Early Diagnosis of Smear Negative Pulmonary Tuberculosis: A Two Year Study from Tertiary Care Center

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ABSTRACT

Introduction: Globally pulmonary tuberculosis as well as extra-pulmonary tuberculosis is a major public health issue, all age groups are at high risk to acquire infection. Multi-drug resistant (MDR) and extensively-drug resistant (XDR) cases are increasing day by day, which is an alarm for the Government organization to improve the control program. Smear negative pulmonary tuberculosis (SNPT) is also an issue, which can be solved by using a Line probe assay. Success of any control program depend on early diagnosis and proper treatment, which will help to control transmission of disease in society. Aim of the study was to evaluate the efficacy of LPA in diagnosis of smear negative pulmonary tuberculosis cases and to detect mono-resistance and multidrug resistant.

Material and Methods: This was a laboratory based observational study, which were conducted in department of microbiology, IGIMS, Patna and TBDC, Agamkuan, Patna, for the period of two years. Sputum specimens were collected from clinically suspected cases of pulmonary tuberculosis. Smear negative suspects were included in study, which were subjected to culture and LPA.

Results: A total of 3729 sputum specimens were collected from suspected cases of PTB, which were subjected to sputum smear microscopy. A total of, 1611 smear negative samples were included in study, which were subjected to LPA and culture. Out of these, 904 (56%) were positive using culture and/or LPA, and 707 (44%) were negative by both diagnostic tests. Whereas in diagnosed cases of PTB using LPA, 558 samples were sensitive to rifampicin and isoniazid, and 195 samples were resistant to rifampicin and/or isoniazid.

Conclusion: It has been seen that SNPT cases were increasing day by days, therefore LPA can be a reliable diagnostic tool to overcome these issue, which can reduce the treatment delay and transmission. However, culture must be followed in diagnosis of TB along with other test.

Keywords: Line Probe Assay, Culture, Smear Negative Pulmonary Tuberculosis, Mono-Resistant, Multi-Drug Resistant

INTRODUCTION

Tuberculosis is an infectious disease, which is caused by genus mycobacterium, mainly due to *Mycobacterium tuberculosis* and other species is less common. *M. tuberculosis* mainly infect lungs, which is known as pulmonary tuberculosis, but it can infect other organs also such as gastrointestinal tract, central nervous system, lymph nodes, bones, joints, urinary tract and other sites, which is known as extra pulmonary tuberculosis. Tuberculosis is the second leading cause of death worldwide, after the human immunodeficiency virus.[1] Evidence of tuberculosis found from the Neolithic period in 5800 BCE and in Egyptian mummies to 2400 BCE.[2] Infection is acquired by inhalation of infectious droplets nuclei, which is released in air by pulmonary tuberculosis infected individuals during coughing, sneezing, or talking, which remain suspended for long periods. Coughing or talking up to 5 minute can produce 3,000 infectious droplet, and sneeze can generate up to 40,000 droplets.[3] Smear negative pulmonary tuberculosis patients are less infectious than smear positive pulmonary tuberculosis patients.[4] Those who are infected with HIV are susceptible to acquire disease, whereas other condition such as chronic renal disease, neoplastic disorders and those receiving immunosuppressive therapy are also susceptible.[5,6] Sputum smear microscopy is simple and rapid diagnostic method, which is still used in many developing countries as the only test to confirm the diagnosis of TB. Overall sensitivity of the sputum smear microscopy has been reported to range from 22 to 80 percent.[7] Smear microscopy will come positive if one ml of sputum will contain ≥5000 acid fast bacilli (AFB).[8] Therefore, false negative diagnosed cases based on sputum smear microscopy are also responsible to transmit infection in society and subsequent development of active disease.[9] It is estimated that approximately, 20% of TB transmission is due to smear negative pulmonary tuberculosis cases.[10] Conventional culture method is more sensitive compare to smear microscopy, but it takes 2 to 8 weeks to growth, which can hampers the early diagnosis and treatment. Line probe assay (LPA) is a rapid diagnostic technique, which is based on polymerase chain reaction, identify *Mycobacterium tuberculosis* (MTB) complex and mutations to genes associated with rifampicin (*rpoB*) and isoniazid (*katG* and *inhA*) resistance within 24 hours.[11] Drug resistant tuberculosis cases are problem in high TB burden countries, such as India. Keeping in view of all the above issue present study were designed to evaluate the efficacy of

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How to cite this article: Rohit Kumar, Rakesh Kumar, Shivendra Kumar Shahi, Swati Salila, Shailesh Kumar, Balkrishna Mishra. Early diagnosis of smear negative pulmonary tuberculosis: a two year study from tertiary care center. International Journal of Contemporary Medical Research 2018;5(2):B1-B4.

LPA in diagnosis of smear negative pulmonary tuberculosis cases and to detect mono-resistance and multi-drug resistant pulmonary tuberculosis cases.

MATERIALS AND METHODS

This was a laboratory based observational study conducted in the department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna and TBDC, Agamkuan, Patna from January 2016 to December 2017. All the suspected pulmonary tuberculosis patients attending OPD were included. All the specimens were subjected to smear microscopy, culture and LPA.

Inclusion criteria: i) Signs and symptoms associated with PTB, cough ≥2 weeks, haemoptysis, weight loss, fever, chest pain and abnormal chest X-ray ii) Smear negative cases iii) Patient of all age group and iv) Both sexes.

Exclusion criteria: i) Sputum mixed with blood ii) Macroscopic and microscopic examination revealed saliva not sputum.

Specimen collection: Sputum sample were collected from suspected cases of PTB. Standard protocols, as per RNTCP were followed for sample collection. Patients were provided a sterile, wide mouth, leak-proof plastic container, they were educated to cough deeply to produce sputum specimen and how to collect without contaminating the collection container.

Processing of samples: Direct smears were prepared from sputum samples for ZN staining and it was examined in bright field microscope. Lowenstein-Jensen (LJ) medium were used for cultivation of acid fast bacilli (AFB). Firstly specimens were decontaminated by NALC-NaOH (N-acetyl L-cysteine-Sodium hydroxide) method and concentrated thereafter.[12] Hundred µL of sediments were inoculated on two slopes of LJ medium and incubated at 37°C. They were examined once a week for up to eight weeks. On the basis of colonies morphology and ZN staining reveal the presence of AFBs, culture were reported as positive.[13]

Line probe assay: Test were performed according to the manufacturer's instruction. Direct sputum specimen after decontaminant were used to perform LPA.[14] Test is based on DNA strip technology and it has three steps, which includes DNA extraction, multiplex PCR amplification, and reverse hybridization.[15] The test was performed in three different rooms with restricted access and unidirectional workflow. *Mycobacterium* DNA were extracted in Biosafety level-3 laboratory using DNA extraction kit (Genolyse®- Hain Lifescience). A final volume of 50 µL master mixture were used for DNA amplification. An initial step of denaturation at 95ºC for 15 minute followed by 30 cycle at 95ºC for 25 second, annealing at 50°C for 40 second, extension at 70°C for 40 second and a final extension at 70°C for 8 minute. Amplified product were analysed by 'Reverse Hybridization' technique using DNA strip technology, which is pre-attached with 27 different reaction zones consisting of controls and mutant probes (rpoB, katG and inhA gene).

Sputum smear microscopy	Number of Samples (percentage)
Positive:	2118 (57%)
Negative:	1611 (43%)
Total:	3729

Table-1: Sputum smear microscopy results

Diagnostic tests	Number (percentage)
Culture (+ ve), LPA (+ ve)	673 (74%)
Culture (+ ve), LPA (- ve)	151 (17%)
Culture (- ve), LPA (+ ve)	80 (9%)
Total:	904

Table-2: Diagnosis as pulmonary tuberculosis based on diagnostic tests used

	Drug resistance pattern				
	RIF^S INH^S	RIF^R INH^R	RIF^R INH^S	RIF^S INH^R	Total
Smear negative	558 (74%)	121 (16%)	50 (7%)	24 (3%)	753

Table-3: Drug sensitivity testing result using LPA.

RESULTS

A total of 3729 suspected cases of PTB were screened using sputum smear microscopy examination. Out of these, 1611 (43%) were smear negative [Table 1]. In smear negative group of patients, 1095 (68%) were male and 516 (32%) were female.

Among the smear negative specimens, 904 (56%) patients were diagnosed as PTB, using culture and/or LPA diagnostic tests, and 707 (44%) were negative by both diagnostic tests. Whereas in diagnosed cases of pulmonary tuberculosis, 673 (74%) were culture positive and LPA positive, 151 (17%) were culture positive and LPA negative, and 80 (9%) were culture negative and LPA positive [Table 2].

Interpretation of LPA: Using LPA, 753 patients were diagnosed as smear negative pulmonary tuberculosis, out of these, 558 (74%) were sensitive to rifampicin and isoniazid and 195 (26%) were resistant to rifampicin and/or isoniazid. Whereas in drug resistant cases, 121 (16%) were MDR, 50 (7%) were rifampicin mono-resistant and 24 (3%) were isoniazid mono-resistant [Table 3].

DISCUSSION

Knowledge of drug resistant profile in *Mycobacterium tuberculosis* from different parts of Country will help the Government organization to control the transmission of tuberculosis as well as drug resistant tuberculosis in community. By conducting the present study we had made an attempt to know the prevalence of pulmonary tuberculosis and drug resistant pulmonary tuberculosis in smear negative suspected cases, in eastern part of India by using LPA. Result presented in the present study indicated that among the diagnosed cases of smear negative pulmonary tuberculosis, 83% were diagnosed based on LPA. Out of these, 16% were MDR, 7% were rifampicin mono-resistant and 3%

were isoniazid mono-resistant. Another study indicated that among the clinically suspected, smear negative pulmonary tuberculosis cases, 38% were diagnosed based on LPA. They also found that among the smear negative drug resistant pulmonary tuberculosis cases, 28% were rifampicin resistant and 24% were isoniazid resistant. In comparison to present study they found higher number of drug resistant smear negative pulmonary tuberculosis cases.[16] In a study by Abyot M, et al. found that among the diagnosed cases of tuberculosis, 53% were diagnosed as smear negative tuberculosis based on LPA result.[17] With good routine reporting systems, the national tuberculosis programmes of countries such as Malawi and the United Republic of Tanzania have reported a larger increase in new cases of smear negative than of smear-positive pulmonary tuberculosis in the last 10 years.[18] A study from Mexico had reported that among the culture confirmed pulmonary tuberculosis of cases, 80% of patients were smear negative and 20% of patients were smear positive.[19] Another study from China indicated that among the diagnosed cases of smear negative tuberculosis, 26% were found MDR-TB.[20] Present study and a study by Sunita T, et al found similar result of smear negative drug resistant tuberculosis cases, which were 26% in both study. Researcher also found that prevalence of MDR-TB were, 15% from North Bihar.[21] A study from AIIMS, Delhi reported that among the diagnosed cased of tuberculosis, 26% were MDR-TB, 10% and 22% were INH and RIF mono-resistant tuberculosis respectively, using LPA.[22] In clinical practice significance of INH mono-resistance and effect on TB treatment outcomes, is still a topic of debate. However, a meta-analysis study and a study from South Africa were reported poor outcome in treatment of isoniazid mono-resistant tuberculosis.[23,24] It is reported that latest version of LPA is having 72% of sensitivity to detect *Mycobacterium tuberculosis* complex in smear-negative sputum samples.[16] Advantage of LPA in diagnosis of smear negative tuberculosis as well as drug resistant tuberculosis is that it is sensitive and it also provides rifampicin and isoniazid resistant pattern in *Mycobacterium tuberculosis* complex. Test can be directly performed on clinical specimens.

CONCLUSION

In present situation where SNPT as well as drug-resistant cases is increasing day by day, LPA can play an important role in finding SNPT and rapid screening of drug resistance TB. However, culture must be followed in diagnosis of TB along with other test. LPA will reduce the turnaround time, which will help in early management of SNPT and drug-resistant cases. WHO recommendations must be followed to ensure high quality results. The results presented in the present study suggest the use of LPA for rapid and reliable methods for diagnosis of SNPT and drug-resistant TB. This will help the clinician to start appropriate treatment regimens, thereby improving treatment outcome and reducing transmission.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 08-02-2018; **Accepted:** 04-03-2018; **Published:** 16-03-2018