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## Comparative evaluation of in-house Real time IS 6110, nested MPT 64 PCR and Roche AMPLICOR 16s rRNA PCR for diagnosing tuberculous meningitis

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**Background**: Early diagnosis of tuberculous meningitis (TBM) is still a diagnostic challenge due to paucibacillary nature of the disease and conventional methods being quite insensitive and time consuming. We evaluated performance of three real time PCR assays targeting MPT64, IS6110 and 16s rRNA gene sequences for early diagnosis of TBM

**Methods & Materials**: A total of 100 consecutive probable TBM patients and 50 non TBM patients were enrolled from an ongoing prospective study on tuberculous meningitis (July 2012 to Dec 2014). CSF specimens were subjected to microscopy and automated BACTEC MGIT culture. In-house Real time nested MPT64 and IS6110 PCR were standardized using H37Rv spiked CSF and evaluated for diagnostic utility along with commercially available Roche Amplicor Real time PCR assay.

**Results**: Out of 100 clinical specimens processed, the sensitivity of smear microscopy and culture was 4% and 42% respectively. The sensitivity, specificity of Real time IS6110 PCR, Nested MPT 64 assay and Roche Amplicor Real time PCR assay was 71%, 98%; 69%, 98% and 25%, 100% respectively against probable TBM as reference standard. The Real time IS6110 PCR, Nested MPT 64 assay and Roche Amplicor Real time PCR assay could detect M. tuberculosis in only 84%, 77% and 30% of culture positive patient's respectively

**Conclusion**: Real time IS6110 PCR proved to be a simple and rapid method to diagnose TBM with sensitivity, specificity of 71%, 98% only. Nested MPT 64 assay though had comparable sensitivity and specificity was much more difficult due to nested design and higher risks of contamination. None of the PCR was 100% sensitive for detecting all culture positive samples suggesting that for TBM diagnosis there is no single rule out test and all the tests are contingent upon their ability to pick the target in tested volume of CSF.

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## Evolutionary patterns of T cell epitopes in mycobacterium tuberculosis strains isolated in India

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**Background**: Mycobacterium tuberculosis (Mtb) is an obligate, persistent, intracellular human pathogen. Host immune pressure and associated Mtb immune evasion that drive the evolution of Mtb, is crucial for diagnosing and designing effective vaccine. Human T cell responses are essential for containment of Mtb through secretion of IFNγ and TNFα, previous studies have shown that Mtb T cell epitopes (TCEs) are hyperconserved, suggesting there is little evidence for immune selection pressure and indicating that antigenic variation may contribute to bacterial persistence. Thus, the aim of this study was to provide an in-depth analysis of the pattern of changes in TCEs in circulating Indian Mtb strains.

**Methods & Materials**: We analyzed 79 Mtb whole genome sequences generated from strains isolated from South Indian pulmonary TB patients. Extensive bioinformatic analyses were employed, but not limited to determine i) phylogenetic relationships ii) extent and nature of mutations harbored within TCEs and iii) binding affinity of novel mutated TCEs (mTCEs).

**Results**: Phylogenetic trees revealed clustering of 79 strains to three lineages: EAI, CAS and Beijing. We identified that 13% of 1101 examined TCEs within the Mtb genome were mutated in atleast one strain, with 67% of mutations resulting in single amino acid changes. We report for the first time, focused mutations in 16 of 66 highly immunodominant CD4 T cell antigens comprising experimentally verified epitopes. More than 80% of the sequenced strains had common mutations across this antigenic group, with 4-6 mutated immunodominant antigens occurring per strain. The most impacted functional category of mutated immunodominant antigens belonged to the cell wall and cell processes. Furthermore, we identified three mTCEs (one each in mce2A, hemK and espA antigens) have not previously been identified to be immunodominant, but present in all the strains studied. Binding affinity of the majority of mTCEs to common Indian HLA alleles was marginally higher than the corresponding parent TCEs.

**Conclusion**: Presently, investigating the impact of these novel mutants and their corresponding parent epitopes in CD4 T cell functional assays that enable the evaluation of the immunogenicity of candidates peptides. Together, our data provide novel insights into the importance of immune selection pressure in Mtb evolution in India.

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