# Herpes Simplex Encephalitis Cases with Typical and Atypical Symptoms Confirmed by PCR-Amplification of the DNA Polymerase Gene

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**Objective: We have confirmed by PCR-amplification of the DNA polymerase gene, three cases of Herpes Simplex encephalitis (HSE) with atypical clinical symptoms.**

**Methods: Cerebrospinal fluid (CSF) samples were collected by lumbar puncture during the course of testing from 23 patients suspected to have viral encephalitis based on commonly encountered symptoms, cytochemical analysis and neurological testing. PCR was done on CSF using specific primers that amplify a 290 base pair sequence on the DNA polymerase gene common to both HSV-1 and HSV-2 strains.**

**Results: Only 3 of the 23 CSF samples collected showed the expected amplicon size on the target sequence. One case had conventional symptoms of encephalitis and 2 cases showed atypical symptoms from the conventional ones. All 3 patients recovered after administration of acyclovir. Fourteen PCR-negative patients were shown to have non-HSE viral encephalitis and the remaining 6 patients were shown later to have other neurological diseases.**

**Conclusion: PCR was efficient in elucidating the atypical cases of HSV encephalitis.**

***Key words:*** *HSV, encephalitis, PCR*

Herpes Simplex Encephalitis (HSE) is the most common cause of fatal viral encephalitis and hence it constitutes a clinical problem if prompt therapy is delayed. However, unlike most viral encephalitis for which there is only supportive management, HSE responds to specific drugs, mainly acyclovir (1). The outcome of the disease is influenced by how early in the illness acyclovir treatment is initiated (2). While the clinical presentation enables some physicians to treat empirically, it can hardly be considered specific for HSE (3). The typical clinical presentation of herpes encephalitis includes: alteration of consciousness, fever, headache, focal and generalized seizures, focal motor weakness, aphasia and personality changes. Polymerase chain reaction (PCR) of HSV DNA polymerase gene (4) was found to be sensitive, specific and rapid and consequently made a significant impact on management of HSE (5,6,7,8). PCR confirms within hours the clinical picture and other neurological and radiological tests (computed tomography (CT), electroencephalography (EEG) and magnetic resonance imaging (MRI) of the brain as well as cytochemical analysis of CSF that detect certain characteristic inflammatory changes (9).

In this study we used the PCR-amplification of the Herpes Simplex virus DNA polymerase gene to detect the virus in CSF specimens from patients suspected to have viral encephalitis and showing 1) a typical clinical picture, or 2) atypical symptoms. The PCR amplifies a sequence of the order of 290-bp present on the DNA polymerase gene of both HSV-1 and HSV-2. The primers used for the detection of HSV were designed by Espy et al. (4) and they amplify the two strains with equal sensitivity. As for their specificity, they only detect HSV and do not cross-react with varicella-zoster virus, cytomegalovirus, or Epstein-Barr virus (4).

## Material and Method

CSF was collected from 23 patients suspected to have viral encephalitis an admision based on the clinical picture which included: seizures, irritability, high fever, and altered consciousness. CSF for PCR analysis were collected with disposable tap needles. The specimens were aliquoted into cryovials (NUNC, Roskidle, Denmark) sterile tubes and stored at -20$^0$C until use. Additional clinical and laboratory information on patients with encephalitis or another neurological disorder was collected from the medical records or clinicians. The information included: 1) clinical picture of the patient on admission, 2) abnormalities on CT scan and/or EEG, 3) a characteristic pattern of CSF cytochemical changes, 4) the presence of specific antibodies at a significant level in CSF and blood, 5) response to acyclovir therapy when administered. Status of patients with HSE are presented in table I.

*PCR*: Ten microliters of untreated CSF was used directly in PCR after being boiled for 15 minutes. HSV-DNA, which served as positive control, was extracted according to the method of Van Ketel et al. (9). Two negative controls were employed in each PCR run to avoid contamination: 1) the amplification negative control, consisting of autoclaved UV radiated distilled water and 2) the extraction negative control. PCR-amplification was done on 10 µl of DNA lysate (positive control), the nega-

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tive controls, and the boiled CSF (4) in a MiniCycler (MJ Research, Watertown, Mass., USA). PCR primers, mix and PCR conditions used were done according to Epsy et al (4). Reverse transcription PCR (RT-PCR) using universal primers for enteroviruses were done on all CSF samples to rule out enteroviral infections (10). Amplicons were detected after electrophoresis in a 1.5% agarose (Sigma, St. Louis, Mo.) gel stained with (5mg/ml) ethidium bromide, observed under UV light, and photographed with type 667 Polaroid films. HSV PCR products were confirmed by Southern hybridization using a digoxygenin-labeled internal probe (4).

## Result and Discussion

Our data have shown that 3 of the 23 CSF samples have HSV-DNA prior to acyclovir treatment resulting in the expected 290-bp amplicon by PCR. All other patients were PCR-negative for HSV-DNA. All CSF samples were negative by RT-PCR using enteroviruses primers. Figure 1 shows a representative amplicon of HSV polymerase gene amplicon from the CSF of a patient. One case that was PCR positive for HSV was clinically suggestive of having HSV encepahlitis (Table I). However, the other 2 cases were suspected of having viral encephalitis, however their symptoms were atypical as compared to the conventional ones and these 2 were PCR positive for HSV (Table I).

Patient 1 was a ten-year old boy with coma, fever and

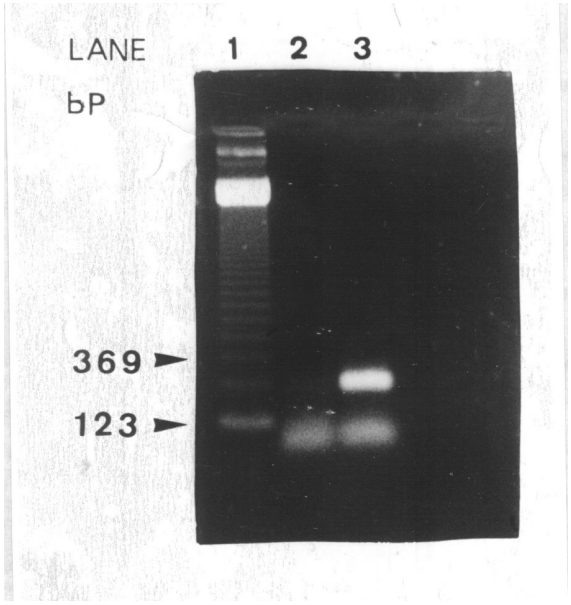


Figure 1. HSV DNA polymerase gene amplicons. Lane 1: 123-bp ladder, lane 2: negative control, lane 3: representative HSV DNA polymerase gene amplicon from CSF of a patient.

right focal seizures; his EEG showed left temporal spikes and his brain CT scan was normal. His CSF showed lymphocytosis. He responded to acyclovir and now has minimal residual behavioral problems. Patient 2 was a six and a six months old girl who presented with headache and vomiting. Her CT scan was normal and her EEG showed right temporal spikes. Her CSF showed lymphocytosis.

Table I. Status of three patients with Herp Simplex encephalitis

| Age | Clinical picture | CSF analysis | Neurediagnostik tests | Specific Ab for HSV | PCR of HSV | Response to acylovir therapy | Clinical diagnosis |
|---|---|---|---|---|---|---|---|
| 10 Y | Fever coma Right focal seizures | Glucose 87 mg/dl (NI) Protein 0,67 g/l (high) 36 WBC (all lymph) | CT scan normal EEG: Left temproral Spikers | ND | Positive | Good | Herpes Simplex Encephalitis |
| $6^{1/2}$ Y | Headache vomiting | Glucose 71 mg/dl (NI) protein 0.63 g/l (high) 96 WBC (all lymph) | ND | Positive | Good | Good | Herpes Simplex Encephalitis |
| 9 Y | Ataxia lethargy spasticity | Glucose 76 mg/dl (NI) protein 0.33 G/l (high) 2 WBC (all lymph | CT: adema, multiple areas of white and gray matter hypointsities MR: Areasof increased signal EEG: Slow bacground, no paroxysmal activity | ELISA for HSV IgG positive | Positive | Good | Herpes Simplex Encephalitis |

She improved on acyclovir treatment. Patient 3 was a nine-year old girl with ataxia, lethargy, then coma, one seizure, but no focal findings. Her EEG showed generalized slowing and her CT scan indicated diffuse brain edema and multiple areas of white and gray matter hypodensities. Her CSF showed lymphocytosis. She received acyclovir and her condition improved markedly. Later in follow-up, her serum was positive for HSV specific IgG antibodies.

Of the remaining 20 PCR-negative patients, 14 were diagnosed to have a non-HSV encephalitis, and 6 had other neurological disorders. These served as negative controls. Out of the 14 non-HSE cases, who ranged in age between 6 months to 12 years, two of the etiologies of viral encephalitis were known. One patient was diagnosed clinically, along with demonstration of elevated IgM titers by ELISA, to have Cytomegalovirus encephalitis. The patient showed improved clinical response only when gancyclovir treatment was given. The other patient had chicken pox symptoms in addition to encephalitis, and accordingly the final clinical diagnosis was Varicella encephalitis. In the remaining twelve cases, HSE was not confirmed by the information reported in the patients' records. They had symptoms of acute viral encephalitis such as a combination of fever, lethargy, and seizures, with no specific features for HSE. When available, the neurological test done showed non-specific changes such as non-specific generalized edema by CT scan of the brain. Six other patients were confirmed by their clinical records to have other neurological disorders. These had CNS involvement with leukemia or lymphoma, febrile seizures, brain malformation, peripheral neurological problems, and tuberculous meningitis.

In conclusion, three cases of HSE, one with typical and two with atypical clinical spectrum were uncovered by PCR of the HSV DNA polymerase gene. Detection of HSV DNA is considered to be associated with viral replication and HSV infection of the CNS (1,2). This indicates that since these patients harbored the virus as detected by PCR and responded to acyclovir treatment, the atypical symptoms they show may have to be considered in future cases as suggestive of HSE.

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