Prevalence of Herpes Simplex Virus (Types 1 and 2), Varicella-Zoster Virus, Cytomegalovirus, and Human Herpesvirus 6 and 7 DNA in Cerebrospinal Fluid of Middle Eastern Patients with Encephalitis

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HSV-1 DNA was detected in 32 (30%) of 106 cerebrospinal fluid samples from patients with encephalitis. Cytomegalovirus, varicella-zoster virus, and human herpesvirus 6 (HHV-6) DNAs were each detected in three patients (3%); herpes simplex virus type 2 (HSV-2) and HHV-7 PCRs were negative. HSV detection was associated with seizure ($P = 0.02$), especially focal seizure ($P = 0.0002$), and pathological computed tomography ($P = 0.02$) with focal lesions ($P = 0.0004$).

Herpesviruses, especially herpes simplex virus (HSV), varicella-zoster virus (VZV), and human herpesvirus 6 (HHV-6), play a major role as a cause of meningitis or encephalitis (8, 9, 13). Viral encephalitis is associated with significant mortality and morbidity, and its outcome is directly correlated with the rapid onset of antiviral therapy (12, 15). Therefore, the appropriate management of encephalitis needs techniques suitable for a rapid diagnosis, such as PCR (1, 10).

The aims of this study were to evaluate the prevalences of HSV-1, HSV-2, VZV, cytomegalovirus (CMV), HHV-6, and HHV-7 in cerebrospinal fluid (CSF) samples from Middle Eastern patients with encephalitis or meningitis, as well as in controls, and to correlate virus detection with clinical symptoms.

CSF was obtained from 106 patients with encephalitis and 11 patients with meningitis, and the patients had a mean age of 4 years (1 month to 40 years). Sixty-one samples (52%) were taken 1 to 5 days after the onset of symptoms, 33 samples (28%) were taken 6 to 10 days after symptom onset, and 23 samples (22%) were taken after 11 days after symptom onset. Patients were considered to have meningitis when at least one of the following symptoms was present and other causes were ruled out: nuchal rigidity, Kernig's sign, Brudzinski's sign, and Lasegue's sign. Encephalitis was suspected when an altered level of consciousness or significant change in personality or cognitive dysfunction or focal neurological symptoms not explained by cranial nerve paralysis persisted for ≥ 24 h and other causes were excluded. In all patients, the disease was accompanied by at least two of the following symptoms: headache, nausea, temperature of $\geq 38^\circ\text{C}$, and pleocytosis (>5 white blood cells/ μl CSF). As controls, 35 patients (mean age, 2 years [2 months to 12 years]) with noninfectious neurological disorders or with infectious but nonneurological diseases were enrolled.

DNA was extracted by use of the QIAamp tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA was detected by a hot-start real-time PCR on the LightCycler instrument (Roche, Mannheim, Germany), as previously described in detail for HSV-1, HSV-2, and CMV (5). For VZV, HHV-6, and HHV-7 PCRs, a similar PCR was performed using the following primers and probes: for HHV-6, primers 5'-GCTTTTCTAGCCGCTCTTC3' and 5'-CCACATCTATAATTTTAGACGATCCC3', fluorescein probe 5'-CCA TATCTACAAACTTAGGTGCTGGGTGA3', and Red640 probe 5'-GTTGTAAGTGTTGCGATATCTTTAGCTTCC3'; for HHV-7, primers 5'-TATCCAGCTGTTTTTCATATAGTAAC3' and 5'-TTGCGGTAGCACTAGATTTTTTG3', fluorescein probe 5'-CATTTGTACTTCAAAGTAGCCTTCATTAGGATAGCT3', and Red640 probe 5'-GACACCAACATCTGTCTATCATTTCTGGATGAA3'; and for VZV, primers 5'-TTGAGGAAGTTGAAGCCAGATCA3' and 5'-TCCAGTTC CAACCAACCGTTA3', fluorescein probe 5'-TGAAAACGA TCCATTGCATAATTGGAAT3', and Red640 probe 5'-TTC TCTGTATGCACCATCCCGTAGG3'. Annealing temperature was 64°C for VZV and HHV-6 and 61°C for HHV-7. Inhibition and extraction control was performed by a second extraction and PCR with the same sample spiked with positive control before extraction. Statistical analysis was done by Fisher's exact test and the Mann-Whitney U test.

HSV-1 DNA was detected in 32 patients out of 106 with encephalitis (30%); none of the patients with meningitis alone was HSV DNA positive. Other herpesviruses were present at a much lower rate. VZV DNA was detected in three patients (3%) with encephalitis and in one with meningitis (9%). DNAs of CMV and HHV-6 were each found exclusively in three patients with encephalitis (3%). HSV-2 and HHV-7 were not detectable. Double infections were found in two patients (HSV/CMV and HSV/HHV-6). Together, herpesviral DNA was found in 39 patients (37%) with encephalitis and at a much lower frequency in patients with meningitis (1 patient, 9%). By contrast, all 35 samples of the control group were negative. When the sampling time was taken into consideration, the rate of PCR-positive samples was highest within days 6 through 10

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TABLE 1. Historical, clinical and neurodiagnostic data of 32 HSV-1 PCR-positive patients compared to 74 HSV-1 PCR-negative patients, both groups with encephalitis

Clinical parameter	Value ^a for indicated group		<i>P</i>
	HSV-1 DNA positive (<i>n</i> = 32)	HSV-1 DNA negative (<i>n</i> = 74)	
Epidemiological data			
Age (range)	4.8 yrs (2 mos–40 yrs)	4.4 yrs (8 mos–24 yrs)	0.54
No. of males	14 (44)	41 (55)	0.30
Clinical presentation			
Fever of $\geq 38^{\circ}\text{C}$	29 (91)	58 (78)	0.17
Alteration of consciousness	21 (66)	56 (76)	0.34
Vomiting	11 (34)	37 (50)	0.20
Upper respiratory infection	12 (38)	28 (38)	1.00
Labial herpes	3 (9)	3 (4)	0.36
Seizure	24 (75)	36 (49)	0.02 ^d
Focal vs. generalized	13/11 (54)	6/30 (17)	0.004 ^d
Focal	13 (41)	6 (8)	0.0002 ^d
Brain computed tomography ^a			
Abnormalities (total)	22 (85)	30 (52)	0.02 ^d
Focal vs. generalized	16/6 (73)	9/21 (30)	0.005 ^d
Focal lesions	16 (62)	9 (16)	0.0004 ^d
CSF findings			
WBC ^b /mm ³			
0–5	17 (53)	36 (49)	0.83
6–100	10 (31)	28 (38)	0.66
>100	5 (16)	10 (14)	0.77
Erythrocytes	8 (25)	21 (28)	0.82
Protein, >0.4 g/liter	12 (38)	26 (35)	0.83
Therapy (acyclovir therapy)	18 (56)	40 (54)	1.00
Outcome ^c			
Death vs. survival	13/19 (41)	13/56 (19)	0.03 ^d
Survival with neurological sequela vs. full recovery	4/15 (21)	15/41 (27)	0.76

^a No CT was performed for 6 patients with HSV DNA detection and for 16 patients without HSV DNA detection.^b WBC, white blood cell count.^c Five patients without HSV DNA in CSF were not followed up.^d Significant results were *P* values of <0.05.^e Unless otherwise indicated, values are numbers of patients (percentages of total).

after the onset of symptoms (48%). Sampling at days 1 to 5 revealed a much lower rate (25%) (*P* = 0.02), as did sampling >10 days after the onset of symptoms (35%).

To identify historical, clinical, and laboratory abnormalities associated with herpes simplex-positive encephalitis, the data from the 32 patients with HSV DNA in CSF were compared with those of 74 HSV-negative patients with encephalitis (Table 1). The clinical presentations differed significantly only in the numbers of patients with seizures (*P* = 0.02) and in particular, seizures of the focal type (*P* = 0.0002). This is also reflected by the higher number of pathological computed tomography scans (*P* = 0.02), and again, the focal appearance of lesions (*P* = 0.0004). In addition, the outcome of the encephalitis was worse in patients with HSV-1 encephalitis. All other clinical, historical, and laboratory data were not significantly different.

The present study covered mainly pediatric patients from Syria. Herpesviral DNA was strongly predominant (37% in patients with encephalitis). HSV encephalitis is known to occur at any age; however, it has a bimodal distribution, with one-third occurring at an age of less than 20 years (14). The prev-

alence of HSV-1 is high in Syria, reaching 80% even at a young age of 6 to 10 years (6), explaining the elevated numbers of HSV encephalitis in these pediatric patients. The failure to identify HSV-2 likewise reflects the epidemiology of rarely seen HSV-2 infections in Syria (6). Although HHV-6 and CMV are known to cause encephalitis in rare cases (7, 11), it cannot be excluded that contamination by leukocytes may account for the few PCR-positive subjects. Of note in this context, HHV-6 genome and antigens are frequently detected in patients with multiple sclerosis, suggesting that the virus may persist in brain tissue even without causing encephalitis (2). The low prevalence of VZV in CSF is most likely reflected by the very low number of patients with symptoms compatible with chickenpox or herpes zoster (two patients, 2%).

Importantly, several investigators have reported patients with HSV encephalitis that were negative by CSF PCR (1, 4, 10). Obviously, different factors, including the time of sampling of the CSF specimens, may be critical. We have observed that HSV DNA may be more readily detectable in the later phases after the onset of symptoms and not in the very acute phases, in accord with earlier reports (1, 3). It might be speculated that

especially focal lesions in the very early course do not necessarily result in viral DNA in CSF.

In conclusion, we have identified HSV-1 as an important agent of viral encephalitis in mainly pediatric patients from Syria, whereas VZV, CMV, and HHV-6 were rarely seen, and the two herpesviruses HSV-2 and HHV-7 were never detected. Seizures of the focal type and focal lesions in the computed tomography scan were significantly more frequently associated with PCR-established HSV encephalitis than with DNA-negative encephalitis.

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