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COMPARITIVE STUDY OF DIAGNOSTIC EFFICACY OF LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) TEST WITH LIQUID CULTURE IN TUBERCULOSIS

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INTRODUCTION:
Globally in 2023,10.8 million people fell ill with TB, incidence rate increased by 4.6% between 2020 and 2023.TB caused 1.25 million deaths. WHO's End TB Strategy calls for the early diagnosis of TB and for universal drug susceptibility testing.Given the prevalence and lethality of tuberculosis (TB) in developing countries,there is an ongoing need for rapid, simple, and low-cost detection method that is sensitive and highly specific.The most common method for diagnosing TB is sputum smear microscopy which offers the advantages of rapid detection time and simplicity. However, the sensitivity of smear microscopy is relatively low in EPTB and it requires well-trained laboratory staff. Culture methods can detect as few as 100 Mycobacterium cells per 1 ml specimen and TB culture is still considered the gold standard for the detection of TB. However, the culture methods require up to several weeks in sophisticated facilities.In 2000, a novel nucleic acid amplification method known as loop-mediated isothermal amplification (LAMP) was developed and considered a breakthrough technique for the diagnosis of TB and other infectious diseases.

AIMS AND OBJECTIVES :
1.Compare LAMP test with liquid culture (gold standard) in the diagnosis of tuberculosis.
2.To evaluate the performance characteristics of the LAMP TB detection assay based on the principle of loop mediated isothermal amplification.

METHODOLOGY:
Facility based study was done during march 2023 to march 2024 at Government chest hospital among 50 patients who attended outpatient and inpatient at department of Respiratory Medicine.Patients included in the study were selected according to the inclusion and exclusion criteria. After taking the written consent of the patient, detailed history of present and past illness was recorded. Thorough clinical examination was done. All necessary investigations were carried out.

RESULTS:. When compared to gold standard liquid culture for MTB, overall sensitivity and specificity of LAMP in combined pulmonary and extrapulmonary samples are 81.8% and 85.1 % respectively. Among extrapulmonary samples, LAMP detected MTB in 28.5 % cases (10 samples) and did not detect MTB in 71.4% cases (25 samples).The sensitivity of LAMP in sputum samples (pulmonary tuberculosis) is 85.7% .Among smear negative samples, LAMP showed a sensitivity of 87.5% .The sensitivity of LAMP in extrapulmonary samples is 75% compared to 85.7% sensitivity in pulmonary samples.LAMP detected MTB in one endometrial sample which was positive for liquid culture for MTB among 5 endometrial samples.The sensitivity and specificity of LAMP in pus samples are 83.3% and 40% respectively.The sensitivity of LAMP in pleural fluid samples is negligible.

CONCLUSION: In the present study, LAMP showed good sensitivity in sputum samples (pulmonary tuberculosis) compared to extra-pulmonary samples. LAMP showed good sensitivity in both pus samples from cervical lymph nodes and endometrial samples. Thus, LAMP can be used as a diagnostic tool in sputum samples from pulmonary tuberculosis, pus samples from cervical lymph nodes and endometrial samples from female genital tuberculosis

KEY WORDS: Loop mediated isothermal amplification (LAMP),Tuberculosis, Liquid culture, Extra pulmonary tuberculosis

INTRODUCTION:

Tuberculosis is one among the most important health problems worldwide[1].The lifetime risk of breaking down to disease among those infected with TB is 10 -15%, which gets increased to 10% per year among those co-infected with HIV[2].It is also estimated that about 2.2 lakhs of people die due to TB annually[2].The burden of EPTB is high, ranging from 15–20% of all TB cases in HIV-negative patients while in HIV-positive people it accounts for 40–50% of new TB cases[3]. All efforts should be made to establish microbiological confirmation in case of presumptive EPTB[4].Lymphnode TB is the most common followed by pleural TB form of EPTB in India[3] .The most common method for diagnosing TB in developing countries is sputum smear microscopy; however, the sensitivity of this test is relatively low in EPTB and it usually requires well-trained laboratory staff. Cultures of Mycobacterial Tuberculosis Complex require up to several weeks in sophisticated facilities.[5] Conventional NAATs, which are comparable with the culture confirmation method, have a high sensitivity and specificity for the detection of MTC. However, they require exclusive equipment, and the use of complicated techniques and procedures (Aryan et al., 2010).Therefore, they are not suitable for resource-limited laboratory settings in developed and developing countries.[5]

Loop-mediated isothermal amplification (LAMP) is a unique, temperature independent technique for amplifying DNA that is simple to use, providing a visual display that is easy to read; additionally, the technique is robust and can be used at peripheral health centres, where microscopy is performed.[6] TB-LAMP requires minimal laboratory infrastructure and has few Biosafety requirements; and it has been evaluated for use as a rapid alternative to sputum smear microscopy, which remains the primary diagnostic test for pulmonary TB in resource-limited settings.[6] Extra-pulmonary tuberculosis is difficult to diagnose by conventional methods, because they are less sensitive and more time consuming.[7] Loop-mediated Isothermal Amplification (LAMP) is a novel gene amplification method that has been developed to diagnoseTB in pulmonary and paucibacillary extra-pulmonary specimens even in resource poor settings[7] .

The present study aims at evaluating the efficacy of LAMP to detect Mycobacterium tuberculosis complex in paucibacillary extra-pulmonary specimens and pulmonary specimens and to compare the results with culture methods.

AIMS AND OBJECTIVES :
1.Compare LAMP test with liquid culture (gold standard) in the diagnosis of tuberculosis.
2.To evaluate the performance characteristics of the LAMP TB detection assay based on the principle of loop mediated isothermal amplification.

INCLUSION CRITERIA:
1. All newly diagnosed tuberculosis patients above 15years
2. All patients with clinical picture suggestive of tuberculosis
3. Patients with cervical lymphadenopathy and clinical picture suggestive of EPTB
4. Patients presenting with pleural effusion and clinical picture suggestive of EPTB
5. Female patients with infertility and clinical picture suggestive of genital tuberculosis.

EXCLUSION CRITERIA:
1. Age <15years.
2. Previously treated cases of pulmonary and extrapulmonary tuberculosis.

PATIENTS AND METHODS
It is a cross sectional study of 50 Randomly selected patients with clinical picture suggestive of pulmonary and extrapulmonary tuberculosis, attending Government Chest Hospital from March 2023 to March 2024 who fulfil the inclusion and exclusion criteria are included in the study. After taking the written consent of the patient, detailed history of present and past illness was recorded. Thorough clinical examination was done. All necessary investigations were carried out.

OBSERVATION AND RESULTS
The present study was conducted in Government Chest Hospital to study the performance characteristics of the LAMP (loop mediated isothermal amplification test) and to compare with liquid culture in the diagnosis of tuberculosis.

TABLE 1: DESCRIPTIVE ANALYSIS

1.	**GENDER**	**No.of patients**	**% of patients**
	male	30	60%
	female	20	40%
	total	50	100%
2	**Age groups**		
	<20yrs	6	12%
	21-30yrs	15	30%
	31-40yrs	18	36%
	41-50 yrs	7	14%
	51-60yrs	2	4%
	>60yrs	2	4%
3	**symptoms**		
	fever	40	80%
	Loss of appetite	43	86%
	Loss of weight	43	86%
	Generalised weakness	50	100%
	cough	33	66%
	dyspnoea	28	56%

	Chest pain	25	50%
	haempotysis	4	8%
	Cervical lymphnodes	14	28%
	Abdominal pain	1	2%
	infertility	4	8%
4	**Contact history for TB**		
	yes	9	18%
	no	41	82%
5	**Comorbidities**		
	Diabetes mellitus	4	8%
	Hypertension	2	4%
	NIL	44	88%
6	**Cervical lymphnodes**		
	yes	15	30%
	no	35	70%
7	**HIV**		
	reactive	4	8%
	Non reactive	46	92%

TABLE 2. TYPE OF SAMPLES IN THE STUDY:

samples	No.of patients	% of patients
Pulmonary(sputum)	15	30%
Extra pulmonary	35	70%
Total	50	100%

TABLE 3: EXTRAPULMONARY SAMPLES IN THE STUDY

samples	No.of patients	% of patients
Pleural fluid	19	54.2%
pus	11	31.4%
Endometrial washings	5	14.2%
total	35	100%

TABLE 4: LAMP AND LIQUID CULTURE IN PULMONARY AND EXTRAPULMONARY SAMPLES

Liquid culture	LAMP				total	
	Detected		Not detected			
	count	%	count	%	count	%
Growth at the end of 1 week	9	39.1%	2	7.4%	11	22%
Growth at the end of 2 week	7	30.4%	2	7.4%	9	18%
Growth at the end of 3 week	2	8.9%	0	0.0%	2	4%
No Growth at the end of 6 week	5	21.6%	23	85.2 %	28	56%
total	23	100%	27	100 %	50	100%
Chi-square value =23.91 P-value<0.01*						

TABLE 5: LAMP AND LIQUID CULTURE IN PULMONARY AND EXTRAPULMONARY SAMPLES

Sample	Liquid culture	LAMP				Total	
		detected		Not detected			
		count	%	Count	%	count	%
Extra pulmonary	Growth at the end of 1 week	1	10.0 %	0	0.0 %	1	2.9%
	Growth at the end of 2 week	5	50.0 %	2	8.0 %	7	20.1 %
	No Growth at the end of 6 week	4	40.0 %	23	92.0 %	27	77.1 %
	Chi-square value =12.42 P-value<0.01*						
pulmonary	Growth at the end of 1 week	8	66.7 %	2	100. 0%	10	71.4 %
	Growth at the end of 2 week	2	16.6 %	0	0.0 %	2	14.2 %
	Growth at the end of 3 week	2	16.6 %	0	0.0 %	2	14.3 %
	Chi-square value =0.94 P-value=0.82						

TABLE 6: LAMP AND LIQUID CULTURE IN EXTRAPULMONARY SAMPLES

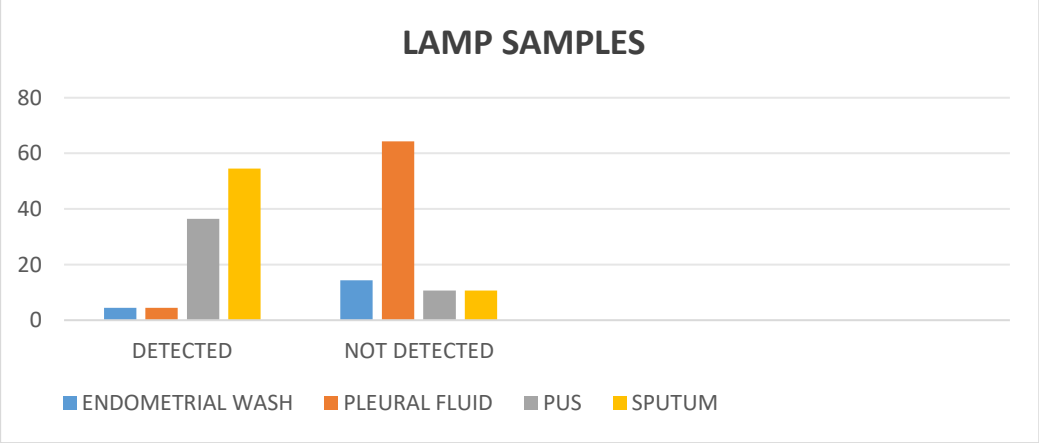
sample	Liquid culture	LAMP				total	
		detected		Not detected			
		count	%	count	%	count	%
Endometrial washings	Growth at the end of 2 weeks	1	100.0 %	0	0.0%	1	20.0 %
	No Growth at the end of 6 weeks	0	0.0%	4	100.0 %	4	80.0 %
	Chi-square value =5 P-value=0.02*						
Pleura fluid	Growth at the end of 2 weeks	0	0.0%	1	5.6%	1	5.3%
	No Growth at the end of 6 weeks	1	100.0 %	17	94.4%	18	94.7 %
	Chi-square value =0.06 P-value=0.81						
pus	Growth at the end of 1 weeks	1	12.5%	0	0.0%	1	9.1%
	Growth at the end of 2	4	40.0%	1	33.3%	5	45.5

	weeks						%
	No Growth at the end of 6 weeks	3	37.5%	2	66.7%	5	45.5 %
	Chi-square value =0.92 P-value=0.63						

TABLE 7 : LAMP IN PULMONARY AND EXTRAPULMONARY SAMPLES

sample	LAMP				total	
	Detected		Not detected			
	count	%	count	%	count	%
Endometrial washings	1	4.5%	4	14.3%	5	10.0%
Pleural fluid	1	4.5%	18	64.3%	19	38.0%
pus	8	36.4%	3	10.7%	11	22.0%
sputum	12	54.5%	3	10.7%	15	30.0%
total	22	100%	28	100%	50	100%
Chi-square value =23.91 P-value<0.01*						

GRAPH 1:LAMP IN PULMONARY AND EXTRAPULMONARY SAMPLES



DISCUSSION

In the present study males (60%) were more commonly affected than females (40%) which is comparable to a study by Abdul Qayyum khan et al [8] which showed a male preponderance of 67.2% and females of 32.7 %. The greater mobility of males could possibly lead to getting a higher infection by coming into contact with the TB patients and/ or the males were more prone to infection of the disease[8].The majority of cases (36%) were in the age group of 31-40yrs. Which is comparable to a similar study conducted by Lydia Nakiyingi et al [9], the mean age of presentation was 40.

Fever was seen in 80% cases and cough is seen in 66% cases which is comparable to a study by Barman TK, et al[10], which showed chronic cough in 73.7% cases, followed by low grade fever in 70.8% cases. The chest pain was seen in 50% cases comparable to the study conducted by Bhatt CP et al[11], which showed chest pain in 41% cases.

Infertility (in females) is the presenting symptom in 8% cases in the present study population. According to a study published by Jai B sharma et al [12], the incidence of FGTB (female genital tuberculosis) is 1-19% in various parts of India and incidence of infertility in FGTB to be 42.5 %.
In the present study 18 % cases had a contact H/O PTB, among which 22% cases had active PTB. According to a study conducted by M Cailleaux Cezar et al [13], 47% cases develop tuberculosis infection and 5% develop active tuberculosis disease within 2 years of exposure.

LAMP:
In the present study, 50 patients were included in which, 15 sputum samples from patients of pulmonary tuberculosis (30%) and 35 samples from the sites suggestive of extrapulmonary tuberculosis were included (70%).In the present study,out of 35 extrapulmonary tuberculosis samples, 19 pleural fluid samples (54.2% of EPTB samples), 11 pus samples from cervical lymph nodes (31.4% of EPTB samples) and 5 endometrial washings (14.2% of EPTB samples) were included in the study.

LAMP AND CULTURE:
A.Among all 22 culture positive cases for MTB, (14 sputum,6 pus,1 pleural fluid,1 endometrial sample) LAMP detected MTB in 18 samples (12 sputum,5pus,1 endometrial sample,i.e. combined sensitivity of 81.8%) and LAMP did not detect MTB in 4 samples (2 sputum sample,1 pleural fluid,1 pus sample- i.e.,14.2%). Among 27 culture negative cases for MTB, LAMP detected MTB in 4 samples (3 pus samples, 1 pleural fluid sample-i.e.,14.8% of culture negative samples) and LAMP did not detect MTB in 23 samples (17 pleural fluid samples, 4 endometrial samples,2 pus samples-combined specificity of 85.1%).
B.Among 14 sputum samples (pulmonary tuberculosis) positive for MTB culture,LAMP detected MTB in 12 samples (sensitivity of 85.7%) and did not detect MTB in 2
samples (14.2% cases).
C.Among extrapulmonary samples, positive for MTB culture (8 cases- 6 pus, 1 endometrial sample, 1 pleural fluid sample), LAMP detected MTB in 6 samples (5 pus samples, 1 endometrial sample) with a sensitivity of 75% and LAMP did not detect MTB in 2 samples (1 pleural fluid and pus sample- 25%)
D.Among culture negative extrapulmonary samples (27 samples), LAMP detected MTB in 4 samples (14.8%- 3 pus samples, 1 pleural fluid sample) and did not detect MTB in another 23 samples (17 pleural fluid samples,4 endometrial samples,2 pus samples) with a specificity of 85.1%
E. Among the 5 endometrial samples, LAMP detected MTB in one endometrial sample only which was also positive for liquid culture.
F. Among 6 pus samples, positive for MTB culture LAMP detected MTB in 5 samples (sensitivity of 83.3%) and did not detect MTB in 1 sample positive for AFB culture.
G.Among 5 pus samples, negative for MTB culture, LAMP detected MTB in 3 samples (60%) and did not detect MTB in 2 pus samples (specificity of 40%).
H. Among 19 pleural fluid samples, LAMP detected MTB in one pleural fluid sample negative for MTB culture. Among pleural fluid samples, liquid culture was positive for 1 pleural fluid sample in which LAMP did not detect MTB.

LAMP IN PULMONARY AND EXTRAPULMONARY SAMPLES:
IN THE PRESENT STUDY:
1. Out of 50 pulmonary and extrapulmonary samples, LAMP detected MTB in 44% cases (22 samples) and LAMP did not detect MTB in 56% cases (28 samples). The combined sensitivity and specificity in pulmonary and extrapulmonary samples is

81.8% and 85.1%, respectively (with liquid culture as the gold standard).
2. Among sputum samples (pulmonary tuberculosis), LAMP detected MTB in 80% cases (12 samples) and did not detect MTB in 20% cases (3 samples). The sensitivity of LAMP in sputum samples (pulmonary tuberculosis) is 85.7%.
3. Among sputum smear negative samples, LAMP showed a sensitivity of 87.5%.
4. Among extrapulmonary samples, LAMP detected MTB in 28.5 % cases (10 samples) and did not detect MTB in 71.4% cases (25 samples).
5. The sensitivity of LAMP in extrapulmonary samples is 75% (compared to 85.7% sensitivity in pulmonary samples) and specificity is 85.1%.
6. The sensitivity and specificity of LAMP in pus samples is 83.3% and 40% respectively.
7. Among the 5 endometrial samples, LAMP detected MTB in one endometrial sample positive for MTB liquid culture
8.Among 19 pleural fluid samples, LAMP detected MTB in one pleural fluid sample which was negative for MTB culture. The sensitivity of LAMP in pleural fluid samples is negligible.

In a Study by Kenjiro Nagai et al [14], for sputum samples, 26 studies yielded the summary estimates of sensitivity of 89.6% (95% CI 85.6–92.6%), specificity of 94.0% (95% CI 91.0–96.1%).One study by Joon50 *et al*, evaluated the diagnostic test accuracy of the LAMP MTBC assay for culture-proven TB using extra-pulmonary specimens. Sensitivity of 93.3% (95% CI 77.9–99.2%), and a specificity of 91.9% (95% CI 88.1– 94.8%)was observed.

CONCLUSION:

Thus, in the present study, LAMP showed good sensitivity in sputum samples (PTB) compared to extrapulmonary samples.Among the extrapulmonary samples, LAMP showed good sensitivity in both pus samples from cervical lymph nodes and endometrial samples.Thus, LAMP can be used as a diagnostic tool in sputum samples from pulmonary tuberculosis, pus samples from cervical lymph nodes and endometrial samples from female genital tuberculosis.

LIMITATIONS OF THE STUDY
1)The present study may not be the representative of the actual population, because the study population included patients attending tertiary care centre limited sample size.
2) The sample size is limited.
3) Management aspects of the patients have not been followed up

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