International Journal of Advances in Medicine
Singh JP. Int J Adv Med. 2019 Feb;6(1):182-185
http://www.ijmedicine.com
pISSN 2349-3925 | eISSN 2349-3933
DOI: http://dx.doi.org/10.18203/2349-3933.ijam20190127

Original Research Article

A study of efficacy of phage amplification technique in diagnosis of pulmonary and extra-pulmonary tuberculosis

Jai Pratap Singh*

Department of Pulmonary Medicine, Prasad Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

Received: 22 November 2018
Accepted: 02 January 2019

***Correspondence:**
Dr. Jai Pratap Singh,
E-mail: drjaipratapsingh97@gmail.com

ABSTRACT

Background: Tuberculosis (TB) is a leading infectious disease in India. Diagnosis of TB has always been a problem due to slow rate of growth of *Mycobacterium tuberculosis*. In this study, author had compared the conventional tools for diagnosis of TB with the new Fast Plaque TB™.
Methods: The study was conducted at Dr. ML Chest Hospital, Department of Tuberculosis and Respiratory Diseases, G.S.V.M. Medical College, Kanpur. Specimens were collected after taking informed consent from patients attending outpatient and indoor patients admitted in the hospital. Study consisted of cases having suspected tuberculous exudation both pulmonary and extra pulmonary.
Results: Most of the patients in this study were between 21-40years of age. Most of them were male (78%). Most of the patients came from urban areas and middle socioeconomic strata. Among them 68% were smokers and 32% were non-smokers. Comparison of phage assay with clinical evidence of disease has been done and results were sensitivity 85.7%, specificity 100%, PPV 100%, NPV 84.6% found.
Conclusions: Delay in diagnosis resulting in further delay to initiate drug therapy. In these circumstances the rapid detection of mycobacteria by phage amplification technique could lead to earlier institution of antitubercular treatment.

Keywords: Bacteriophage assay, Mycobacterium tuberculosis, Tuberculosis

INTRODUCTION

Tuberculosis (TB) is a leading health problem worldwide and remains one of the leading causes of death from infectious diseases. An estimated 2billion people (i.e., one third of the world's population) are infected with M. *tuberculosis*. Each year, approximately 9million people suffer from the disease, and approximately 2million die as a result.[1] Tuberculosis kills more adults in India than any other infectious disease. More than 1,000 people a day or one in every minute die of TB in this country.[2] The prevalence of all forms of TB in India is estimated to be 5.05 per thousand, prevalence of smear positive cases 2.27 per thousand and average incidence of smear positive cases is 84 per 100,000 annually.[3] The incidence of TB is expected to increase substantially worldwide because of the interaction between TB and human immunodeficiency virus (HIV)/AIDS epidemic. Nearly 1.8million Indians get infection every year. Every day, about 5000 people develop the disease and around 1000 die.[4] In India, TB kills more in the younger age group thus compounding to the economic loss of the country. The direct cost of the disease in India annually is estimated at US$300million, the annual indirect cost is US$3billion.[4] In this country with a high prevalence of tuberculosis, diagnosis is mainly based on the conventional methods like clinical assessment, radiology, sputum microscopy and culture in Lowenstein Jensen (LJ) media. New diagnostics approaches, including nucleic acid amplification, antibody detection, liquid culture, cellular immune response, antigen capture, and chemical and physical detection tests have been

developed.[5] Many molecular methods have been developed for direct detection, species identification, and drug susceptibility testing of mycobacteria.[6] These require expertise and finance and are not easily affordable in low income countries. The sensitivity of smear microscopy has been between 20%-80% in culture confirmed TB cases.[7] Though smear microscopy can detect positive cases if properly performed, it can miss quite a number of paucibacillary cases. The quality of results with smear microscopy is heavily dependent on the workload, skill and motivation of the technician reading the slides.[8] Culture techniques are available but the time required and negative results in paucibacillary cases are important limitations.[9] Chest X-ray is commonly used to aid the diagnosis of TB. However, since radiological changes are not specific for TB and do not always reflect active disease, overreliance on chest X-ray can lead to misdiagnosis.[8] Therefore, there is need for a rapid, reliable and sensitive method for diagnosis of pulmonary tuberculosis so that early treatment can be started and the disease can be contained. In this study, author had tried to find efficacy of phage amplification technique for diagnosis pulmonary and extra pulmonary tuberculosis.

METHODS

The study was conducted at Dr. ML Chest Hospital, Department of Tuberculosis and Respiratory Diseases, G.S.V.M. Medical College, Kanpur from September 2016 to September 2017.

All cases attending outdoor and indoor Department of Tuberculosis and Respiratory Diseases, G.S.V.M. Medical College, Kanpur having suspected tuberculous exudation both pulmonary and extra pulmonary were included. Those patients who did not give consent were excluded.

Specimens were collected after taking informed consent from patients attending outpatient and indoor patients admitted in the hospital. Study consisted of cases having suspected tuberculous exudation both pulmonary and extra pulmonary. All exudates were investigated for smear microscopy and phage amplification technique (FAST PLAQUE TB™).

A complete clinical history and examination were done. Those suspected of having pulmonary tuberculosis were subjected to chest X-ray PA view and investigations are advised such as Hb%, TLC, DLC, ESR, Mantoux Test, Sputum for AFB, any other investigation found necessary, phage amplification technique (FAST PLAQUETB™).

The chemical used and utility includes sodium hydroxide-sodium citrate mixture to decontaminate the sample N-Alanine L- Cysteine (NALC) and to liquefy the sputum samples and phosphate buffer to neutralize the NaOH-NaCitrate mixture.

Sediment from processes was washed in 15ml FPTB medium plus and centrifuged at 3000rpm for 20minutes. Pellet was suspended in ml. FPTB medium plus. Overnight incubation done at 37ºC. Infect with Phage (Actiphage™) for 60minutes at 37ºC. Virucide (Virusol™) added. Mix thoroughly and incubate for 5minutes at room temperature. Neutralize with 5ml. FPTB medium plus. Add 1ml. Sensor™ cells (Nonpathogenic Mycobacteria) Add 5ml. Molten FPTB Agar to petridish. Add reaction mixture and mix. Allow to set, then invert and incubate overnight at 37ºC.

RESULTS

All patients were investigated for smear microscopy (ZiehlNeelsen Method) and Bacteriophage Amplification Technique (FAST Plaque TB™) and if necessary additional investigations have been done. All 50 patients were divided into two groups viz., disease present or disease absent. The presence of disease was determined by positive AFB smear, positive culture report or those with clinical, radiological and other laboratory findings suggestive of tuberculosis.

It was observed that maximum number of patients were between 21-30years of age (30%) followed by 61-70years (22%), 31-40years (16%), 51-60years (16%), above 70years (10%), and 41-50years (6%) (Table 1).

Table 1: Distribution of cases according to age.

Age groups (years)	Total	
	No.	%
21-30	15	30
31-40	8	16
41-50	3	6
51-60	8	16
61-70	11	22
70 and above	5	10
Total	50	100

Table 2: Distribution of cases according to demographic factors and smoking.

Factors	Number	%
Gender		
Male	39	78
Female	11	22
Residence		
Urban	38	76
Rural	12	24
Socioeconomic status		
Upper	4	8
Middle	36	72
Lower	10	20
Personal habit		
Smoker	34	68
Non-smoker	16	32

Majority of the patients were males (78%) and belonged to urban areas (76%) in the study population. Most of the patients belonged to middle socio-economic status (72%) followed lower (20%) socio-economic status and upper (8%) socio-economic status as per modified Prasad's classification. A higher proportion of patients admitted having the habit of smoking (68%) (Table 2).

A higher proportion of samples of pulmonary tuberculosis using the phage amplification technique were of sputum (66%) followed by bronchial wash (2%) while most samples of extra pulmonary tuberculosis taken were of pleural fluid (18%), aspirate (6%), ascitic fluid (4%), urine (2%) and synovial aspirate (2%) (Table 3).

Table 3: Distribution of clinical samples using the phage amplification technique (fast plaque tb™ test).

Tuberculosis	Sample	No.	%
Pulmonary (N=34)	Sputum	33	66
	Bronchial wash	1	2
	Pleural fluid	9	18
	Ascitic fluid	2	4
Extra pulmonary (N=16)	Urine	1	2
	Aspirate (lymph node, abscess)	3	6
	Synovial aspirate	1	2

Twelve tuberculous cases were found to be smear positive and 16 tuberculous cases were found smear negative while two non-tuberculous cases were smear positive and 20 non-tuberculous cases were found smear negative using the Ziehl Neelsen staining, the difference being statistically significant (P<0.05). The sensitivity (smear microscopy with comparison of clinical evidence of disease) was found to be 42.86%, Specificity was 90.9%, positive predictive value was 85.7%, negative predictive value was found to be 55.56% (Table 4).

Table 4: Comparison of smear microscopy with clinical evidence of disease.

Investigation (Ziehl Neelsen staining)	Tuberculous	Non-tuberculous	Total
Smear +ve	12	2	14
Smear –ve	16	20	36
Total	28	22	50

Chi-square $X^2 = 6.96$, P <0.05.

Comparison of bacteriophage amplification technique (fast plaque tb™) with smear microscopy shows that 12 smear positive cases were phage +ve and 12 smear -ve cases were phage +ve while two smear positive cases were phage -ve and 24 smear -ve cases were phage -ve, the difference being statistically significant (<0.05). The sensitivity (phage assay with comparison to smear microscopy) was found to be 85.71%, specificity was 66.67%, positive predictive value was 50%, and negative predictive value was found to be 92.30% (Table 5).

Table 5: Comparison of bacteriophage amplification technique (fast plaque tb™) with smear microscopy.

Phage amplification test results (FAST PLAQUE TB™)	Smear microscopy (Ziehl Neelsen)		Total
	Smear +ve	Smear -ve	
Phage +ve	12	12	24
Phage –ve	2	24	26
Total	14	36	50

Chi-square X2 = 11.08, P <0.05.

DISCUSSION

Diagnosis of tuberculous exudates constitute a difficult problem in many cases. The vague and largely nonspecific symptomatology in early stage of tuberculosis, variable investigatory findings and difficulty in demonstration of *Mycobacterium* from exudates are major obstacle in firm diagnosis of tuberculosis. Conventional methods for isolation of *Mycobacterium tuberculosis* from exudative samples have given disappointing results. So, the present study was carried out on clinical suspected cases of tuberculous exudates in whom confirmation of diagnosis by conventional method was extremely difficult. Newer method phage assay technique was applied on them to evaluate the diagnostic potential of this technique.

In present study, the patients came from all age group. A total of 50 patients were taken, majority of patients, 23 (46%) out of 50 were between age group 21-40years. Nagpaul DR et al, found larger proportion of patients were in 20-30years of age group.[10] In this study male, female ratio was 3.6:1, 78% male and 22% female. In this study, 76% patients came from urban population, 24 came from rural population, 72% patients were of middle socio-economic status and 68% patient were smoker. 38% of patients were having the total duration of illness more than 60months. In present study, patients were divided into pulmonary (N=34) and extra pulmonary (N=16) cases. Twelve patients who were smear microscopy negative but phage assay positive. All 12 patients who were diagnosed to have tubercular exudative lesion but none of them could be identified by smear microscopy but phage assay was able to detect all of them. These patients were followed up and they responded to anti tubercular treatment. Patients in present study were having smear microscopy positive but phage assay negative. Out of them, one was taking anti-tubercular drugs and in smear microscopy dead bacilli could have been seen. Another patient was clinico-radiologically having destroyed lung and adequately treated with anti-tubercular drugs in recent past. The performance of FAST Plaque TB™ was compared with smear microscopy and conventional culture using Lowenstein-Jensen medium or a combination of solid and

liquid culture methods. Giving a high degree of confidence that a positive FASTPIaque TB™ result was representative of active disease. High specificity was reported even in the presence of a high number of non-tubercular mycobacteria isolates (32% of total isolates) in one of the populations studies.[11] Overall the test detected 65-83% of confirmed TB cases within two days, compared to culture, which took up to eight weeks. FAST Plaque TB™ sensitivity of 48% to 67% and specificity of 98% was reported for detection of smear negative when the use of smear microscopy and FASTPlaqueTB™ were combined an overall sensitivity of 90% reported.[12]

CONCLUSION

The ability of the test to detect *Mycobacterium tuberculosis* rapidly in AFB smear negative exudative fluid is of obvious importance. Currently, microscopic examination of stained smear was the only rapid way to detect *Mycobacterium tuberculosis* in exudative fluids. This causes delay in diagnosis resulting in further delay to initiate drug therapy. In these circumstances the rapid detection of mycobacteria by phage amplification technique could lead to earlier institution of antitubercular treatment.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Cdc.gov. Atlanta, Centers for Disease Control and Prevention, World TB Day-March 24, 2006 MMWR, 2006; 55. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5511a1.htm. Accessed 23 March 2006.
2. Tuberculosis- a guide for practicing physicians. Available at: http://www.trc-chennai.org/Rntcp/GuideforPrPhysicians.pdf.
3. Chakraborty AK. Epidemiology of tuberculosis: current status in India. Ind J Res. 2004;120(4):248.
4. TB India 2006. RNTCP Status Report. Available at: https://tbcindia.gov.in/. Accessed 24 March 2006.
5. Perkins MD. New diagnostic tools for tuberculosis [The Eddie O'Brien Lecture]. Inter J Tubercul Lung Dis. 2000;4(12):S182-8.
6. Soini H, Musser JM. Molecular diagnosis of mycobacteria. Clin Chem. 2001;47(5):809-14.
7. Colebunders R, Bastian I. A review of the diagnosis and treatment of smear-negative pulmonary tuberculosis. Inter J Tubercul Lung Dis. 2000;4(2):97-107.
8. Seaman T, Trollip A, Mole R, Albert H. The use of a novel phage-based technology as a practical tool for the diagnosis of tuberculosis in Africa. African J Biotechnol. 2003;2(2):40-5.
9. Katoch VM. Newer diagnostic techniques for tuberculosis. Ind J Med Res. 2004;120:418-28.
10. Nagpaul DR, Vishwanath MK, Dwarakanath G. A socio-epidemiological study of out-patients attending a city tuberculosis clinic in India to judge the place of specialized centres in a tuberculosis control programme. Bulletin World Health Org. 1970;43(1):17.
11. Orus P, Berlanges P, Gali N, Alcaide F, Blanco S, Benitez MA, et al. Usefulness of a new mycobacteriophage-based technique for the rapid diagnosis of pulmonary tuberculosis. 11th ECCMD, Istanbul, Turkey; 2001.
12. Muzaffar R, Batool S, Aziz F, Naqvi A, Rizvi A. Evaluation of the FASTPlaqueTB assay for direct detection of Mycobacterium tuberculosis in sputum specimens. Inter J Tubercul Lung Dis. 2002;6(7):635-40.

Cite this article as: Singh JP. A study of efficacy of phage amplification technique in diagnosis of pulmonary and extra-pulmonary tuberculosis. Int J Adv Med 2019;6:182-5.