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EFFICACY OF LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY IN DETECTING MYCOBACTERIUM TUBERCULOSIS IN EXTRAPULMONARY SAMPLES

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Abstract

Introduction: Despite the availability of highly efficacious treatments for decades, tuberculosis remains a major public health issue worldwide. In 2017, WHO reported that 10.0 million people developed TB, mainly in developing countries, and that approximately 3.6 million (36%) were not diagnosed or notified to the national authorities. The most widely used method for TB diagnosis is sputum smear microscopy. This test is simple, rapid, and inexpensive but has a relatively low sensitivity, particularly in patients with extrapulmonary TB, children, and in people with HIV. Hence, the development of new diagnostic tools suitable for use in low- and middle-income countries is one of the top priorities for TB control.

Materials and Methods: A Prospective study conducted from January 2023- December 2023 (12 months) in the Department of Respiratory Medicine, Gayatri Vidya Parishad Institute of Health Care and Medical Technology, Visakhapatnam). Sputum samples collected from all patients suspected to have pulmonary tuberculosis due to Mycobacterium tuberculosis complex. Samples were collected from those patients who fulfilled the following clinical criteria. Data were analysed using SPSS to determine specificity and sensitivity of LAMP in comparison to culture for detection of Mycobacterium Tuberculosis. The sputum specimens that full filled the criteria were included in this study. Around 50 sputum samples were collected and processed.

Results: Hundred sputum samples which were fitted into the criteria were processed. 50 sputum samples male patients 65%, and the remaining 35% were obtained from female patient. It shows majority of presumptive pulmonary tuberculosis patients were males. Among the 50 sputum samples that were received in the laboratory, maximum number of 15 patients fall under the age group 51-60 years, followed by 13 patients in 61-70 years and 9 patients between 41-50 years. Least incidence was seen under the extreme age groups less than 10 years, and greater than 81 years.

Conclusion: The sensitivity and specificity of LAMP and Gene Xpert MTB/RIF were almost equal in our study. In comparison to LAMP, Gene Xpert MTB/RIF machine and its consumables (cartridge) are very expensive. Moreover Gene Xpert MTB/RIF requires air conditioning; fluctuation in temperature adversely affects its performance, so it is not feasible to implement in peripheral health centres. LAMP is cost effective (50% lesser cost than Xpert), less time consuming (1.5 hour, faster than Xpert), requires minimal amount of sputum and is not adversely affected by temperature fluctuation, making it ideal for use in peripheral poor resource health centres.

Key Words: Tuberculosis, HIV, SPSS, MTB/RIF machine.

INTRODUCTION

Despite the availability of highly efficacious treatments for decades, tuberculosis remains a major public health issue worldwide. In 2017, WHO reported that 10.0 million people developed TB, mainly in developing countries, and that approximately 3.6 million (36%) were not diagnosed or notified to the national authorities.[1] The most widely used method for TB diagnosis is sputum smear microscopy. This test is simple, rapid, and inexpensive but has a relatively low sensitivity, particularly in patients with extrapulmonary TB, children, and in people with HIV. Hence, the development of new diagnostic tools suitable for use in low- and middle-income countries is one of the top priorities for TB control.[2]

Among the technologies developed and implemented over the past decade, nucleic acid amplification technologies hold the greatest promise of substantial gains in turn-around-time (compared with culture) and in sensitivity and specificity (compared with smear microscopy).[3]

Loop-mediated isothermal amplification (LAMP) has several advantages: no need of sophisticated instrumentation, DNA amplification of partially processed or unprocessed samples, and visual readout of the amplified products.[4] Therefore, in 2016, WHO recommended using the Loopamp™ MTBC assay (TB-LAMP) for the detection of *Mycobacterium tuberculosis* complex (MTBC) in sputum as a replacement of smear microscopy and as a follow-on test after negative smear microscopy.[5] Despite these conditional recommendations, further data are required to establish TB-LAMP usefulness in the TB diagnostic pathway in developing countries.

MATERIALS AND METHODS

Type of study: Prospective study

Study period: January 2023- December 2023 (12 months)

Study area: Hospital based study (Department of Respiratory Medicine, Gayatri Vidya Parishad Institute of Health Care and Medical Technology, Visakhapatnam)

Study population:

Inclusion criteria: Sputum samples collected from all patients suspected to have pulmonary tuberculosis due to Mycobacterium tuberculosis complex. Samples were collected from those patients who fulfilled the following clinical criteria.

World Health Organization (WHO) criteria were included:

1. Cough for 2 weeks or more

2. Chronic fever more than 2 weeks

3. Night sweats

4. Weight loss (unintentional)

Exclusion criteria:

- Patients with extra pulmonary tuberculosis

- Patient with infections due to non- Tuberculous Mycobacteria

Sample size: 50 samples

Data Management and Analysis

Data were analysed using SPSS to determine specificity and sensitivity of LAMP in comparison to culture for detection of Mycobacterium Tuberculosis. The sputum specimens that full filled the criteria were included in this study. Around 50 sputum samples were collected and processed. Smears were made from all 50 samples and performed Ziehl neelsen stain, fluorescence staining and looked for presence of Mycobacterium tuberculosis (acid fast bacilli). Simultaneously Gene xpert MTB/RIF was also performed. After decongestion and decontamination samples were inoculated in to solid, liquid culture. DNA extraction was performed simultaneously. Loop mediated isothermal amplification were performed from the extracted sample. Finally the sensitivity, specificity, positive and negative predictive values were analysed using SPSS software.

RESULTS

50 sputum samples male patients 65%, and the remaining 35% were obtained from female patient. It shows majority of presumptive pulmonary tuberculosis patients were males.

Table 1: Gender distribution

S.No	Gender	N (%)
1	Male	33 (65%)
2	Female	17 (35%)

Table 2: Age distribution

S.No	Age group	N (%)
1	21-30	1 (2%)
2	31-40	5 (10%)
3	41-50	9 (18%)
4	51-60	15 (30%)
5	61-70	13 (26%)
6	71-80	5 (10%)
7	81-90	2 (4%)

Among the 50 sputum samples that were received in the laboratory, maximum number of 15 patients fall under the age group 51-60 years, followed by 13 patients in 61-70 years and 9 patients between 41-50 years. Least incidence was seen under the extreme age groups less than 10 years, and greater than 81 years.

S.No	Sex wise distribution in LAMP positive population	N (%)
1	Male	10 (71%)
2	Female	4 (29%)

Table 4: Age wise distribution of LAMP positive Tuberculosis patients

S.No	Age wise distribution of LAMP positive Tuberculosis	N
1	21-30	1
2	31-40	2
3	41-50	3
4	51-60	4
5	61-70	4
6	71-80	1

Table 5: Comparative evaluation of ZN smear with BD MGIT 960 smear

ZN Smear	LJ/BDMGIT960 CULTURE		Total	P value
	Positive	Negative		
Positive	12	1	13	
Negative	3	34	37	

Total	15	35	50	0.000

Table 6: Comparative evaluation of LJ (Lowenstein Jensen) medium with MGIT

MVL FM Smear	LJ/BDMGIT960 CULTURE		Total	P value
	Positive	Negative		
Positive	11	1	12	
Negative	3	35	38	
Total	14	36	50	0.000

Table 7: Comparative evaluation of Gene Xpert MTB/RIF with MGIT

Gene Xpert MTB/RIF	LJ/BDMGIT960 CULTURE		Total	P value
	Positive	Negative		
Positive	14	1	15	
Negative	1	34	35	
Total	15	15	50	0.000

Table 8: Comparative evaluation of LAMP with MGIT

Nu-TB LAMP	BDMGIT960 CULTURE		Total	P value
	Positive	Negative		
Reactive	14	1	15	
Non reactive	1	34	35	
Total	15	35	50	0.000

Table 9: Sensitivity and specificity of LAMP by smear positive and culture positive patients

LAMP	SMEAR POSITIVE		Total
	Culture Positive	Culture Negative	
Reactive	11	0	11
Non reactive	1	1	2
Total	12	1	13

Sensitivity of LAMP in smear positive, culture positive cases = 12/13 =95.65%

Specificity of LAMP in smear positive, culture positive cases=1/1 = 100% Table (9) showed sensitivity of LAMP in smear positive, culture positive population was 95.65%, specificity was 100%.

DISCUSSION

TB is the major public health problem in developing countries like India. WHO‟S End TB strategy along with UN Sustainable Development Goals (SDGs) targets 80% reduction in TB incidence (new case per one lack population per year), and 90% reduction in the absolute number of TB deaths.[6]

In 2017 most high income countries were having fewer than 10 new cases per one lack population; in low income high burden countries had 150- 400 new cases per one lack population. Drug resistance also play a critical role in developing countries. Half of the world‟s MDR RR/TB cases were occupied by 3 countries like India 24%, China 13%, Russian Federation 10%. To reduce the number of new cases, MDR RR/TB and to prevent death from TB cases, early diagnosis and treatment is important. So WHO endorsed NAAT, for rapid and accurate diagnosis of TB with drug resistance and better sensitivity and specificity than conventional methods (microscopy, culture).[7] Unlike routine PCR tests, Gene Xpert MTB/RIF detected Mycobacterium tuberculosis within 2 hours. But it is expensive, requires elaborate infrastructure and requires expertise. So in developing countries like India where there are large number of tuberculosis patients it may not be feasible to have such technically demanding and labour intensive tests. To fulfil these gaps it requires simple , rapid, cost effective diagnostic methods that can be performed by individuals with minimum training and should have better sensitivity and specificity than conventional methods commonly used in laboratories.[8]

So in 2012 WHO recommended LAMP (Loop Mediated Isothermal Amplification)) to fulfil these gaps. It is an isothermal molecular test which requires regular water bath or heating block. It is more economical and practical than Conventional PCR, which requires expensive Gene Xpert MTB/RIF instrument and high technical expertise; NAAT like Xpert is easier to perform with very low turnaround time (2 hours) by anybody with minimal training but requires expensive instruments and consumables of high cost. LAMP is an innovative molecular technique developed by Notomi et al which can detect MTB complex within 1.5 hour and is cost effective.[9]

In our study we evaluated the sensitivity and specificity of LAMP in comparison with conventional methods like ZN smear microscopy, Fluorescent microscopy, solid culture ,liquid culture and NAAT (Gene Xpert MTB/ RIF).[10]

CONCLUSION

The sensitivity and specificity of LAMP and Gene Xpert MTB/RIF were almost equal in our study. In comparison to LAMP, Gene Xpert MTB/RIF machine and its consumables (cartridge) are very expensive. Moreover Gene Xpert MTB/RIF requires air conditioning; fluctuation in temperature adversely affects its performance, so it is not feasible to implement in peripheral

health centres. LAMP is cost effective (50% lesser cost than Xpert), less time consuming (1.5 hour, faster than Xpert), requires minimal amount of sputum and is not adversely affected by temperature fluctuation, making it ideal for use in peripheral poor resource health centres. WHO‟s dream of replacing less sensitive sputum microscopy by NAAT test in resource poor settings can be fulfilled by Loop Mediated Isothermal Amplification (LAMP).

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