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RESEARCH ARTICLE

Diagnostic value of mycobacteriophage based fast plaque technique for early detection of pulmonary tuberculosis

Mikobakteriyofaja dayalı hızlı plak yönteminin akciğer tüberkülozunu erken saptamadaki değeri

Servet Kayhan

Department of Chest Diseases, Medical Faculty of Ondokuz Mayıs University, Samsun, Turkey

ABSTRACT

Objectives: The aim of this study was to evaluate diagnostic performance of FASTPlaqueTB™ (FPTB) test and to compare with mycobacterial Löwenstein-Jensen (LJ) culture and smear test.

Materials and methods: Respiratory samples were obtained from 60 patients with suspected tuberculosis and 20 control patients who had any diagnosis other than tuberculosis. One sample of case with suspected tuberculosis was excluded from the study due to contamination. All specimens were examined by smear, LJ culture and fast plaque test for Mycobacterium tuberculosis.

Results: A total of 54 specimens of 59 patients with clinically and radiologic tuberculosis grew on LJ media and all the isolates were M. tuberculosis complex. *M. tuberculosis* was isolated by LJ culture from 42 smear-positive and 12 smear-negative specimens. Smear and LJ culture negative five cases were diagnosed as tuberculosis with clinical and radiologic findings. Fast plaque method determined M. tuberculosis in 36 (61%) specimens of 59 cases with tuberculosis. The sensitivity, specificity, positive predictive value, negative predictive value and efficiency of prediction were calculated as 61%, 100%, 100%, 46% and 0,70 respectively in all cases. We found the performance of fast plaque test with a high (73,8%) sensitivity in smear-positive cases and low sensitivity (29,4%) in smear-negatives.

Conclusion: Fast plaque assay is not a preferable diagnostic test to LJ culture or smear in M. Tuberculosis and It may be used for a rapid detection of pulmonary tuberculosis. *J Clin Exp Invest 2012; 3(2): 189-193*

Key words: Mycobacteriophage, Mycobacterium tuberculosis, diagnostic test, pulmonary tuberculosis

ÖZET

Amaç: Bu çalışmada, iki günde sonuç alınabilinen hızlı plak (FASTPlaqueTB™) yönteminin Löwenstein-Jensen (LJ) kültürü ve yayma incelemesi sonuçlarıyla karşılaştırılması amaçlanmıştır.

Gereç ve yöntem: Tüberküloz olduğu düşünülen 60 hastadan ve tüberküloz dışı akciğer hastalığı tanısı olan 20 kontrol hastasından solunum materyali alındı. Kontaminasyon nedeniyle tüberküloz şüphesi olan olgulardan bir tanesi çalışmadan çıkartıldı. Alınan tüm örneklere yayma, LJ kültürü ve hızlı plak yöntemiyle *Mycobacterium tuberculosis* incelemesi yapıldı.

Bulgular: Klinik ve radyolojik olarak tüberküloz olduğu düşünülen 59 olgunun 42'si yayma pozitif, 17'si ise yayma negatifti. Yayma pozitif olan 42 olgunun tamamında ve yayması negatif olan 17 olgunun 12 tanesinde olmak üzere toplam 54 örnekte kültür pozitifliği ile M.tuberculosis kompleksi izole edildi. 5 örnekte ise LJ kültür negatifliği saptandı. Hızlı plak yöntemiyle 59 tüberküloz olgusunun 36 (%61) örneğinde M. tuberculosis saptanabildi. Hızlı plak yönteminin duyarlılık, özgüllük, pozitif prediktif değer, negatif prediktif değer ve doğruluk düzeyleri sırasıyla %61, %100, %100, %46 ve 0,70 olarak hesaplandı. Hızlı plak yönteminin duyarlılığı; yayma pozitif olgularda %73,8 yayma negatif olgularda ise %29,4 bulundu.

Sonuç: Hızlı plak yöntemi, M. tuberculosis tanısında LJ kültürü ve yayma yöntemine tercih edilemez fakat akciğer tüberkülozunun hızlı tanısı için kullanılabilir.

Anahtar Kelimeler: Mikobakteriyofaj, Mycobacterium tuberculosis, hızlı tanı, akciğer tüberkülozu

INTRODUCTION

Tuberculosis (TB) is a common worldwide infectious disease that is preventable and curable. The World Health Organisation estimated the global burden of tuberculosis disease in 2009 as 9.4 million incident patients, 14 million prevalent cases and 2.38 million deaths and 11-13% of incident cases were HIV-positive.[1] Diagnosis of TB remains problematic due to slow rate of growth of Mycobacterium tuberculosis. With the increasing incidence of TB, many studies have been performed to find a rapid, handy and

Correspondence: Dr Servet Kayhan
Dept. Chest Diseases, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey Email: servet-kayhan@hotmail.com
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reliable diagnostic test. An array of new diagnostic approaches, including nucleic acid amplification, antibody detection, liquid culture, cellular immune recognition, antigen capture, and chemical and physical detection, have been developed. However, many of these techniques are expensive, complex and often require sophisticated equipment, making them unsuitable for routine application especially in low-income countries. Ziehl-Neelsen (ZN) staining and culture of microorganisms are traditional methods in the laboratory diagnosis of mycobacterial infections. The FASTPlaqueTB (FPTB) test, a rapid test (48 h) based on bacteriophage amplification technology to reflect the presence of viable M. tuberculosis, is used in the diagnosis of TB in respiratory specimens. We aimed in this study to evaluate diagnostic performance of FPTB and to compare it with mycobacterial LJ culture and smear test by using bronchial fluid and sputum.

MATERIALS AND METHODS

Patients

Tuberculosis "suspect" patients are those who present with symptoms and signs suggestive of TB, in particular cough of long duration and with specific findings including cavitation, infiltration, nodularity in their chest X-rays. Patients were classified into three groups (A, B and C) according to radiological findings. Group-A included the minimal advanced cases having non cavitary or minimal infiltration. Moderately advanced cases with cavitation smaller than 4 cm or smaller infiltration area than one third of the lungs were included in group-B. Far advanced tuberculosis with cavitation greater than 4 cm or widely infiltration was included in group-C.

Samples

A total of 80 sputum and bronchial fluid samples of different patients with pulmonary disease who were hospitalized in a tuberculosis referral hospital (İstanbul, Turkey). Respiratory samples were obtained from consecutive 60 patients with suspected tuberculosis and 20 control patients who had any diagnosis other than tuberculosis. All respiratory samples were examined by smear, LJ culture and FPTB assays for M.tuberculosis. Only one sample was excluded from the study for the reason of contamination by candidiasis. So the results of 59 patients with clinically suspected tuberculosis were analyzed for test evaluations.

Homogenisation, decontamination and culture

The bronchioloalveolar lavage fluids (BAL) and sputum samples of suspected tuberculosis patients were subjected to homogenisation and decontamination process by N-Acetyl-L-Cysteine (NALC) and sodium hydroxide (NaOH) mixture and then to LJ culture. Each sample was divided into two portions. While one portion was immediately processed for culture, The processed sediments (0,5 ml) were inoculated in LJ medium. Mycobacterial cultures were incubated at 37°C for 8 weeks. LJ cultures were examined weekly for positive result. The standard biochemical tests were performed to identify M. tuberculosis isolates. Smears were stained by the Ziehl-Neelsen method.

Fast plaque tuberculosis (FPTB) assay and test control

FPTB assay was performed by the principles of Biotec website (Biotec Laboratories, Ipswich, UK; www.biotec.com). This test was carried out using the FPTB kit. Specimens were processed according to manufacturer's recommandations. Both positive and negative controls were tested according to the manufacturer's instructions. Negative controls contained 1 ml of FPTB broth, and 3 positive controls were prepared by serial dilutions (10^{-2}, 10^{-4}, 10^{-6}) of M. smegmatis. These controls were included to assess the integrity of the phage and the effectiveness of the phagicidal agent. For the assay, 1 ml of decontaminated and concentrated sediment was mixed with 1 ml of FPTB broth and incubated at 37°C overnight to enrich viable TB bacilli present in the sample. After enrichment, 100 µl of the mycobacteriophage solution (ActiphageTM) was added and incubated for a further 1 h to allow infection to take place; 100 µl of virucide solution (VirusolTM) was then added to destroy all bacteriophages that had not infected host cells, and incubated at room temperature for 15 min. Then 5 ml FPTB medium was added to neutralise excess virucide, followed by 1 ml of helper cells. After thorough mixing, it was added to the Petri dish and overlayered with 5 ml molten agar. The plates were rotated several times. The plates were then allowed to set and were incubated at 37°C. A sample was considered positive if the number of plaques on the plate was greater than 20. When the number was less than 20, the sample was considered negative. Samples were evaluated only if 20-300 plaques were present on the control plates including organisms, and if less than 10 plaques were present on the control plates without organisms.

Study was performed in accordance with the ethical standards of Declaration of Helsinki and all persons gave informed consents prior to their inclusion in the study.

Statistical analysis

We measured the performance of the diagnostic tests by calculating sensitivity [TP/(TP+FN)], specificity [TN/(TN+FP)], positive predictive value [TP/(TP+FP)], negative predictive value [TN/(TN+FN)] and accuracy (efficiency of prediction) [(TP+TN)/(TP+FP+TN+FN)], (TP: true positive, TN: true negative, FP: false positive and FN: false negative).

RESULTS

All of 59 specimens of patients with clinically tuberculosis were examined for M. tuberculosis by smear, LJ culture and FPTB assays. M. tuberculosis complex were isolated from the samples of 54 patients by LJ culture where 42 of these samples were smear-positive and 12 of them were smear-negative. Both smear and LJ negative 5 cases were named as smear negative tuberculosis and diagnosed by clinical and radiologic findings. M. tuberculosis could be detected by FPTB in 36 (61%) specimens of 59 cases of tuberculosis. In all of 20 cases included in control group, results were reported as negative by three diagnostic methods. Charactheristics of our study group and distribution of results according to smear, LJ culture and FPTB results were shown in Table 1. The sensitivity, specificity, positive predictive value, negative predictive value and efficiency of prediction were respectively 61%, 100%, 100%, 46% and 0,70 in all cases (Table 2). Results of sensitivity and efficiency of prediction for LJ culture were correlated with radiologic findings (LJ values increased with the advancing). But we did not find a correlation between performance of smear, FPTB tests and radiologic findings. We found the performance of FPTB test with a high (73,8%) sensitivity in smear-positive cases and low sensitivity (29,4%) in smear-negatives.

Table 1. Distribution of study group according to smear, LJ culture and fast plaque tuberculosis test results

Patient group	Smear result	Sex (M/F)	Mean age (y)	FPTB (+)	FPTB (-)	LJ (+)	LJ(-)
Tuberculosis	Positive, (n=42)	29/13	34,5	31 (73,8%)	11 (26,2%)	42 (100%)	-
	Negative (n=17)	8/9	35,2	5 (29,4%)	12 (60,6%)	12 (60,6%)	5 (29,4%)
Total (n=59)	59/59	37/22	34,7	36 (61,5%)	23 (29%)	54 (91,5%)	5 (8,5%)
Control (n=20)	negative	14/6	49	-	20 (100%)	-	20(100%)

FPTB= Fast plaque tuberculosis test, LJ= Löwenstein Jensen, M= male, F=female, y=year

Table 2. Performance of fast plaque tuberculosis test according to radiologic manifastations compared to smear and LJ culture

Radiologic class	Diagnostic test	Sensitivity %	Specificity %	PPV	NPV	Efficiency of prediction
Group A (n=4)	Smear	25	100	1	0,86	0,87
	LJ culture	25	100	1	0,86	0,87
	FPTB	50	100	1	0,90	0,91
Group B (n=35)	Smear	77,1	100	1	0,71	0,85
	LJ culture	94,2	100	1	0,90	0,96
	FPTB	45,7	100	1	0,57	0,65
Group C (n=20)	Smear	70	100	1	0,76	0,85
	LJ culture	100	100	1	0,90	0,96
	FPTB	45,7	100	1	0,90	0,95
Total (n=59)	Smear	71,1	100	1	0,54	0,78
	LJ culture	91,5	100	1	0,80	0,93
	FPTB	61	100	1	0,46	0,70

FPTB: Fast plaque tuberculosis test, LJ: Löwenstein Jensen, PPV: positive predictive value, NPV: negative predictive value.

Group-A (minimal advanced): non cavitary or minimal infiltration, group-B (moderately advanced): cavitation smaller than 4 cm or infiltration area smaller than one third of the lungs, group-C (far advanced): cavitation greater than 4 cm or widely infiltration.

Table 3. Study results about performance of FPTB method

Reference	Region and date	Number of isolates	Patient group	Performance of FPTB			
				Sensitivity (%)	Specificity (%)	PPV	NPV
Present study	Turkey	59	smear (-)	29,4	100	1	0,62
			smear (+)	73,8	100	1	0,64
			total	61	100	1	0,46
Albert et al (2)	S. Africa, 2002	1618	smear (-)	48,7	99,5	0,84	0,97
			smear (+)	86,8	83,3	0,94	0,67
			total	72,5	99	0,91	0,96
Prakash et al (3)	India, 2009	68	smear (+,-)	90,7	96	0,97	0,85
Barman and Gadre (4)	India, 2006	212	smear (+)	94,34	93,88	0,94	0,93
			LJ (+)	92,86	97,83	0,98	0,91
Muzaffar et al (5)	Pakistan, 2002	514	smear (+)	87,4	88,2	0,99	0,40
			LJ (+)	81,6	97,7	0.97	0,85
Çavuşoğlu et al (6)	Turkey, 2006	63	smear (+)	27	97	0,90	0,55
		45	LJ(+)	53	97	0,89	0,81

LJ: Löwenstein Jensen, PPV: positive predictive value, NPV: negative predictive value.

DISCUSSION

Diagnostic techniques utilized in developed countries to obtain pulmonary specimens (such as bronchoscopy, diagnostic surgical interventions and tissue biopsy) are more sensitive and faster analytic methods but often unavailable in resource-poor countries. In undeveloped or developing countries with a high prevalence of TB, the diagnosis of disease is generally based on smear examination of sputum, radiographic and clinical assessment.[7] There is often a delay in diagnosis in smear negative TB patients as no rapid definitive test is available, which has important consequences in terms of transmission, increased morbidity and mortality for the individual patient, and costs of a prolonged hospitalization while establishing the diagnosis.[8] Recently, further diagnostic assays, including nucleic acid amplification, mycobacterial antibody detection, liquid cultures like BACTEC, cellular immune recognition, antigenic detections have been developed.[9] However, these techniques are generally expensive, often require experienced persons and sophisticated equipment, making them unsuitable for routine application in low-income countries. The sensitivity of sputum smear microscopy has been reported to be between 22-80% of culture-confirmed TB cases.[10] Considering these entire suggestions FPTB test has been evaluated for the diagnosis of pulmonary TB in different regions such as Pakistan, India, South Africa and Turkey. FPTB detected 65-83% of TB cases within two days and with a wide range of sensitivity between 27- 92% and specificity of 88-100% in previous studies.[2,3,4,6,11,12,13] Studies analyzed the performance of FPTB method and we summarized these results in Table 3. FPTB gives quick results compared with smear microscopy and conventional culture, using LJ medium which took up to eight weeks. Smear positive patients have higher numbers of TB bacilli in their sputum, and are therefore more likely to infect close contacts. Smear negative patients may also lead to extended opportunities for transmission because of delays in diagnosis. FPTB assay could be useful where the disease is highly prevalent and a prompt diagnosis is important from both health and economic points of view since it gives result within 2 days.

Limitations of the FPTB method

FPTB test results should be interpreted with clinical and radiological findings. FPTB positivity represents only M.Tuberculosis Complex (M.tuberculosis, M.Africanum, M.bovis and M. microti). FPTB method can detect only live basilli. Inappropriate conservation of samples and starting antituberculous treatment up to test time affects the performance of FPTB test. Decontamination of the respiratory specimens should be made only by NALC-NaOH for test preparartions.

In conclusion, FPTB may be used for a rapid detection of M. tuberculosis in pulmonary tuberculo-

sis but it is not a preferable diagnostic test to LJ culture or smear examinations. Respirarory materials should be followed by LJ cultures for M.tuberculosis growth in all cases with suspected tuberculosis.

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REFERENCES

1. WHO, The Global Plan to stop TB 2011-2015, Global report on surveillance and response. Geneva, World Health Organization, 2010 (WHO/HTM/STB/2010-2 and 3).
2. Albert H, Heydenrych A, Brookes R, et al. Performance of a rapid phage-based test, FASTPlaqueTB™, to diagnose pulmonary tuberculosis from sputum specimens in South Africa. Int J Tuberc Lung Dis 2002;6(6):529-37.
3. Prakash S, Katiyar S, Purwar S, Singh JP. Clinical evaluation of the mycobacteriophage-based assay in rapid detection of Mycobacterium tuberculosis in respiratory specimens. Indian J Med Microbiol 2009;27(2):134-8.
4. Barman P, Gadre D. A study of phage based diagnostic technique for tuberculosis Indian J Tuberc 2007;54(1):36-40.
5. Muzaffar R, Batool S, Aziz F, Naqvi A, Rizvi A. Evaluation of the FASTPlaqueTB assay for direct detection of Mycobacterium tuberculosis in sputum specimens. Int J Tuberc Lung Dis 2002;6(7):635-40.
6. Cavusoglu C, Guneri S, Suntur M, Bilgic A. Clinical evaluation of FASTPlaqueTB for the rapid diagnosis of pulmonary tuberculosis. Turk J Med Sci 2002;32(6):487-92.
7. Harries AD, Banda HT, Boeree MJ et al. Management of pulmonary tuberculosis suspects with negative sputum smears and normal or minimally abnormal chest radiographs in resource-poor settings. Int J Tuberc Lung Dis 1998;2(12):999-1004.
8. Hudson CP, Wood R, Maartens G. Diagnosing HIV-associated tuberculosis: reducing costs and diagnostic delay. Int J Tuberc Lung Dis 2000;4(3):240-5.
9. Perkins MD. New diagnostic tools for tuberculosis. Int J Tuberc Lung Dis 2000;4(12):182-8.
10. Colebunders R, Bastian I. A review of the diagnosis and treatment of smear-negative pulmonary tuberculosis. Int J Tuberc Lung Dis 2000;4(2):97-107.
11. Alcaide F, Gali N, Dominguez J, Berlanga P, Blanco S, Orus P, Martin R. usefulness of a new mycobacteriophage based technique for the rapid diagnosis of pulmonary tuberculosis. J Clin Microbiol 2003;41(7):2867-71.
12. Phulpoto MA, Qayyum S, Rizvi N, Khuhawar SM. Diagnostic yield of fast plaque TB test for rapid detection of Mycobacterium in tuberculosis suspects. J Pak Med Assoc 2005;55(2):57-60.
13. Shenai S, Rodrigues C, Mehta A P. Evaluation of a new phage amplification technology for rapid diagnosis of tuberculosis. Indian J Med Microbiol 2002;20(4):194-9.