Differences in Laboratory Findings for Cerebrospinal Fluid Specimens Obtained from Patients with Meningitis or Encephalitis Due to Herpes Simplex Virus (HSV) Documented by Detection of HSV DNA

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Laboratory findings for cerebrospinal fluid (CSF) specimens were correlated with clinical presentations and histories in 55 cases of encephalitis or meningitis due to herpes simplex virus (HSV), as determined by polymerase chain reaction (PCR)–based detection of HSV DNA. Sixteen patients (29%) had HSV encephalitis (HSVE), 3 had mild or “atypical” meningoencephalitis, 34 (64%) had HSV meningitis (HSVM), and 1 had disseminated neonatal HSV infection. CSF findings included elevated leukocyte counts and/or elevated CSF protein levels in all HIV DNA–positive specimens. CSF leukocyte and protein abnormalities were more pronounced in cases of HSVM than they are in cases of HSVE. Patients with HSVE who had only mild CSF abnormalities also had minimal numbers of erythrocytes in the CSF. Patients with HSVM were younger than were patients with HSVE and were predominantly female. Eleven patients with HSVM reported having prior episodes, and 5 reported a history of recurrent headaches. These findings suggest that milder forms of HSV infection of the central nervous system may be identified by PCR for HSV. Prescreening of CSF specimens for the presence of leukocytes or elevated protein level may improve test utilization.

The rapid diagnosis of CNS infection with herpes simplex virus (HSV) is important because of the potential morbidity and mortality associated with HSV encephalitis (HSVE) and the availability of antiviral therapy (acyclovir) that is proven to ameliorate this disease. Without therapy, >70% of HSVE cases are fatal and ~11% of surviving patients recover normal function [1, 2]. Treatment with acyclovir has been shown to reduce the mortality rate to ~20% for patients of all ages [3, 4].

The broad spectrum of clinical syndromes associated with HSV infection of the CNS most commonly presents as 1 of 3 general entities: neonatal infection, encephalitis, or meningitis. Neonatal HSV infection, usually with dissemination and a mortality rate similar to that for HSVE, is most often caused by HSV type 2 (HSV-2) transmitted vertically during delivery [5]. HSVE presents with an acute onset, localized CNS findings, and necrosis of brain parenchyma (classically of the temporal and/or frontal lobes) and may occur in patients of any age beyond the neonatal period. It is the most common cause of sporadic fatal encephalitis in the United States (an incidence of 1 case per 250,000–500,000 persons per year) [6] and is predomi-
inantly caused by HSV type 1 (HSV-1; >85% of cases) [7–9].
Viral meningitis caused by HSV (HSVM) is a self-limited illness
of 2–5 days’ duration characterized by CSF findings of pleo-
cytosis and an elevated protein level with a normal glucose
level. The number of cells in the CSF may have a range of
5–3000 cells/mm³, but it is usually 100–400 cells/mm³ with
lymphocyte predominance. HSVM is commonly caused by
HSV-2 infection, and a recent study demonstrated a predomin-
inance in young adult women, with a ratio of affected women
to affected men of 6:1 [9]. It is also reported to be a sequela
of primary genital herpes in 36% of affected women and 13% of
affected men [10], but only 25%–33% of patients with
HSVM ever report a history of genital lesions [11, 12]. A subset
of these patients, possibly as many as 25% [9, 13], have re-
current bouts of HSVM separated by asymptomatic periods
that last from months to years, a syndrome first described by
Mollaret in 1944 [14].

Traditional methods of detection for laboratory diagnosis of
HSV infection of the CNS are either extremely invasive (brain
biopsy with culture or antigen detection in tissue specimens
[8, 15]) or insensitive (culture of CSF specimens yields positive
results in only 4% of biopsy-proven cases of HSVE [8]) or they
lack clinical significance (HSV antibody levels in CSF take 1–4
weeks to reach diagnostic levels [8, 13, 16, 17]). Since the initial
PCR-based detection of HSV DNA in CSF specimens obtained
from patients with HSVE by Rowley et al. [18] in 1990, nu-
merous reports of the utility of PCR for the diagnosis of HSVE
[19–27] and HSVM [9, 12, 27–32] have been published. In
patients with HSVE, PCR-based detection of HSV DNA in CSF
specimens has sensitivity and specificity comparable or superior
to those of brain biopsy, and it is now considered the reference
standard for the laboratory diagnosis of HSVE [19, 27]. With
PCR-based detection, HSV-2 DNA has also been reported in
an increasing number of patients with recurrent meningitis [7,
9, 12, 13, 27–29].

PCR has been used to detect HSV DNA in the CSF of patients
who present with “atypical” clinical syndromes. PCR detection
has facilitated the diagnosis of mild atypical meningenceph-
alitis, a syndrome possibly due to HSV infection and/or re-
activation that is characterized by meningeal and neurological
signs that spontaneouly resolve without sequelae [7, 33, 34].
PCR-based detection of HSV DNA in CSF specimens obtained
from patients with radiculomylitis has also been reported [35,
36]. Bell’s palsy [37, 38], cluster headaches [39, 40], migraines
[41], and even common recurrent headaches [42] all have been
suggested to have HSV infection or reactivation as a cause.
PCR-based detection may identify those patients who could
benefit from antiviral therapy.

This study reports the experience of PCR-based detection of
HSV DNA in CSF specimens with an assay developed “in-
house” at a large, urban general hospital. The purpose was to
correlate laboratory findings in CSF with clinical presentations
and histories to better define CNS disease due to HSV infection
and to improve laboratory diagnosis.

METHODS

Samples were submitted from 3 hospitals in the Partners
Healthcare System (Boston): 2 large, urban academic medical
centers and 1 suburban community hospital. The method used
for HSV DNA amplification was modified from the method of
Espy et al. [43] with use of primers complementary to a region
of the DNA polymerase gene specific for HSV: 5′-TAGATCGG-
CGTCATCTGCAGGG-3′ and 5′-CAGTTCGGCCTGAGAGAA-
CAAAG-3′. The primers produce a 291-bp amplicon that
cannot discriminate between HSV-1 and HSV-2 [44]. DNA
was isolated from 200 μL of a CSF specimen by use of the QIAamp
DNA isolation kit (Qiagen). Negative controls (water as DNA
template) and positive controls were included with each assay.
Positive controls were a mixture of either HSV-1 or HSV-2
DNA (VR-539 or VR-540, respectively; American Type Culture
Collection) diluted to the detection limit of the assay (60 copies
of template DNA per reaction). Sample specimens spiked with
positive control DNA were also run in parallel to control for
the presence of PCR inhibitors in human CSF, but this pro-
cedure was discontinued in January 1999 because of the low
level of inhibition detected (only 3 [0.67%] of 446 samples).

Amplicons were electrophoresed in 2% agarose gels and
stained with ethidium bromide. Amplicons comparable in size
to the positive control (291 bp) were processed for confirmation
by Apa I restriction digestion. A positive result for HSV-1 or
HSV-2 digested by Apa I yielded 96-bp and 195-bp fragments.
Once the test yielded a confirmed positive result, clinical and
laboratory data for the case were reviewed retrospectively. Clin-
ical data were obtained from discharge summaries written by
the admitting house staff and by telephone when the positive
results were reported to the clinician. Clinical and laboratory
data were obtained for 54 cases. On the basis of the clinical
diagnosis, cases were divided into 4 categories: neonatal infec-
tion, severe encephalitis (HSVE), a milder atypical meningo-
encephalitis, and meningitis (HSVM). Differences of the mean
values between the HSVE and HSVM groups were analyzed
with a 2-tailed t test.

Most CSF specimens submitted to the laboratory for HSV
PCR were assayed without established acceptance criteria. This
policy was changed on 1 September 2000 to a requirement of
a CSF leukocyte count of >5 cells/mm³ and/or an abnormally
elevated CSF protein level (>55 mg/dL). This policy was im-
plemented after a retrospective review of local hospital cases
and publication of a report that failed to demonstrate HSV
DNA–positive cases without abnormal CSF findings [45]. If
clinical suspicion of virus-associated CNS infection was an ur-

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gent consideration, the physician contacted the laboratory, and HSV PCR amplification was done despite the patient having normal CSF parameters.

Protocols for review of medical records and laboratory data were approved by the Human Research Committee of the Massachusetts General Hospital (IRB 2001-P-000828/1).

RESULTS

From November 1996 through February 2001, 2759 CSF samples were submitted for HSV PCR analysis. All requested tests were done until 1 September 2000, when test criteria (i.e., CSF WBC count of $>5$ cells/mm$^3$ and/or CSF protein level of $>55$ mg/dL) were implemented. Before 1 September 2000, 2353 CSF specimens were tested, and 45 (1.9%) were positive for HSV DNA. From 1 September 2000 through February 2001, 406 specimens were submitted and 153 were rejected on the basis of test criteria. Ten of the specimens tested positive for HSV DNA, which is 2.5% of the submitted specimens and 4.0% of the tested specimens, and which represents a 2-fold increase in the positivity rate after implementation of the test criteria. Test utilization improved, with a 38% reduction in the number of specimens analyzed after implementation of these acceptance criteria.

Of the 55 HSV DNA–positive cases, 20 (36%) occurred in male patients and 35 (64%) occurred in female patients. The age range was 0.1–86 years (mean age, 44.1 years). Patient outcomes and a synopsis of herpes-related and CNS-related medical histories are shown in table 1 for HSVE compared with HSVM. Only 1 case of disseminated neonatal infection was reported (see below).

Sixteen cases of HSVE (29% of all positive cases) were identified, and patients had an age range of 1.3–86 years (mean, 57.2 years) and a ratio of male to female patients of 1:1.3. Clinical information was available for 15 of the 16 cases of HSVE. All of these patients presented with mental status changes and/or seizures, and 14 of 15 also had documented fever. CT or MRI of the brain also demonstrated abnormalities consistent with HSV infection in 14 of 15 cases. Culture of CSF specimens for virus were performed for 5 patients, but only 1 specimen yielded virus (HSV-1 in the patient with herpes labialis). All of the patients with HSVE received acyclovir, and 3 died despite receiving therapy. Ten of the 13 survivors required transfer to rehabilitation facilities when they were discharged from the hospital.

Thirty-four cases of HSVM (62% of HSV DNA–positive cases) were documented in 33 patients. Ages ranged from 20 to 66 years (mean, 37.9 years) with a ratio of male to female patients of 1:2.8. All patients with HSVM presented with headaches, and a majority also presented with fever and/or men-

| Table 1. Clinical data retrospectively obtained from medical records of patients with herpes simplex virus (HSV) DNA in CSF. |
|---------------------------------|-----------------|-----------------|-----------------|
| Finding or history              | HSVE$^a$ (n = 16) | HSVM (n = 34) | Total$^b$ (n = 55) |
| Cutaneous lesions               | 1 / 16           | 5 / 34         | 8 / 55          |
| Positive CSF culture            | 1 / 16           | 1 / 34         | 3 / 55          |
| Positive results of CT or MRI of head | 14 / 15$^c$      | 0 / 12         | 15 / 31         |
| HIV type 1 positive             | 0 / 2            | 2 / 15         | 2 / 19          |
| History                         |                  |                |                 |
| Herpes genitalis                | NA               | 10             | 11              |
| Herpes labialis                 | 1                | 1              | 2               |
| Recurrent meningitis            | NA               | 11             | 11              |
| Outcome                         |                  |                |                 |
| Died                            | 3                | 0              | 3               |
| Transferred to other hospital or rehabilitation unit | 10 | NA | 13 |
| Discharged to home              | 3                | 31             | 35              |
| Received acyclovir therapy      | 16               | 23             | 43              |

NOTE. Data are no. of patients or no. of patients with characteristic/no. tested. HSVE, HSV encephalitis; HSVM, HSV meningitis; NA, not available.

$^a$ There was no history available for 1 case.

$^b$ There was no clinical information available for 1 case, 1 case occurred in a neonate (described in text), and 3 cases were mild “atypical” meningoencephalitis (described in text). No outcome data were available for 4 cases.

$^c$ HSV type 1.

$^d$ HSV type 2.

$^e$ All patients showed temporal and/or frontal lobe involvement, except 1 that showed thalamic involvement.
ingismus (n = 22 and 20, respectively). Photophobia and/or nausea or vomiting were reported in 35% and 44% of patients, respectively. Only 5 patients had skin lesions suggestive of herpes simplex (4 patients had genital lesions and 1 patient had oral lesions). Five cultures of CSF specimens for virus were performed, and 1 specimen yielded virus (HSV-2 in a patient with no history or symptoms of genital herpes simplex). Twelve patients underwent brain imaging studies, but in contrast to the results for patients with HSVE, none of the results were positive. Fourteen patients with HSVM (41% of all HSVM cases) reported recurrent headaches, “migraines,” or aseptic meningitis. One patient (a 32-year-old woman) had HSV DNA–positive CSF specimens during her second and third episodes of meningitis, which were 7 months apart. She was not tested for HSV DNA during her first episode 2 years before and had no history of genital herpes.

A single case of disseminated neonatal infection (data not shown) occurred in a baby born during the 33rd week of gestation after preterm premature rupture of membranes. He developed a vesicle on his scalp a few days after delivery that culture verified was due to HSV-2 infection. He developed apnea at 8 days of age, and PCR of a CSF specimen yielded positive results for HSV DNA. Laboratory studies of CSF specimens also revealed an elevated leukocyte count and protein level (WBC count, 570 cells/mm³; protein level, 242 mg/dL). This patient survived after receiving acyclovir therapy.

Three cases with positive results of PCR for HSV DNA in CSF were diagnosed as mild atypical meningeecephalitis. Laboratory analysis of CSF specimens obtained from all 3 patients showed elevated leukocyte counts with lymphocyte predominance and elevated protein levels (CSF WBC count, 300–730 cells/mm³ [mean, 506 cells/mm³]; CSF protein level, 103–930 mg/dL [mean, 379 mg/dL]). All patients with mild atypical meningeecephalitis who received acyclovir therapy survived.

A comparison of the laboratory findings for CSF specimens obtained from patients with HSVM and HSVE documented by HSV DNA–positive CSF specimens is shown in table 2. All patients with HSV DNA–positive CSF specimens had elevated CSF leukocyte counts and/or protein levels. For patients with HSVE, CSF leukocyte counts had a range of 2–667 cells/mm³ (mean, 202 cells/mm³), whereas the mean CSF leukocyte count for all patients with HSVM was 484 cells/mm³ (P < .004). Three patients with HSVE (19%) had CSF leukocyte counts of <10 cells/mm³; 4 patients with HSVM (12%) had CSF leukocyte counts of <100 cells/mm³ (range, 58–98 cells/mm³). CSF protein levels were also lower in patients with HSVE (mean, 73 mg/dL) than they were in patients with HSVM (mean, 129 mg/dL; P < .0001). Four patients with HSVE (25%) had CSF protein levels of <55 mg/dL (range, 22–48 mg/dL), which is the upper limit of normal for this laboratory. There was no significant difference in the number of RBCs in CSF between the 2 groups: although several patients with HSVE had markedly elevated RBC counts (>5000 cells/mm³), for 6 patients, the RBC count was ≤3 cells/mm³.

**DISCUSSION**

This study represents the first direct comparison of CSF laboratory findings for HSV encephalitis and meningitis in patients with known HSV DNA status. The results demonstrate that elevations in CSF WBC cell counts and protein levels are greater in cases of HSVM than they are in cases of HSVE. CSF RBC counts were unreliable in distinguishing patients with HSVE from those with HSVM: although RBC counts were markedly elevated (>5000 cells/mm³) in CSF specimens obtained from several patients with HSVE, 6 had RBC counts of ≤3 cells/mm³.

In all HSV DNA–positive cases, patients had elevated leukocyte counts or protein levels in CSF, regardless of clinical presentation. This suggests that prescreening of CSF specimens may be used to improve test utilization. The subtle CSF abnormalities seen in some cases of HSVE (especially those with minimal numbers of CSF erythrocytes) indicate that caution should be exercised when screening specimens obtained from patients with suspected cases of HSV infection prior to PCR amplification. This cautious approach is particularly important earlier in disease presentation or among immunocompromised patients, because HSV with normal CSF parameters has been reported in immunocompromised patients [7]. In 1999, Tang et al. [45] first demonstrated the use of CSF parameters to

| Table 2. Laboratory findings for CSF specimens obtained from patients positive for the presence of herpes simplex virus (HSV) DNA in CSF by PCR. |
|-----------------|-----------------|-----------------|-----------------|
| Finding         | HSVE group      | HSVM group      | P               |
| Leukocyte count, cells/mm³ | 202 (2–667) | 484 (58–1888) | <.004           |
| Percentage of leukocytes | 76 (16–97) | 87 (43–100) | <.11            |
| Erythrocyte count, cells/mm³ | 2518 (0–27,566) | 54 (0–711) | <.21           |
| Protein level, mg/dL | 73 (22–146) | 129 (75–281) | <.0001         |

**NOTE.** HSVE, HSV encephalitis; HSVM, HSV meningitis.
screen specimens before PCR analysis for HSV DNA detection. Of 723 CSF specimens tested for HSV DNA, none of the 24 positive specimens had CSF leukocyte counts of <5 cells/mm³ and CSF protein levels of <45 mg/dL [45]. They also showed that, by screening CSF specimens a priori for leukocyte counts and protein levels with use of these criteria, they could reduce their workload by 29% without missing any HSV-positive CSF specimens [45]. Our experience showed that, after introduction of test criteria, a 38% reduction in the number of specimens analyzed was realized without a proportional decrease in the number of positive results. The positivity rate was 4.0%, approaching the rates (7.1% and 7.6%) obtained when the test was specifically used for cases with a high degree of suspicion for HSV in prior studies [7, 27].

Comparison of CSF laboratory findings demonstrates milder abnormalities in HSVE than in HSVSM and suggests that care should be taken when interpreting CSF findings in patients with encephalitis. A prior report correlated CSF findings with HSV type among patients with HSV DNA detected in CSF specimens by PCR and demonstrated similar results [32]. They detected HSV DNA in 33 (1.5%) of 2233 specimens submitted; 20 specimens had HSV-1, 6 had HSV-2, and 7 had HSV-1 or -2. From prior experience [7–9], it can be assumed that the cases due to HSV-1 were predominantly encephalitis, and CSF leukocyte counts ranged from 0 to 575 cells/mm³ (mean, 102 cells/mm³) and CSF protein levels ranged from 30 to 130 mg/dL. Cases due to HSV-2, which are most likely HSVM, were associated with CSF leukocyte counts ranging from 116 to 1188 cells/mm³ (mean, 553 cells/mm³) and CSF protein levels ranging from 86 to 170 mg/dL (mean, 110 mg/dL) [32].

PCR-based detection of CNS infections with HSV has yielded increased appreciation of mild CNS disease, including HSVSM. Almost one-half (41%) of the patients with HSVSM described here had prior CNS symptoms in the form of aseptic meningitis and/or recurrent headaches or “migraines,” even though only 15% reported a history of herpetic rash. With the identification of more patients having recurrent meningitis associated with HSV infection by use of PCR of CSF to detect HSV, it is possible to identify patients with mild disease and to determine whether antiviral therapy offers any therapeutic advantage to such patients. Anecdotal reports have already suggested that acyclovir may reduce recurrences of symptoms in such patients [46, 47].

In summary, abnormalities (elevated CSF leukocyte count and/or protein level) were present in all CSF specimens that were positive for HSV DNA by PCR. These CSF abnormalities were milder in cases of HSVE than in cases of HSVSM, especially when minimal numbers of CSF erythrocytes were also present. Nevertheless, the findings indicate that CSF parameters may be used to screen specimens before analysis for HSV DNA by PCR. However, caution should be exercised when the patient is immunocompromised, because the patient may have HSVE with normal CSF parameters.

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