LOOP MEDIATED ISOTHERMAL AMPLIFICATION FOR THE RAPID DIAGNOSIS OF PULMONARY TUBERCULOSIS

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ABSTRACT

BACKGROUND

Control of tuberculosis is often challenged by diagnostic methods which are time consuming, often less sensitive, expensive and inaccessible to most patients especially in the developing countries. The characteristics of loop-mediated isothermal amplification (LAMP) method make it a promising platform for the molecular detection of tuberculosis in developing countries. We wanted to evaluate the loop mediated isothermal amplification method for the rapid diagnosis of pulmonary tuberculosis (PTB) in low resource settings.

METHODS

We conducted an observational study in the Microbiology department for a period of eighteen months on sputum samples collected from suspected cases of pulmonary tuberculosis which satisfied the inclusion as well as exclusion criteria. All these samples after processing were subjected to smear microscopy by acid fast staining, culture on Lowenstein-Jensen (LJ) medium and LAMP assay and results were compared to derive sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

RESULTS

A total of 110 samples were included in the study. Using culture as the reference standard, we found that the sensitivity and specificity for TB-LAMP assay were 98.3% and 98.03% respectively and PPV and NPV were 98.3% and 98.03% respectively. The AFB staining had a sensitivity of 91.5% and specificity of 98%.

CONCLUSIONS

Hence LAMP method is a reliable test for the diagnosis of tuberculosis which can be suited to resource limited settings. The method has very good positive and negative predictive value for pulmonary samples. It is a rapid technique when compared to conventional culture and simple and less expensive than other molecular based methods. Therefore, it can be applied as a point of care testing (POCT) method in resource limited settings.

KEY WORDS

Diagnosis of Tuberculosis, Loop Mediated Isothermal Amplification

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BACKGROUND

Tuberculosis (TB) still remains one of the major health problems facing mankind, particularly in developing countries even though the tubercle bacillus was discovered more than a hundred years ago. In 2015, 10.4 million incident cases of tuberculosis (TB) were reported globally while the estimate of incidence (New TB cases per year) was 2.8 million cases in India.[1] China, India and Indonesia alone accounted for 45% of global cases in 2015.

Inappropriate diagnostic practices may lead to delay in diagnosis, increasing the TB transmission. TB diagnosis, even today, continues to rely mainly on direct smear microscopy, solid culture, chest radiography, and tuberculin skin testing: tools that often perform poorly with delayed reports, and require infrastructure frequently unavailable in most of the routine diagnostic centres. Several molecular methods like polymerase chain reaction (PCR) have come up to address the need of rapid and sensitive diagnosis of TB. But they are not routinely applied especially in developing countries due to their high cost, need for skilled technicians and sophisticated equipment. Very few published data are available from Kerala on the feasibility of novel diagnostic modalities of tuberculosis.

Recently, a novel technique for the amplification of nucleic acid has been described, loop-mediated isothermal amplification (LAMP) that can be used in poor resource settings, because it does not require expensive or complicated instruments. Recently WHO expert group has recommended that more research is needed for the evaluation of LAMP methods in different geographical settings.[2] This study is attempted to evaluate Loop mediated isothermal amplification assay using conventional culture as gold standard in the diagnosis of pulmonary tuberculosis.

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	LAMP		AFB Staining	
	Positive	Negative	Positive	Negative
Culture positive(59/110)	58	1	54	5
Culture negative(51/110)	1	50	1	50
Sensitivity %	98.3		91.5	
Specificity%	98.03		98	
Positive predictive value %	98.3		98.18	
Negative predictive value %	98.03		90.90	

Table 1. Comparison of LAMP Assay with Culture Among Pulmonary Samples

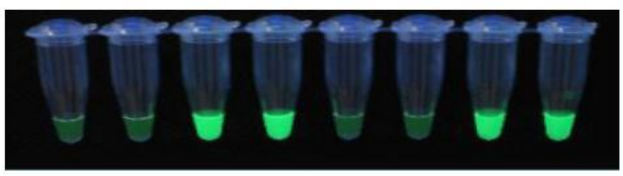

Figure 1. Vials 3,4,7,8 Shows Fluorescent Green Colour Which Indicates Positive by LAMP Method. Vials 1,2,5,6 Shows no Fluorescence Which Indicates Negative by LAMP

METHODS

This is a descriptive study conducted in the department of microbiology, Jubilee Mission Medical College & Research Institute, Thrissur, Kerala. The study was started after getting institutional ethical clearance. Based on a previous study[3] the minimum number of samples required for the study to be statistically significant was calculated to be 98.

The study was conducted on patients with clinical suspicion of pulmonary Tuberculosis like those with prolonged cough, evening rise of temperature, loss of weight, laboratory findings like high ESR and suggestive chest X ray findings attending various clinical specialities and DOTS centre of Jubilee Mission Medical College, Thrissur which is a 1800 bedded tertiary care teaching hospital. Samples were collected for a period of 18 months from December 2016 to June 2018. An informed consent was obtained from each patient. Non consenting patients and were excluded from the study. Clinical and demographic details of the patients were collected including age, sex, clinical history including past tuberculosis, treatment history, clinical, laboratory and radiological findings.

Specimen collection and processing: Early morning sputum, induced sputum or broncho alveolar lavage (BAL) were collected from suspected patients and were immediately processed in the laboratory. All the processing were done in biosafety cabinet Class II using appropriate personal protective equipment (PPE).

A direct smear was made from the samples for AFB staining. The samples were then subjected to homogenization & decontamination with standard N-acetyl-L-cysteine-NaOH method. The pellets were again subjected to AFB staining and culture on Lowenstein Jensen (LJ) medium.

The same pellets were subjected to Loop mediated isothermal Amplification method using the kit procured commercially from RAS Life sciences. After DNA extraction using the kit provided by the manufacturer (RAS DNA Extraction Kit) the solution was subjected to LAMP (Nu-LAMP MTB Kit). All the instructions and guidelines by the manufacturer were strictly followed throughout the procedure.

Cultures were observed for 12 weeks and the growth was identified as Mycobacterium tuberculosis by the time taken for growth to appear, colony morphology and acid-fast staining.

Statistical Analysis

Sensitivity, specificity as well as positive predictive value (PPV) and negative predictive value (NPV) were calculated for the LAMP method and smear microscopy with results of culture as the gold standard.

RESULTS

We received 110 non-duplicate specimens from suspected patients of pulmonary tuberculosis during the study period. Among the 110 samples, 74 (67.30%) samples were from males and 36 (32.70%) from females. Maximum number (25) of samples belonged to the age group between 60 and 70 years followed by 50-60 (24%), 30-40 (17%).

Fifty-nine (53.63%) specimens yielded growth in culture on Lowenstein Jensen medium and 51(46.36%) samples were culture negative. Of these 59 culture positive samples, 54 were AFB staining positive and 5 negative and among 51 culture negative samples one was AFB staining positive and 50 AFB negative. Thus, considering culture as gold standard the sensitivity of AFB staining was found to be 91.5% and specificity was 98.0% with 98.18 % PPV and 90.90% NPV(kappa value of 0.891).

Among the 59 culture positive samples, LAMP was positive in 58 samples and negative in one. Out of 51 culture negative samples, one was LAMP positive and the remaining 50 were LAMP negative. Thus, comparing with conventional culture, the sensitivity and specificity of LAMP method is 98.30% and 98.03% respectively with PPV of 98.30% and NPV of 98.03% (kappa value 0.963).

DISCUSSION

Tuberculosis (TB) is a major public health issue worldwide. Early and accurate diagnosis is of utmost importance in the effective control of Tuberculosis. The absence of a cost effective, rapid and accurate TB point of-care diagnostic test especially in poor resourced settings have been an obstacle for the control of the TB diseases. The sputum ZN microscopy even being less sensitive remains the main stay diagnostic method available for the detection of TB at peripheral laboratories in developing countries.[4] Culture is considered as the gold standard method for the diagnosis of tuberculosis. However due to the slow growth of mycobacteria it may require 8 to 12 weeks to yield results. Nucleic acid amplification (NAAT) testing provides fast, sensitive and specific TB diagnosis but the utility of current NAAT methods are limited by their cost and complexity, particularly in TB high burden developing countries.[5]

Loop mediated isothermal amplification (LAMP), is a new NAA method, that can be performed in one tube under isothermic conditions and with high amplification efficiency in a very short time i.e. 30 to 35 minutes. Globally, two companies: Eiken Chemical Ltd, Japan and Optigene, UK, have developed LAMP based point-of- care tests for the detection of various pathogens.[2,6]

In the present study a total of 110 pulmonary samples from suspected cases of tuberculosis were subjected to acid fast staining, culture on LJ medium and isothermal loop mediated amplification (LAMP) method. The mean age of study subjects was 53.45 (± 16.917 SD). In the suspected PTB patients, predominant clinical manifestations were cough more than 2 weeks followed by fever, haemoptysis, breathlessness and chest pain.

Among the 110 pulmonary samples, 59 (53.63%) specimens yielded growth in culture on Lowenstein Jensen medium and 51 (46.36%) samples were culture negative. Acid fast staining was positive in 55(50%) samples and negative in 55 (50%). Thus, smear microscopy when compared with culture as gold standard showed a sensitivity of 91.5% and specificity of 98% with kappa value at 0.891. Sensitivity of acid-fast staining in our study showed a higher value than a previous study conducted by Sarokhalil et al in 2013(78.57%)[7]. The specificity in the present study was similar with that of a multi-centre study (97.3%).[2] Paramjith et al from Uttar Pradesh in 2017 has reported the sensitivity and specificity of smear microscopy in comparison with culture as 78.6% and 87.5% respectively[8]. Another study which compared smear microscopy with Mycobacterial growth indicator tube (MGIT) culture reported a sensitivity of 60.5% and specificity of 98.5% for pulmonary samples.[9] Sputum ZN microscopy is the main stay diagnostic method available for the detection of TB at peripheral laboratories in developing countries. This technique seems to be simple, rapid and inexpensive but has the inconvenience of being less sensitive and the need for repeated patient's sputum examinations.

In our study, among the 110 pulmonary samples, LAMP was positive in 59(53.63%) samples and negative in 51(56.36%) samples. Thus, we found that the sensitivity and specificity of LAMP when compared with culture was 98.30% and 98.03% respectively with kappa value 0.963. Sensitivity of LAMP in our study was better (98.30%) compared to a similar study which has reported it as 89%.[2] Kohan L et al 2011 has reported the sensitivity of in-house LAMP assay in culture positive samples as 100%[10]. Bojang and co-workers (2016) from Gambia[11] has reported a higher sensitivity (99%) and a slightly lower specificity (94%) compared to our results for LAMP method. The specificity of LAMP method in a multicentre study was similar to our findings (97.6%).[2] Joon et al in 2017 reports that IS6110 LAMP assay shows a sensitivity of 94.4% and specificity of 97.2%[12] among pulmonary samples. Another study conducted by George et al in 2011 arrived at a sensitivity of 79.5% and specificity of 93.8%[13] for LAMP method among pulmonary samples. In 2007, Boehme et al reported the sensitivity of LAMP among culture positive samples as 88.2%.[14]

In the present study, one sample showed acid fast staining and LAMP positive, but culture was negative. But later we found that the same patient was positive by GeneXpert also and had started on antituberculous treatment. However, since we have taken culture as the gold standard in our study this case was considered as false positive.

We found that overall sensitivity was slightly lower in smear negative/culture positive (80.0%) samples than smear positive / culture positive (100%) samples. A similar study carried out in Nepal[15] has shown sensitivity of LAMP as 96.1% in smear positive culture positive and 85% in smear negative culture positive samples, illustrating that LAMP can be used to provide confirmation of clinically suspected pulmonary TB cases where smear is negative. M. tuberculosis was detected by LAMP in all the 56 smear-positive and even in 5 smear negative samples which were also confirmed by culture. For smear negative specimens, a positive LAMP assay greatly increased the probability of culture proven TB and can be used as a rule-in test.

Conventional culture-based methods, because of their high specificity remains the gold standard test for the diagnosis of pulmonary TB. Despite its outstanding performance (100 times more sensitive than smear microscopy), the Mycobacterial culture is notoriously known as time-consuming, dangerous to handle and costly in terms of investment in infrastructure and equipment.

Early diagnosis of TB facilitates appropriate treatment initiation that interrupts the transmission of the agent. The development of nucleic acid-based tests has provided novel avenues for generation of highly sensitive point-of-care tests. While most of TB diagnostic NAATs use Real Time -qPCR technology, which needs precision, thermal cycler and sophisticated optical detection systems that add complexity and cost to the instrumentation. LAMP gives faster results than conventional PCR. In PCR, nearly 3 hrs. is required for the detection and post PCR analysis, while LAMP assay require less than 2 hours.

A key gap in the fight against TB is the availability of cheap, fast and accurate diagnostic tests that can be used in resource-limited settings. Loop-mediated Isothermal Amplification (LAMP) remains an accessible and cost-effective alternative assay to rapidly detect Mycobacteria in pulmonary samples. Indeed, LAMP method requires just constant incubation temperature, and therefore can be implemented with simplified instrumentation. With the features that it can be performed in simple laboratories, small-scale hospitals and primary care facilities in developing countries, LAMP assays appear to be a promising technique suitable for diagnosis of TB as a point of care (POCT) test

LAMP has inherent properties that make amplification and detection possible in one uninterrupted process, with no need to open the amplification vessel or any need for a luminometer or other detection device. It requires only a heating block, robust to inhibitors which interfere with PCR method, and can be visualized with naked eye. The assay utilizes a single polymerase enzyme that is active at relatively high isothermal amplification temperatures, diminishing the likelihood of nonspecific priming. Technicians without molecular training could perform the test with high reproducibility in a simple laboratory space without specialized equipment. Reader-to-reader variability was negligible.

The drawbacks of the study were the limited sample size which was an obstacle to arrive at statistically significant estimates of efficacy. Speciation of the cultured samples was also not performed.

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CONCLUSIONS

Timely and accurate diagnosis of TB is critical in the early initiation of therapy. The loop-mediated isothermal amplification (LAMP) assay is a nucleic acid amplification technique which is feasible in resource limited settings. In our study, the results of LAMP method have shown good sensitivity and specificity considering culture as the gold standard. It is a rapid technique when compared to culture. LAMP is simple and less expensive outweighing PCR. Thus, it can be used as a point of care testing in resource poor settings.

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