	16	**16**
	TOTAL	**5**	**17**	**22**

Table 3. The results of Xpert MTB/RIF test in sputum and bronchial lavage samples

	Sputum		**Bronchial lavage**	
Calculated value	**Result**	**%95 CI**	**Result**	**%95 CI**
Sensitivity	% 89,47	%(75,2 – 97,1)	% 100	%(47-100)
Specifty	% 100	%(86,8 – 100)	% 94,1	%(71,3-99,8)
PPD	% 100	%(89,7 – 100)	% 83,3	%(35,8-99,5)
NPD	%86,66	%(69,3 – 96,2)	%100	%(79,4-100)

Table 4. Sensitivity, specifity, positive predictive value and negative predictive value of XpertMTB/RIF method in sputum and bronchial lavage samples

Drug susceptibility testing was performed in 76 of 83 who had positive cultures. Rifampicin resistance was detected in 11 of 76 cases. With XpertMTB/RIF test 9 of 11 resistant to rifampicin confirmed as resistant but in 2 resistant cases, XpertMTB/RIF test showed as rifampicin sensitive (False negativity). Within 65 rifampicin sensitive samples, 63 of them identified as rifampicin-sensitive by XpertMTB/ RIF test but 2 sample was determined as resistant to rifampicin by XpertMTB/RIF test (false positivity). (Table 5) Sensitivity, specifity, positive predictive value and negative predictive value of XpertMTB/RIF test for rifampicine resistance was shown in table 6.

XpertMTB/RIF	Drug susceptibility test		Total
	Resistant	Sensitive	
Resistant	9	2	**11**
Sensitive	2	63	**65**
Total	**11**	**65**	**76**

Table 5. The results of XpertMTB / RIF test for rifampicine resistance

Calculated value	Result	%95 CI
Sensitivity	% 81,81	%(48,2 – 97,7)
Specifity	%96,92	%(89,3 – 99,6)
PPD	%81,81	%(48,2 – 97,7)
NPD	%96,92	%(89,3 – 99,6)

Table 6. Sensitivity, specifity, positive predictive value and negative predictive value of XpertMTB / RIF test for rifampicine resistance

DISCUSSION

Ineffective tuberculosis detection and increase in multidrug resistance lead to investigate new rapid molecular tests in the world. X-pert MTB/RIF test is a real time PCR-based assay which can detect both TB and rifampicin resistance directly in clinical specimens. In this study we studied sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of X-pert MTB/RIF testand this assay appeared to be a reliable method for the diagnosis of TB and resistance to rifampicin.

Early and accurate diagnosis of TB is a global priority for control of disease. Direct examination of the smear by microscope is the first step in the diagnosis of M. tuberculosis. It is rapid, easy and inexpensive method, but it has low sensitivity (20-80%) (6,7,8). Smear-positivity can be detected only 44% of new tuberculosis cases and spread of the disease continues until diagnosis (9). Culture (Löwenstein Jensen medium) which is the basic diagnostic method for detection of tb takes a long time like 4-6 weeks. This delay in diagnosis can cause delay in treatment, progression and transmission of disease.

X-pert MTB/RIF test provided detection ofTB and rifampicin resistance in less than 2 hours and easily performed(5). Diagnostic accuracy of X-pert MTB/RIF test investigated and different results were reported. Sensitivity of test vary between 58-100% and specificity of the test vary between 86-100% in different studies(10). This difference can depend on sample size, tb incidence of population, type of sample like sputum or other and variation in bacilli load (11,12). In metaanalysis sensitivity and specificity of X-pert MTB/RIF testwere reported as 90.4% and 98.4% respectively (11). In our country Ozkutuk reported sensitivity and specificity of X-pert MTB/RIF test as 73,9 and 98,6% respectively for all types of samples. The sensitivity of the test reached 100% for smear positive

cases (10). In another study from our country, specificity of the testwas found similar but sensitivity was found as high as 96% (13). Cavusoglu reported sensitivity of X-pert MTB/RIF test as 93.8%, specificity of 98.8% , positive predictive value 86.5%, negative predictive value 99.5% for respiratory specimens. Sensitivity of smear positive 30 cases were 100% and 83.3% for smear negative 18 cases(4).

In our study, all patients with smear-positive bacilli were identified by GeneXpert MTB/RIF test, sensitivity was 100%. X-pert MTB/RIF test could not identify the presence of M. tuberculosis in 4 of 41 smear negative, culture positive cases. In one of the four remaining negative sputum samples, atypical mycobacteria were isolated. Non tuberculosis mycobacterial infections have been reported as the cause of negative results from other nucleic acid
amplification tests like our test (14). Center for Disease Control and Prevention (CDC) in 2000, has created a new algorithm for the use of molecular methods. According to this, in smear positive but nucleic acid amplification test is negative situations, if the inhibitory substance is not to detected then non-mycobacterium M. tuberculosis can be described (15). In this study, we found that Xpert MTB/RIF method has 95.1% sensitivity, 97.8% specificity, 97.5% positive predictive value, 91.8% negative predictive value and 94.3% correct diagnosis rate in the diagnosis of smear-negative pulmonary tuberculosis. In multicentered study Xpert MTB / Rif assay identified 551 of 561 cases with smear positive tuberculosis (98.2%) and 124 of 171 (72.5%) with smear negative tuberculosis (5).

Brown and colleagues has been studied GeneXpert MTB/RIF test with respiratory specimens and they found sensitivity, specificity, NPV and PPV as 94.05%, 97.83%, 94.74% and 97.53% as respectively (16). Sensitivity of the test has been different according to sample type and higher in sputum, sensitivity was found 86.2% in sputum, 79% in bronchoalvelar lavage and bronchial aspirate, 60% in endotracheal and transtracheal aspirate (10). Sensitivity of Gene Xpert MTB/RIF test was reported as %58.2 and specifitiy was 98.4%. In a meta-analysis of seven studies the sensitivity of the test was reported 25-95%, specifity 73-100% in the diagnosis of extrapulmonary tuberculosis (11). The overall sensitivity of calculated as 80 %, specificity as 86 % as a result of meta-analysis. Reliability of GeneXpert MTB/RIF test was very low in diagnosis of extrapulmonary tuberculosis compared to the the pulmonary tuberculosis, suggesting that this test is more suitable for respireatory samples (11). In our study most of the samples were consisted of sputum and there was no difference between bronchial lavage and sputum.

Detection of rifampicin resistance is considered as a good indicator of multidrug-resistant tuberculosis (17,18,19). The sensitivity, specifity, NPV and PPV of the Xpert MTB/RIF test for the detection of rifampicin resistance were found as 100%, 98.68%, 100%, and 84.62% (16). In a meta-analysis of seven studies the sensitivity and specifity of the XpertMTB/RIF test for the detection of rifampicin resistance, were reported as 17-98% and 72-100% respectively. The overall calculated sensitivity and specifity of meta-analysis were 94.1% and 97%. (11). In another multicentered study MTB/RIF testing correctly identified rifampicine resistance in 209 of 211 cases (99.1% sensitivity) and in all 506 patients with rifampicine susceptibility (100% specifity) (5). So the Xpert MTB/RIF assay is highly sensitive and specific for the detection of RIF resistance (5).

In this study drug susceptibility test was performed to 76 Lowenstein-Jensen culture positive, specimens. 53 samples were sensitive to all drugs. Gene Xpert MTB/RIF system correctly identified 9 of total of 11 RIF resistance, one of these false sensitive patients was MOTT and the other one had MOTT + Mycobacterium tuberculosis complex (mix infection). In

our study, the determination of RIF resistance with Xpert MTB/RIF, the sensitivity was 81.8%, specificity was 96.9%, positive predictive value was 81.8%, and negative predictive value was 96.92%.

Among these 11 MDR-TB patients, 6 cases were recurrent TB, and 4 were new cases. According to reports from our country, MDR-TB ratio were 3.1% in new cases, and 17.7% in previously treated cases (20). These results are parallel with increasing spread of the MDR tuberculosis disease in the community, they should be diagnosed as early as possible and treatment of these patients should be monitored closely.

Rapid and accurate diagnosis of tb is the most effective way to control the disease and interrupt transmission. Rifampicin resistance is an indicator of MDR and early detection of resistance has a critical role for appropriate treatment and management of drug resistant tuberculosis. X-pert MTB/RIF is an automated moleculer test which can detect Mycobacterium tuberculosis and resistance to rifampicin in clinical samples. It is a real time PCR assay which is superior in sensitivity than microscopic examination. The results can be obtained within 2 hours of receipt and easily performed.

We concluded that the X-pert MTB/RIF is a useful assay for rapid detection of Mycobacterium tuberculosis and rifampicin resistance. Complete costing and cost effectiveness of the X-pert MTB/RIF test can be studied in future publications.

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