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Herpes Simplex Virus 1&2 Genotyping Characterization and *Mycobacterium tuberculosis* Complex Detection in Neurological Disorders

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Original Research Article

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Abstract: Herpes simplex virus (HSV) causes a wide spectrum of clinical manifestations in the central nervous system (CNS) along with *Mycobacterium tuberculosis* complex (MTC). Current study focuses on the molecular characterization of MTC and HSV1&2 in the cases with neurological disorders. 60 clinical samples with clinical manifestations for neurological disorders were collected, MTC and HSV genotyping. DNA was isolated from all the CSF samples and was used further for molecular characterization. Out of 30 clinical samples, 06 CSF yielded positive amplicon with yielding 123 bp product for MTC infection accounting for 20% while 80% samples were found to be negative. ZN staining was performed for all the cases for CSF as non came positive by microscopy. For HSV genotyping, 02 cases came positive where those were the cases of viral meningitis.
Keywords: Tuberculosis Meningitis, *Mycobacterium tuberculosis* complex, *IS6110* gene, Viral Meningitis, PCR, genital herpes.

INTRODUCTION

Meningitis is a disease caused by the inflammation of the protective membranes covering the brain and spinal cord known as the meninges [1]. The inflammation is usually caused by an infection of the fluid surrounding the brain and spinal cord. Meningitis may develop in response to a number of causes, usually bacteria or viruses, but meningitis can also be caused by physical injury, cancer or the use of certain drugs [2, 3]. Bacterial meningitis is an inflammation of the meninges, including the pia, arachnoid, and subarachnoid space that occurs in response to infection with bacteria and/or bacterial products.

Meningitis is a significant cause of mortality and morbidity worldwide, with considerable variation in incidence depending on age and geographic location of the patient and causative agent [4-6]. Members of the *Mycobacterium tuberculosis* complex (MTC) contain the transposable element IS*6110* that, due to its high numerical and positional polymorphism, has become a widely used marker in epidemiological studies [7]. Herpes simplex viruses (HSV) persist in various cells of the host following primary infection [8]. Infection of Central Nervous System (CNS) by HSV is often associated with severe disease including encephalitis and meningitis. Meningitis resulting from infection with HSV-2 is usually self-limiting and seldom causes severe CNS symptoms as compared to HSV-1 induced encephalitis, the symptoms associated with HSV-2 encephalitis have been described as milder, often diagnosed as meningoencephalitis. Current study focuses on the molecular characterization of MTC and HSV1&2 in the cases with neurological disorders [9, 10].

MATERIALS AND METHODS

In this study, a total of 60 clinical samples with clinical manifestations for neurological disorders with symptoms which includes Partial or complete paralysis in some cases, muscle weakness, partial or complete loss of sensation, seizures, difficulty in reading and writing, poor cognitive abilities, altered sensorium, unexplained pain, hydrocephalus etc. were collected, 30 for MTC and 30 samples for HSV genotyping. Samples were collected from different departments of Shri Mahant Indresh Hospital, Dehradun that include neurosurgery, medicines, pediatrics. For the genotyping of herpes simplex virus 1 and herpes simplex virus 2 and *mycobacterium tuberculosis* complex characterization, isolation of DNA from all the CSF were done by silica column method. Isolated DNA was used as the template for the genotyping as both the method Real time PCR and conventional PCR method were considered for the genotyping of herpes simplex virus. In case of HSV 1 and 2, master mix was prepared for the glycoprotein gene. For *mycobacterium tuberculosis, IS6110* gene was amplified.

RESULTS

Out of 30 clinical samples, 06 CSF yielded positive amplicon with yielding 123 bp product for MTC infection. Accounting for 20% while 80% samples were found to be negative as shown in figure-1. ZN staining was performed for all the cases for CSF as non came positive by microscopy. For HSV genotyping., 02 cases came positive where those were the cases of viral meningitis.

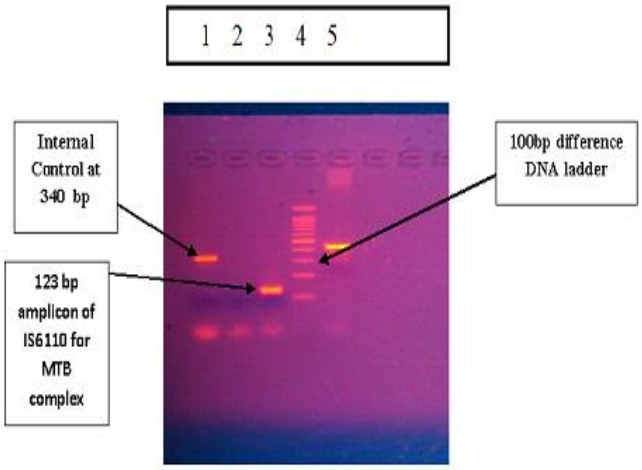

Fig-2: Agarose gel picture for MTC complex yielding amplicon of 123bp.

DISCUSSION AND CONCLUSION

Tuberculous meningitis (TBM) is the most severe form of infection caused by mycobacterium tuberculosis [11]. Causing death or disability in more than half of those affected, TBM is a form of meningitis characterized by inflammation of the membranes (meninges) around the brain or spinal cord and caused by a specific bacterium known as Mycobacterium tuberculosis [12]. TBM is usually found in children aged one to five years although it may occur at any age. HSV encephalitis is a serious infection but diagnosis previously required brain biopsy in certain cases due to low sensitivity of CSF culture and serology. PCR now allow the detection of HSV DNA from CSF with 95% sensitivity thus avoiding invasive brain biopsy. Viral meningitis, commonly caused by either enteroviruses or HSV is more reliably detected by PCR when compared to culture and in a shorter time (one verses up to 5 days). HSV PCR can be multiplexed with other pathogens responsible for meningitis. Genital ulceration due to HSV, usually due to HSV type II infection is now routinely detected by PCR in many clinical microbiology laboratories due to its increased sensitivity over viral culture. Genital herpes is the most common causes of genital ulcer disease in the developed world [13-15]. HSV 1 classically presents as herpes gingivostomatitis an infection of the oral mucosa. It can also cause conjunctivitis, keratitis, and herpetic whitlow. HSV 2 is most common cause of genital ulcer in the United States. More than 95% of recurrent disease is due to HSV 2. The main application for HSV subtyping is with regard to the clinical issue of recurrent infection. Most painful and annoying recurrent genital herpes is due to HSV 2, and almost all recurrent cold sores or fever blisters are due to HSV 1. However, genital herpes also can be caused by HSV 1. This type of genital herpes is much less frequently recurrent and each recurrence usually last only a few days. It has been documented that as many as one third of herpes infections are due to HSV 1, particularly in adolescent and young adult.

Conflict of Interest: None

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