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Research Article

MOLECULAR DETECTION OF HERPES SIMPLEX VIRUSES (HSV) AMONG ENCEPHALITIS PATIENTS IN CHENNAI

Saran N[1,2*] and Anandaraj B[2]

[1]Department of Virology, King Institute of Preventive Medicine and Research, Guindy, Chennai 600 032, TamilNadu, India
[2]Department of Microbiology, M.R.Govt Arts College, Mannargudi- 614001, TamilNadu, India

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A B S T R A C T

Human Herpes Simplex Viruses 1 and 2 (HSV-1 and HSV-2) are large genome DNA viruses that establish a persistent infection in sensory neurons and commonly manifest as recurring oral or genital erosions. Major infection is usually acquired in childhood and is most often asymptomatic, subsequent to which virus becomes latent in neurons of cranial nerve ganglia (HSV-1) or sacral ganglia (HSV-2). The present study aimed at the preliminary screening of suspected HSV encephalitis cases. A total of 100 cerebrospinal fluid (CSF) specimens were collected from clinically suspected HSV encephalitis cases representing various government and private hospitals of Chennai and were subjected to molecular detection by nested real-time Polymerase Chain Reaction (PCR) at the Virology Laboratory, King Institute of Preventive Medicine and Research (KIPM & R). The detection of HSV glycoprotein gene confirmed a total of 15 (15% of 100) samples as positive for HSV infection by PCR. In addition, three cases were also confirmed to be positive based on their cultivation in vero cell line.

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INTRODUCTION

The HSV-1 and HSV-2 are members of the subfamily *Alphaherpesvirinae* and encode 12 predicted glycoproteins that serve various functions, including cellular attachment, entry, and egress. Glycoprotein G is currently the target of an antibody test to differentiate HSV-1 from HSV-2 (Paz-Bailey *et al.*, 2007). Virus isolation by cell culture or rapid shell vial culture combined with immune fluorescence detection of virus antigen using type-specific monoclonal antibodies have been the methods of choice (Ashley, 1993). HSV can be isolated from various specimens such as swabs from mucocutaneous lesions, vesicle fluid, less frequently sputum, broncho-alveolar lavage, cerebrospinal fluid and conjunctival fluid or tissue biopsies. The highest isolation rates are obtained upon immediate inoculation of specimens on cell cultures. Although it is the gold standard, culture-based diagnostic tests are generally laborious and take longer duration to detect and may yield a high frequency of false-negative results due to the stage of the clinical lesion, inadequate collection of specimen, improper transport and storage conditions or inhibitory substances in the specimen.

Corresponding author:* **Saran N
Department of Virology, King Institute of Preventive Medicine and Research, Guindy, Chennai 600 032, TamilNadu, India

Direct virus antigen detection in clinical specimen either by ELISA or immune fluorescence is rapid but has a sensitivity of 80-90% compared to culture (Moseley *et al.*, 1998).

Serological detection of virus specific antibodies can provide retrospective information on a primary or recurrent infection. Further, the use of serological assays specific for glycoprotein G (IgG) could even distinguish between HSV-1 and HSV-2 infections (Lafferty *et al.,* 1983). However, the role of serological detection in rapid diagnosis is limited, as weeks have to elapse before a significant rise in antibodies is detectable. The use of PCR techniques has been largely restricted to the diagnosis of HSV encephalitis. Although numerous PCR assays have been established for detection of HSV and these assays have demonstrated to be significantly more rapid, sensitive and specific than virus culture (Wiedbrauk, 1993) and antigen assays, their implementation in routine diagnosis of HSV infections has been hindered by the high demands on laboratory logistics and personnel and the high contamination risks. This situation has been changed with the development of the real-time PCR, which has overcome the need for post amplification manipulation of the sample; it has a rapid turn-around time and high capacity.

Real-time PCR assays demonstrated to have sensitivity comparable to conventional PCR systems for detection of various viruses. In addition, real-time PCR assays can be combined with automated nucleic acid extraction systems

thereby providing rapid and highly standardized molecular diagnosis of HSV infections. In the present study, molecular characterization based HSV detection was done to confirm infection by the virus among suspected encephalitis cases. The specimens were also confirmed based on their culture in cell line.

MATERIALS AND METHODS

Study population

In this study, a total of 100 CSF samples were collected from highly suspected HSV encephalitis cases who reported at various government as well as private hospitals in and around Chennai in 2014 (ethical clearance number). The inclusion and exclusion criteria were suitably followed during the study. Similarly, the % positivity of HSV caused encephalitis was checked by employing appropriate controls. With the help of lab request form (LRF), complete details of patients such as the details on physical examination, their age, sex, contact history, date of onset, occupation were recorded and evaluated (Lafferty, 2000).

Virus and cell culture

HSV strain was propagated in Vero cells maintained in Eagle Minimal Essential Medium (EMEM) containing 2mM L-glutamine, 0.1mM essential amino acids, 1mM sodium pyruvate,10% fetal bovine serum (PR omega Scientific), penicillin (100 U/ml), and streptomycin (100 mg/ml) (Sigma, St. Louis, MO).

DNA extraction and Nested real-time PCR

The DNA extractions from CSF samples were done by using QIA amp Viral DNA KIT (Qiagen, USA). The extracted DNA (5µl) was detected with first set of HSV specific primers (OR-5'TCCGG GGCA GCAGGGTGCT3' and OF-5'ATCCGAACGCAGCCCCGCTG 3') for G gene by conventional nested PCR that yield a 320bp product. Subsequently, 2 µl of the PCR product was used for a second in circles of extension using the HSV specific nested oligo nucleotide primers IR-5'AGCTGTATASGGCGACGGTG3' and IF-5'GCGCCGTCAGCGA GGATAAC3'that yield a 282 bp of the first amplicons. The amplification was performed as follows - early on denaturation step at 94^{o} C for 5 minutes, followed by 45 cycles (each 94^{o}C for 45 seconds; 57^{o}C for 45 seconds and 72^{o}C for 1 minute and a final extension step at 72^{o} C for 10 minutes. The second round PCR followed by the first round amplification included 35 cycles with the same cycling conditions.

The PCR products were analyzed in 1.2% agarose gel and were viewed using Alpha Imager (Alpha InfoTech San Diego, California, USA) after staining with ethidium bromide and the follow- on bands were captured with a Polaroid camera.

Virus isolation by culture

The PCR detected positive cases were then proceeded with viral isolation. The samples were infected in the Vero cell line (African Green monkey kidney cell) to assure the presence of virus in the infected sample. The collected specimens were processed and 20 µl of viral sample was added in the Vero cells and was observed periodically for the viral infectivity.

RESULTS AND DISCUSSION

The molecular markers developed from known genomic sequence play a milestone in the diagnosis of infectious diseases. In this context, the present study portrayed the detection of HSV based on its glycoprotein gene which is involved in the replication and transcriptional signaling of HSV in host using PCR as well as viral culture methods. Accordingly, the % positivity of encephalitis by HSV was determined to be 15%. As a part of the study, three cases were also confirmed to be HSV positive based on viral growth in cell line.

Detection of HSV infection has been facilitated by the development of an antibody detection assay, but the utility of this assay was reported to be limited due to the window period of 2 to 6 months between infection and sero-conversion (Esteban *et al.*, 1996). Reverse transcriptase PCR (RT-PCR) has been used for effective means of detecting specific HSV viral sequences. It has also been reported that higher sensitivity could be reached in nested real- time PCR for HSV DNA detection and by following reverse transcription in which the amplified cDNA (using an outer set of primers) has been subjected to a second round of amplification with an internal set of primers, greatly increasing the amount of amplicons available for the HSV genome detection by ethidium bromide staining (Nakatsuji *et. al.,* 1992). In the present study, followed by the nested real-time PCR of the HSV samples, viral cell culture was also performed and analyzed.

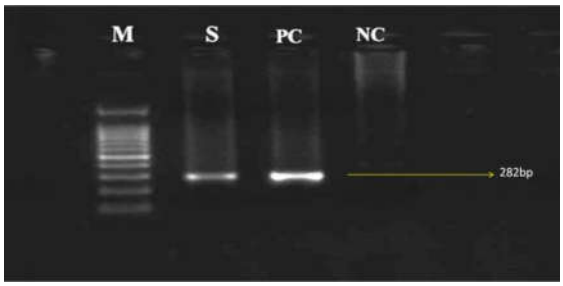

Fig 1 Molecular detection of HSV glycoprotein G gene (282bp) using nested real- time PCR

Lanes: M- Marker DNA ladder100-1000bp, S - CSF sample derived 282 bp HSV DNA product, PC - Positive control, NC - Negative control The HSV detection at molecular level was done by conventional nested real – time PCR and the oligonucleotide primers ultimately amplified a 282-bp region sequence of the glycoprotein (gp) G gene. In this analysis, the primers OR-5'TCCGGSGGCAGCAGGGTGCT3' (Sense) OF- 5'ATC CGAAC GCAGC CCCGCTG3' (Antisense) yielded a 380 bp product whose subsequent amplification using IR-5'AGCTGTATA SGGCG ACGGTG3' (sense) and IF-5'GCGCCGTCAGCGAGGATAAC3' (antisense) primers yielded the 282 base pairs product (figure 1) confirming the positivity of CSF samples for HSV among the encephalitis cases. In this context, the primers employed in the present study were highly helpful to detect the virus as well as to employ the target sequence as a marker for HSV diagnostics using conventional PCR.

The inoculation of viral specimen in Vero cell lines assisted in reconfirming the HSV in the test specimens. Upon 72 hours, the infected cells were observed with cell gap, bubbling in morphology with shrinkage of cells assured a cytopathic effect and the establishment of HSV in the Vero cells. The Herpes

simplex virus induced cytopathic effects (CPE) in the Vero cell lines on 3rd day are shown in figure 2. Finally, the Herpes simplex viruses were isolated and to the samples were preserved for additional characterization studies.

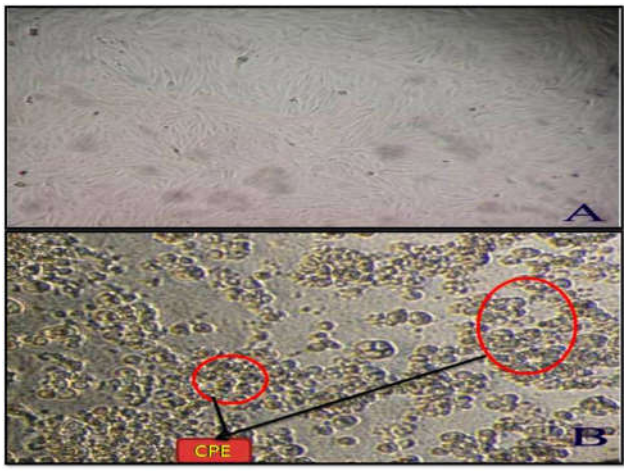

Fig 2 HSV virus in Vero cell line.

A - Normal Vero cells; B - HSV induced CPE (encircled) - cells with gaps, bubbling morphology and with shrinkage.

CONCLUSION

In the present study, a preliminary attempt was made as to detect the local strains of HSV at molecular level based on the primers designed and developed for the HSV envelope glycoprotein gene. It is optimistically expected that this would extremely be helpful to provide a promising diagnosis of infections caused by HSV circulating endemically in this region.

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