Loop Mediated Isothermal Amplification (LAMP) for rapid diagnosis of Tuberculous meningitis (TBM) in HIV positive patients

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Introduction

- Most common presentation of neurotuberculosis in Indian subcontinent is **tuberculous meningitis (TBM)**

- Early and rapid diagnosis is crucial for successful disease management - **neurological sequelae in 20-25% due to delay in diagnosis**

*J Neurol Sci 2007;252: 163-168*
Introduction

• Microscopy and culture (Gold standard) - quite inadequate

• IGRAs can not differentiate latent from active infection

• **Nucleic acid-based amplification (NAA) tests:** important tools - high specificity but low sensitivity in TBM (Metaanalysis)

  *Lancet infect Dis 2003; 3:633-643*

• Real time PCR is beneficial but requires expensive equipment and expertise, limiting its use to highly sophisticated facilities
LAMP
(loop mediated isothermal amplification)

• Highly sensitive and specific

• **Six primer pairs** recognize **eight distinct regions** in the target DNA

• Reaction takes only **one hour**, and can be conducted **under isothermal conditions (ranging from 60-65°C)**, eliminating the need for **specialized equipment or expertise**

• Can be performed even in rural setting
• Positive reaction detected by a color change of the reaction mixture in ambient light when DNA binding dye (Sybergreen. HNB) is used or by ladder like banding pattern on gel

• The present study was undertaken to-
  ➢ explore the utility LAMP for rapid diagnosis of TBM
  ➢ compare LAMP with IS6110 PCR for diagnosis of TBM
Material & Methods:

- CSF samples from total of 105 patients received in Mycobacteriology lab of Medical microbiology, PGIMER, Chandigarh were tested.
- The relevant history and other details of patients were noted and they were divided into 3 groups on the basis of following criteria:

  - TBM patients  
    - N=65
  - Disease Control 
    - N=20
  - Non TBM Non-infectious 
    - N=20
Group I: **Tuberculous meningitis group** (n= 65)

(a) **Confirmed TBM** (n=5): culture/smear positive.

(b) **Suspected TBM** (n= 60): Smear/culture negative, Fever, S/S meningeal irritation, CSF: Increased Protein level (>40 mg/ dl), Deceased glucose,>10WBC/ml & 80% lymphocytes, Good response to ATT.

Group II: **Non TBM infectious meningitis** (n=20)

Pyogenic meningitis (n=10)
Viral meningitis (n=8 )
Fungal meningitis (n=2)

Group III: **Non-infectious neurological disorders** (n=20)

HI-10
LGBS-5
MS-3
Tumors-2
Processing of CSF: 200µl of samples were used for Culture, smear LAMP, PCR with IS6110

DNA Extraction: QIAGEN KIT

LAMP: Primers specific for IS6110 (123bp), MPB 64 were used

PCR: IS6110

Results of LAMP were compared with simple IS6110PCR,
Results of LAMP

L1- MM, L2- Positive control with bending pattern on gel, L3-L4: Positive samples, L5- Negative control, L6-L7; negative samples
T1- Positive Control (Green color) , T2-T6: Clinical samples with positive amplification, T7-Negative control (Sybers green dye with no change in color), T8-T10: negative clinical samples with no amplification
Improved LAMP test for diagnosis of TBM

T1 – Purple color no amplification by using HNB dye, T2-T6 – Blue color amplification and positive for MTB DNA. T2- Positive control, T2-6, -clinical samples
<table>
<thead>
<tr>
<th>TYPE</th>
<th>SUB TYPE</th>
<th>NO. OF PATIENTS</th>
<th>SMEAR (+) %</th>
<th>CULTURE (+)%</th>
<th>IS6110 PCR N (%)</th>
<th>LAMP N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP-1</td>
<td>TBM</td>
<td>Confirmed TBM Patients</td>
<td>5</td>
<td>-</td>
<td>5 (100)</td>
<td>4 (80)</td>
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<tr>
<td></td>
<td></td>
<td>Suspected TBM Patients</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>44 (73.33)</td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
<td>65</td>
<td>-</td>
<td>18</td>
<td>48 (73.84)</td>
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<tr>
<td>Control group</td>
<td>Non TB</td>
<td>40</td>
<td>-</td>
<td>-</td>
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</table>
## Sensitivity & Specificity of LAMP, IS6110 PCR compared to culture /AFB smears

<table>
<thead>
<tr>
<th>Test</th>
<th>Test Results</th>
<th>TBM cases (N=65)</th>
<th>Control Group (N=40)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LAMP</strong></td>
<td>Positive</td>
<td>57</td>
<td>-</td>
<td><strong>87.69%</strong></td>
<td>100%</td>
<td>100%</td>
<td>66.44%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>8</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>IS6110 PCR</strong></td>
<td>Positive</td>
<td>48</td>
<td>-</td>
<td><strong>74.16%</strong></td>
<td>100%</td>
<td>100%</td>
<td>49.18%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>17</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Culture</strong></td>
<td>Positive</td>
<td>5</td>
<td>-</td>
<td><strong>7.69%</strong></td>
<td>100%</td>
<td>100%</td>
<td>21.92%</td>
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<tr>
<td></td>
<td>Negative</td>
<td>60</td>
<td>40</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Microscopy</strong></td>
<td>Positive</td>
<td>-</td>
<td>-</td>
<td><strong>18.33%</strong></td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>65</td>
<td>40</td>
<td></td>
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</table>
Comparison of LAMP & IS6110 PCR Results with response to ATT in cases of TBM

<table>
<thead>
<tr>
<th>Response to ATT</th>
<th>IS6110 PCR  n (%)</th>
<th>LAMP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=65</td>
<td>48 (74.16%)</td>
<td>57(87.69%)</td>
</tr>
<tr>
<td>Study</td>
<td>PCR target</td>
<td>No. of cases</td>
</tr>
<tr>
<td>------------------------------</td>
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<tr>
<td>Halder et al, 2009</td>
<td>IS6110</td>
<td>167</td>
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<tr>
<td></td>
<td>Dev R</td>
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</tr>
<tr>
<td>Dil-Afrose et al, 2008</td>
<td>MPB64</td>
<td>27</td>
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<td>Sumi et al, 2002</td>
<td>IS6110</td>
<td>37</td>
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<tr>
<td>Sharma K et al 2011</td>
<td>MPB64, Protein b, IS6110 MPCR</td>
<td>210</td>
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<tr>
<td>Nagdev et al, 2011</td>
<td>IS6110 LAMP</td>
<td>27</td>
</tr>
</tbody>
</table>
Conclusions:

• LAMP is more sensitive than IS6110PCR in diagnosing TBM

• HNB is better than Syber green in diagnosing TBM
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