Comparative evaluation of a multiple- antigen (GlcB, HspX, MPT51,Ag 85B and PstS1) based diagnostic protocol versus Polymerase Chain Reaction assays (qRT-PCR and gel-based duplex PCR) for Rapid and Efficient diagnosis of childhood tuberculous meningitis.

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Abstract

Comparative evaluation of a multiple- antigen (GlcB, HspX, MPT51,Ag 85B and PstS1) based diagnostic protocol versus Polymerase Chain Reaction assays (qRT-PCR and gel-based duplex PCR) for Rapid and Efficient diagnosis of childhood tuberculous meningitis.

Keywords: Tuberculous Meningitis; PCR; ELISA.

Introduction

Tuberculosis is a global public health hazard. Tuberculosis meningitis (TBM) is the most severe form of extrapulmonary tuberculosis. Prompt diagnosis is crucial for successful TBM management ; the case fatality rate for untreated TBM is almost 100% and delay in treatment often leads to permanent neurological damage. The difficulty in obtaining a precise history and collecting an adequate volume of cerebrospinal fluid (CSF) makes the diagnosis of TBM a daunting challenge especially in pediatric subjects. PCR and ELISA based assays for detecting antigens are fast and reliable but have not yet been amalgamated into routine diagnostics .Thus, there is an overall need for improving TB diagnostics by the development of cost-effective and robust tools.

Material & methods

One hundred and thirty tow CSF samples collected from pediatric wards were included in the study. A pediatric adaptation of Ahuja's criteria was used as the gold standard to classify subjects as TBM and Non-TBM. An ELISA-based assay was performed for the detection of mycobacterial antigens (GlcB, HspX, MPT51, Ag 85B and PstS1) and its efficacy was compared with quantitative real-time PCR and gel-based duplex PCR. All statistical analyses were performed with SPSS, version 11.5. Standard curve was generated for antigen detection and Receiver-Operating Characteristic (ROC) curves were generated to establish cut offs that distinguished between TBM and non-TBM samples.

Results

GlcB and HspX ELISA had the highest sensitivities of 95% and 93% respectively with specificities of 96% and 97%. The sensitivity of MPT51, Ag85B and PstS1 ELISA ranged between 90-93% with specificities ranging between 92-96%.qRT-PCR was the best performing assay with sensitivity and specificity of 97% and 99% respectively. Gel-based duplex PCR had a diagnostic accuracy of 84% vs. 98% for the qRT-PCR assay.In this study all tests had area under ROC curve ranging from 0.94-0.99. A 0.99 value implies that a randomly selected value from TBM group has a test value larger than that from a randomly chosen individual from the control group 99% of the time.

Conclusion

The diagnosis of pediatric TBM is a problem both by conventional microbiological methods and radiological techniques. Present study concludes that in addition to qRT -PCR , GlcB and HspX can serve as potentially useful markers for rapid, user friendly, inexpensive diagnosis of childhood tubercular meningitis. Since no commercial test is licensed for diagnosis of TBM, present study provides a better insight into development of rapid tests for better TBM diagnosis.