

Original Research Article

***IS6110* gene in *Mycobacterium tuberculosis* complex in Tuberculosis meningitis- Clinical Applications for Disease Monitoring**

Bhavana Singh¹, Vishal Khandelwal², Urvashi Agarwal², Sandhya Mangla², Uma Shankar², Anjana Goel², Aditya Saxena², A.K. Bhatia², Meenakshi Bahuguna¹, Narotam Sharma^{1*}

¹Central Molecular Research Laboratory, Department of Biochemistry, Shri Guru Ram Rai Institute of Medical & Health Sciences, Patel Nagar, Dehradun-248001, Uttarakhand, India

²GLA University, Mathura, Uttar Pradesh, India

***Corresponding author**

Dr. Narotam Sharma

Email: sharmanarotam5@gmail.com

Abstract: Tuberculosis meningitis (TBM) is the most common form of chronic infection of central nervous system. Regardless of the extent of the problem, the general diagnostic outlook is discouraging specifically; there is no generally accepted early confirmative diagnostic protocol available for TBM. In the current work, 16 Cerebrospinal Fluid (CSF) cases were considered for the molecular characterization. 02 CSF came positive for the Nested PCR targeting *IS6110* gene, considered to be positive for the presence of *mycobacterium tuberculosis* DNA. It was also seen that the positive cases were with increased ADA titer. The clinical manifestations of the positive cases were matching with those for the tuberculosis meningitis.

Keywords: Nested PCR, Amplimer, Uracil DNA glycosylase, Extra Pulmonary Tuberculosis, Tuberculosis Meningitis.

INTRODUCTION:

Tuberculosis meningitis (TBM) is also known as TB meningitis or tubercular meningitis, which is the infection of meninges [1]. The system of membranes which envelop the Central Nervous System. Tuberculosis meningitis is the severe form of tuberculosis. It causes severe neurological defects or death more than half of the cases. The pattern of tuberculosis meningitis in the population is different in different areas of the world. In areas with much tuberculosis, tuberculosis meningitis usually affects young children. It develops typically 3-6 months after primary tuberculosis infection. Tuberculosis meningitis (TBM) is the most common form of chronic infection of central nervous system [2, 3]. Despite the magnitude of the problem, the general diagnostic outlook is disappointing purposely; there is no usually conventional early confirmative diagnostic protocol available for TBM [4-6]. Thus in the current study we considered the cases of meningitis which were processed for Molecular approaches for further characterization.

MATERIALS AND METHODS:

The samples were collected from the patients suspected for Tuberculosis meningitis from the different Departments of Shri Mahant Indresh Hospital, which includes; Ortho pediatrics, Neurosurgery and Pediatrics.

Clinical symptoms include; fever and headache for more than 2 weeks, vomiting, neck stiffness, altered sensorium, seizures or focal neurologic deficit. The Nucleic Acid (DNA) is extracted from the clinical specimen (CSF) by spin column based Nucleic Acid Extraction method. Column-based nucleic acid purification is a solid phase extraction method to quickly purify nucleic acids. The method of serum ADA estimation was done based on the principle of Guisti G Galanti methods of enzymatic analyses. Further Nested PCR was performed on the DNA template isolated from CSF specimens. This test is based on the principles of single-tube nested PCR method, which is a powerful and sensitive diagnostic tool for the identification of *Mycobacterium Tuberculosis* complex. This assay is a two-step sequential assay [7]. In the first step, the Insertion sequence region of *Mycobacterium tuberculosis* complex DNA sequence, a 220 bp is amplified by specific external primers. In the second step, the nested primers are added to further amplify a 123 bp amplification product. In this assay, false positive reactions that may be caused by previous amplicon contamination are prevented by the use of uracil DNA glycosylase (UDG) and dUTP instead of dTTP added in the premix. Nested PCR [8-10]. An amplimer of size 123 bp is indicative of infection with *Mycobacterium tuberculosis* complex. The amplification product of

internal control DNA is 340 bp which is used for the validation of the results (as depicted in figure1).

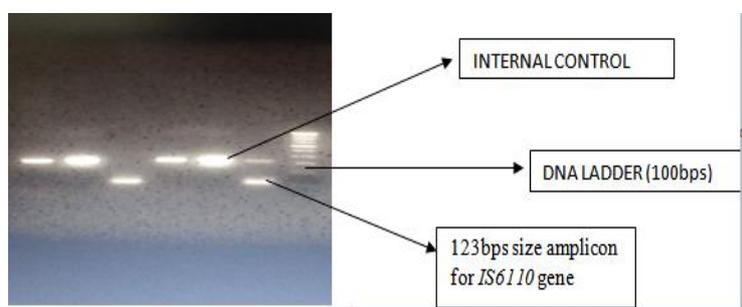


Fig 1: Gel picture depicting the *IS6110* gene band for TB at 123bp

Well 1,2,4,5 and 6 – Internal control at 340bps

Well 3 and 6- 123bp size amplicon of *IS6110* gene

Well 7 – DNA Ladder (100bps)

RESULTS:

A total of 16 CSF cases were considered for the proposed study. Out of 16 cases 02 came positive for the Nested PCR targeting *IS6110* gene, considered to be positive for the presence of *mycobacterium tuberculosis* DNA. It was also seen that the positive cases were with increased ADA titer. The clinical manifestations of the positive cases were matching with those for the tuberculosis meningitis.

DISCUSSION AND CONCLUSION:

The study signifies about the usage of Nested PCR as a diagnostics tool for the characterization of *Mycobacterium tuberculosis* complex in CSF in symptomatic cases for Tuberculosis meningitis. The two cases which turned out to be positive fulfill the entire criterion for the meningitis. As PCR offers rapid sensitive and accurate method for timely diagnosis of the, disease, thus can be utilized as one of the most important diagnostic tool to screen out the patients with symptoms for tuberculosis meningitis. Despite the difficulties in diagnosing TB in children and the low number of cases evaluated in the present study, Nested PCR in CSF specimens proved to be a rapid and specific technique, with high sensitivity.

Conflict of Interest: None

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